Research and Reviews in Parasitology, 58 (3-4): 203-208 (1998)

Published by A.P.E.
Printed in Barcelona, Spain

BITING PHYSIOLOGY OF *ANOPHELES* AFFECTING *PLASMODIUM* TRANSMISSION

V. ROBERT[1] & P.T. BREY[2]

[1]*Laboratoire de Paludologie, Institut de Recherche pour le Développement (IRD-ORSTOM), B.P. 1386, Dakar, Sénégal*

[2]*Laboratoire de Biochimie et Biologie Moléculaire des Insectes, Institut Pasteur, 25 rue du Docteur Roux, 75724 Paris Cedex 15, France*

Received 4 February 1998; accepted 7 April 1998

REFERENCE: ROBERT (V.) & BREY (P.T.), 1998.– Biting physiology of *Anopheles* affecting *Plasmodium* transmission. *Research and Reviews in Parasitology*, 58 (3-4): 203-208.

SUMMARY: In humans, infection by *Plasmodium* starts with the infectious bite of an *Anopheles* mosquito. The parasitic cycle requires the interaction of three unrelated organisms: the parasite, the vector and the vertebrate host. The bite of the *Anopheles* harbouring sporozoites in its salivary glands involves three distinct, but linked processes: penetration and probing by the mouth parts into the skin, injection of saliva containing sporozoites, and blood feeding. Recent information relative to *Plasmodium* transmission via an infectious bite concerns principally four points: 1) the number of sporozoites actually injected into the host is now considered to be low, on the average of about 10, but can occasionally reach a few hundred; this number is surprisingly low and represents about 1% of the sporozoites stored in the salivary glands; 2) the sporozoites injected into the skin during probing seem to be the only ones able to infect the hepatic cells of the vertebrate host; the sporozoites injected into the vertebrate blood stream during feeding are not considered important for transmission of the parasite because they are ingested with the blood meal and are carried into the lumen of the midgut; 3) the success of one mosquito bite to infect the non-immune vertebrate host is difficult to evaluate; however, even though the estimate is approximate, chances are now considered to be around one out of two; 4) the biting behaviour of *Anopheles* species seems to be modified when sporozoites are present in the salivary glands provoking an increased time of skin penetration by the mouth parts during the bite and an increased average number of hosts being bitten in order to achieve blood meal repletion; this would of course augment the chances of parasite transmission. These behavioural differences depend on the vector species, but they could also depend on the *Plasmodium* species.

KEY WORDS: *Plasmodium*, *Anopheles*, sporozoite, saliva, malaria transmission, review.

INTRODUCTION

Malaria infection requires the precise encounter of three organisms: the *Plasmodium* parasite, the vertebrate host and the mosquito vector. Their interaction occurs when the female mosquito bites the vertebrate host in order to take a blood meal requisite for egg formation. During the bite, the asexual and sexual forms of the parasite circulating in the blood stream of the vertebrate host are ingested with the blood meal while the infectious form of the parasite (sporozoite) stored in the mosquito's salivary apparatus are injected into the skin of the vertebrate host to initiate another round of the parasite cycle. The severe consequences of these infections in terms of public health justify a careful examination of the steps which initiate the process. It is essential to better understand the biological interplay going on between the members of this triad in order to decrease their interaction and by the same token to avoid infection.

Apart from the vectorial transmission, malaria infections can also occur by congenital vertical transfer and by transfusion of *Plasmodium*-contaminated blood. These marginal means of transmission unrelated to the bite of infected anophelines will not be considered here.

BACKGROUND KNOWLEDGE

Biting occurs shortly after the mosquito has landed on the skin of its host. The tips of the proboscis, measuring about 1500 mm long and comprised of a labium (1), labrum (1), hypopharynx (1), mandibules (2), and maxilles (2), come into contact with the skin. This also brings the sensillae of the labellar lobes into contact with it. With alternating movements of the terminally serrated maxilles and mandibules, the stylet fascicule (i.e. all the mouthparts minus labium) penetrate the host skin.

Mosquitoes search for blood by repeatedly thrusting the fascicule into a host's dermal network of blood vessels until a blood vessel is pierced (GORDON & CREWE, 1948).

Blood can then be ingested either by direct cannulation of a blood vessel or by pool feeding from a microhemorrhage induced by the probing (GORDON & CREW, 1948; GRIFFITHS & GORDON, 1952). The blood is imbibed by action of the cibarial and pharyngeal pump muscles via the food canal formed by the labrum and hypopharynx. Blood then passes into the midgut.

Saliva is synthesised by a pair of salivary glands, each consisting of three lobes, two lateral and one median. The saliva is ejected from glands via two ducts which fuse into a common salivary duct, that continues in the central part of hypopharynx and finishes at the very distal part of it.

Salivation occurs continuously from the beginning of probing to withdrawal of the mosquito mouthparts upon repletion (GOLENDA *et al.*, 1995). Discharge of saliva into the bloodstream is observed only under exceptional circumstances, and then only when the biting fascicule is lying in an unusually small capillary (GRIFFITHS & GOR-

DON, 1952). The saliva contains numerous bioactive proteins (KERLIN & HUGUES, 1992) that counteract components of the vertebrate haemostatic response. The anopheline saliva contains anticoagulating factors (CHRISTOPHERS, 1960), factors which prevent platelet aggregation (such as apyrase), inflammatory factors, digestive enzymes (phosphatase, esterase, aminopeptidase, glycosidase), and vasodilatators such as peroxidase (JAMES & ROSSIGNOL, 1991; CHAMPAGNE, 1994). For three species of *Anopheles* the speed of locating blood was positively correlated with the titer of stored apyrase in the salivary glands (RIBEIRO, ROSSIGNOL & SPIELMAN, 1985). A classical experiment carried out by HUDSON, BOWMAN & ORR (1960) on *Aedes stimulans*, showed that if the main salivary duct is cut at the anterior region of the mosquito neck (that is to say no salivary secretions can be ejected when biting), the lack of saliva does not affect the intake or movement of blood into the midgut. On the contrary, comparable experiments conducted on *Aedes aegypti* showed that some of the duct-cut mosquitoes have difficulty in probing (HUDSON, 1964) and took longer to feed (RIBEIRO, ROSSIGNOL & SPIELMAN, 1984), showing that saliva is important in maintaining the blood in a fluid state for transport into the gut.

The life-cycle of the malaria parasite in the mosquito vector was proposed a century ago (ROSS, 1898; GRASSI, 1898). The sporogonic cycle begins in the mosquito midgut following the ingestion of gametocytes in the blood meal. The midgut environment elicits the transformation of the gametocytes into female and male gametes that fuse to form a zygote. The zygote then develops into a mobile ookinete that crosses the midgut epithelium to the space between the basal membrane of the epithelial cell and the lamina, where it will settle to give rise to an oocyst. Intense mitotic divisions within the oocyst give rise to sporozoites; when mature, they are liberated into the haemocoel and migrate to and invade the salivary glands to await injection into a vertebrate host.

Sporozoites released from mature oocysts are distributed throughout the haemocoelic cavity, but they present a high affinity for salivary glands (ROBERT *et al.*, 1988). The numbers of *P. falciparum* sporozoites estimated to be present in the salivary glands of experimentally infected *Anopheles maculipennis* vary greatly (range 6 - 213450). The same observation was made with field-collected *A. gambiae* and *A. funestus* (range 82 - 245760) (PRINGLE, 1966). However, about half of the infected anophelines harbour sporozoite loads of <1000 (BEIER *et al.*, 1991b; KABIRU *et al.*, 1997). *P. berghei* sporozoites tend to accumulate in the cells and secretory cavities of the distal portion of salivary gland lobes (STERLING, AIKAWA & VENDERBERG, 1973), on the contrary *P. falciparum* sporozoites are randomly distributed among the lobes of the salivary glands (PONNUDURAI *et al.*, 1991). Sometimes the sporozoites are observed throughout one entire lobe, commonly the median one, while the other lobes are without any sporozoites (PONNUDURAI *et al.*, 1991).

Transfer of sporozoites from the mosquito to the vertebrate host is considered to be a passive and random process totally dependent on access of the parasites to the salivary duct and the secretion and flow of saliva. The motility of sporozoites, which was certified for several parasite species, including *P. falciparum* (VANDERBERG, 1974; VANDENBERG & GWADZ, 1980), was up until recently not considered to have a role in its transfer from mosquito to vertebrate. An elegant study by SULTAN *et al.* (1997) strongly suggests that the surface protein TRAP is important for sporozoite motility and cell invasion. The introduction of sporozoites into the skin and subcutaneous tissues of the host has been certified by histology by showing extravascular sporozoites in the skin of human volunteers fed upon by *P. vivax*-infected mosquitoes (BOYD & KITCHEN, 1939).

THE NEW KNOWLEDGE

Quantification of the sporozoite number delivered during a bite

Only 25% of the sporozoites released into the haemocoel are estimated to reach the salivary glands (ROSENBERG, 1991).

How do the sporozoites locate, recognise and invade the salivary glands? Virtually nothing is known about the migration of the sporozoites through the haemocoel, or salivary invasion (BREY, 1991).

To know the exact number of sporozoites delivered during a bite is a requisite for advancing fundamental biology concerning host/parasite interactions, but also for malaria control. The dose of sporozoites inoculated during a bite might be related to the clinical outcome of infection among endemic populations (MARSH, 1992). In monkeys, intradermal inoculation of *P. coatneyi* sporozoites by means of a syringe gave a negative correlation between sporozoite number and the prepatent period (BROWN, ROSENBERG & NGAMPOCHJANA, 1992).

Only a very small percentage of the sporozoites in the salivary glands are actually injected during the bite. Laboratory studies prove that most infected mosquitoes transmit few sporozoites, generally <10, during the blood feeding process (ROSENBERG *et al.*, 1990; PONNUDURAI *et al.*, 1991; BEIER *et al.*, 1991a, 1992a). These observations were indirectly confirmed in a field study (KABIRU *et al.*, 1997). Maximum numbers of injected sporozoites were about 70 (BEIER *et al.*, 1992a), 400 (BEIER *et al.*, 1991a), 500 (PONNUDURAI *et al.*, 1991) or 1000 (ROSENBERG *et al.*, 1990).

Most infective anophelines transmit <1% of the total sporozoites in the salivary glands. This important discrepancy between the number of sporozoites within salivary glands and the number of sporozoites actually injected during a bite is puzzling.

Several considerations can be made:

- *The salivary gland morphology*: many of the sporozoites are within the cytoplasm of the gland cells. Those located in the proximal portion of the lobes, where the salivary duct is lined by a cuticle, seemingly have no possibility to reach the secretory duct. Only sporozoites which manage to enter this secretory duct in the distal portion of the gland have a chance to be injected with the saliva (PONNUDURAI *et al.*, 1991).
- *The relatively large size of the sporozoite*: the inner diameter of the salivary duct is 1.8 mm for *A. stephensi* (WRIGHT, 1969) and 1.0 for *A. gambiae* and *A. freeborni* (BEIER *et al.*, 1992a). Hence, the dimentions of the sporozoite, about 11 mm in length and 1.0 in diameter (AIKAWA, 1988) are just enough for the sporozoite to squeeze through. Thus spatial constraints could be problematic for sporozoites to leave the glands via the duct; however, we do not find any mention in the literature concerning «sporozoite-clogged» secretory ducts.
- *The salivation behaviour*: the feeding behaviour of mosquitoes is complex and blood feeding must not be considered alone (FRIEND & SMITH, 1977; BEIER, 1996). Females probably feed more often on nectar than on blood; water is also another source of feeding. Sporozoite ejection occurs normally during sugar feeding (SHUTE, 1945; BEIER et al., 1991a). Restrictive sugar access increases the blood feeding frequency and number for long-lived *A. gambiae* (STRAIF & BEIER, 1996); these mosquitoes present the highest probability to have sporozoites in their salivary glands. Furthermore, discharge and subsequent swallowing of saliva occurs frequently, even when the mosquito is not feeding (FÜLLEBORN, 1932 in CLEMENTS, 1992; HABLUTZEL *et al.*, 1992). Thus, the probable fate of some sporozoites located in the salivary duct is direct passage into the midgut, excluding any contact with the vertebrate host. Hence, if the female mosquito is prone to sustained salivation, a high number of residual sporozoites may be required to maintain the infectivity of the mosquito.
- *Sporozoite density factor*: this is pure speculation, but it could be possible that a high resident sporozoite population is necessary to provide the enviroment necessary for sporozoite survival in the glands. Interestingly, electron micrographs of sporozoites in salivary glands show them arranged in orderly packets often in contact with one another (STERLING *et al.*, 1973).

Transmitting *Anopheles* contain significantly more sporozoites in their salivary glands than non-transmitters (PONNUDURAI *et al.*, 1991; BEIER *et al.*, 1991a). Whether the salivary gland sporozoite load is related with the number of sporozoites ejected during the bite is under debate at that moment. This important epidemiological question has received a negative answer from some authors (PONNUDURAI *et al.*, 1991; BEIER *et al.*, 1991a), a weak positive from others (BEIER *et al.*, 1992a) and a positive one from others (ROSENBERG *et al.*, 1990; GOLENDA, BURGE & SCHNEIDER 1992). It may be stressed that the observations of PONNUDURAI *et al.* (1991) appear credible because the mosquitoes were fed on freshly removed mouse skin, which approaches as closely as possible the normal feeding behaviour of a mosquito.

The innate potential for sporozoite transmission depends on the vector species; for instance *A. gambiae* transmits more than twice as many sporozoites than *A. freeborni* (BEIER *et al.*, 1992a).

The fate of sporozoites delivered during the bite

Mosquitoes tend to reingest their saliva while taking a blood meal. This is not surprising; among several fonctions of the saliva, one is clearly digestive. The diameter of the salivary channel is about 1% of the food canal. During the bite the flow rate of saliva through the salivary channel of *Aedes aegypti* is estimated to be of the order of 104 - 105 times lower than the rate of the counterflow in the food canal (HURIBUT, 1966). Under these conditions the reingestion of sporozoites ejected with saliva into the tip of the food canal is attempted. Indeed, about two-thirds of the sporozoites ejected during blood feeding are ingested and could be found in the midgut of the fully engorged female mosquitoes (BEIER *et al.*, 1992b); the other one-third remains in the host. The numbers of sporozoites in the midguts increases as a linear function of sporozoite loads in the salivary glands (BEIER *et al.*, 1992a).

Whether many of the sporozoites injected by mosquitoes go immediately into circulating blood or remain at the bite site has been difficult to ascertain. Recently, this question has made some progress. *Anopheles stephensi* infected with *P. yoelii* were allowed to feed on the ears of mice. When the ear tissue around the biting site was excised immediately or 5 minutes postfeeding, the percentage of mice which developed parasitaemia was smaller than the control. However, if the tissue is left 15 minutes after the bite and then removed, the percentage is the same as the control. This finding shows that mosquito-injected sporozoites tend to remain at the bite site for at least 5 min after the mosquito bite. It suggests that malaria infection due to direct injection of sporozoites into circulating blood is a relatively rare event (SIDJANSKI & VANDERBERG, 1997). This conclusion is in full agreement with the observations of PONNUDURAI *et al.* (1991), who showed that zero or very few sporozoites are present in the blood reservoir of a Parafilm® membrane feeder after an infectious blood feeding. It has been suggested that the preferred route of sporozoite migration from bite site to host liver would be via the lymphatics rather than the blood capillaries (GRIFFITHS & GORDON, 1952).

Infected bite efficiency

Mosquito bites may not always be infective, even when apparently mature sporozoites are present in the salivary glands. A single bite from an *Aedes aegypti*

mosquito infected with *P. gallinaceum* infected more than 85% of the chicks challenged (COATNEY, COOPER & MILES, 1945). Forty-three % of *Anopheles stephensi* with *P. yoelii* sporozoites in their salivary glands succeed in infecting mice following single-mosquito/single-mouse feedings (PUMPINI, MENDIS & BEIER, 1997).

The bites of 5 *A. stephensi* infected with *P. falciparum* reproductibly infected volunteers (HERRINGTON *et al.*, 1988). With the same couple of vector/parasite species, 3 of 5 volunteers developed parasitaemia after being bitten by 1 infected mosquito, and 2 of 5 volunteers after being bitten by 2 infected mosquitoes (RICKMAN *et al.*, 1990). This inconsistency to initiate a new infection at each bite can be due either to the failure of some mosquitoes to transmit sporozoites, or the lack of infectivity of the sporozoites which are actually injected.

However the failure of 26% (PONNUDURAI *et al.*, 1991) and of 20% (ROSENBERG *et al.*, 1990) of infected *A. stephensi* to eject *P. falciparum* sporozoites is already established.

A relationship is established between the number of *P. yoelii* sporozoites present in the salivary glands of individual *A. stephensi* and sporozoite infectiousness as measured by infection in mice (PUMPINI, MENDIS & BEIER, 1997). Furthermore, large *Aedes aegypti* infected with *P. gallinaceum* are more infective for naive chicks than small *Aedes aegypti*.

Better knowledge of the infectious bite efficiency, e.g., number of sporozoites required to initiate an infection, is necessary not only for a more accurate calculation of the vectorial capacity, but also to estimate the quantity of challenge infections while conducting malaria vaccination trials on humans.

Biting behaviour of infected anophelines

The biting time and the biting rate of *Aedes aegypti* infected with sporozoites of *P. gallinaceum* is twice as long as that of uninfected *Aedes aegypti* (ROSSIGNOL *et al.*, 1984, 1986). The initiation of the probing, the number of probes and the biting times of wild-caught P. *falciparum*-infected *Anopheles gambiae s.l.* are also longer that uninfected mosquitoes (WEKESA, COPELAND & MWANGI, 1992). A natural population of human-feeding *A. punctulatus* present a larger amount of blood in their midgut after blood-feeding when infected by *P. falciparum* and *P. vivax* (KOELLA & PARKER, 1996).

In Gambian children living in the same house and presenting clinical symptoms of malaria at the same time, the *P. falciparum* isolates were found to belong to the same genotype in approximately 50% of them, compared to approximately 1% of randomly chosen children presenting clinical symptoms of malaria from the study area as a whole (CONWAY & MCBRIDE, 1991). This finding underlines the importance of the interrupted feeding process for anophelines with sporozoites in their salivary glands.

In Tanzania, Koella and colleagues (MORELL, 1997), placed human volunteers, each with a distinct blood phenotype, with unfed infected/uninfected anophelines in a house during one night. In the morning, mosquitoes that had fed were collected and the origin of the blood was attributed individually to the various volunteers. Only 18% of the 111 uninfected mosquitoes had fed on more than one person, compared to 34% of the 62% infected mosquitoes. This result shows that an interrupted blood meal occurs more frequently with *Plasmodium*-infected anophelines.

All these findings suggest that sporozoite stage in the salivary glands modifies the feeding behaviour of *Anopheles* females and by the same token increase the parasite's chances of being transmitted.

Nevertheless, the biting behaviour of *A. stephensi*, measured by the total duration of probing (including feeding), was not affected by the presence of *P. berghei* sporozoites in their salivary glands (LI, SINA & ROSSIGNOL, 1992) nor by those of *P. yoelii* (PUMPINI, MENDIS & BEIER, 1997).

CONCLUDING REMARKS

Information concerning the biting behaviour and mechanisms of *Plasmodium* infected mosquitoes has increased substantially during the last few years. A continued research effort in this field will certainly shed new light on the *Plasmodium* transmission and will perhaps provide implementation of novel operational tools to control morbidity and mortality due to malaria.

REFERENCES

AIKAWA (M.), 1988.– Fine structure of malaria parasites in the various stages of development. In: *Malaria, principles and practice of malariology* (W.H. Wernsdorfer & Sir I. McGregor edit), 1: 97-129.

BEIER (J.C.), 1996.– Frequent blood-feeding and restrictive sugar-feeding behaviour enhance the malaria vector potential of *Anopheles gambiae s.l.* and *An. funestus* (Diptera: Culcidae) in Western Kenya. *Journal of Medical Entomology*, 33: 613-618.

BEIER (M.S.), DAVIS (J.R.), PUMPUNI (C.B.), NODEN (B.H.) & BEIER (J.C.), 1992b.– Ingestion of *Plasmodium falciparum* sporozoites during transmission by anopheline mosquitoes. *American Journal of Tropical Medicine and Hygiene*, 47: 195-200.

BEIER (J.C.), BEIER (M.S.), VAUGHAN (J.A.), PUMPUNI (C.B.), DAVIS (J.R.) & NODEN (B.H.), 1992a.– Sporozoite transmission by *Anopheles freeborni* and *Anopheles gambiae* experimentally infected with *Plasmodium falciparum*. *Journal of the American Mosquito Control Association*, 8: 404-408.

BEIER (J.C.), ONYANGO (F.K.), KOROS (J.K.), RAMADHAN (M.), OGWANG (R.), WIRTZ (R.A.), KOECH (D.K.) & ROBERTS (C.R.), 1991a.– Quantitation of malaria sporozoites transmitted *in vitro* during salivation by wild Afrotropical *Anopheles*. *Medical and Veterinary Entomology*, 5: 71-79.

BEIER (J.C.), ONYANGO (F.K.), RAMADHAN (M.), KOROS (J.K.), ASIAGO (C.M.), WIRTZ (R.A.), KOECH (D.K.) & ROBERTS (C.R.), 1991b.– Quantitation of malaria sporozoites in the salivary glands of wild Afrotropical *Anopheles*. *Medical and Veterinary Entomology*, 5: 63-70.

BOYD (M.F.) & KITCHEN (S.F.), 1939.– The demonstration of spo-

rozoites in human tissues. *American Journal of Tropical Medicine and Hygiene*, 19: 27-31.

BREY (P.T.),1991.– «The taming of *Anopheles*»: current trends in malaria vector research. *Research in Immunology*, 142: 712-722.

BROWN (A.E.), ROSENBERG (R.) & NGAMPOCHJANA (M.), 1992.– The infective efficiency of intradermally inoculated *Plasmodium coatneyi* sporozoites. *Annals of Tropical Medicine and Parasitology*, 86: 193-195.

CHAMPAGNE (D.E.), 1994.– The role of salivary vasodilatators in bloodfeeding and parasite transmission. *Parasitology Today*, 10: 430-433.

COATNEY (G.R.), COOPER (W.C.) & MILES (V.I.), 1945.– Studies on *Plasmodium gallinaceum*. I. The incidence and course of the infection in young chicks resulting from single mosquito bites. *American Journal of Tropical Medicine and Hygiene*, 41: 109-118.

CONWAY (D.J.) & MACBRIDE (J.S.), 1991.– Genetic evidence for the importance of interrupted feeding by mosquitoes in the transmission of malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 85: 454-456.

CHRISTOPHERS (Sir S.R.), 1960.– *Aedes aegypti (L.), the yellow fever mosquito; its life history, bionomics and structure*. Cambridge University Press.

CLEMENTS (A.N.), 1992.– *The biology of mosquitoes*. Chapman & Hall.

FRIEND (W.G.) & SMITH (J.J.B.), 1977.– Factors affecting feeding by bloodsucking insects. *Annual Review of Entomology*, 22: 309-331.

GOLENDA (C.F.), BURGE (R.) & SCHNEIDER (I.), 1992.– *Plasmodium falciparum* and *P. berghei*: detection of sporozoites and the circumsporozoite proteins in the saliva of *Anopheles stephensi* mosquitoes. *Parasitology Research*,78: 563-569.

GOLENDA (C.F.), KLEIN (T.), COLMAN (R.), BURGE (R.), WARD (R.A.) & SEELEY (D.C.), 1995.– Depletion of total salivary gland protein in blood-fed anopheles mosquitoes. *Journal of Medical Entomology*, 32: 300-305.

GORDON (R.M.) & CREWE (W.), 1948.– The mechanism by which mosquitoes and tsetse flies obtain their blood meal, the histology of the lesions produced, and the subsequent reactions of the mammalian host, together with some observations on the feeding of *Chrysops* and *Cimex*. *Annals of Tropical Medicine and Parasitology*, 42: 334-356.

GRASSI, B. (1898).– Rapporti tra la malaria e peculiari insetti. *Policlinico*, 5: 469-476.

GRIFFITHS (R.B.) & GORDON (R.M.), 1952.– An apparatus which enables the process of feeding by mosquitoes to be observed in tissues of a live rodent, together with an account of the ejection of saliva and its significance in malaria. *Annals of Tropical Medicine and Parasitology*, 46: 311-319.

HABLUETZEL (A.), MERZAGORA (L.), JENNI (L.), BETSCHART (B.), ROTIGLIANO (G.) & ESPOSITO (F.), 1992.– Detecting malaria sporozoites in live, field-collected mosquitoes. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 86: 138-140.

HERRINGTON (D.A.), CLYDE (D.F.), MURPHY (J.R.), BAQAR (S.), LEVINE (M.M.), DO ROSARIO (V.) & HOLLINGDALE (M.R.), 1988.– A model for *Plasmodium falciparum* sporozoite challenge and very early therapy of parasitaemia for efficacy studies of sporozoite vaccines. *Tropical and Geographical Medicine*, 40: 124-127.

HUDSON (A.), 1964.– Some functions of the salivary glands of mosquitoes and other blood-feeding insects. *Canadian Journal of Zoology*, 42: 113-120.

HUDSON (A.), BOWMAN (L.) & ORR (C.W.M.), 1960.– Effects of absence of saliva on blood feeding by mosquitoes. *Science*, 131: 1730-1731.

HURIBUT (H.S.), 1966.– Mosquito salivation and virus transmission. *American Journal of Tropical Medicine and Hygiene*, 15: 989-993.

JAMES (A.A.) & ROSSIGNOL (P.A.), 1991.– Mosquito salivary glands: parasitological and molecular aspects. *Parasitology Today*, 7: 267-271.

KABIRU (E.W.), MBOGO (C.M.), MUIRURI (S.K.), OUMA (J.H.), GITHURE (J.I.) & BEIER (J.C.), 1997.– Sporozoite loads of naturally infected *Anopheles* in Kilifi District, Kenya. *Journal of the American Mosquito Control Association*, 13: 259-262.

KERLIN (R L.) & HUGHES (S.), 1992.– Enzymes in saliva from four parasitic arthropods. *Medical and Veterinary Entomology*, 6: 121-126.

KOELLA (J.C.) & PARKER (M.J.), 1996.– Malaria parasites enhance blood-feeding of their naturally infected *Anopheles punctulatus*. *Parasitology*, 113: 105-109.

LI (X.), SINA (B.) & ROSSIGNOL (P.A.), 1992.– Probing behaviour and sporozoite delivery by *Anopheles stephensi* infected with *Plasmodium berghei*. *Medical and Veterinary Entomology*, 6: 57-61.

MARSH (K.), 1992.– Malaria - a neglected disease? *Parasitology*, 104: S53-S69.

MORELL (V.), 1997.– How the malaria parasite manipulates its hosts. *Science*, 278: 223.

PONNUDURAI (T.), LENSEN (A.H.W.), VAN GEMERT (G.J.A.), BOLMER (G.) & MEUWISSEN (T.J.H.E.), 1991.– Feeding behaviour and sporozoite ejection by infected *Anopheles stephensi*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 85: 175-180.

PRINGLE (G.), 1966.– A quantitative study of naturally-acquired malaria infections in *Anopheles gambiae* and *Anopheles funestus* in a highly malarious area of East Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 60: 626-632.

PUMPUNI (C.B.), MENDIS (C.) & BEIER (J.C.), 1997.– *Plasmodium yoelii* sporozoite infectivity varies as a fonction of sporozoite loads in *Anopheles stephensi* mosquitoes. *Journal of Parasitology*, 83: 652-655.

RIBEIRO (J.M.C.), ROSSIGNOL (P.A.) & SPIELMAN (A.), 1984.– Role of mosquito saliva in blood vessel location. *Journal of Experimental Biology*, 108: 1-7.

RIBEIRO (J.M.C.), ROSSIGNOL (P.A.) & SPIELMAN (A.), 1985.– Salivary gland apyrase determines probing time in anopheline mosquitoes. *Journal of Insect Physiology*, 31: 689-692.

RICKMAN (L.S.), JONES (T.R.), LONG (G.W.), PAPARELLO (S.), SCHNEIDER (I.), PAUL (C.F.), BEAUDOIN (R.L.) & HOFFMAN (S.L.), 1990.– *Plasmodium falciparum* infected *Anopheles stephensi* inconsistently transmit malaria to humans. *American Journal of Tropical Medicine and Hygiene*, 43: 441-445.

ROBERT (V.), VERHAVE (J.P.), PONNUDURAI (T.), LOUWE (L.), SCHOLTENS (P.) & CARNEVALE (P.), 1988.– Study of the distribution of circumsoporozoite antigen in *Anopheles gambiae* infected with *Plasmodium falciparum*, using the enzyme-linked immunosorbent assay. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 82: 389-391.

ROSENBERG (R.), 1991.– Salivary glands and the transmission of malaria: a brief review. In: *Prospects for malaria control by genetic manipulation of its vectors, Abstracts*, WHO/World Bank/Tropical Diseases Research Program Scientif Working Group on Biological Control of Vectors, January 27-31, Tucson, Arizona, USA.

ROSENBERG (R.), WIRTZ (R.A.), SCHNEIDER (I.) & BURGE (R.), 1990.– An estimation of the number of malaria sporozoites ejected by a feeding mosquito. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 84: 209-212.

ROSS (R.), 1898.– Report on the cultivation of *Proteosoma*, Labbé, in grey mosquitoes. *Indian Medical Gazette*, 33: 401-408; 448-451.

ROSSIGNOL (P.A.), RIBEIRO (J.M.C.) & SPIELMAN (A.), 1984.– Increased intradermal probing time in sporozoite-infected mosquitoes. *American Journal of Tropical Medicine and Hygiene*, 33: 17-20.

ROSSIGNOL (P.A.), RIBEIRO (J.M.C.) & SPIELMAN (A.), 1986.– Increased biting rate and reduced fertility in sporozoite-infected mosquitoes. *American Journal of Tropical Medicine and Hygiene*, 35: 277-279.

SHUTE (P.G.) 1945.– An investigation into the number of sporozoites found in the salivary glands of *Anopheles* mosquitoes. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 38: 493-498.

SIDJANSKI (S.) & VANDERBERG (J.P.), 1997.– Delayed migration of *Plasmodium* sporozoites from the mosquito bite site to the blood. *American Journal of Tropical Medicine and Hygiene*, 57: 426-429.

STERLING (C.R.) AIKAWA (M) & VENDERBERG (J.P.) 1973.– The passage of *Plasmodium berghei* through the salivary glands of *Anopheles stephensi*: an electron microscope study. *Journal of Parasitology*, 59: 593-605.

STRAIF (S.C.) & BEIER (J.C.), 1996.– Effects of sugar availability on the blood-feeding behavior of *Anopheles gambiae* (Diptera: Culcidae). *Journal of Medical Entomology*, 33: 608-612.

SULTAN (A.A.), THATHY (V.), ROBSON (K.J.H.), CRISANTI (A.), NUSSENZWEIG (V.), NUSSENZWEIG (R.S.) & MENARD (R), 1997.– TRAP is necessary for gliding motility and infectivity of *Plasmodium* sporozoites. *Cell*, 90: 511-522.

VANDERBERG (J.P.), 1974.– Studies on the motility of *Plasmodium* sporozoites. *Journal of Protozoology*, 21: 527-537.

VANDERBERG (J.P.) & GWADZ (R.W.), 1980.– The transmission by mosquitoes of plasmodia in the laboratory. In: *Malaria* (J.P. Kreier edit), 2: 153-235.

WEKESA (J.W.), COPELAND (R.S.) & MWANGI (R.W.), 1992.– Effect of *Plasmodium falciparum* on blood feeding behaviour of naturally infected *Anopheles* mosquitoes in Western Kenya. *American Journal of Tropical Medicine and Hygiene*, 47: 484-488.

WRIGHT (K.A.), 1969.– The anatomy of salivary glands of *Anopheles stephensi* Liston. *Canadian Journal of Zoology*, 47: 579-587.