Gametocyte and gamete development in *Plasmodium falciparum*

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[Plates 1–6]

The ultrastructural organization of the mature gametocytes of *Plasmodium falciparum* isolated from the peripheral circulation of naturally infected Gambia is examined and compared with immature forms obtained from the peripheral circulation of a chloroquine treated patient. The latter are recognized as the stage 2 and 3 developmental forms (Hawking, Wilson & Gammage 1971 Trans. R. Soc. trop. Med. Hyg. 65, 549–559) observed by light microscopy and are distinguished in the electron microscope by three characters; (i) they do not fill the host cell, (ii) they contain few, if any, osmiophilic bodies, (iii) they possess an extensive subpellicular tubule system. Maturation (capacitation) of these immature parasites takes many days and is followed by an extended period of maturity during which the gametocytes will exflagellate.

Mature macro- and microgametocytes have numerous characters in common with the gametocytes of avian and reptilian Plasmodiidae, namely a tripartite pellicle, cristate mitochondria, a comparatively high density of osmiophilic bodies in the macrogametocyte, cytostomal feeding, Golgi body, and persistent nucleolus in the female gametocyte. These similarities together with the unexpected nuclear changes detected in macrogametogenesis suggest that *P. falciparum* is best considered as pre-dating the ‘malariae’ and ‘vivax’ groups and not as having evolved from them.

Light microscopy, scanning and transmission electron microscopy and videotape analyses of gamete formation were undertaken. Nuclei in the mature gametocytes are Feulgen negative but upon activation rapidly become Feulgen positive. The gametes also are Feulgen positive. The crescentic parasites swell to become large spherical cells and escape from the host cell by osmotic or enzymic activity. The microgametocyte undergoes three mitotic divisions during which the chromosomes are sequentially reduced in number such that ca. 7 are incorporated into each gametic nucleus. The microtubule organizing centre (m.t.o.c.), which in the mature gametocyte is associated with the intranuclear body, is attached to the centriolar plaque of the first division spindle. There it differentiates into kinetosomes which act as foci for the polymerization of axonemes. The
Kinetosomes and axonemes remain attached to the centriolar plaques during division and are segregated synchronously with the genome at each division. Subsequently one axoneme enters each haploid gamete at exflagellation. Exflagellation is accompanied by a significant reduction in microgametocyte volume which is associated with an increase in density of the cytoplasm. The female gametocyte does not decrease in volume but undergoes nuclear changes in which a single pole of an intranuclear spindle is detected. Comparisons are made with macrogametogenesis in avian malarial parasites from which it is suggested that this spindle, if not half of a normal mitotic spindle, is an atavistic trait. The possibility of a meiotic gametic division is discussed but discounted.

The activity pattern of the microgamete was found to be similar to that of other malarial parasites, with states of high and low activity or immobility. High activity, which results in rapid movement through the medium, is produced by long wavelength (12 μm), low amplitude (1.1 μm) waves generated at ca. 12 waves per second; low activity, which results in contorted gyration on the spot, is produced by long wavelength (14.1), high amplitude (2.3) waves produced at ca. 1 wave per second. Following an initial period of continuous activity the gamete usually alternates between high and low activity states. Subsequent low activity and immobility is in turn followed by death. Microgamete activity was profoundly affected by the plasma of some patients, presumably as a result of the antigametocyte antibodies present.

The microgamete contains a single axoneme, at one end of which lies the kinetosome with the juxtakinetosomal sphere and granule. It is this end which emerges first from the parental gametocyte. A single nucleus is centrally located in many microgametes although 23% are anucleate.

**Introduction**

The gametocytes of *Plasmodium falciparum* contrast markedly with those of the majority of other mammalian malarial parasites, because of their unusual crescentic shape and prolonged development in the visceral blood (Hawking et al. 1971; Smalley 1976), which is followed by the appearance of a wave of mature gametocytes in the peripheral circulation of the host.

Gametocytogenesis has been examined cytologically in the blood of naturally infected patients (Thomson 1914; Garnham 1931; Thomson & Robertson 1935; Field & Shute 1956) and *in vitro* (Mitchell, Butcher & Cohen 1976; Philips, Wilson & Pasvol 1976; Smalley 1976).

Cytological studies of the mature crescents have drawn attention to the unusual nuclear organization of the parasite, the appearance of a darkly staining capsule, and to large parasite-induced erythrocytic inclusions, the so-called ‘Garnham bodies’ (Garnham 1931; Thomson & Robertson 1935). The apparent occurrence of more than one nucleus in some gametocytes led to conflicting opinions on the position of the meiotic division in the cell cycle, some suggesting that it occurred during gametocytogenesis (Garnham 1931; Thomson & Robertson 1935), with even the possibility of a parthenogenetic origin of schizonts being entertained and
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others holding that there was a gametic meiosis (MacDougal 1947; Lüdicke & Piekarski 1951).

Gametocyte ultrastructure has been examined only in the mature intraerythrocytic forms of *P. falciparum* (Smith, Theakston & Moore 1969; Kass et al. 1971; Aikawa & Ward 1974) the first of the two studies commenting upon the unexpected similarity between these gametocytes and those of avian malarial parasites.

In this study the ultrastructural organization of immature and mature gametocytes of *P. falciparum* has been examined in the blood of naturally infected patients. Cytological studies of micro- and macrogametogenesis *in vitro* have been related to behavioural studies of gametocyte activation and gamete movement.

The phylogenetic relation of *P. falciparum* with other malarial parasites of mammals and birds are considered in the light of present findings and the significance of nuclear behaviour in micro- and macro-gametogenesis are discussed.

**Materials and methods**

Carriers of *P. falciparum* gametocytes were identified among patients attending the outpatients' department at the M.R.C. Laboratories, Fajara. With one exception (H.N.) blood samples were taken into heparinized syringes before oral administration of a therapeutic dose of chloroquine.

**Examination of immature gametocytes**

Light microscope specimens were examined as Giemsa-stained thin films. Sequential observation of gametocyte maturation in patent H.N. were made every 6 h on samples taken by thumb prick. Gametocyte density and exflagellation were estimated by the methods of Smalley & Sinden (1977). For transmission electron microscopy (t.e.m.), blood was dispensed immediately into cooled fixative. Fixation was for 1 h at 4 °C in 2.5% (by vol.) glutaraldehyde in 0.05 M phosphate buffer pH 7.4, containing 4 g/100 ml sucrose. The suspension was then collected on a Millipore swinnex filter (Millipore G.S.W.P. 02500) and the sample subsequently processed on the filter. It was washed for 1 h in 0.05 M phosphate buffer pH 7.4, then postfixed in 1 g/100 ml osmium tetroxide in the same buffer. Tertiary fixation, dehydration and block staining were as described previously (Sinden, Canning & Spain 1976).

**Mature gametocytes**

Light and electron-microscope specimens were prepared as above, particular attention being paid to rapid fixation after withdrawal of blood from the patient, in order to prevent activation of gametocytes before fixation. Scanning electron microscope (s.e.m.) preparations were made, by using the methods of Sinden (1975). Feulgen staining of thin smears followed the techniques of Canning & Sinden (1975).
Gametogenesis

Measures of 5 ml of blood were taken into a heparinized syringe and transferred to a deep watchglass in a moist chamber at 29 °C. One sample was taken immediately and was observed continuously under a sealed coverslip and the events of gametogenesis were recorded on videotape as described previously (Sinden & Croll 1975). Thin smears of other samples were made at 1 min intervals. At 5 min intervals further samples were prepared for transmission and scanning electron microscopy. The observations made by the various techniques were later correlated in time.

Indirect fluorescent antibody techniques

Antigens were prepared as thick smears of washed erythrocytes infected with gametocytes, or of gametocytes which had been allowed to undergo gametogenesis (as described above). Following dehaemoglobinization, the antigens were exposed to a known antigametocyte serum taken from a naturally infected patient. After washing in 0.1 M phosphate buffered saline pH 7.4, it was labelled with fluorescent antihuman globulin as described by Smalley & Sinden (1977).

Results

(a) Immature gametocytes

A single 5-year-old patient (H.N.), who had received doses of 60 mg (intramuscularly), 37.5 mg (orally) and 61 mg (i.m.) of chloroquine 27, 4 and 2 days respectively before examination, had a mixture of immature and mature gametocytes in the peripheral circulation. The course of the gametocytaemia over a 2-week period is shown in figure 1.

When examined initially 50% of the gametocytes in the peripheral circulation were immature. Examination of blood taken from the bone marrow of the iliac crest showed the same ratio of immature to mature forms. Of the gametocytes, 21% contained no pigment and, of the remainder, a majority showed prominent clumping of pigment induced by the chloroquine treatment (Warhurst 1973). The immature gametocytes were mostly in the stage categories 2 and 3 as described by Hawking et al. (1971), namely small rounded or wheat-shaped parasites with a small nucleus containing a red-staining inclusion and with cytoplasm staining intensely mauve, and long spindle-shaped cells (Field & Shute 1976). The deeply stained peripheral 'capsule' described by Garnham (1931) and Thomson & Robertson (1935) was frequently recognized in both types of cell. The gradual transformation from immature to mature gametocytes as indicated by morphology and ability to exflagellate is illustrated in figure 2.

In electron microscope samples taken on days 1 and 2 of the period of observation (24 and 25 September), it was often difficult to distinguish the immature from mature gametocytes, only the spindle-shaped immature parasites were readily recognizable (figures 3 and 4, plate 1). All gametocytes were surrounded by a triple
Figure 1. Plot showing total gametocyte density (○—○) and asexual parasitaemia (■—■) in patient H. N. throughout period of observation, and times of recent chloroquine administration.

Figure 2. The relative distribution of immature female (○——○) and male (□——□) and mature female (●——●) and male (■——■) gametocytes in the total gametocyte population in patient H. N. following chloroquine treatment. Maturity as indicated by the ability of each sample to exflagellate is indicated on the horizontal axis as + or −. Arrows point to the times at which preparations of ‘immature chloroquine treated’ gametocytes were made for electron microscopic examination.
layered membrane complex (figure 5, plate 1). The outermost layer was the 7 nm thick plasmalemma of the parasitophorous vacuole, and beneath this and separated from it by an amorphous matrix, was the plasmalemma of the gametocyte. The innermost layer, 14 nm thick, was a continuous trilaminar structure lining the whole parasite formed from two tightly associated unit membranes. In a significant fraction (up to one third) of gametocytes, particularly the spindle-shaped parasites of both sexes, a prominent system of subpellicular tubules was found. Tubules were present in single and doublet form (figure 5, arrowed). Each tubule measured 32.9 nm in outer diameter (o.d.) with a wall thickness of 5 nm when fixed by the method described, and 51.25 nm o.d. with a wall thickness of 9 nm when fixed by a modified Karnovsky method (Sinden et al. 1976). The presence of these tubules, the frequent absence of pigment and osmiophilic bodies and the fact that these parasites did not always fill the erythrocytes were the only ultrastuctural features which distinguished these gametocytes from mature parasites in the absence of chloroquine pressure.

(b) Mature gametocytes

The immature and mature gametocytes induced numerous modifications to the host erythrocytes which differed from those seen in cells infected by asexual parasites (Miller 1972). The erythrocyte cytoplasm was no longer a homogeneous electron-dense matrix but appeared ‘coagulated’ with electron-dense and electron-lucent regions. A prominent electron-lucent cavity immediately surrounded the parasite. The most characteristic modification was the formation of Laveran’s ‘bib’ readily seen in light and scanning microscope preparations (figures 6–8, plate 1). The ‘bib’ had either a smooth edge (figures 6, 7) or a fluted one (figure 8). The bib extension (figure 9, plate 2) sometimes contained an extension of the

DESCRIPTION OF PLATE 1

FIGURE 3. Longitudinal section (l.s.) of ‘immature chloroquine treated’ female gametocyte of Plasmodium falciparum. Note the extensive tubule system (arrows) lying beneath the trilaminate pellicle. (Magn. x 24000.)

FIGURE 4. Photomicrograph of a large spindle-shaped ‘immature, chloroquine treated’ gametocyte. Note that the straight parasite has noticeably pointed ends which distort the host cell. (Magn. x 1700.)

FIGURE 5. Electron micrograph of a transverse section (t.s.) of an ‘immature, chloroquine treated’ microgametocyte showing the large individual, and occasionally double subpellicular (arrowed) tubules. The relatively thick duplex inner pellicular membranes are evident. (Magn. x 79000.)

FIGURE 6. Photomicrograph of mature microgametocyte (top) and macrogametocyte (bottom) showing host cell distortion as Laveran’s ‘bib’. (Magn. x 1700.)

FIGURE 7. Scanning electron micrograph of mature gametocyte illustrating the flattening of the host cell as Laveran’s ‘bib’. (Magn. x 12000.)

FIGURE 8. Scanning electron micrograph of mature gametocyte where the ‘bib’ of the enveloping host cell has an unusual scalloped appearance (arrowed). (Magn. x 9000.)
Figures 3–8. For description see opposite.

(Facing p. 380)
FIGURE 9. T.s. of mature macrogametocyte. The host cell is modified to Laveran's 'bib' (b), the cytoplasm is 'coagulated' and leached. Cytostome is situated close to the base of the 'bib'. Osmiophilic bodies (o), Pigment (p), mitochondria (m). (Magn. x 30000.)

FIGURE 10. Host cell containing extensive gametocyte-induced membranous whorls assumed to be 'Garnham bodies'. (Magn. x 28000.)

FIGURE 11. Multilamellate capsule in host cell cytoplasm closely applied to the parasitophorous vacuole membrane (p.v.m.). (Magn. x 144000.) (Inset) Caveola-like modification (arrow) of erythrocyte occupied by an immature gametocyte. (Magn. x 24000.)

FIGURE 12. T.s. of mature microgametocyte showing food vacuole (f) still attached to cytostome in which numerous membranous whorls have developed. Nucleus (n). (Magn. x 30000.)
Figures 9–12. For description see opposite.
Figures 13–17. For description see opposite.
parasitophorous vacuole and was then composed of two very thin sheets of erythrocyte cytoplasm each bounded on either side by a unit membrane. Some host cells contained up to five membranous whorls each measuring up to 0.6 μm in diameter (figure 10, plate 2). They were not revealed in Giemsa-stained preparations but were none the less believed to be identical to the elongate ‘Garnham bodies’ (Garnham 1931). Other host cells contained a different multilamellate system of 1–5 unit membranes which lay in the erythrocyte cytoplasm parallel to, and spaced 60 nm from, the parasitophorous vacuole membrane (figure 11, plate 2) covering up to two thirds of the parasites’ surface. A rare modification of the erythrocyte was a small cleft in the plasmalemma (figure 11, inset) strikingly similar to the caveolae in cells infected with *P. vivax*, *P. cynomolgi* (Aikawa, Miller & Rabbage 1975) and *P. simium* (Sterling, Seed, Aikawa & Rabbage 1975).

The sex of mature gametocytes was readily determined by light microscopy. The cytoplasm of the macrogametocyte was strongly basophilic, that of the microgametocyte was less so. Pigment which occupies a perinuclear position, was more tightly clumped in the female than the male, reflecting the comparatively small size of the macrogametocyte nucleus. Within the macrogametocyte’s nucleus lay a compact chromatin mass which stained intensely with Giemsa stain, but only faintly with Feulgen’s stain. The microgametocyte’s nucleus did not contain the intensely staining intranuclear mass and was Feulgen negative. Nuclei of schizonts and merozoites were by comparison strongly Feulgen positive.

In the transmission electron microscope mature gametocytes were seen to have the same pellicular structure as immature stages, and the inner double membrane was often involuted as densely staining irregular sacs. Similar involutions have been observed in the saurian parasite *P. mexicanum* (Moore & Sinden 1974). A micropore was invariably found in the pellicle of the gametocyte, frequently

**Description of Plate 3**

**Figure 13.** L.s. of mature macrogametocyte with small centrally situated nucleus with prominent nucleolus (n.n.), numerous osmiophilic bodies (O), mitochondria (m) and pigment crystals (p), cistena of endoplasmic reticulum dilated with electron-dense matrix. (Magn. × 16 000.)

**Figure 14.** L.s. of mature microgametocyte. Highly lobed nucleus (n), with pigment crystals in cytoplasmic pockets between nuclear lobes. Intranuclear body (i.n.b.) with kinetochores around its edges. Electron-dense cytoplasmic microtubule organizing centre (m.t.o.c.) in close proximity to i.n.b. The cytoplasm contains few polyribosomal clusters, little endoplasmic reticulum and a single osmiophilic body. (Magn. × 21 000.)

**Figure 15.** Female gametocyte in host cell with Laveran’s ‘bib’ (b) and membranous capsule modifications (m.c.). Within the nucleus lies a single i.n.b. exhibiting a hemispherical organization; the m.t.o.c. is on the cycloplasmic face of the nuclear envelope adjacent to the i.n.b. (Magn. × 24 000.)

**Figure 16.** M.t.o.c. adjacent to nucleus of a mature microgametocyte. The organelle appears to contain two electron-dense components. (Magn. × 44 000.)

**Figure 17.** Mature macrogametocyte with electron-dense i.n.b. around which lie a few kinetosome-like structures (arrows). (Magn. × 36 000.)
situating opposite the base of Laveran's 'bib' (figure 9). The dimensions of the
micropore during food vacuole formation were $64 \pm 15$ nm inner diameter (i.d.)
and $154 \pm 25$ nm o.d., and when inactive, the dimensions were $77.5 \pm 6$ nm and
$134 \pm 22$ nm respectively. The food vacuoles contained numerous whorled mem-
branes (figure 12, plate 2) probably derived from ingested parasitophorous vacuole
membranes. No lysosome-like structures, similar to the dense spherules seen by
Aikawa & Sterling (1974), were observed.

In the macrogametocyte the endoplasmic reticulum, in both rough and smooth
forms, was continuous with the inner pellicular membranes and the nuclear
envelope. The cisternae of the endoplasmic reticulum were dilated and filled with
an amorphous electron-dense matrix (figure 13, plate 3). There was also a discrete
region of cytoplasm occupied by a collection of small membranous vesicles.
Similar vesicles have been considered to be the Golgi body in the saurian parasite
Plasmodium gaba1doni (Garnham 1977). In contrast the microgametocyte was
virtually devoid of endoplasmic reticulum.

Osmiophilic bodies were numerous in the female cell but almost entirely absent
in the male (compare figures 13 and 15 with 14, plate 3). These ovoid, membrane-
limited organelles, which measured 280 by 80 nm were occasionally attached to
the endoplasmic reticulum, which was presumed to be their site of synthesis.
Some were attached by small ducts, to the inner pellicular membranes, which
were presumed to be the site of their ultimate activity.

Mitochondria were present in equal numbers clustered about the nuclear
envelope in gametocytes of both sexes. They measured 280 nm in diameter and
extended up to 1.3 $\mu$m in sections. The tubular cristae were bathed in an electron-
dense matrix within which lay numerous ribosomes and large 170 nm diameter
electron-dense bodies. A spherical body was occasionally seen next to the nuclear
envelope; this association has also been noted in the avian parasite P. gallinaceum
(Sterling & Aikawa 1973).

The nucleus of the macrogametocyte was small, centrally placed and rounded.
In the microgametocyte the nucleus was markedly digitate, the fingers extending
towards the poles to occupy as much as two thirds of the cell. The nucleus was
bounded by a typical bilamellate nuclear envelope punctured by complex nuclear
pores similar to those described in P. yoelii nigeriensis (Sinden et al. 1976). A micro-
tubule organizing centre (m.t.o.c.) was present in both gametocytes adjacent to
the nuclear envelope. It was an electron-dense structure 0.35 $\mu$m in diameter com-
posed of particles 20 nm in diameter (figures 14–16, plate 3). There was no evidence
of microtubules or centriolar pinwheels within the m.t.o.c. of mature inactivated
gametocytes, but in one cell (figure 16) the m.t.o.c. contained two paired parallel
electron-dense bodies 0.1–0.2 $\mu$m long. The nucleoplasm was homogeneous except
for randomly dispersed 20 nm and 10–12 nm particles. In the macrogametocyte
only, adjacent to the nuclear envelope, was a large 0.5 $\mu$m diameter nucleolus
composed of closely aggregated 20 nm diameter ribonucleoprotein particles
(figure 13). In the nuclei of both gametocytes was a single intranuclear body
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(i.n.b.) 0.3–0.44 × 0.5 μm, in which the electron-dense nucleoplasmic particles were closely aggregated (figures 14, 15, 17, plate 3). At the periphery of the i.n.b. in the microgametocyte nucleus lay ca. 17 (15–25) kinetochores each 50 × 83 nm and made up of 2 pairs of electron-dense plates. As with other malarial parasites the i.n.b. was closely associated with the m.t.o.c. (Sterling & Aikawa 1973; Sinden et al. 1976).

Pigment crystals were clustered around the nucleus as dense membrane-bound inclusions or, where dissolved by lead citrate, as vacuoles not greater than 0.2 μm in diameter and 0.5 μm long. Their striking association with the nuclear envelope was particularly marked in the microgametocytes where crystals usually lay in the cytoplasmic pockets between the lobes of the nucleus (figure 14). The different sizes of the nuclei between the male and female gametocytes accounts for the comparatively restricted distribution of pigment in the macrogametocyte compared to the microgametocyte.

(c) Gametogenesis

(i) Microgametogenesis

The periods of activation, exflagellation, dispersal and fertilization described by Sinden & Croll (1975) and Sinden et al. (1976) were recognized on videotape recordings of gametogenesis of P. falciparum (figure 18).

Activation was first recognized by the appearance of at least two centriolar pinwheels within the m.t.o.c. (figure 19, plate 4). These contained a single central tubule attached by radial linkages to nine peripheral single microtubules. Their appearance was followed by the dissolution of the i.n.b. and the synthesis of the first intranuclear spindle in the central lobe of the nucleus (figure 20, plate 4). Each spindle pole was based on an electron-dense centriolar plaque which was situated on the inner face of the nuclear envelope and extended through a nuclear pore into the cytoplasm where it was contiguous with a single kinetosome. The spindle was 1.45 μm long and contained about 20 microtubules which were of the interzonal and kinetochore types. Occasionally a long double-barred structure of unknown function was found attached to the i.n.b. in some activated gametocytes (figure 21, plate 4).

Simultaneously with the first nuclear division the microgametocyte became spherical. This process was completed in 5.07 ± 2.63 min and was paralleled by a reduction in staining intensity with Giemsa stain and a fall-off in electron density of thin sectioned material. All observations suggested there was a fluid uptake by the parasite. Expansion of the parasite within the erythrocyte resulted in the loss of Laveran’s ‘bib’, but surprisingly the erythrocyte cytoplasm, far from being compressed, was itself increased in volume and less electron dense (compare figures 14 and 20, plates 3 and 4).

There was a marked increase in the activity of the cytoplasm and pigment at 7.98 ± 5.98 min, which may have been associated with the process of rounding up of the nucleus (figure 22, plate 4). Two spindles, marking the second gamete
Figure 18. A synopsis of timing of the events occurring during gametogenesis.
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division, each made up of 12–15 microtubules, were formed in pockets of the nuclear envelope at this time. Feulgen positive material has been observed as condensations near the periphery of the nucleus but the chromatin has not been identified in the electron microscope. Axonemes, which had polymerized in the cytoplasm on the kinetosomes, were associated with the poles of the spindles. Axoneme synthesis was identical to that described in *P. y. nigeriensis* (Sinden et al. 1976) where the A subfibres were laid down first and the B subfibres polymerized on to them (figures 22, 25, plates 4 and 5).

![Diagram](http://rspb.royalsocietypublishing.org/)

**Figure 23.** Plot showing changes in the volume (○—○) and surface area (●—●) of a single microgametocyte, following activation, and their relation to the emergence from the host cell, and exflagellation.

Videotape analysis of the changes in volume of the parasite during microgametogenesis showed that an initial increase in volume was followed by an equally marked decrease, with a peak volume at about the time of the second nuclear division. These changes for a single parasite are illustrated in figure 23. Three phenomena were consistently recorded: the erythrocyte remnants were lost during the period of parasite expansion at $8.37 \pm 2.87$ min (this process involved the progressive dissolution of the entire erythrocyte plasmalemma, rather than a localized puncturing of an otherwise intact host cell membrane); exflagellation occurred during a period of parasite contraction; the loss of parasite volume in the final period was greater by an order of magnitude than the volume of the gametes released and was accompanied by an increase in the cytoplasmic density of the residuum (figure 26, plate 5).

Changes in volume were of course accompanied by changes in surface area (figure 23), which were most easily calculated during the period when the gametocytes were spherical. During the period of expansion the plasmalemma increased in surface area. However t.e.m. revealed that the inner pellicular doublet membrane remained of constant surface area and was therefore disrupted at intervals (figure 25, plate 5). At the time of exflagellation only 40% of the membrane enveloping the emergent gametes can be accounted for by membrane made avail-
able as a result of the reduction in surface area of the shrinking microgametocyte residuum. The remainder was presumably synthesized de novo.

Preliminary immunological comparisons of the plasmalemma of the free microgamete and the intraerythrocytic gametocyte, by indirect fluorescent antibody technique, have suggested differences in membrane organization of these cells:

Antiserum from naturally infected patients which gave a strong positive fluorescence with the mature gametocyte gave a negative reaction with the free gamete.

Exflagellation was first recognized by the agitation of the intracytoplasmic axonemes at $9.58 \pm 3.37$ min. The emergence of the gametes began $0.21 \pm 0.05$ min

**Description of Plate 4**

**Figure 19.** Activated microgametocyte in which the m.t.o.c. has partially transformed into two parallel kinetosomes (k) made of single peripheral tubules organized by radial linkages about a single control tubule. The i.n.b. has not yet begun transformation into the first gametic spindle. (Magn. $\times 67,000$.)

**Figure 20.** Activated microgametocyte showing first gametic spindle stretching between centriolar plaques on opposing sides of central lobe of the still digitate nucleus. Kinetosomes (k) are on the cytoplasmic side of each centriolar plaque. Parasite and host cell have begun to increase in volume and become spherical. (Magn. $\times 17,000$.)

**Figure 21.** Microgametocyte with prominent cytoplasmic m.t.o.c. connected to i.n.b. within which lies a long double-barred electron-dense organelle (arrow). (Magn. $\times 44,000$.)

**Figure 22.** Activated, near spherical microgametocyte in which the nucleus has begun to round up. Multiple intranuclear spindles are seen in t.s. (arrows). Numerous axonemes (a) are seen developing in the cytoplasm, one of which shows the partly polymerized B microtubules. (Magn. $\times 28,000$.)

![Graph showing the variability in the rate of extension of a single microgamete](image-url)
Figures 19–22. For description see opposite.

(Facing p. 386)
**Description of Plate 5**

**Figure 25.** Activated but still intracellular microgametocyte undergoing third and final microgametic division. Budding gamete nucleus (g.n.) contains condensed nucleic acid. Adjacent to the lobe lies the electron-dense juxtakinetosomal sphere and granule within the perikinetosomal tubular basket (arrow). (Magn. × 21,000.)

**Figure 26.** Microgametocyte residium with a very dense cytoplasm bounded by a discontinuous plasmalemma. Adjacent to the residuum lies the tip of a free gamete (g) showing the juxtakinetosomal sphere and granule situated in the rounded tip. (Magn. × 19,000.)

**Figure 27.** Exflagellating microgametocyte with dense cytoplasm and highly disorganized plasmalemma and numerous free emergent gametes, each with a single axoneme and often devoid of a plasmalemma. A single gamete shows the electron-dense nucleus and axoneme bounded by a unit membrane. (Magn. × 20,000.)

**Figure 28.** Scanning electron micrograph of free gamete, showing the distinct rounded and pointed ends with bulbous nuclear swelling. (Magn. × 6,000.)

**Figure 29.** Aberrant exflagellating microgametocyte still retained within host erythrocyte. Parasite cytoplasm is highly lobed with numerous abnormal axoneme configurations in the lobes (arrows). (Magn. × 29,000.)

**Figure 33.** Activated female gametocyte in the process of rounding up. The trilaminate pellicle is still intact. A hemispindle (s) is seen radiating from an electron-dense centriolar plaque in a nuclear pore, a hemispherical m.t.o.c. is situated on the cytoplasmic face of the plaque (arrow). (Magn. × 17,000.)

**Figure 34.** Activated macrogametocyte showing the inner pellicular membranes which are continuous with the endoplasmic reticulum (arrow), being spaced apart by the expanding parasite plasmalemma (double arrow). (Magn. × 19,000.)
Figures 23–24. For description see opposite.
Figures 35–40. For description see opposite.
later. For a given gametocyte all gametes elongated at a similar rate, e.g. in one particularly slow exflagellation sequence the rates calculated were 0.35–0.43 μm/s. However, the rate of growth between gametocytes varied widely. The rate of gamete extension was not constant with time (figure 24), but was synchronized between gametes emerging from a single gametocyte. The rate of extension of the gametes may be directly related to the subsequent cyclical activity patterns of gamete movement. The pattern of microgamete escape differed between gametocytes; in some all gametes escaped simultaneously, in others only a few escaped and in yet others all the gametes died very rapidly even before becoming free.

The final microgametic nuclear division occurred during exflagellation. Four intranuclear spindles were situated in pockets of a persistent nuclear envelope. Each spindle was not less than 1.2 μm long and contained at least seven microtubules which terminated in a large centriolar plaque. The chromosomes condensed rapidly within each nuclear pocket as the final spindle separated and the genome was drawn into the emergent microgametes (figure 25, plate 5). On the cytoplasmic face of each centriolar plaque the kinetosome developed a small juxtkinetosomal sphere and granule (figure 25) which was later found in the tip of the emergent gamete (figures 26, 28, plate 5). The extensive subpellicular tubular system present in P. y. nigeriensis (Sinden et al. 1976) which proliferated as the perikinetosomal basket was not found in this study, although apparent vestiges of the system were seen in some exflagellating cells (figure 25). This difference may be related to the difference in fixatives used in the two studies (see below).

Free microgametes had a single axoneme, nucleus and little cytoplasm. In s.e.m. preparations they were at least 10 μm long and 0.28 μm in diameter, except in the central nuclear region, where there was enlargement to 0.42 μm. Each had a rounded emergent end, in which lay the juxtkinetosomal sphere and granule, and a pointed trailing end (figure 28). The nucleus was packed with electron-dense

**Description of Plate 6**

**Figure 35.** Mature macrogamete following escape from host cell. Expanded pellicle has a fibrous coating and shows discontinuities in the inner membrane sacs (arrows). Cytoplasm is noticeably less-electron dense. Nucleus contains a single hemispindle (s.). (Magn. × 14 000.)

**Figure 36.** Micrograph showing initial binding of microgamete (m.g.) to macrogamete. (Magn. × 16 000.)

**Figure 37.** Detailed micrograph of the binding of microgamete and macrogamete showing continuity of the fibrous coats about each gamete. Microgamete nucleus (g.n.). (Magn. × 81 000.)

**Figure 38.** Section showing the surface interaction between microgametes and erythrocyte. (Magn. × 104 000.)

**Figure 39.** Extracellular macrogamete in the process of being ingested by a phagocyte. (Magn. × 15 000.)

**Figure 40.** An apparently intracellular macrogametocyte that has been ingested by a polymorphonuclear leucocyte. (Magn. × 6 000.)
Feulgen-positive chromatin and was bounded by a nuclear envelope devoid of nuclear pores. The gamete plasmalemma was covered by a buffy coat 21 μm thick, composed of long fibrous molecules.

Aberrant exflagellating gametocytes and gametes were very frequently encountered. Such gametocytes either possessed a fragmented and punctured plasmalemma (figures 26, 27, plate 5) or became lobed and distorted during exflagellation (figure 29, plate 5). Aikawa, Huff & Strome (1970) described similar cytoplasmic lobes in exflagellating *Leucocytozoon simondii*. Aberrant gametes were manifested as forms with multiple (2–4) axonemes, disorganized axonemes, bulbous cytoplasmic inclusions, large nuclei with partially condensed chromatin, and anucleate gametes. Of 118 gametes observed by light microscopy, 23% were anucleate. Additionally 5% of the population appeared to have a free flagellum as described by Raffaele (1939). The latter observation was, however, not confirmed by either t.e.m. or s.e.m. studies and was therefore believed to be caused by adherent fibrin stands.

The activity patterns of free microgametes were analysed from videotape recordings. They moved by the generation of sinusoidal waves which passed along the flagellum at a changeable rate. As in earlier studies (Anderson & Cowdry 1928; Sinden & Croll 1975) three activity states were recognized, namely fast movement, slow movement, and rest. During fast movement waves, of wavelength (λ) $11.99 \pm 4.85 \mu m$ and amplitude $1.08 \pm 0.62 \mu m$, were generated at a minimum of 4.5 waves per second – which was the limit of resolution of our equipment. (Analysis of the cinefilm of Ferguson, Wolcott & Young (1954) suggests rates of 12.5 waves per second.) Slow waves were generated at $1.04 \pm 0.23$ waves per second with a λ of $14.09 \pm 4.48 \mu m$ and an amplitude of $2.34 \pm 1.34 \mu m$ (figure 30). The gamete’s lifespan usually began with an initial period of continuous rapid move-
ment, which was followed by a phase in which fast bursts of activity were interspersed with slow movement or pauses. This in turn was followed by a phase of cyclic slow movement and immobile periods, and then death (figure 31). Occasional gametes, although displaying a cyclic activity and a progressive reduction in activity, were never immobile even over periods as long as 42 min (figure 32). Others exhibited only slow movement and died almost immediately. Translocation of the gametes through the plasma at speeds of ca. 100 μm/s only occurred during periods of rapid wave production. Slow activity gave rise to writhing movements on the spot, and on resumption of fast movement, the gametes

Figure 31. Microgamete activity: the relative proportion of each successive 1 min period occupied by each of the three activity states: fast (■), slow (□) and immobile (■), showing a progressive reduction in activity with increasing age of the preparation.

Figure 32. Microgamete activity: analysis of a single exflagellation sequence showing a gamete alternating between short bursts of rapid (○) and slow (●) movement. With increasing age there is a progressive increase in the length of the individual slow periods. Data taken from a single gamete which remained attached to the microgametocyte residuum.
darted off in a new direction. There was no evidence for the attraction of the microgamete to the macrogamete. However, the existence of gradients about a macrogametocyte were revealed by the purposeful migration of macrophages toward the parasite which was subsequently engulfed. Light microscopic examination of engulfed gametocytes suggested that only extracellular parasites were ingested (Sinden & Smalley 1976). However, t.e.m. studies revealed that both extra- and apparently intracellular gametocytes may be engulfed (figures 39, 40, plate 6). In the latter case, however, the possibility cannot be excluded that the host erythrocyte had been partly disrupted out of the plane of the section.

During fertilization the microgametes were closely applied over a considerable proportion of their length to the macrogamete surface and the buffy coats of the two gametes became continuous (figures 36, 37, plate 6). A similar binding of the microgamete to the plasmalemma of erythrocytes was also detected (figure 38, plate 6); this probably accounted for the apparent 'stickiness' of microgametes to erythrocytes, the binding being strong enough to allow a free gamete to tow an erythrocyte about the preparation. Fusion of the gametes and zygote development have not been seen in this study.

(ii) Macrogametogenesis

The activated macrogametocyte increased in cell volume, and surface area at the same times as the microgametocyte. Similar pellicular changes were observed (figures 13, 33–35, plates 3, 5 and 6). The initial changes in shape, associated with the increasing cell volume, were often irregular, the parasites occasionally resembling the 'herniated' forms illustrated by Field & Shute (1956, pl. XVI, figs. 5 and 6), and the column-isolated gametocytes of Kass et al. (1971).

Some aspects of nuclear organization during macrogametogenesis were unexpected. Feulgen-stained preparations showed that while the majority had a single nucleus containing a prominent Feulgen-positive body, some had lobed or apparently divided nuclei each containing a chromatic inclusion.

At the ultrastructural level a single large nucleolus was invariably detected against the peristent nuclear envelope. In the majority of cells there was no sign of division, but in seven activated macrogametocytes (see for example, figures 33, 35, plates 5 and 6) and in a single mature gametocyte, presumed not to be activated (figure 15, plate 3), a radially symmetrical structure lay adjacent to the inner side of the nuclear envelope. Irrespective of the stage of development of the macrogametocyte or macrogamete, only one such structure was ever seen in each nucleus. Within this structure, which is believed to be a hemispindle, two to eight arms, which although indistinct might well be microtubules, radiated 0.15 μm into the nucleoplasm from an electron-dense centriolar plaque situated in one or two adjacent nuclear pores. At their nucleoplasmic end the radiating arms terminated in a structure made of three or four electron-dense bars 43 nm × 43 nm, similar to the kinetochores seen in the dividing microgametocyte.

Upon activation there was a marked reduction in cytoplasmic density which
revealed a network of fibrous molecules, some of which extended across polyribosome clusters and were therefore interpreted as messenger RNA in the process of translation. The cisternae of the endoplasmic reticulum also became less dense which suggested that the cell has secreted a protein or glycoprotein into the environment. The external surface of the extracellular macrogamete was covered by a 16–26 nm thick fibrous coat, similar to the matrix formerly found in the cisternae of the reticulum. The osmiophilic bodies were reduced in number and occupied a superficial position in the cell, but were not observed to fuse extensively with the plasmalemma as in *P. y. nigeriensis* (Sinden *et al.* 1976).

**Discussion**

**Gametocyte development**

The current study of gametocyte development in the peripheral circulation of a patient following chloroquine treatment has reaffirmed that this process takes many days *in vivo* (Garnham 1966) and *in vitro* (Smalley 1976). Furthermore, these results confirm that the gametocytes are long lived (Field & Shute 1956; Jeffery & Eyles 1955; Jeffery, Young & Eyles 1956; Smalley & Sinden 1977), and, contrary to the suggestion of Hawking and co-workers (Hawking, Worms & Gammage 1968; Hawking *et al.* 1971), show that mature gametocytes have a long-lived capability of exflagellation. The continued development of the young gametocytes despite chloroquine pressure suggests that these parasites had passed the chloroquine sensitive period (0–4 days) demonstrated in cultured gametocytes (Smalley, unpublished observations). It is therefore assumed that the three rounds of genome replication required for the development of the mature microgametocyte occur during this period (0–4 days) and that subsequent development, for which the term ‘capacitation’ is suggested, required predominantly the re-organization of existing materials, rather than the significant uptake of new nutrients by phagotrophy.

While gametocytes were readily identified by light microscopy as immature, mature or chloroquine damaged, such distinctions were less apparent by t.e.m. where only two classes of gametocyte were recognized: mature and those which were spindle shaped, possessed subpellicular tubules, and were almost totally devoid of osmiophilic bodies. Aikawa & Ward (1974) have demonstrated a similar subpellicular microtubule system in a chloroquine resistant line (Vietnam) of *P. falciparum* but not in a chloroquine sensitive line and it is possible that similar changes had been induced in the spindle-shaped gametocytes in the present study, by the chloroquine treatment. Nevertheless the ultrastructural features of these stages may equally well be normal characteristics of immature stages. Further experiments are in progress to determine the significance of this subpellicular system.
Of the host cell modifications induced by the gametocytes of *Plasmodium falciparum* most have been found only in this parasite. The flattening of the erythrocyte, which gives rise to Laveran's 'bib', is most probably caused by the re-distribution of the host cell cytoplasm into the growing crescent-shaped parasite, this change being most apparent in cells parasitized by the mature gametocytes. Ingestion of the host cell cytoplasm via the micropore (cytostome) was readily demonstrated in this study.

As suggested by Kass *et al.* (1971) the multilamellate whorls found in the cytoplasm of infected erythrocytes are probably equivalent to 'Garnham bodies'. However, we were unable to see them in Giemsa-stained bloodfilms of the same blood in which they were demonstrated by electron microscopy. Also we were not able to confirm Garnham's observations (1931) and those of Field & Shute (1956) that they were more common in erythrocytes infected by immature gametocytes. Similarly, Bray (unpublished observations) was unable to detect these structures in blood of chimpanzees infected with immature gametocytes of *P. falciparum*.

Light microscopic descriptions of gametocytes, particularly immature forms, of *P. falciparum* have drawn attention to the presence of a capsule around the parasite (Garnham 1931; Thomson & Robertson 1935). As noted by Kass *et al.* (1971) this capsule is probably represented by the multilamellate proliferation of the parasitophorous vacuole membrane. There is no recognizable structure within the parasite to account for such a capsule.

The mature gametocyte, shown by s.e.m. to be sausage-shaped with a central nuclear bulge, is invested by a pellicle composed of the plasmalemma and two inner membranes formed from a single sac-like expansion of the endoplasmic reticulum. This triple-membraned organization has also been described by Kass *et al.* (1971) and Aikawa & Ward (1974) and contrasts with that of other mammalian malarial parasites. It closely resembles the pellicle of avian malarial parasites. While subpellicular tubules are present in the immature parasites and may be responsible for the shape of the developing cell, no such tubular system is found in the mature gametocyte and the characteristic shape of these parasites is presumably determined by the elaborate pellicular membrane architecture.

Nuclear organization of the male and female gametocytes differs dramatically. In the macrogametocyte, but not the microgametocyte, the persistent nucleolus might reflect a continuation of synthesis of ribosomal RNA. However, the absence of a nucleolus even in immature (stage 2 or 3) microgametocytes suggests that rRNA-coding genes are repressed early in male gametocyte development, leading to a loss of the nucleolus and consequent reduction in the cyttoplasmic ribosome population. Those ribosomes remaining in the mature microgametocyte are predominantly stabilized in a polyribosomal form and are responsible for the limited *de novo* protein synthesis shown to be essential to microgametogenesis (Toye, Sinden & Canning 1977).
Nuclei of both gametocytes contain an intranuclear body (i.n.b.), which is associated with the cytoplasmic microtubule organizing centre (m.t.o.c.). The nature of the small barred structures in the i.n.b. remains uncertain. The attractive suggestion of Sterling & Aikawa (1973) that these are kinetochores was questioned by Sinden et al. (1976) who estimated that the number in *P. y. nigeriensis* was too low for the number of chromosomes in the cell. However, in this capacity they would serve, in an enlarged and irregular nucleus, to localize the multiple copies of the genome in the region of the m.t.o.c. and thus ensure the apposition of the genome replicates and axonemes. Much larger bodies, reminiscent of a sinuous synaptonemal complex (Sotello 1969) were also found in the i.n.b. As in *P. y. nigeriensis* (Sinden et al. 1976) centriolar pinwheels appear in the m.t.o.c. only when the microgametocyte is activated, and the subsequent development of the m.t.o.c. through the three successive microgamete divisions is in accord with their observations. Hence, upon activation, the single m.t.o.c. gives rise to multiple kinetosomes, which are connected through a nuclear pore to the dividing centriolar plaque of the first intranuclear spindle. Subsequent divisions across evaginated pockets of the nuclear envelope, involve a step by step reduction in both spindle microtubule number (which faithfully reflects the chromosome number (Petersen & Ris 1976)), and kinetosome number, in such a way that ultimately each gamete receives a single kinetosome (and the axoneme synthesized upon it) and a haploid genome.

Mitochondria are prominent organelles which, in contrast to *P. y. nigeriensis* (Sinden et al. 1976), are found in equal numbers in macro- and microgametocytes. As found in *P. brasiliannum* (Sterling, Aikawa & Nussenzweig 1972) they contain tubular cristae, an electron-dense matrix, and dense inclusions. The characters are most unusual for gametocytes of mammalian malarial parasites but typical for malarial parasites of birds and reptiles. Sterling et al. (1972) regard this morphological organization as slim evidence for the presence of an active TCA cycle.

The morphological similarity of the osmophilic bodies to the rhoptry microneme complex of the invasive stages of the malaria parasite, and their fusion with the gametocyte plasmalemma during escape from the host cell, prompted Sinden et al. (1976) to suggest that these organelles were responsible for either enzymic dissolution of the host cell, or the osmotic expansion of the parasite. In *P. falciparum* the rare occurrence of the osmophilic bodies in the microgametocyte makes such conclusions less clear cut. However, the observation that gametocytes invariably escape from the host erythrocyte during a period of expansion of cell volume suggests that an increase in cell volume plays an important role in this process. This must occur by the uptake of plasma components due to the osmotic activation of molecules within the parasite, and possibly also in the host cell cytoplasm. The host cell plasmalemma becomes less refractile as the parasite expands, but has never been seen to burst suddenly, suggesting that the membrane is modified by the emerging parasite. The possibility remains therefore that both osmotic and enzymic mechanisms are employed, and that either may be based on the activity of the osmophilic
bodies. The eventual reduction in volume of the microgametocyte, but not of the macrogametocyte, during gamete formation is of unknown significance. It has, however, also been recorded for *Plasmodium* (= *Hepatocystis* kochi) (Anderson & Cowdry 1928).

**Immunological considerations**

The expansion of the parasites during gametogenesis necessitates an increase in surface area of at least 30%. The plasmalemma of the activated gametocyte must therefore be a mosaic of old and newly synthesized membranes. The microgametes also may be enveloped by a mixture of membrane types, or perhaps exclusively by new membrane synthesized at their sites of emergence. While our preliminary indirect fluorescent antibody technique results suggest that antiserum which binds to gametocytes is incapable of binding to free gametes, Gwadz (1976) has found evidence that there are immunological similarities between the membranes of gametocytes and gametes. He found that antibodies produced against formalinized or X-irradiated gametocytes are capable of immobilizing and agglutinating microgametes and preventing the infection of mosquitoes by gametocyte-infected blood. Should the antibodies induced by Gwadz be capable of binding both to gametocytes and microgametes, it is difficult to understand how the lethal effects of the antiserum are apparently confined to the liberated gametes or exflagellating gametocytes, and not to the earlier events of gametogenesis – unless the microgamete bears a higher density of antigenetic sites on its plasmalemma than the gametocyte. The present observations on the variability of exflagellation of *P. falciparum* gametocytes in the serum of naturally infected patients do however concur with Gwadz’s results. Carter & Chen (1976) have recently demonstrated that immunization of chickens with gametes of *P. gallinaceum* induces a massive immune response which will inhibit transmission of the parasite to mosquitoes completely. Additional factors affecting the survival of extracellular (and possibly intracellular) gametocytes and the development of gametes of *P. falciparum* in the mosquito are phagocytic and cytotoxic cells in the bloodmeal (Sinden & Smalley 1976).

**Microgamete behaviour**

The behaviour of the exflagellating microgametocyte, although occasionally modified by the presence of immune serum, was similar to that of *Plasmodium* (= *Hepatocystis* kochi) (Anderson & Cowdry 1928) and *P. y. nigeriensis* (Sinden & Croll 1975), with the single exception that the quiescent period before exflagellation was not detected. Anderson & Cowdry (1928) related this phase to changes in the viscosity of the cytoplasm, but in all probability it merely represents a short period of nuclear immobility.

Motility of the liberated microgamete was similar to that described in *P. (= Hepatocystis) kochi* and *P. y. nigeriensis* (Anderson & Cowdry 1928; Sinden & Croll 1975) with three activity states: fast, slow and immobile. The former authors
suggested that during fast movements, propulsive waves of long wavelength were produced. We found the converse, i.e. waves of shorter wavelength and lower amplitude when compared to periods of slow movement. Anderson & Cowdry (1928) also described a polarization of activity, with slow movement in a 'cephalic' direction, and fast movement with a rotational component in a 'caudal' direction. Our observations suggest that the gamete may move in either direction irrespective of the activity state. The cyclical activity, interpreted as a mechanism for enhancing the probability of fertilization (Sinden & Croll 1975) was again evident. The linear decline in activity seen in the rodent parasite was not always detected; presumably the life of the microgamete of *P. falciparum* was more frequently cut short by the presence of antibodies.

**Cytogenetics**

Early cytological studies on gametocytes of *P. falciparum* were made at a time when it was believed that gametic divisions were necessarily meiotic. Consequently an apparent reduction in chromosome number during the successive microgametic divisions was described by MacDougal (1947) and Lüdicke & Piekarski (1951). The chromosome complement of mature gametocytes was variously estimated as from 4 to 12, and of gametes, 2 to 4 (Anderson & Cowdry 1928; Thomson 1932; MacDougal 1947; Lüdicke & Piekarski 1951). However, the unusual condensation cycle of chromosomes of malarial parasites (Canning & Sinden 1975; Sinden et al. 1976) precludes their detection as discrete structures in division. The bodies seen in the early studies may therefore have been aggregated but nevertheless diffuse chromosomes localized to the intranuclear body or spindle poles.

In this investigation we have been unable to obtain accurate counts of the number of kinetochores present in each of the three microgametic spindles, but the number of microtubules in each spindle has been determined. If it is assumed that a constant proportion of microtubules on each spindle are attached via kinetochores to the chromosomes (Petersen & Ris 1976), we estimate that the mature microgametocyte contains at least 20 chromosomes (there are probably many more) and the microgamete about 7, the reduction in number being achieved by three mitotic divisions, in the absence of replication. The mature macrogametocyte however, contains a maximum of eight chromosomes and is presumably therefore haploid (see below).

The present finding of an elongate electron-dense structure in the i.n.b., highly reminiscent of a synaptonemal complex between meiotic bivalents (Sotello 1969) might suggest a meiotic microgametic division. However, evidence to the contrary is overwhelming: the immediate precursors of the gametocyte, the asexual stages and merozoites, are haploid (Walliker 1976); other coccidia have a zygotic reduction (Grell 1973; Sinden 1977). The function of this sinuous barred organelle therefore remains enigmatic.

Nuclear organization in macrogametocytes of malarial parasites varies widely. There are species in which there is no evidence of nuclear changes, beyond variable
reactivity of the chromatin with Feulgen stain during gametocytogenesis, e.g. *P. y. nigeriensis* (Canning & Sinden 1975; Sinden et al. 1976). There are species in which nuclear fragmentation has been detected by light or electron microscopy, e.g. *Haemoproteus lophortyx* (O'Roke 1930), *Hepatozoon* sp. (Canning, Killlick-Kendrick & Garnham 1975), *Haemoproteus columbae* (Gallucci 1974) and *P. falciparum* (Thomson 1932; Garnham 1931; Thompson & Robertson 1935; Lüdicke & Piekarzki 1951). Finally there are those species in which nuclear spindles and an associated m.t.o.c. have been described, e.g. *Haemoproteus metchnikovi* (Sterling 1972), *H. columbae* (Gallucci 1974) and *P. gallinaceum* (Sterling & Aikawa 1973).

In the present study of *P. falciparum* nuclear fragmentation has only rarely been seen in macrogametocytes by light microscopy and never by t.e.m. Evidence of nuclear activity in macrogametocytes of *P. falciparum* has been provided by earlier workers, some of whom considered this as evidence of a parthenogenetic development of asexual blood stages (see Garnham 1931; Thomson & Robertson 1935; Thomson 1932). Bishop (1955) reviewed the evidence for the formation of ‘Polar nuclei, assumed to be reduction bodies’, but cautiously concluded ‘the function of these bodies is enigmatic’. Gallucci (1974) similarly remained undecided as to the possible rôle of these nuclear fragments in *Haemoproteus*.

Ultrastructural evidence for macronuclear activity in the macrogametocytes of *P. falciparum* has been obtained in this study. There is an intranuclear spindle-like body based on a centriolar plaque, which extends through a nuclear pole to an m.t.o.c.-like structure on the cytoplasmic side of the nuclear envelope. Similar structures have been described in *H. columbae* (Gallucci 1974), *P. gallinaceum* (Sterling & Aikawa 1973) and *H. metchnikovi* (Sterling 1972). These authors also described a centriolar pinwheel within the m.t.o.c., which, although not found in the present study, clearly establishes the identity of these organelles and their homology with the m.t.o.c. and mitotic spindle of the microgametocyte. While these observations suggest that mitosis is occurring in macrogametocytes, we uphold the observation of Gallucci (1974) that two opposing spindle poles have never been found in a macrogametocyte nucleus. If, as is suspected, there is no mitotic division, the hemispindle may serve only to localize the genome in the haploid cell, in which case this hemispindle could be an atavistic trait as suggested by Canning & Morgan (1975) for *Hepatozoon domerguei*, a relic from sporozoan ancestors where macrogametogenesis, like microgametogenesis, involved the production of numerous gametes.

**Phylogeny**

Two malarial parasites, *P. falciparum* and *P. reichenowi* are distinguished from other mammalian Plasmodidae by their crescentic gametocytes and have been placed in the subgenus *Laverania* (Garnham 1966; Peters et al. 1976). This subgenus is regarded by some as the most recently evolved of the mammalian malarial parasites (Garnham 1966), although others, e.g. Landau, Miltgen & Chabaud (1976), found it difficult to place *P. falciparum* on a phylogenetic tree.
because of the archetypal 'reptilian-avian' nature of the gametocytes. They suggested a polyphyletic origin for mammalian Haemosporina.

Electron microscope studies on the gametocytes of *P. falciparum*, such as Smith *et al.* (1969), Kass *et al.* (1971), Aikawa & Ward (1974) and the present study, together with reviews on gametocyte organization, such as Aikawa, Huff & Sprinz (1969), Aikawa (1971), Sterling *et al.* (1972), Sterling & Aikawa (1973), have revealed at least one of the following, typically avian, characteristics of gametocyte ultrastructure of *P. falciparum*:

1. the prominent pellicular organization comprising three virtually continuous unit membranes;
2. a persistent nucleolus in the mature macrogametocyte;
3. numerous mitochondria, with well developed tubular cristae and an electron-dense matrix, in gametocytes of both sexes (also described in *P. brasilianum* and *P. malariae* (Sterling *et al.* 1972);
4. the disparity in the number of osmophilic bodies between the two sexes of gametocyte;
5. the occurrence of cytostomal feeding;
6. the presence of a vesicular Golgi body; and
7. the production of an intranuclear hemispindle during macrogametogenesis. These observations strongly support the conclusion of Landau *et al.* (1976) that the gametocytes of *P. falciparum* bear a stronger resemblance to the archetypal avian malarial parasites, than to other mammalian Haemosporina. Consequently, if a monophyletic origin of malarial parasites is favoured, the subgenus *Laverania* must be considered as ancestral to the 'vivax' and 'malariae' groups. Alternatively a polyphyletic origin must be supported.

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Gametogenesis in Plasmodium


