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An Ultrastructural Study on the Sporogony of *Plasmodium vivax* in *Anopheles stephensi*

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The present study was carried out to investigate the ultrastructural features of the successive stages involved in sporozoite formation from the solid oocyst in case of *P. vivax*. Appearance of vacuoles just beneath the oocyst capsule marks the beginning of sporoblast segregation. From the sporoblast cytoplasm the sporozoites are budded off in a manner similar to merozoite formation. The budding sporozoites show rudiments of the pellicular components and apical complex. Further development is accompanied by the migration of nuclear material from the sporoblast to the sporozoite. After dehiscence of the oocyst, the free sporozoites are released into the haemocoelomic fluid and they invade the acinal cells of the salivary glands and mature into infective forms. The developmental sequence observed is compared with that of other mammalian and avian malaria parasites.

INTRODUCTION

Plasmodium vivax causing the benign tertian malaria in man is the most prevalent species in the Indian subcontinent. It is known to be transmitted from one vertebrate host to another by several species of anopheline mosquitoes. In the stomach of the insect vector, only the gametocytes survive and all other stages of schizogony taken along with the blood meal are digested away. The gametocytes, male and female, produce the gametes which fuse to form the zygote cell, which as ookinete actively penetrates through the stomach wall and forms oocyst. The oocyst enlarges gradually and through a series of complex changes, results in the formation of a large number of sporozoites. The sporozoites eventually become free and emerge into the haemocoelomic cavity and finally invade the acinal cells of the salivary glands.

Ultrastructural changes during the formation of sporozoites have been studied in various species of malaria parasites viz., *P. berghei* (Vanderberg and Rhodin, 1967; Vanderberg *et al.*, 1967), *P. gallinaceum* (Terzakis *et al.*, 1966, 1967), *P. cathemerium* (Duncan *et al.*, 1960). Among the less studied forms are the primate parasites, *P. cynomolgi* (Terzakis, 1971) and parasites of man *P. falciparum* (Sinden and Strong, 1978). There are many descriptions of the fine structure of the sporozoites from the salivary glands of many plasmodial species including *P. gallinaceum* (Garnham *et al.*, 1960), *P. falciparum* (Garnham *et al.*, 1961), *P. brasilianum*, *P.c. bastianellii*, *P.*

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ovale and *P. vivax* (Garnham *et al.*, 1963), *P. cathemerium* and *P. relictum* (Stebbens and Schmidt, 1968), *P. berghei nigeriensis, P.vinkei chabaudi* and *P. cynomolgi* (Sinden and Garnham, 1973). However, the mechanism of invasion of host cells by sporozoites and the details of the host cell–parasite interactions remain to be clarified. Thus it appears that, except for the studies on *P. falciparum* there has been little work on the development of human malaria parasites in their natural insect vectors. In the present study we have traced the sporogonic development of *P. vivax* at ultrastructural level.

MATERIAL AND METHODS

Anopheles stephensi (Liston) mosquitoes were fed on the blood of patients showing high *P. vivax* gametocytaemia (1–1.5%). The mosquitoes were maintained in a closed insectary at 28 ± 1°C and R.H. 60–80%. At different time intervals after the blood meal, mid guts or salivary glands of the mosquitoes were dissected into the ice cold fixative, 3% glutaraldehyde in 0.2M cacodylate buffer (pH 7.4). Guts with oocysts or salivary glands with sporozoite infection were then transferred to a large volume of fixative at 4°C. The specimens were post-fixed in 2% osmium tetraoxide in 0.2M cacodylate buffer and dehydrated in an ethanol–propylene oxide series before being embedded in Epon–araldite medium. Sections were cut with Porter–Blum MT-2 ultramicrotome, mounted on formvar carbon covered 200 mesh copper grids, stained with saturated alcoholic solution of uranyl acetate (BDH) and alkaline lead citrate (Reynolds, 1963) and examined at 80 KV in Siemens Elmiskop IA. For scanning electron microscopy of the oocysts, the midguts of the infected mosquitoes were fixed in 3% glutaraldehyde in 0.2M cacodylate buffer (pH 7.4), post osmified in 2% osmium tetraoxide in 0.2M cacodylate buffer and dehydrated in an ethanol series, then into 50/50 v/v absolute alcohol/amyl acetate and into 100% amyl acetate. It was finally critical — point dried in liquid CO_2. Dried specimens were coated with silver and scanned in Philips EM-501 stereoscan scanning electron microscope.

RESULTS

The solid and vacuolated forms of oocyst: In the solid form of oocyst, the capsule is in contact with the basal lamina of the mosquito midgut epithelium. Its external surface is smooth, however, on the internal surface, the capsule material often invaginates deeply into the oocyst cytoplasm (Fig. 1). Nuclei in the solid oocyst are randomly dispersed. They are roughly oval or irregular polygons in shape (Fig. 1). Within the nucleus, heterochromatin and euchromatin as such are not easily distinguished. There are prominent, dense roughly circular areas containing granules present in the nuclei (Fig. 1). Appearance of vacuoles in the oocyst marks the beginning of sporoblast segregation (Fig. 2). It seems that these vacuoles become large, coalesce and form large clefts subdividing the oocyst cytoplasm into sporoblasts.

Sporoblast and sporozoite formation: After cleft formation in solid oocyst, broad peninsulae of sporoblast cytoplasm can be seen. The nuclei, previously randomly distributed, now occupy peripheral position just below the sporoblast plasma membrane (Fig. 3). The central portion of the sporoblast is occupied by free ribosomes and small dense granules.

From the sporoblast cytoplasm, sporozoites are budded off in a manner similar to merozoite formation. As the budding sporozoite begins to bulge away from the sporoblast cytoplasm, the rudiments of the pellicular complex are visible (Fig. 4). At this stage, precursor of apical complex of the sporozoite can also be seen (Fig. 4). Degenerative constituents outside the sporoblast and the capsular invaginations indicate that the sporozoite formed by budding from the sporoblasts are bathed in this enriched medium and may serve as a nutrient source for the further development of sporozoites (Fig. 4). The nucleus migrates from the sporoblast into the budding

sporozoites (Fig. 4). In the single sporozoite nuclear material is in the form of dense chromatin. The nucleus conforms to the elongate shape of the sporozoite and is quite slender as compared to its previous form in the sporoblast (Fig. 5). Fig. 6, 7 and 8 show the advanced stages of sporogony in the oocyst. (A centrally located residual body and completely formed sporozoites surrounding it can be seen in these figures).

Scanning electron microscope studies show that the mature oocysts are spherical in shape and they have smooth surface texture (Fig. 9). Oocysts from which the sporozoites are liberated, are irregular in shape and their surfaces show several folds (Fig. 10). Musculature of the midgut is well preserved in these preparations and appears as circular bands of cells bulging from the surface (Fig. 10). The tracheal system appears very prominent and its branches can be seen around the oocyst (Fig. 10).

Sporozoites from mature oocysts and salivary glands: Sporozoites obtained from oocysts on the tenth day after feeding show complete differentiation of subpellicular organelles. Electronmicrographs (Figs. 11 to 15) show sections of fully formed sporozoites at various planes. At this time there is very little of sporoblast cytoplasm (Figs. 11 & 12). The structural organization of the sporozoite can be reconstructed from these electronmicrographs.

Fully formed sporozoites within the mature oocyst, are surrounded by a pellicle of two membranes and a row of subpellicular microtubules (Figs. 11, 14 & 15). A thin fibrillar coat surrounds the outer membrane of the mature sporozoite (Figs. 14 & 15). At this stage, the bipolar organization of the sporozoites is very clear. The truncated anterior end is demarcated by polar rings (Fig. 12). Electron dense rhoptries and micronemes are not only present in the anterior portion of the sporozoite but they extend to the mid-region (Figs. 11 & 12). At the anterior portion of these rhoptries, a slender extension or conductule leads to the region of conoid (Figs.11 & 13). The rhoptries are much larger and there are more micronemes in the sporozoites as compared to those in merozoites (Aikawa, 1977). Free ribosomes and granular endoplasmic reticulum are also present in the mature sporozoites. A structure that could be identified as typical protozoan mitochondria is not seen in these motile forms. A large elongated nucleus is present in the middle portion of the sporozoite (Figs. 11 & 13).

After dehiscence of the oocyst, the free sporozoites are released into the haemocoelomic fluid and they concentrate in the salivary glands. Firstly they pass through the basal lamina of the salivary gland and later they invade the membrane lined vacuoles in the secretory cells (Figs. 16, 17 & 19). Their invasion is associated with the invagination of the membrane lining these vacuoles (Figs. 16 & 17). The sporozoites in these vacuoles are separated from the proteolytic secretion of the vacuoles by a definite space similar to that found in the parasitophorous vacuole surrounding the erythrocytic forms (Fig. 17). The sporozoites may be found in the intracellular spaces separating these vacuoles (Fig. 18). In case of heavy infection they are also lodged in the peripheral portion of the salivary gland lobes (Fig. 20). Then they penetrate further into the salivary gland lumen and appear in the ramifications of the salivary duct and are injected into the blood of man when the mosquito bites.

The basic structural organization of the sporozoite in the salivary gland is similar to that of a mature sporozoite in the oocyst. The pellicle of these motile forms is composed of an outer and two closely associated inner membranes (Figs. 21 to 23). It appears more pronunent as compared to that of sporozoites in the oocyst. Inside the pellicle run the hollow peripheral fibrils, arranged asymmetrically around the circumference of the sporozoite, and possibly extending from one extremity to the other (Figs. 21 & 22). The pellicle is broken at one point anterior to the nucleus by the micropore. At this point the pellicle dips at a right angle into the interior, its two

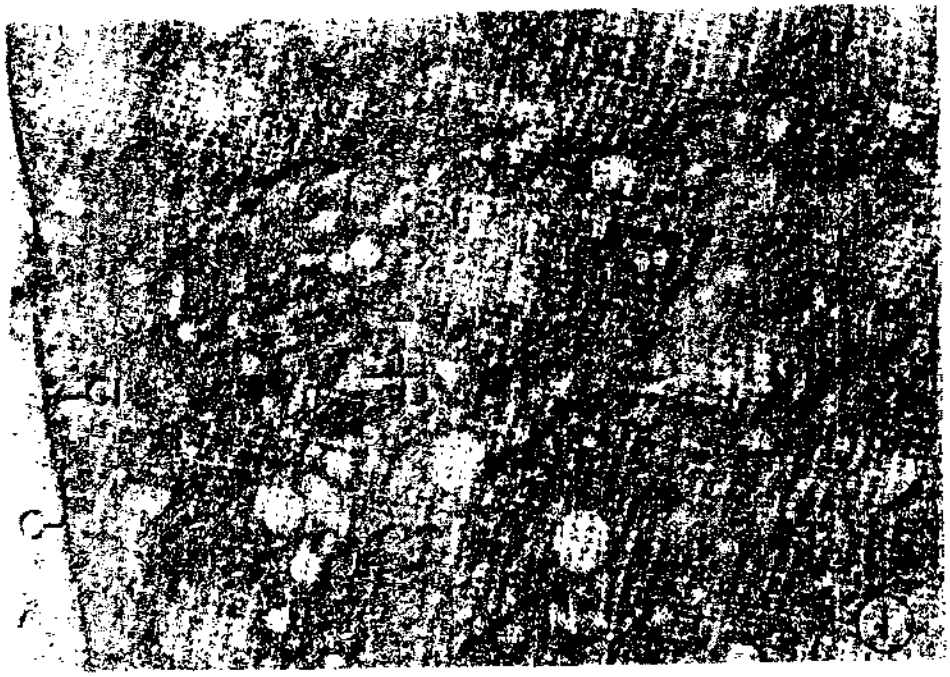


Fig. 1: Electronmicrograph of the solid form of oocyst of *P. vivax* showing capsule (C) with spinous invaginations (Cl) and randomly distributed nuclei (N). [×8,000].

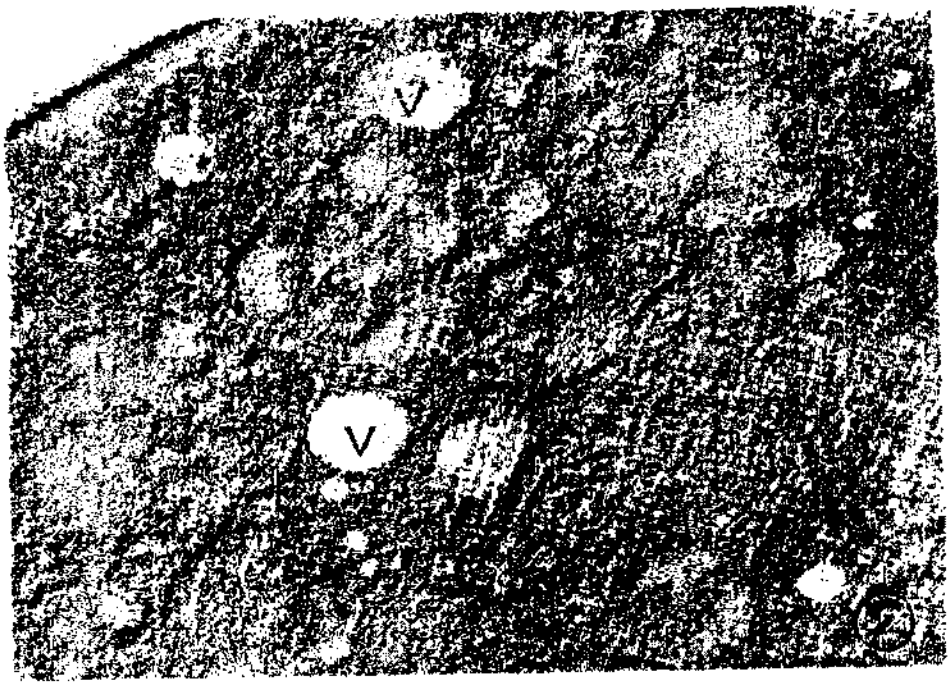


Fig. 2: Electronmicrograph showing subcapsular vacuolization (V) in the oocyst. [×20,000].

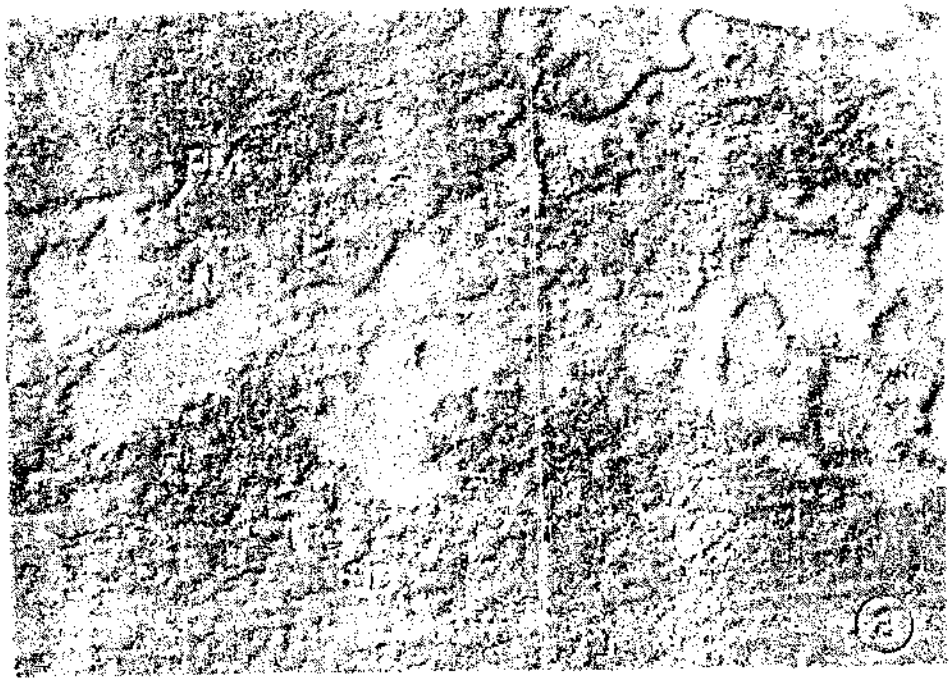


Fig. 3. Electronmicrograph showing part of the sporoblast (SB). The nuclei (N) previously randomly distributed, now occupy peripheral position just beneath the sporoblast plasma membrane (PM). [× 20,000].

Fig. 4. Electronmicrograph showing the onset of sporozoite budding (BS). Sporozoite pellicle (P) precursor for apical complex (AC) and the ingress of nuclear material (N) can be noted in budding sporozoites. [×16,000].

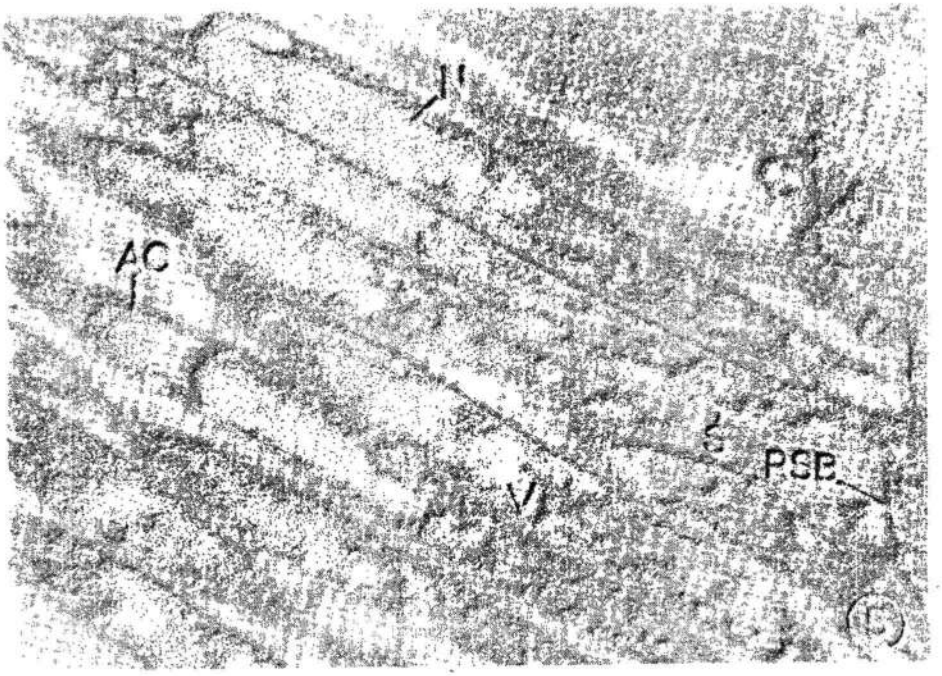


Fig. 4: Electronmicrograph showing sporozoites (S) attached to the residual sporoblast (RSB). At this stage, precursor for apical complex (AC), the elongated nucleus (N) and few vacuoles (V) can be seen in the sporozoites. [× 20,000]

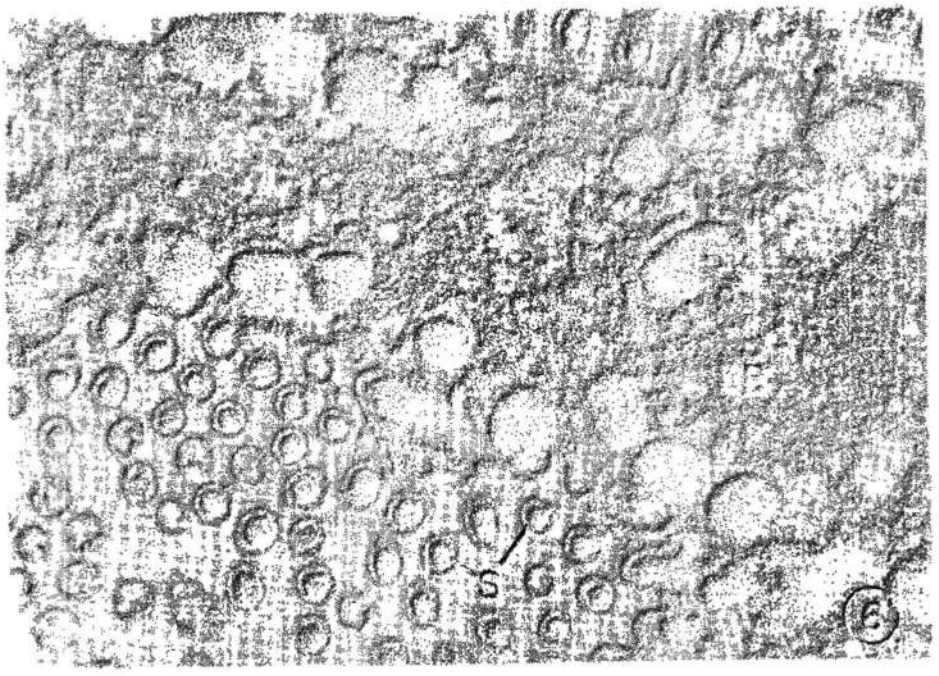


Fig. 5: Electronmicrograph showing part of sporoblast (SB) and the numerous completely formed sporozoites (S). [× 14,000].

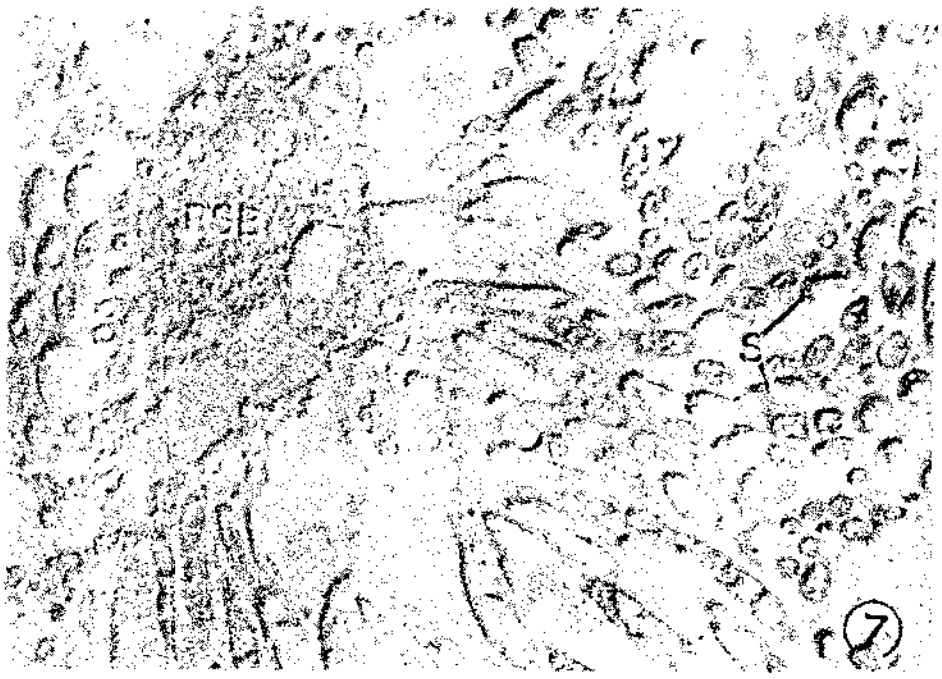


Fig. 7: Electronmicrograph showing advanced sporogony in the oocyst of *P. vivax*. There is a centrally located residual body (RSB) and completely formed sporozoites (S) are present all around it. (× 2,000).

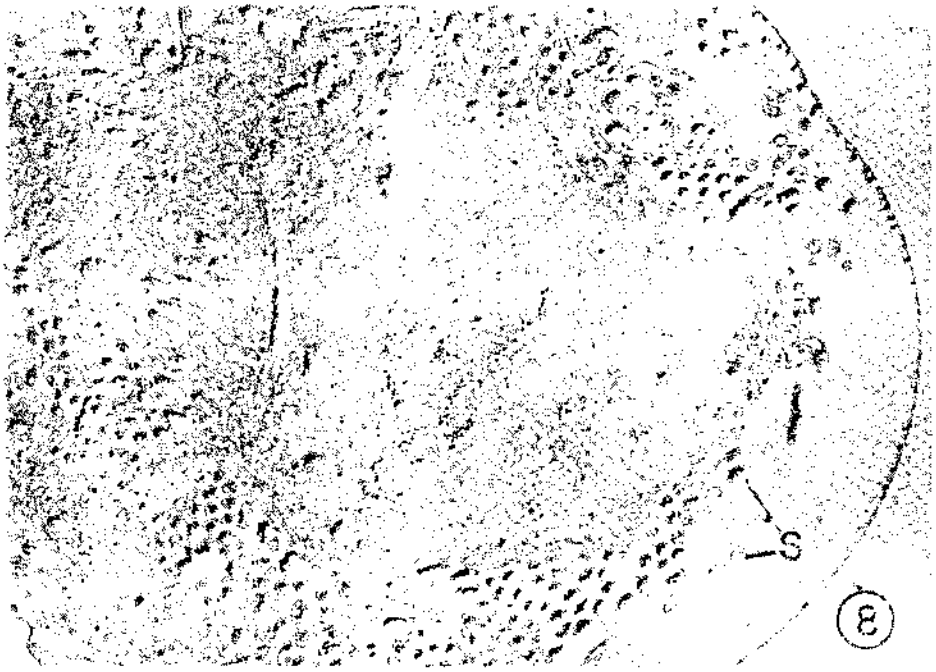


Fig. 8: Section of mature oocyst of *P. vivax*. The sporozoite (S) formation is almost complete. × 4,000).

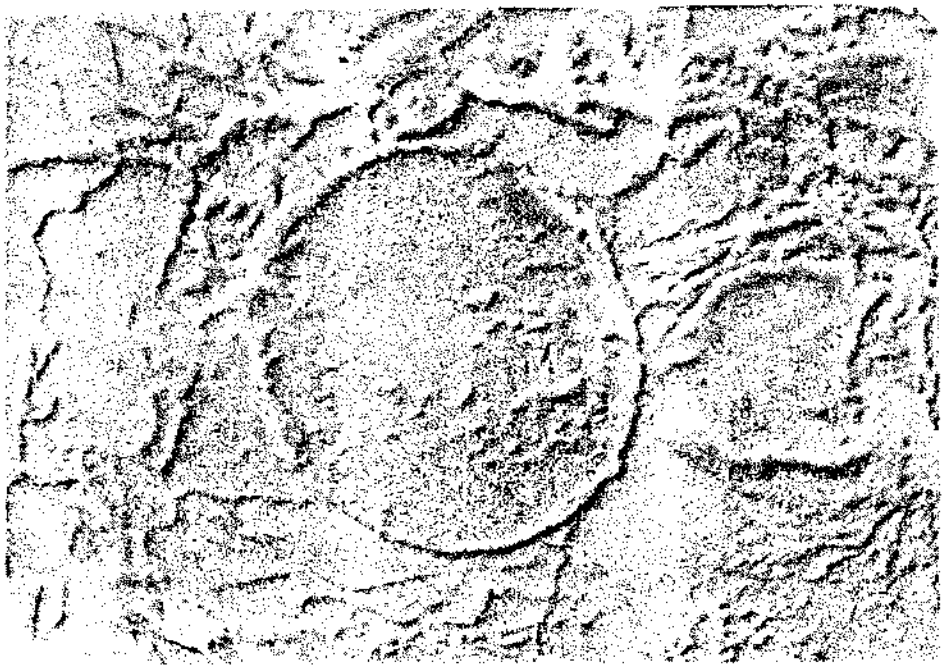


Fig. 9: Scanning electronmicrograph of mature oocyst (*MO*) of *P. vivax* showing spherical shape with smooth surface texture.[×c.4,200].

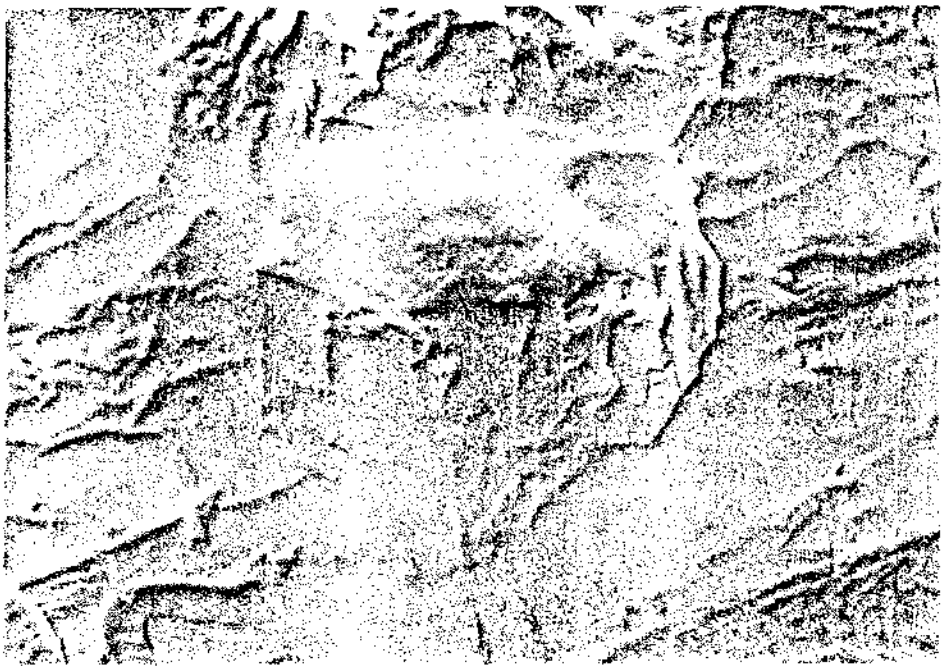


Fig. 10: Oocyst of *P. vivax* from which the sporozoites have been liberated, appears irregular in shape with several folds (F) on its surface. Musculature (*M*) of the midgut and tracheal supply (*T*) around oocyst can be seen.[×c.4,200].

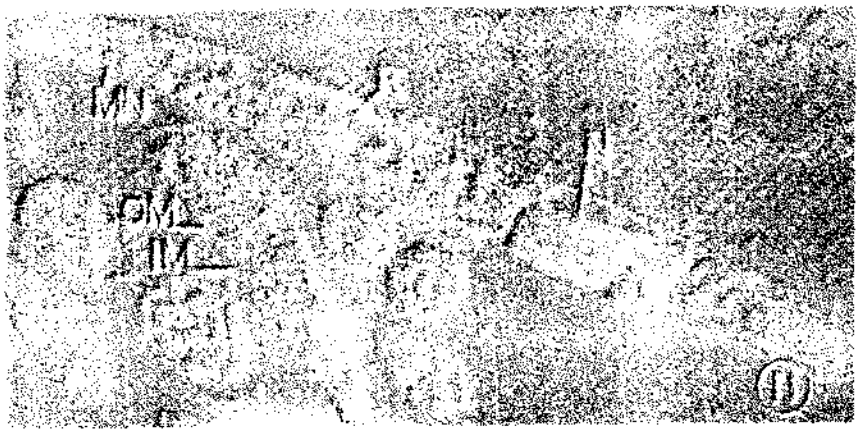


Fig. 11: Longitudinal sections of the sporozoite showing bipolar organization. The anterior region bears rhoptries (R) and micronemes (MN) and the posterior portion contains an elongated nucleus (N). The two layered pellicle (OM, IM) of the sporozoite is clearly visible. [×20,000].

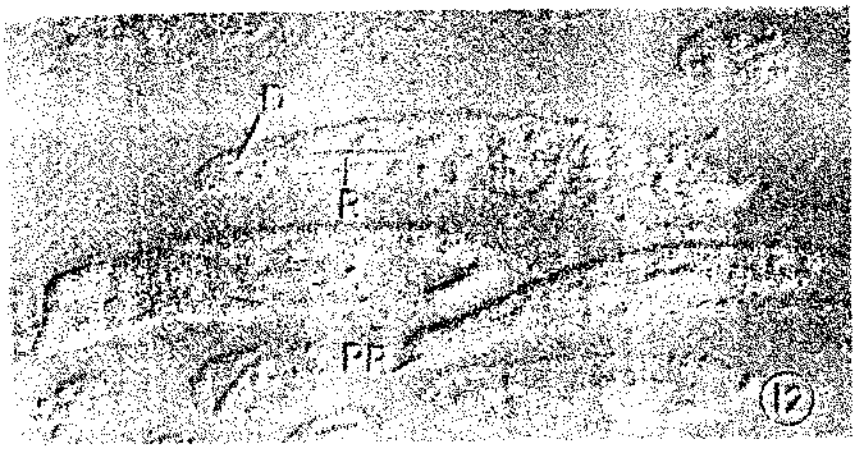


Fig. 12: Sporozoites showing truncated anterior end demarcated by polar ring (PR). Electron dense rhoptries (R) with their ducts (D) leading to conoid can be seen. [×20,000].

Fig. 13: Longitudinal and transverse sections of the sporozoites passing through the nucleus (N). A layer of microtubules (MT) beneath the pellicle (P) is visible in some sections. [×20,000].

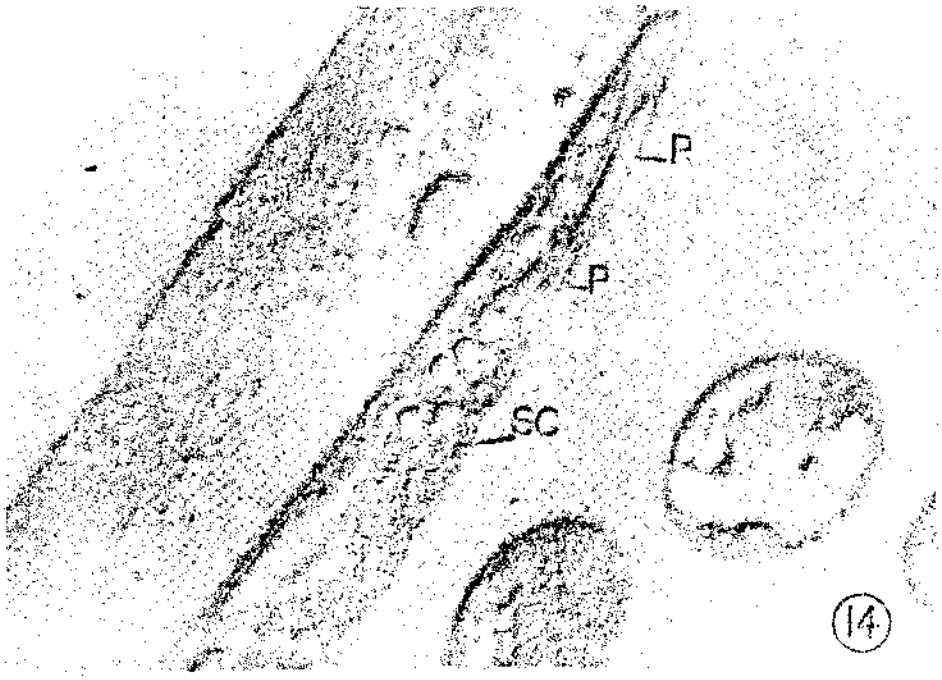


Fig. 14: Longitudinal section of the anterior region of the sporozoite showing a fibrillar surface coat (SC) on its pellicle (P) and rhoptries (R) cut in section. [×20,000].

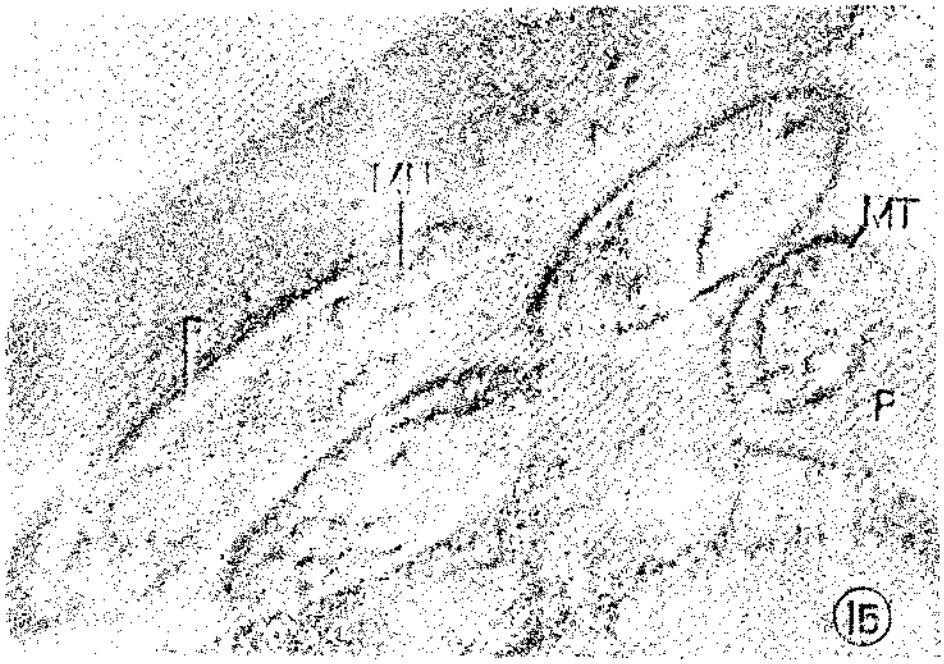
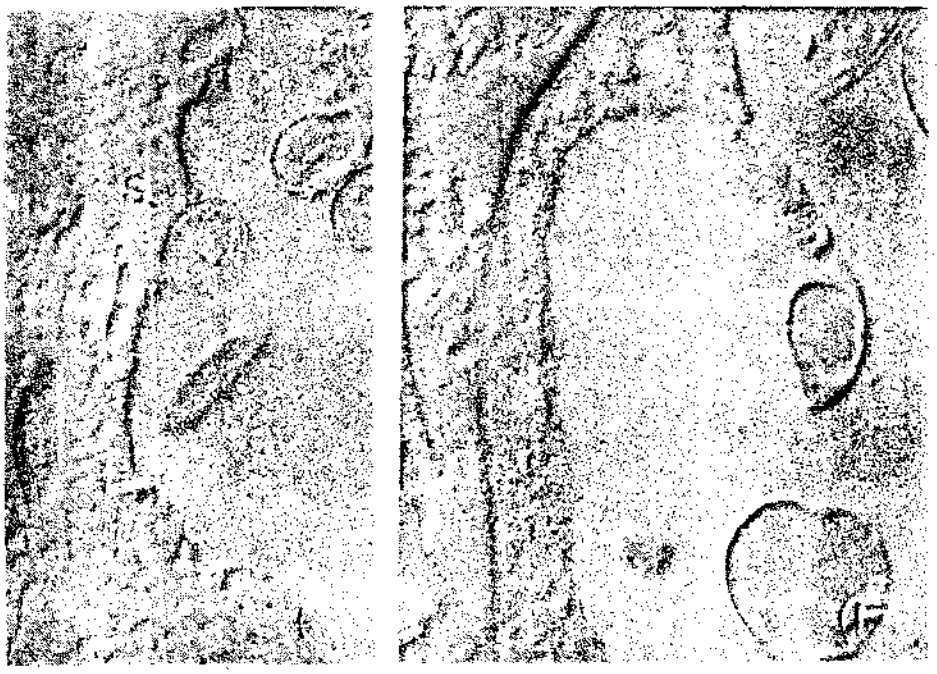


Fig. 15: Tangential sections of the sporozoites through anterior region showing a layer of microtubules (MT) beneath the pellicle (P). Electron dense rhoptries (R) and micronemes (MN) can also be seen. [×40,000]



Figs. 16 and 17: Electronmicrograph showing sporozoites (S) invading the membrane lined secretory vacuoles (SV) of the acinal cells through resulting invaginations. [×20,000].

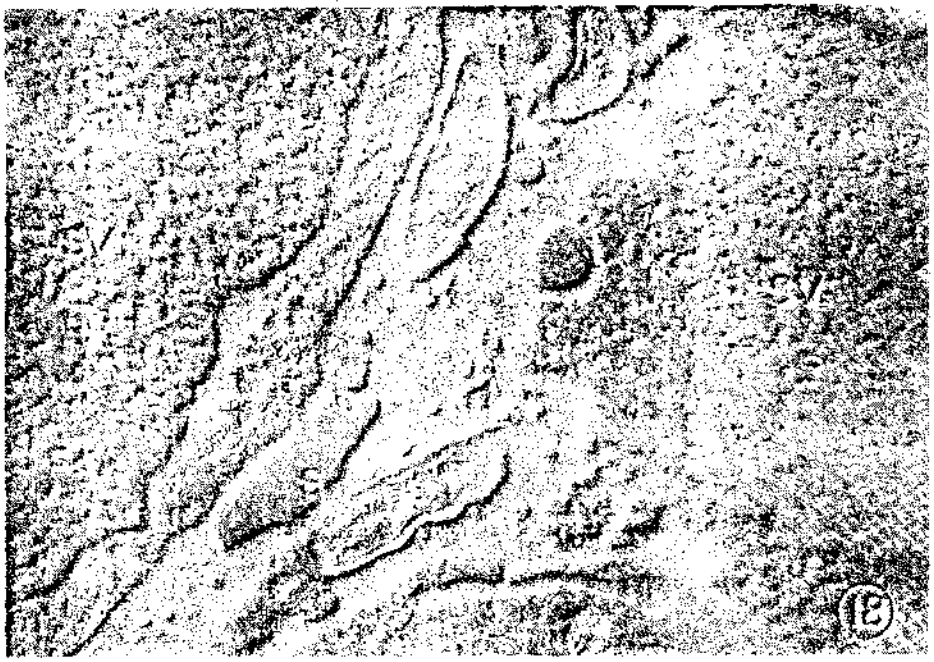


Fig. 18: Electronmicrograph showing sporozoites (S) in the intracellular space separating the secretory vacuoles (SV). [×20,000].

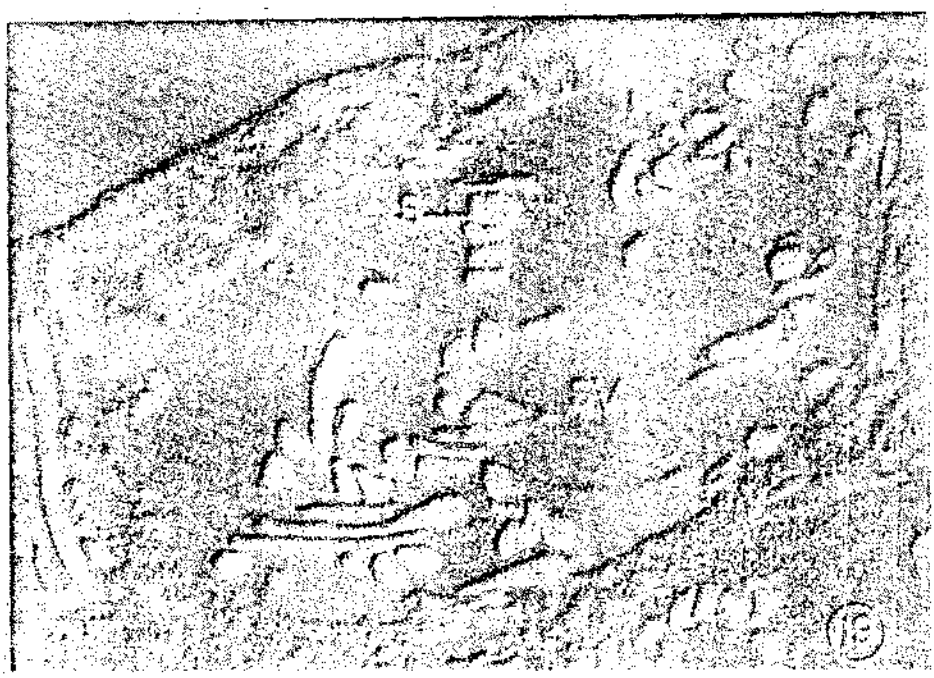


Fig. 19: Electronmicrograph showing large number of sporozoites (S) in the secretory vacuole (SV) of acinal cell. [×9,200].

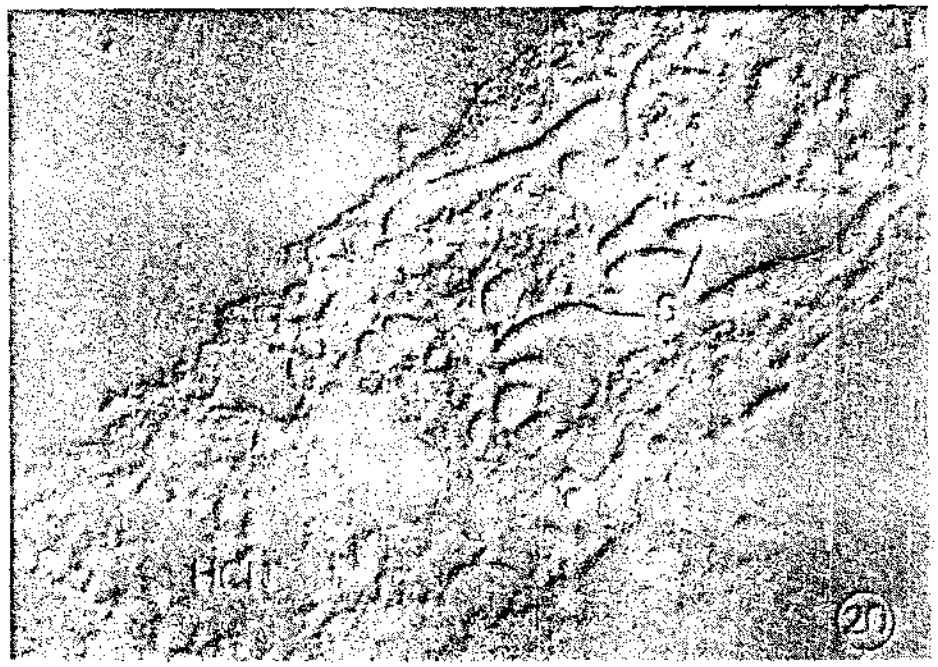


Fig. 20: Part of one infected salivary gland lobe showing longitudinal and transverse sections of the sporozoites (S) and host cell nuclei (*HCN*). [×9,500].

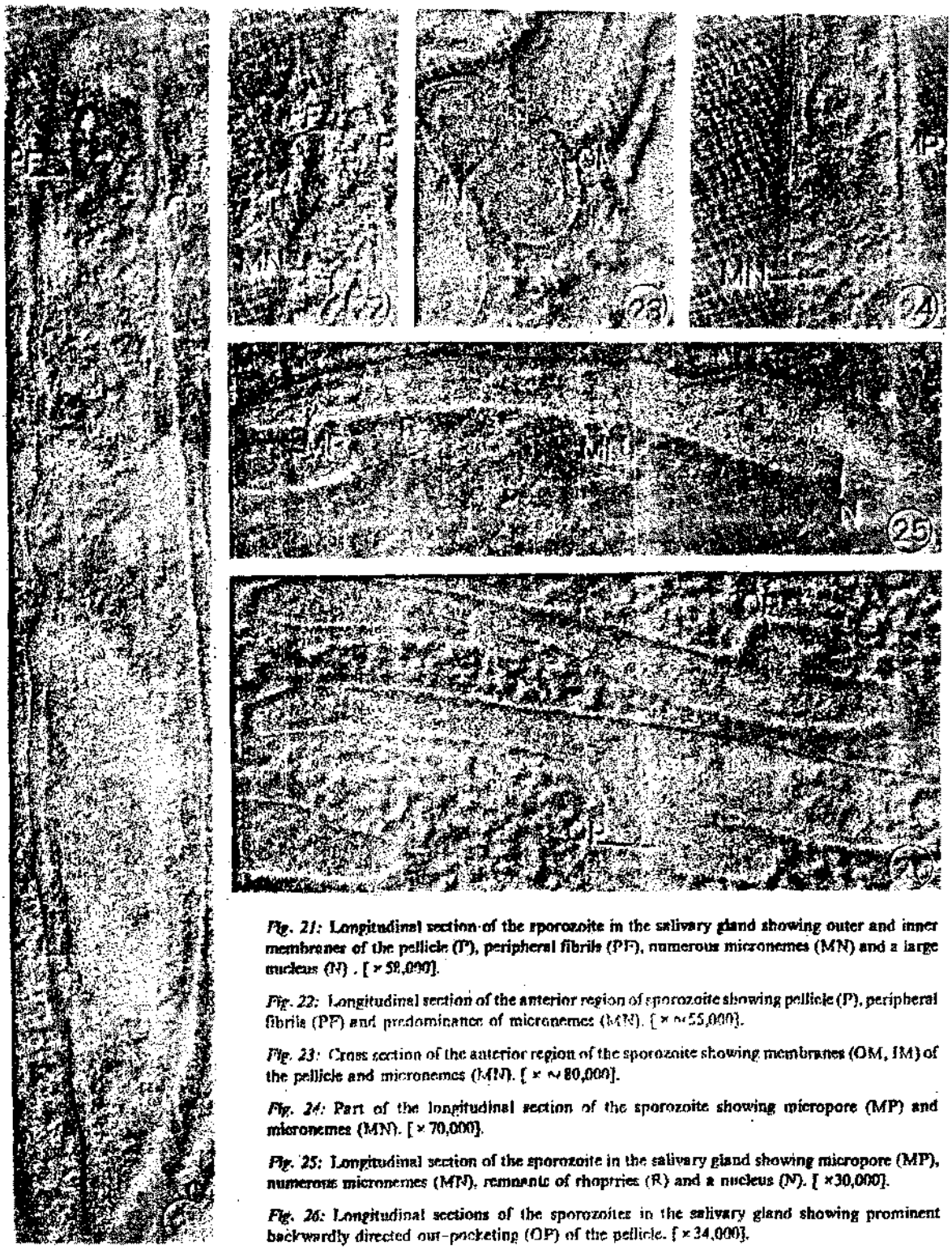


Fig. 21: Longitudinal section of the sporozoite in the salivary gland showing outer and inner membranes of the pellicle (P), peripheral fibrils (PF), numerous micronemes (MN) and a large nucleus (*N*) . [× 58,000].

Fig. 22: Longitudinal section of the anterior region of sporozoite showing pellicle (P), peripheral fibrils (PF) and predominance of micronemes (MN). [× ~55,000].

Fig. 23: Cross section of the anterior region of the sporozoite showing membranes (OM, IM) of the pellicle and micronemes (*MN*). [× ~80,000].

Fig. 24: Part of the longitudinal section of the sporozoite showing micropore (MP) and micronemes (MN). [× 70,000].

Fig. 25: Longitudinal section of the sporozoite in the salivary gland showing micropore (MP), numerous micronemes (*MN*), remnants of rhoptries (R) and a nucleus (*N*). [×30,000].

Fig. 26: Longitudinal sections of the sporozoites in the salivary gland showing prominent backwardly directed out-pocketing (OP) of the pellicle. [× 34,000].

dense layers thickening to form a stout wall to form the micropore. In the longitudinal sections, the micropore shows a smooth oval or circular contour (Figs. 24 & 25).

Rhopiries show further signs of morphogenesis after the release of sporozoites from the oocyst. Within the oocyst the sporozoites have prominent, well defined rhoptries extending to the mid portion, and few micronemes, whereas in the salivary gland forms the micronemes predominate and the rhoptries are less apparent suggesting their role in the invasion of acinal cells (Figs. 21, 22 & 25). The electron dense micronemes though more numerous in front of the nucleus, also exist posteriorly (Fig. 21). The nucleus has a granular structure and it almost fills the whole width of the sporozoite (Fig. 21). A prominent out-pocketing of the pellicle, some times backwardly directed, is seen near the middle portion, in some of the sporozoites (Figs. 25 & 26). This out-pocketing is filled with the granular cytoplasm and appears different from the blebbing of the outer membrane as noticed in some degenerating sporozoites of *P. vinkei chabaudi* (Sinden and Garnham, 1973).

DISCUSSION

In case of heavy infections the number and size of oocysts vary widely. The variation is assumed to result from competition for limited supply of nutrients. The oocysts deeply embedded in the gut wall developed more slowly than those adjacent to the haemocoele confirming the observations of Vanderberg *et al.* (1967) and Bafort (1971) in case of rodent malaria parasites. Such differential development may give rise to separate broods of sporozoites as also suggested by Bafort (1971). However, according to Sinden and Strong (1978), such competition does not result in the oocysts differing in their rates of maturation in case of *P. falciparum*.

As the oocyst grows the cyst wall is stretched and becomes thinner due to marked increase in the volume of oocyst which is not accompanied by significant wall synthesis. Sporoblast formation within the oocyst seems to increase the available surface area of the parasite and facilitates the uptake of metabolites. The mitotic activity of the nucleus which begins in the ookinete, accelerates rapidly and the nucleus becomes large multilobed structure and finally gives rise to large number of nuclei. This change in the nucleus occurs due to several fold increase in the quanity of DNA because of replication. After DNA synthesis the nuclear material segregates into a large number of nuclei presumably with haploid DNA content. From the sporoblast cytoplasm, sporozoites are budded off in a manner similar to merozoite formation in erythrocytic and exoerythrocytic phases of malaria infections (Aikawa, 1977). After the migration of nuclei from the sporoblast to the budding sporozoites, the precursor for apical complex gives rise to elongated rhoptries which become electron dense after maturation.

During the differentiation of the sporozoites, from the time they are budded off from the sporoblasts, several structures viz., polar rings, microtubules, rhoptries etc. are formed and their distribution imparts a definite polarity to the sporozoites. The question is at what point of time these structures are synthesized and assembled. It is possible that as a part of the activity of the ookinete, the sporoblast cytoplasm synthesizes the macromolecules to be assembled in the sporozoite or stable messages are synthesized and are passed on along with the cytoplasm into the sporozoites, or that both transcription and translation takes place in the sporozoite. The last possibility would mean that the sporozoite nucleus would become transcriptionally active. This has to be established experimentally.

Another interesting aspect is the manner in which sporozoites released into the haemolymph of the mosquito by dehiscence of the oocyst, make their way into the lumen of the salivary gland. The mechanism of penetration would be associated with the occurrence of specific receptors on the surface of the sporozoites for host cell

recognition and also with the secretory function of the rhoptries, producing a proteolytic enzyme to facilitate the sporozoites penetration of the host cell. This is evident from the observations that in salivary glands, sporozoites have more micronemes and the rhoptries are less apparent. This could result from the expulsion of material from the rhoptries during their penetration of the salivary glands. ***In vitro*** experiments are planned to trace the interaction of released sporozoites with the salivary glands cells.

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Mosquitoes of Kutch, Gujarat

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Fourteen species of mosquitoes belonging to three genera i.e., *Anopheles*, *Culex* and *Aedeomyia* were collected during February-March, 1984 from nine Talukas of Kutch (Gujarat). *Anopheles aconitus*, *A. jeyporiensis*, *A. pulcherrimus* and *A. sundaicus* were collected for the first time from this region. The previously recorded species *Anopheles barbirostris* was not found.

INTRODUCTION

Kutch is one of the districts forming part of the northwest region of Gujarat state. It is bound on the north and northwest by Rajasthan, on the east by Banaskantha and Mehsana districts, on the south by the Gulf of Kutch and on the west and southwest by the Arabian Sea. The Rann of Kutch is a salt desert covering an area of about 14490 km. The Great Rann which is situated to the north spans about 258 km from east to west and 129 km from north to south. The Little Rann lies to the east and measures about 129 km from east to west and 16-64 km from north to south. The whole district is barren. The physical terrain of Kutch varies considerably: Bhuj Taluka, the headquarters of Kutch represents the central mountainous region, Mandvi Taluka, the southern coastal alluvial tract, whilst Lakhpat Taluka is an example of barren low-lying terrain.

Information on the mosquito fauna of Kutch is very scanty. Afridi *et al.* (1938) were the first to study the mosquito fauna of Kutch and reported 7 species of anophelines. There has been no new information on the mosquitoes of Kutch for the last 40 years. The present survey was undertaken to update this information, since during the intervening period vast developmental changes have occurred in the country. Results of the study are reported in this paper.

MATERIAL AND METHODS

This survey was carried out in 40 days in nine Talukas of Kutch during February and March, 1984. Adult mosquitoes resting indoors and outdoors, and biting man and cattle were collected by suction tube in the mornings (5.00 A.M. to 11.30 A.M.) and evenings (5.30 P.M. to 10.00 P.M.). Adult collections were made from cattle sheds, human dwellings, mixed dwellings and other man-made structures. Outdoor resting adult mosquitoes were collected from the shrubs around cattle and human dwellings, forests and treeholes. In this survey, 41 villages were covered in nine Talukas — Bhuj, Anjar, Mandvi, Mundra, Lakhpat, Rapar, Bhachun, Nakhtarana and Naliya. The collection sites (villages) are shown in Fig. 1. Mosquito collections were made from each village from a

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RANN OF KUTCH
LITTLE RANN
RAPAR
BHACHUN
ANJAR
BHUJ
MUNDRA
MANDVI
NAKHTARANA
NALIYA
LAKHPAT
KUTCH
GUJARAT
MOSQUITO COLLECTION POINTS

Table 1. Mosquitoes collected from Kutch during February–March 1984

Species collected	TALUKAS								
	Anjar	Bhuj	Bhachau	Lakhpat	Mandvi	Mundra	Nakhtarana	Naliya	Rapar
A. aconitus	0	4	0	0	0	16	0	0	0
	(0)	(0.08)	(0)	(0)	(0)	(1.10)	(0)	(0)	(0)
A. annularis	2	142	12	4	7	23	2	124	2
	(0.10)	(2.94)	(2.61)	(2.19)	(0.67)	(1.59)	(4.54)	(8.41)	(0.64)
A. culicifacies	9	399	25	71	55	17	0	195	12
	(0.47)	(8.27)	(5.44)	(39.01)	(6.29)	(1.17)	(0)	(13.23)	(3.88)
A. fluviatilis	32	418	15	3	111	5	0	100	12
	(1.69)	(8.66)	(3.26)	(1.64)	(10.75)	(0.34)	(0)	(6.78)	(3.83)
A. nigerrimus	0	0	0	0	8	0	0	0	0
	(0)	(0)	(0)	(0)	(0.77)	(0)	(0)	(0)	(0)
A. pulcherrimus	0	29	0	51	0	4	0	47	2
	(0)	(0.60)	(0)	(28.02)	(0)	(0.27)	(0)	(3.19)	(0.64)
A. stephensi	1689	2833	352	45	728	399	35	457	191
	(89.31)	(58.73)	(76.58)	(24.72)	(70.54)	(27.66)	(79.54)	(31.02)	(61.81)
A. subpictus	32	26	0	2	38	277	0	2	2
	(1.69)	(0.53)	(0)	(1.09)	(3.68)	(19.20)	(0)	(0.13)	(0.64)
A. sundaicus	0	0	0	0	24	6	0	0	0
	(0)	(0)	(0)	(0)	(2.32)	(0.41)	(0)	(0)	(0)
A. turkhudi	0	4	0	0	30	82	0	0	0
	(0)	(0.08)	(0)	(0)	(2.90)	(5.68)	(0)	(0)	(0)
Cx. quinquefasciatus	124	905	51	6	17	590	7	520	76
	(6.55)	(18.76)	(11.11)	(3.29)	(1.64)	(40.91)	(15.90)	(35.30)	(24.59)
Cx. tritaeniorhynchus	3	61	4	0	3	23	0	25	12
	(0.15)	(1.26)	(0.87)	(0)	(0.29)	(1.59)	(0)	(1.69)	(3.88)
Ma. longipalpis	0	1	0	0	0	0	0	1	0
	(0)	(0.02)	(0)	(0)	(0)	(0)	(0)	(0.06)	(0)
Ma. uniformis	0	1	0	0	0	0	1	2	0
	(0)	(0.02)	(0)	(0)	(0)	(0)	(2.27)	(0.13)	(0)

Figures in parentheses indicate the percentage.

minimum of 8 cattlesheds and 3 human dwellings. In addition to this pyrethrum space spray collections were made from some huts. These villages were also surveyed for larval breeding sites. Field collected larvae were kept in a laboratory until adult emergence. Newly emerged adults were killed with ether and packed in cellophane paper. All field collected mosquitoes were brought to the Centre for identification and preservation. Mosquitoes were identified using the key of Christophers (1933) and Barraud (1934) and the catalog by Knight and Stone (1977).

RESULTS AND DISCUSSION

During the survey, a total of 11655 mosquitoes, belonging to 14 species of 3 genera — *Anopheles, Culex* and *Mansonia*, were collected. In the collections made, genus *Anopheles* consisted of 10 species (9222 specimens), genus *Culex* of 2 species (2427 specimens) and genus *Mansonia* of 2 species (6 specimens).

Among anophelines, the most common species was *A. stephensi* (57.73%) and a large number of morphological variations were found in this species. *A. culicifacies, A. fluviatilis* and *A. annularis* were collected in considerable number from almost all Talukas (Table 1). In January Afridi *et al.* (1938), recorded 27 specimens of *A. stephensi*, 23 specimens of *A. culicifacies* and 1 specimen of *A. fluviatilis*. *A. annularis* was absent in his collection. *A. barbirostris* which was reported by Afridi *et al.* (*loc. cit.*) was not found in this survey instead four new species viz., *A. pulcherrimus, A. nigerrimus, A. aconitus* and *A. sundaicus* were recorded.

Of the 4 new introductions in Kutch, *A. sundaicus* is a primary vector of malaria throughout its range of distribution. It was responsible for malaria epidemics in Chilka lake area (Covell and Singh, 1942). Recent surveys have revealed that *A. sundaicus* has disappeared from coastal Orissa (Nagpal and Sharma, 1983). During the present survey 30 specimens of *A. sundaicus* were found in Mandvi and Mundra Talukas which are on the sea coast. *A. sundaicus* is a saltwater breeder and the possibility that it may adapt itself to fresh water was suggested by Senior White and Adhikari (1939). In Mandvi and Mundra Talukas, it may be playing some role in malaria transmission. At present, the range of its distribution appears to be very limited, and this is the most opportune time to eradicate this species from Kutch before it is fully established in the coastal areas.

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Immunomodulation of Experimental Malaria by MDP

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Immunization of avians, rodents and primates with respective plasmodia coupled to Freund's complete adjuvant (FCA) has been documented as an effective mode of protecting the host against lethal malarial infections. The adjuvant, however, is unacceptable for human use because of serious side effects and there is a dire need to find a suitable substitute which is safe as well as effective. In the present study, different compounds were assessed for their efficacy in protection against *P. berghei* infection in rodents. Of the compounds tested, only muramyl dipeptide (MDP) along with squalane proved to be an effective adjuvant against *P. berghei* in rats. The same adjuvant, however, could not protect swiss mice against the same parasite.

INTRODUCTION

There has been a phenomenal resurgence of malaria as malaria eradication programmes based on vector control and chemotherapy measures have proved to be of limited success. In India, there is an increasing incidence of falciparum infection in areas presumed to be free from this deadly disease. The situation thus warrants alternative approaches for the control of this infection. In this connection, serious efforts are being made in India and abroad to explore the possibility of developing a vaccine (WHO Tech. Rep. Ser. No. 579, 1975). However, use of a potent and safe adjuvant seems imperative for effective protective immunity.

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The whole mycobacterial cell wall/mineral oil adjuvant (Freund's complete adjuvant, FCA), (Siddiqui, 1977; Mitchell *et al.*, 1977) has been found necessary for successful immunization of primates against *P. falciparum*. FCA has been employed in association with nonliving antigens of avian (Freund *et al.*, 1945; Coffin, 1951), rodent (Zuckerman *et al.*, 1967) and primate (Freund *et al.*, 1945, 1948; Targett and Fulton, 1965; Brown *et al.*, 1970; Schenkel *et al.*, 1973) plasmodia. The results of all these experiments were variable, but in general would indicate that FCA can significantly promote protective immunity. The extreme reactivity of FCA precludes its consideration as a potential clinical adjuvant in any vaccination (WHO Tech. Rep. Ser. No. 595, 1976). Thus, the development of an immunologically satisfactory and pharmacologically acceptable adjuvant is of prime importance. The present experiments involved investigation of non-FCA agents as adjuvants for vaccination with *Plasmodium berghei* in mice and rats.

MATERIAL AND METHODS

Animals: Inbred Wistar rats weighing 80–90 gm and inbred NMRI mice weighing 20–25 gm were included in the study. In all the experiments, statistically adequate number of animals kept on pellet diet (Hindustan Levers) with water *ad libitum* were used.

Parasites: Rodent malarial parasite *Plasmodium berghei* Vincke and Lips strain, 1948) obtained from National Institute of Communicable Diseases, Delhi has been maintained in our laboratory by weekly blood passages using 1×10^6 infected RBCs per mouse and 1×10^7 infected RBCs per rat by the intraperitoneal route.

Blood examination: Thin and thick blood smears stained with Giemsa were examined daily after infection and percentage parasitaemia calculated by counting at least 500 red blood cells per smear.

Antigen: Highly infected blood of mice with parasitaemia of 50–80% was taken in heparin. Cells were washed in PBS (pH 7.2) and resuspended in the original blood volume. The cell suspension was treated twice with 6% dextran (mol. wt. 275,000) to eliminate the leucocytes. The washed packed erythrocytes were suspended in 5 volumes of saponin solution (1:7500 in saline) and the suspension incubated with occasional stirring in a water bath at 37°C for 15 minutes. Following chilling in an ice bath, it was centrifuged at 10,000 rpm in a refrigerated centrifuge for five minutes. The supernatant was discarded and the liberated parasites washed 3 times in large volumes of chilled PBS by centrifugation at high speed. The free parasites were then sonicated twice at 20 kcs for 30 sec and centrifuged at 4,000 rpm for 20 min. Protein content of the supernatant was estimated by the Folin's method and after adjusting its concentration to 1 mg/ml, it was stored at –70°C in small aliquots.

Adjuvants

Exp. I Trehalose dimycolate (Cord factor): This was obtained through the courtesy of Dr. Edgar Lederer, Institute de Biochimie, University de Paris–Sud Orsay, France. A group of 12 mice (20–25 gm each) was taken, of which 6 served as control. 200 µg of TDM in saline was injected per mouse intraperitoneally in a single dose. Three weeks later, the experimental group of animals and the control group of untreated animals were challenged with 10^7 parasitized RBCs (PRBCs) per mouse. Parasitaemia and mortality were observed sequentially.

Exp. II Muramyl dipeptide (MDP): MDP was prepared by one of us (CMG). A group of 16 mice (20–25 gm) was taken of which 8 served as control. 100 µg MDP was injected intramuscularly into each mouse 7 days and 14 days before the parasite challenge. Control and test animals were challenged with 10^6 PRBCs per animal. Duration of immunity, parasitaemia and mortality were sequentially observed.

Exp. III Muramyl dipeptide+squalane+antigen in mice: Another group of 6 mice was given MDP mixed with squalane and antigen. The parasite antigen (600 µg) was mixed thoroughly with 0.6 ml of squalane containing 600 µg of freshly added MDP. Two hundred µl of this emulsion was injected intramuscularly into each mouse. A total of 3 such doses were given at one week intervals followed by a parasite challenge of 10^5 PRBCs per mouse one week later. A group of 6 weight and age matched mice was used as control.

Exp. IV Muramyl dipeptide+squalane+antigen in rats: MDP was also tested for its efficacy in rats. A total of 12 rats weighing between 80–90 gm each were taken. Six of these rats were untreated and served as controls. The other group of six animals was immunized with the emulsion of MDP, squalane and parasite antigen. Number and amount of doses was the same as for mice given above. One week after the

last immunizing dose, the test as well as control animals were challenged with 10^5 PRBCs per rat. Duration of immunity, parasitaemia and mortality were sequentially observed.

RESULTS

1. *Trehalose dimycolate (Cord factor)*

Mice immunized with TDM and the controls did not show much variation in the course of parasitaemia when compared with each other. The prepatent period was the same in both the groups. The mean percentage parasitaemia of both the groups nearly paralleled each other until the 9th post inoculation day (Table 1). The maximum mean percentage parasitaemia was 62.5±17.6 on the 12th day in the TDM group whereas it was 50.0±0 in the control group. All the animals in the TDM group and the control group died due to *P. berghei* infection.

2. MDP (Muramyl dipeptide)

Two doses of 100 µg of MDP each were given intramuscularly to each mouse. The parasitaemia curves in the MDP group and the control group (parasite only) paralleled each other (Fig. 1). The maximum mean percentage parasitaemia of the MDP group was 55.0±21.2 on the 10th day whereas it went up to 42.0±25.4 in the control group on the same day. The difference was statistically insignificant ($p>0.05$). All the control as well as MDP group animals died due to *P. berghei* infection.

3. MDP mixed with squalane and antigen

A. Immunization in rats: MDP mixed with squalane and antigen was given to rats in 3 doses, 1 week apart, followed by a challenge of 10^5 PRBCs per rat. The rats in the control group developed parasitaemia by the 6th day (Fig. 2). The maximum mean percentage parasitaemia on the 9th post inoculation day with 10^5 PRBCs/rat was 4.1±0.14 in the control group of rats. After the 9th day, the mean percentage parasitaemia levels declined to 0.1±0 on the 14th day. However, the immunized animals did not develop any parasitaemia during the entire period of observation after challenge with *P. berghei*.

B. Immunization in mice: A similar experiment using an emulsion of MDP, squalane and antigen was carried out in mice. However, the immunized mice did not show any protection against *P. berghei* and died of the infection (Fig. 3 and Table 2).

Table 1. Behaviour of *P. berghei* in PMPI mice pretreated with TDM (200 µg/mouse) and challenged 3 weeks later with 10^7 PRBC/mouse compared with control animals challenged with the same dose

Days after infection	Test	SD	Control	SD	p-value	Mortality	
						Test	Control
5	Negative		Negative			—	—
6	0.6	0.5	0.42	0.42	$p>0.05$	—	—
7	2.6	1.6	2.8	1.9	$p>0.05$	—	—
8	7.0	3.9	6.2	1.9	$p>0.05$	1	1
9	6.5	2.6	8.75	2.2	$p>0.05$	—	1
10	30.0	26.4	22.5	6.4	$p>0.05$	—	—
11	46.6	28.8	33.3	14.4	$p>0.05$	—	—
12	62.5	17.6	35.0	—		1	3
13	50.0	—	40.0	—		2	—
14	60.0	—	50.0	—		2	1

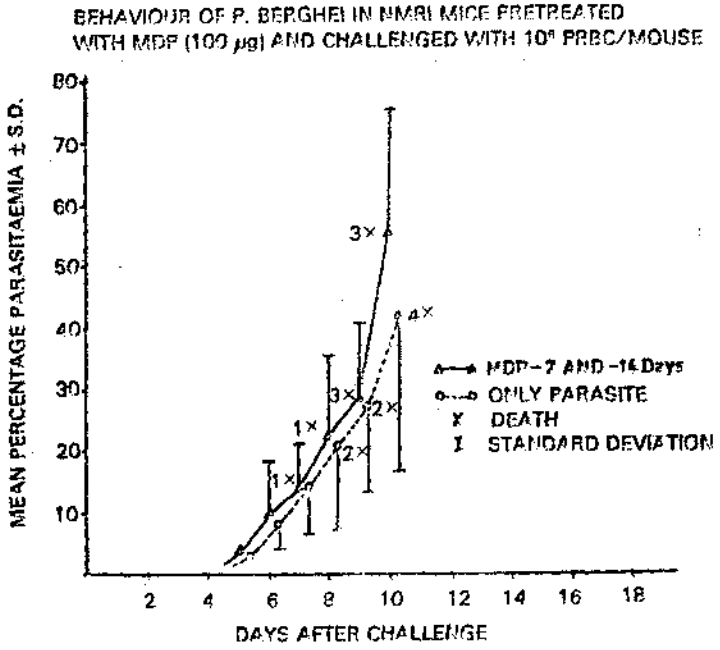

Fig. 1: Course of parasitaemia in NMRI mice pretreated with 2 doses of MDP (100 μg each) & challenged with 10^5 PRBCs per mouse compared with untreated control mice challenged with the same dose.

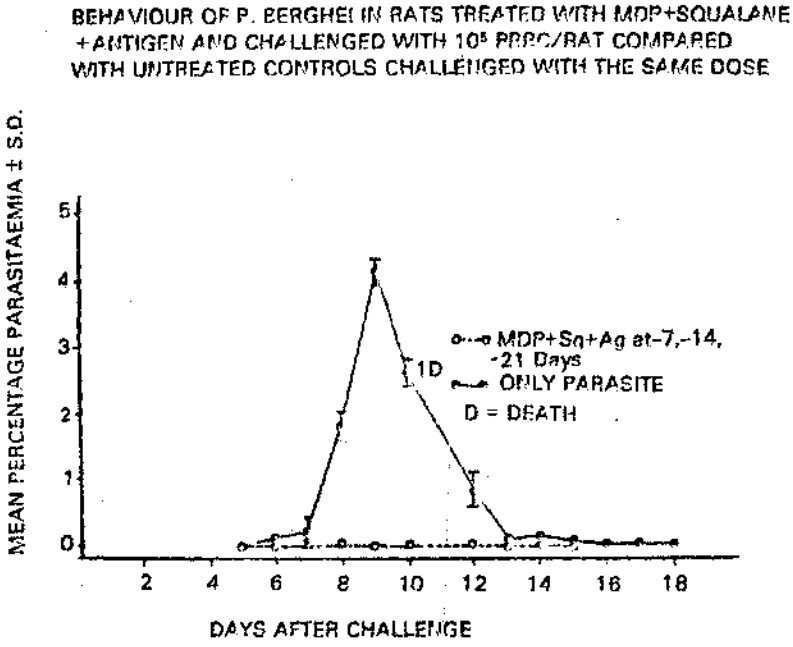

Fig. 2: Course of parasitaemia in Wistar rats pretreated with three doses of MDP mixed with squalane & antigen and challenged with 10^5 PRBCs per rat compared with untreated control rats challenged with the same dose.

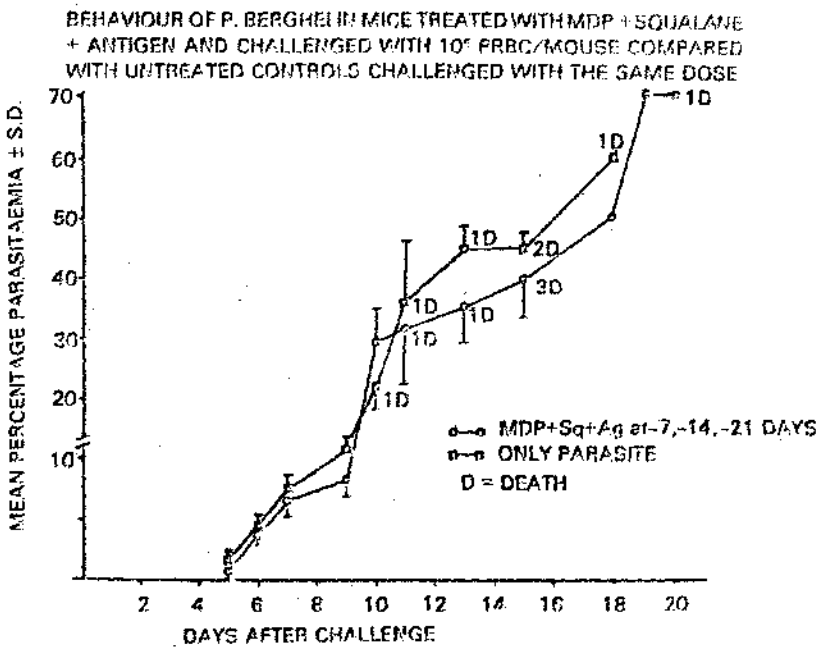

Fig. 3: Course of parasitaemia in NMPI mice pretreated with three doses of MDP mixed with squalane and antigen and challenged with 10^5 PRBCs per mouse compared with untreated control mice challenged with the same dose.

Table 2. Behaviour of *P. berghei* in NMPI mice pretreated with MDP, squalane and antigen (3 doses) and challenged with 10^5 PRBC/mouse compared with control animals challenged with the same dose

Days after infection	Mean percentage parasitaemia				p-value	Mortality	
	Test	SD	Control	SD		Test	Control
4	Negative		Negative			—	—
5	0.83	0.28	1.12	0.62	p>0.05	—	—
6	3.3	0.57	4.0	0.81	p>0.05	—	—
7	6.66	1.15	7.0	1.1	p>0.05	—	—
9	8.0	4.2	12.5	2.51	p<0.05	—	—
10	29.3	11.0	23.7	8.09	p>0.05	—	1
11	32.5	18.9	36.6	20.8	p>0.05	1	1
13	35.0	12.2	45.0	7.07	p>0.05	1	1
15	40.0	14.7	45.0	5.0	p>0.05	3	2
18	50.0	—	60.0	—		—	1
19	70.0	—	—			—	—
20	70.0	—	—			1	—

DISCUSSION

Extensive data pertaining to experiments on mice, rats and guinea pigs have shown that cord factor (TDM) and its synthetic analogues, such as C_{56} are capable of stimulating nonspecific resistance to bacterial infection, to various forms of inoculated cells, and to parasites (Lederer,

1979). Cord factor has been partially successful in affording protection against the parasites *Schistosoma mansoni* and *Babesia microti* in mice (Mahmoud *et al.*, 1977; Clark, 1979).

In the present experiments, TDM failed to protect mice against *Plasmodium berghei*. It is possible that TDM can act against individual parasites and results of one experiment cannot be extrapolated to that of the other and each antigen has to be tested.

Dissection of the components of mycobacterial cells walls responsible for immunoadjuvant effects (reviewed by White, 1976) led to the identification (Ellouz *et al.*, 1974) and synthesis (Merser and Sinay, 1975) of the smallest active moiety, N-acetyl muramyl-L-alanyl-D-iso-glutamine (MDP), which when given with protein antigens in emulsions of water in mineral oil, exhibited an adjuvant potential comparable to that of whole mycobacteria. A synthetic derivative of MDP (nor-MDP) given in mineral oil has proved to be only partially effective as an adjuvant for merozoite vaccination of *Macaca mulatta* against *Plasmodium knowlesi* (Mitchell *et al.*, 1979). MDP whether used as such or emulsified in oil was ineffective in the mouse system used in this study. However, when MDP emulsified in oil was used in the rat model, it could afford 100% protection to the immunized rats, with the antigen MDP mixture. Several reasons account for the above results. It has earlier been documented in the literature that inbreeding increases susceptibility to certain bacterial or parasitic infections (Williams *et al.*,1975). This is borne out by the fact that in our earlier experiments conducted in the same strain, BCG antigen admixture protects the mice but 3 years later, in the same strain the behaviour of the *P. berghei* infection changed considerably with fatalities at a mean parasitaemia of 30% as against 80-90% in earlier studies. It appears that the presently evolved strain is highly susceptible to *P. berghei* and MDP is totally ineffective. In comparison, the infection is not as lethal in rats and hence it is easier to immunize them. There is a great genetic disposition regarding behaviour of the same strain in two genetically different animals and the effect of adjuvant is variable.

In earlier studies, two synthetic adjuvants (MDP+stearoyl MDP) were mixed with peanut oil and antigen and tried in owl monkeys to protect them from a lethal *P. falciparum* infection (Siddiqui *et al.*, 1979). In case of MDP only 1 out of 3 monkeys survived whereas using stearoyl MDP all 4 of the immunized monkeys survived. Our results, however, show that MDP used in rats could confer complete protection against *P. berghei*, while it was ineffective in a mouse system. The experiments suggest that primary compound of MDP is not effective in lethal infection in mice and FCA still remains the most potent of all. Currently, an outbred strain is being tested for the efficacy of MDP. The present data, however, would indicate that further work on newer derivatives of MDP would be most rewarding, particularly with a breakthrough of *in vitro* cultivation (Trager and Jensen, 1976). It is more true when it is realized that the protective antigens of plasmodial merozoites may have too large a molecular weight thus defying easy gene cloning (Freeman *et al.*, 1980) unlike the sporozoite antigens (Yoshida *et al.*, 1981).

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Efficacy of Single Dose Quinine as Presumptive Treatment in *P. falciparum* Malaria

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In vivo test for 7 days was conducted in Diphu Civil Hospital to study the efficacy of a single dose of 20 grains quinine sulphate (adult) orally in 58 *P. falciparum* cases. Six cases were dropped from the study due to unavoidable circumstances. Of the 52 cases followed, 29 cases (59.2%) showed clearance of asexual parasitaemia by day 3 and remained so upto day 7. In 20 cases (40.8%) parasitaemia cleared initially but reappeared by day 7. In 3 cases (5.8%) parasitaemia did not clear at all. Parasite clearance was registered in 76.1% cases by day 4 and by day 5 in 94.2% cases. Adverse side reactions were vomiting, tinnitus, pain in abdomen and temporary deafness of mild to moderate intensity. Children tolerated the drug better.

INTRODUCTION

The system of presumptive treatment, which was adopted from early sixties under the National Malaria Eradication Programme, refers to administration of a single dose (600 mg) of chloroquine after blood smears are collected from fever cases, both through domiciliary services and health agencies. This is based on the assumption that every fever case is due to malaria infection unless proved otherwise.

In case the fever is not due to malaria a single dose of chloroquine does not lead to any adverse side effect and hence no harm is done (NMEP Report).

However, with the emergence of *P. falciparum* strain resistant to chloroquine (Sehgal *et al.*, 1973; Sehgal *et al.*, 1974; Pattanayak *et al.*, 1979; Chakrabarti *et al.*, 1979; Das *et al.*, 1979; Guha *et al.*, 1979) and its spread to many countries, the problem of using presumptive treatment is becoming increasingly difficult. Although amodiaquine may provide temporary relief, development of resistance to this 4-aminoquinoline is also well-known. Hence in some countries a drug combination like pyrimethamine with sulphadoxine or sulphalene has been substituted for chloroquine. This is the policy adopted in this country where definite foci of chloroquine resistant *P. falciparum* have been established.

It should, however, be emphasised that prolonged use of this drug combination is likely to precipitate resistance to these drugs as well, as has been observed in a few countries.

Considering these developments and that several chloroquine resistant foci have been found in the

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district of Karbi Anglong, Assam where *P. falciparum* is the dominant species, the finding of an alternative anti-malarial, to act as a stopgap for a short while has become a vital issue.

Accordingly, studies were undertaken during April-June '83 at Diphu Civil Hospital in Karbi Anglong, using a single dose of quinine sulphate with the following objectives:

1. To collect base line data on the impact of a single dose of quinine on patients with clinical symptoms and parasitaemia of *P. falciparum* within a period of 48 to 72 hrs and then upto day 7 after drug administration.
2. To observe any adverse side symptoms.
3. Should the regime prove effective, whether it could be used as a presumptive treatment.

Incidentally, the slide positivity rate in this area has been from 10 to 12% and the ratio of *P. falciparum* to total cases is about 70 to 75%. The study area has also shown a high percentage (76.4%) of resistance in *P. falciparum* to chloroquine. (Barkakaty *et al.*, Unpublished data).

MATERIAL AND METHODS

Fever cases attending the hospital were subjected to blood examination. *P. falciparum* cases with asexual parasite count above 1000 per cu. mm. of blood were hospitalised. Urine was tested through Dill and Glazko and Lipnin tests (Lelijveld and Kortmann, 1970) to ensure that the persons did not have any 4-aminoquinoline or sulfa drugs earlier. Cases with mixed infection, pregnant women and children below 1 year age were not included in the trial.

Quinine sulphate tablet in the following dosage schedule were given orally on the first day (day 0). Before giving the drug, the patients were advised to have their hospital diet.

Table 1. Dosage schedule of quinine sulphate

Age Groups	Quinine sulphate	
	Base in grains	Base in mg
1–4 yrs.	5	324
5–8 yrs.	10	648
9–14 yrs.	15	972
Above 14 yrs.	20	1296

In younger age groups, quinine was administered mixed with honey. Both thick and thin blood smears were collected from the patients in duplicate on day 0, prior to adminstration of quinine. Follow-up smears were collected daily upto day 7. Urine samples of the study cases were examined on day 2 to see for excretion of quinine (qualitative test) by Mayer tanret reagent (WHO, 1981). Blood smears were stained with 3% Giemsa stain in phosphate buffer (pH 7.2) immediately after collection. Examination and asexual parasite count was done against 1000 WBC.

Total WBC count and haemoglobin estimation was done in each case on day 0 onwards till day 7. Parasite count per cu. mm. of blood was calculated from the total WBC count of the patient on day 0.

The patients were under observation of the same physician from day 0. Daily morning temperature was recorded. Side effects like nausea, vomiting, tinnitus and other toxic symptoms were noted.

Out of the 58 cases initially administered with the single dose of quinine sulphate, four cases could not be followed because of high temperature and deteriorating condition of the patient. Two cases which vomited the drug just after administration were deleted from the trial. Thus the study could be completed in 52 cases. The age and sex of the 58 cases initially considered for trial is shown in Table 2.

Table 2. Age and sex-wise distribution of patients studied

Age group	Male	Female	Total
1–4 yrs.	3 (1)	—	3 (1)
5–8 yrs.	4 (1)	6	10 (1)
9–14 yrs.	2	4	6
Above 14 yrs.	33 (2)	6 (2)	39 (4)
Total	42 (4)	16 (2)	58 (6)

Figures in parentheses indicate the number of cases deleted from the trial.

RESULTS

Clinical symptoms: Out of the 52 cases, 12 (23.1%) cases became afebrile in 24 hours, 18 (34.6%) cases in 48 hours and another 18 cases (34.6%) in 72 hours. In the remaining 4 cases (7.7%) the time taken was 96 hours (Table 3).

Table 3. Showing the rate of clearance of clinical symptoms

Clearance of symptoms in hours	No. of cases	Percentage
24	12	23.1
48	18	34.6
72	18	34.6
96	4	7.7

Thus 92.3 per cent of the cases became afebrile by day 3 and all by day 4.

Fig. 1 shows the mean of temperature of the 52 cases.

Parasitaemia: The effect of administration of single dose of 20 grains quinine sulphate are indicated in Table 4. Out of 52 cases, there was complete clearance of parasitaemia in 49 cases (94.2%) by day 6. The single dose of quinine did not effect clearance of parasitaemia in 3 (5.8%) cases by day 7 i.e., upto the end of the period of studies. The observation of clinical symptoms revealed that even in these 3 cases, there was complete amelioration of clinical symptoms. However, it was observed that in 20 out of 49 cases showing complete clearance of parasitaemia, there was reappearance of asexual stage of malaria parasite by the end of the study period i.e., day 7.

Table 4. Rate of clearance of parasitaemia by day 6

Clearance of parasitaemia in days	No. of cases	Percentage
2	7	13.5
3	13	25.0
4	20	38.5
5	8	15.4
6	1	1.9

Fig. 2 shows the average daily asexual count of the 52 cases. Average asexual parasite count reduced from 31978/cu. mm. of blood on day 0 to 467/cu. mm. of blood on day 3. Even these 23 cases which had low level of parasitaemia from day 4 to day 7, remained afebrile. Two of these patients felt so good that they left the hospital without being discharged on day 7.

Age composition of the cases in which parasite reappeared after the single dose administration of quinine, is shown in Table 5. The youngest patient was four years old and the eldest was 50 years old.

Table 5. Age composition of cases which showed reappearance of parasites

Age group	Days			Total
	5	6	7	
1–4 yrs.	—	—	1	1
5–8 yrs.	3	—	—	3
9–14 yrs.	1	1	1	3
Above 14 yrs.	2	3	8	13
Total	6	4	10	20

Adverse side symptoms: The drug was not well-tolerated in 41 cases out of the 52 in which the trial was completed.

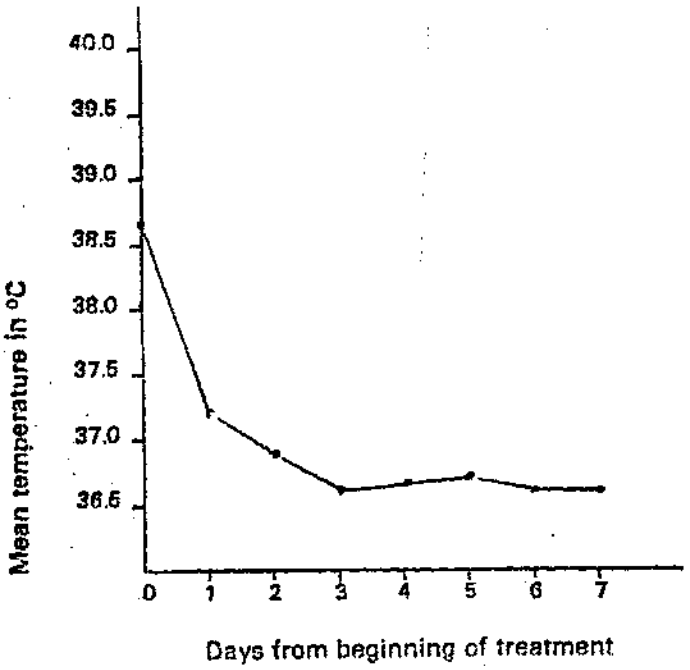

Fig. 1: Fever in 52 cases of acute falciparum malaria treated with quinine at Diphu.

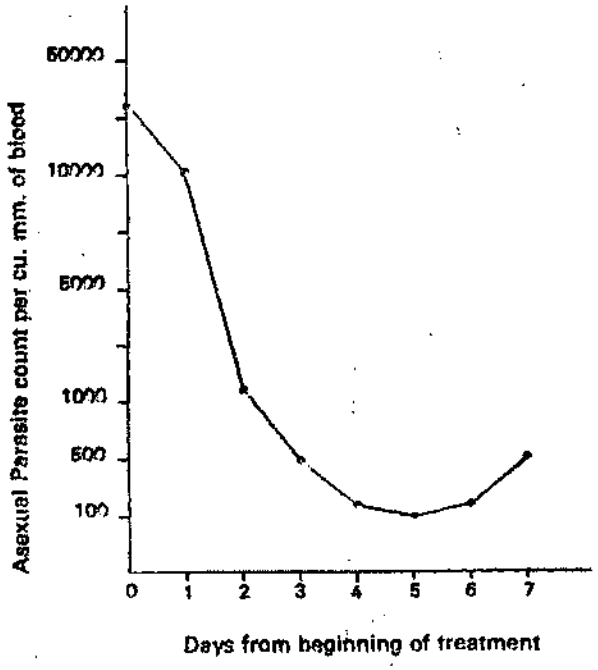

Fig. 2: Parasitaemia in 52 cases treated with single dose quinine at Diphu.

Table 6. Adverse manifestations of single dose oral quinine administration

Age Group	Vomiting	Pain in abdomen	Tinnitus	Shortness of hearing	Giddiness	Blurred vision	Tingling sensation in hands and feet
1-4 yrs.	—	—	—	—	—	—	—
5-8 yrs.	2	—	—	—	—	—	—
9-14 yrs.	2	—	—	—	—	—	—
Above 14 yrs	8	4	13	9	1	1	1
Total	12	4	13	9	1	1	1

Slight tinnitus in 13 cases was noticed. All the cases, however, recovered by day 4.

Vomiting was observed in 12 cases on day 0, mostly 3-4 hours after drug administration. In one patient repeated vomiting occurred on day 1. Since all these patients had high fever, the vomiting that occurred may not be wholly attributable to the drug.

Nine patients in the trial complained of difficulty in hearing on day 1. Normal hearing was restored in 3 individuals by day 3, 4 and 5. Four cases complained of mild pain in the abdomen upto day 4.

One patient each complained of giddiness blurred vision and tingling sensation of hands and feet. Eleven cases were free from side symptoms.

Children tolerated the drug better as compared to the adults under treatment. Total WBC count showed slight improvement on day 7. The mean TC rose from 6322 on day 0 to 6528 per cu. mm. of blood on day 7. Haemoglobin estimation was carried out in Sahli's scale. Mean haemoglobin estimation rose from 64.4% on day 0 to 69.2% on day 7. Urine tests on day 2 gave positive results in all the cases.

DISCUSSION

Resistance to chloroquine in *P. falciparum* strain is emerging as a world-wide phenomenon and poses a serious problem for malaria control activities. In India, from 1977 to 1981, a large number of *P. falciparum* sensitivity tests, including *in vivo* and *in vitro* (both macro & micro) studies were carried out. The results of these studies indicate that the phenomenon is of a higher magnitude level. To meet the anticipated situation, a number of long-acting sulfonamides and pyrimethamine combination drug trials were undertaken. For presumptive treatment this combination is not advisable as *P. falciparum* may develop resistance to these drugs also. Quinine is still considered to be the superior drug, for the treatment of acute malaria.

Encouraging results were obtained in the single dose administration of quinine in *P. falciparum* cases in the present study. Toxic manifestations were mild to moderate upto day 4. As expected, children tolerated the drug better in the present study.

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Effect of a Specific Iron Chelator, Desferrioxamine on the Host Biochemistry and Parasitaemia in mice infected with *Plasmodium berghei*

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Significant changes in the host biochemistry, especially in its ferrokinetics do occur in plasmodium infection. It was conceived that if the biochemical changes in the host due to malaria are reversed or conteined with drugs other than antimalarials, the host would be able to eliminate the infection. In the present study effects of a specific ferric chelator Desferal have been investigated as regards iron, lipid peroxide and protein contents of liver, spleen and brain of mice infected with *Plasmodium berghei* and having 9-13% parasitaemia. The effect of Desferal administration on two drug metabolising enzymes aminopyrine demethylase and aniline hydroxylase in liver have also been studied. Data obtained has been analysed and discussed. It was inferred that comparatively lesser fall in elevated iron contents of liver and spleen in infection suggests that some metabolic alterations of iron into forms which cannot be manipulated very effectively by Desferal takes place during infection.

INTRODUCTION

Significant changes do occur in the host metabolism and biochemistry in malarial infection which are remarkably reflected in the host stress organs, liver and spleen (Bossche van Den, 1972, 1976, 1980; Garnham, 1966; Gutteridge and Coombs, 1977; Hunter *et al.*, 1976; Killick-Kendrick and Peters, 1978; Bruce-Chwatt, 1980; Von Brand, 1973, 1976, 1979; Woodruff and Churchill, 1974). Though extensive studies have been conducted on various aspects of malaria, (Gutteridge and Coombs, 1977; Hunter *et al.*, 1976; Killick-Kendrick and Peters, 1978; Woodruff and Churchill, 1974) it is not known if the metabolic or biochemical changes due to malaria can be reversed or contained with drugs other than the antimalariale (Bossche van Den, 1972, 1976, 1980; Garnham, 1966; Gutteridge and Coombs, 1977; Hunter *et al.*, 1976; Killick-Kendrick and Peters, 1978; Bruce-Chwatt, 1980; Von Brand, 1973, 1976, 1979; Woodruff and Churchill, 1974; Burger, 1979; Roger and Pinder, 1973; Goodman and Gillman, 1980). Some of the key changes which occur in malaria are in the ferrokinetics of the host (Srichaikul *et al.*, 1976) which play an important role in the pathogenesis of the disease. In the present communication the effect of a specific ferric chelating agent (Gross, 1964; Ciba Foundation Symposium: Iron Metabolism, 1977) desferrioxamine (Desferal), has been investigated in *Plasmodium berghei* infection in mice as regards some biochemical changes occurring in liver, spleen and brain during moderate parasitaemia (9-13%).

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MATERIAL AND METHODS

Desferal or Desferrioxamine was obtained as a generous gift from M/s. Ciba-Geigy India Ltd., Bombay/Basel. The drug was dissolved aseptically in normal saline in appropriate concentrations. The tolerance of experimental mice to Desferal was first determined by administering different dose schedules of 300, 800 and 1000 mg/kg body weight intramuscularly daily for ten days. In 1000 mg/kg dose schedule the total daily dose was administered in two equally divided doses. The compound was well tolerated without any adverse effects.

Plan of experiment : For each set of experiments 40, six weeks old male Swiss mice weighing 18-20 gm each were used. These animals were divided in four groups of ten mice each. The animals in the first group (A), were neither infected with *P. berghei* nor treated with Desferal. The second group (B) were not infected with *P. berghei* but treated with Desferal only. The third group (C) were infected with *P. berghei* but not treated with Desferal and the last group (D) was infected with *P. berghei* and treated with Desferal.

The group D animals received their first dose of Desferal one hour after they were inoculated with *P. berghei*. Animals of all the groups were sacrificed when parasitaemia reached a level of 9.0-13.0% in infected groups C and D. The organs—liver, spleen and brain were immediately collected on autopsy, and weighed and frozen for estimation of iron, protein, lipid peroxides etc. Determination of drug metabolizing enzymes was done by pooling the livers of the relevant group of animals. Since the response of animals to the three levels of Desferal was practically similar the data concerning only one dose level of 1000 mg/kg body weight Desferal is being presented in this communication. Three replicates of the experiment were carried out.

Mode of infection: Blood from the orbital plexus of a heavily infected mouse was drawn under aseptic conditions and diluted with 3.8% sodium citrate. Erythrocytes were counted under a microscope with the help of a haemocytometer. Each animal of the relevant group was then injected intraperitoneally with an inoculum containing one million parasitized erythrocytes.

Biochemical parameters : Estimations of iron were carried out as per the method of Sidney and Nathan (1957). Those of lipid peroxide and protein concentration in liver, spleen and brain were carried out by the methods described earlier (Sharma *et al.*, 1978a, 1978b, 1979a, 1979b). Drug metabolizing enzymes in liver were determined according to Sharma *et al.* (1978b).

The values of average content per organ of iron, lipid peroxide and protein were calculated from the mean value of concentration per unit weight. The value of average content thus obtained, if necessary was multiplied by a factor of 10^1 or 10^2 or 10^3 to rationalize it for the purpose of comparative depiction on the histograms.

RESULTS

Table 1 summarises the changes that take place in the various parameters in liver of *P. berghei* infected (parasitaemia 9-13%) and normal mice before and after treatment with Desferal. It is seen that uninfected normal mice when treated with Desferal showed little change in the gross weights of their livers from those of untreated ones. The weights of livers of infected mice (C) were very high. But when, infected mice with same degree of parasitaemia were treated with Desferal (D), their liver weights were observed to be almost like those from uninfected mice (A). Total contents of iron and its concentration, expressed as mg/100 gm were minimum in Desferal treated-uninfected normal mice (B). Total content, though high in both infected-untreated (C) and infected-treated (D) groups, was higher in the former, but its concentration was almost the same in both.

For protein content, there was a fall both in total amount and concentration values in Desferal

Table 1. Effect of Desferal on the Liver of infected and uninfected mice

Groups	Wet tissue wt. in gm	Biochemical Parameters						Enzymes	
		Iron		Protein		Lipid peroxide		Aminopyrine demethylase	Aniline hydroxylase
		Content mg	Concentration mg/100 gm wet tissue	Content mg	Concentration mg/gm wet tissue	Content µmol MDA	Concentration µmol MDA/100 gm wet tissue	nmol CH_2O/hr/mg protein	nmole p-aminophenol/30 min/mg protein
A. Uninfected untreated	1.400±0.134	0.1125	8.04±0.49	235.20	158.00±2.00	0.8421	60.15±1.65	130	9.6
B. Uninfected treated	1.110±0.064	0.0577	5.20±1.12	190.36	162.49±1.16	0.2035	18.34±0.82	100	7.2
C. Infected untreated	2.507±0.348	0.3534	14.10±3.12	362.41	144.56±1.89	2.1201	84.57±2.82	51	2.3
D. Infected treated	1.812±0.114	0.2585	14.27±3.464	267.26	147.50±3.50	1.3542	74.74±5.92	22	4.0

Table 2. Effect of Desferal on the Spleen of infected and uninfected mice

Groups	Wet tissue wt. in gm	Biochemical Parameters					
		Iron		Protein		Lipid peroxide	
		Content mg	Concentration mg/100 gm wet tissue	Content mg	Concentration mg/gm wet tissue	Content µmol MDA	Concentration µmol MDA/100 gm wet tissue
A. Uninfected untreated	0.086±0.012	0.00722	8.40±0.00	12.488	145.22±1.33	0.01248	14.52±1.50
B. Uninfected treated	0.163±0.021	0.00805	4.94±0.64	21.460	131.66±2.12	0.01708	10.48±0.49
C. Infected untreated	0.831±0.191	0.11218	13.50±2.62	108.736	130.85±1.47	0.11294	13.58±2.21
D. Infected treated	0.605±0.137	0.08300	13.72±2.25	82.074	135.66±3.50	0.08203	13.56±2.45

treated-uninfected (B) animals. There were greater falls in infected-untreated (C) and infected-treated (D) groups.

Marked fall in total contents and concentration of lipid peroxide was observed in uninfected-Desferal treated (B) mice. It was very high in infected-untreated (C) ones. In the infected-treated (D) group it was more than in uninfected-untreated (A) ones but less than in infected-untreated (C) group.

The drug metabolizing enzyme aminopyrine demethylase is least in infected-treated (D) mice, the values for uninfected-treated (A) and infected-untreated (C) lying between uninfected-untreated (A) and infected-treated (D) ones. Aniline hydroxylase, the other drug metabolising enzyme is least in infected-untreated (C) group.

Table 2 shows the changes that take place in the spleen of uninfected and infected (with 9-13% parasitaemia) mice before and after treatment with Desferal. Changes were observed in gross weight of spleen. When uninfected mice were treated with Desferal (B) the weight of spleen was seen to increase. It is highest in infected-untreated group (C) and is much less in infected-treated (D) group.

For iron contents, it is seen that in untreated-uninfected (A) and uninfected-treated (B) groups though the total contents were same, the concentration was much less in uninfected-treated (B) group. But the concentrations in infected groups both untreated (C) and treated (D) were similar.

As for protein, the concentrations were much less in all the three groups i.e., uninfected-treated (B), infected-untreated (C) and infected-treated (D).

Data on lipid peroxide shows that concentrations were less in uninfected-treated (B) and infected-treated (D) groups, vis-a-vis corresponding uninfected-untreated (A) and infected-untreated (C) groups.

Results in brain in different groups have been summarized in Table 3. It is seen from the table that, though, practically little change took place in gross brain weights in all the different groups, there was some fall in the two treated groups B and D (uninfected) as compared to the corresponding untreated groups A and C.

As for protein, there was a marked fall in its concentration in the infected-untreated (C) group, with slight improvement on it in the

Table 3. Effect of Desferal on Brain of infected and uninfected mice

Groups	Wet tissue wt. in gm	Biochemical Parameters					
		Iron		Protein		Lipid peroxide	
		Content mg	Concentration mg/100 gm wet tissue	Content mg	Concentration mg/gm wet tissue	Content µmol MDA	Concentration µmol MDA/ 100 gm wet tissue
A. Uninfected untreated	0.495±0.013	0.00831	1.68±0.50	71.111	143.66±1.58	0.14389	29.07±1.32
B. Uninfected treated	0.462±0.036	0.00656	1.42±0.29	69.060	130.00±0.00	0.09896	21.42±3.59
C. Infected untreated	0.431±0.029	0.00840	1.95±2.84	46.849	108.70±4.39	0.11059	25.66±2.14
D. Infected treated	0.465±0.021	0.00767	1.65±0.39	52.307	112.49±5.80	0.11885	25.56±0.11

infected-treated (D) group. There is a fall in protein concentration from uninfected-untreated (A) group to uninfected-treated (B) group.

When lipid peroxide is considered, it is seen that its concentration in uninfected-treated group (B) was very low as compared to that in uninfected-untreated (A) group. Lipid peroxides in infected-untreated (C) and infected-treated (D) groups were, no doubt, lower than in uninfected-untreated (A) group, but between them there was little variation.

DISCUSSION

Iron : The idea of using Desferal as a drug in the containment and reversal of biochemical lesion in malaria was mooted from its chelating property. The objectives were to see if Desferal would help in alteration of iron overloading in the stress organs liver and spleen, a feature of affliction with malaria and to ascertain the form of iron in the host with the help of Desferal which is a specific chelator for Fe^{+++}.

It would be observed from Fig. 1 that in the uninfected animals Desferal exerted a lowering effect on iron content in liver and brain by 48.0% and 25% respectively. The spleen on the contrary recorded a rise, though insignificant, of 11.0% in its iron content under the action of Desferal. This would confirm that Desferal is capable of mobilizing and manipulating iron in the animal body through its chelating or some other as yet undefined action. As would be seen plasmodium infection exerts a tremendous influence on iron contents in liver and spleen as indexed by an increase of 315% and 1550% respectively but not in the brain. Desferal, in infected animals exerted a somewhat modulating effect by lowering the elevated iron content in liver and spleen from 315% to 225% and from 1550% to 1325% respectively. The effect of Desferal on brain was just perceptible. The comparatively lesser fall in iron contents of liver and spleen elevated in infection would indicate that some metabolic alterations of iron into forms, which cannot be manipulated by Desferal so effectively, have taken place. The formation of new pigments and perhaps some other stable iron-bearing complexes in the host body during infection may represent some of these forms of iron (Bossche van Den, 1972, 1976, 1980; Garnham, 1966; Von Brand, 1973, 1976, 1979; Srichaikul *et al.*, 1976), which are not amenable to the action of Desferal.

Protein: Desferal brought about an insignificant lowering in liver protein concentration of normal mice in comparison to the plasmodium infection which caused a perceptible fall (Fig. 2). The administration of this chelator in infection practically did not alter the effect of infection. It is concluded that Desferal when administered during infection could hardly influence the pattern of protein concentration in any of the three organs. When total protein contents were considered, in healthy mice, it caused a lowering by 24.0% in liver and a rise in that of the spleen by 170% indicating thereby an increase in spleen weight, the brain was not influenced perceptibly. The infection brought about a marked increase in the total protein content of the liver (154%) and a large increase (870%) in that of the spleen: as against this, the brain exhibited only a perceptible fall in its protein content of 27.0% (Fig. 3). Desferal, when administered in infection, could modulate the above tendency in a marked manner in liver but only to a much smaller extent in spleen (Fig. 3). The modulating effect of Desferal in brain on the other hand was of a different nature as it slightly raised the protein content, as compared with this biochemical value in infection alone.

Lipid peroxide : In plasmodium infection the liver exhibited a significant increase. Lipid peroxide is an index of the tissue damage to this organ at molecular level caused by the production of free radicals through activation of the xanthine oxidase system (Sharma *et al.*, 1978a). Desferal, which is known to protect against the free radicals (Gross, 1964; Ciba Foundation Symposium: Iron Metabolism, 1977) exhibited a

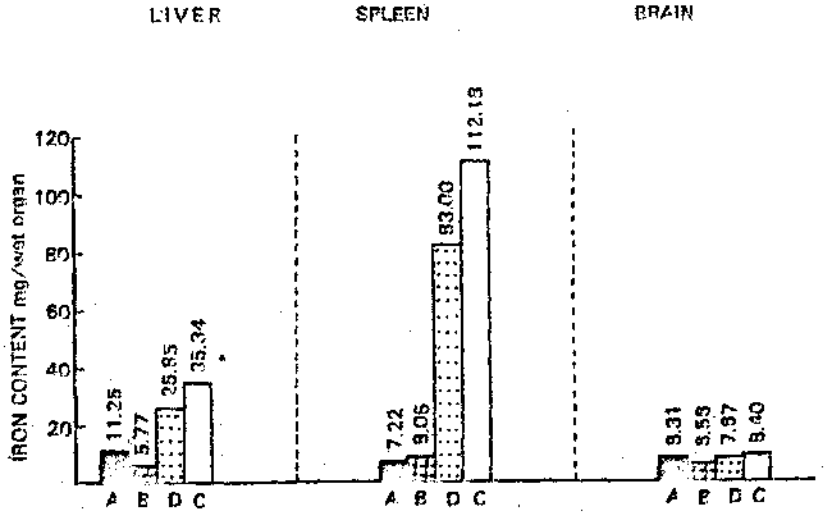

Fig. 1: Influence of Desferal on iron content in stress organs of host in *Plasmodium berghei* infection.
Liver, scale factor: original values × 100.
Spleen, scale factor: original values × 1000.
Brain, scale factor: original values × 1000.
A — Normal group.
B — Drug treated non-infected group.
D — Drug treated infected group.
C — Infected group.

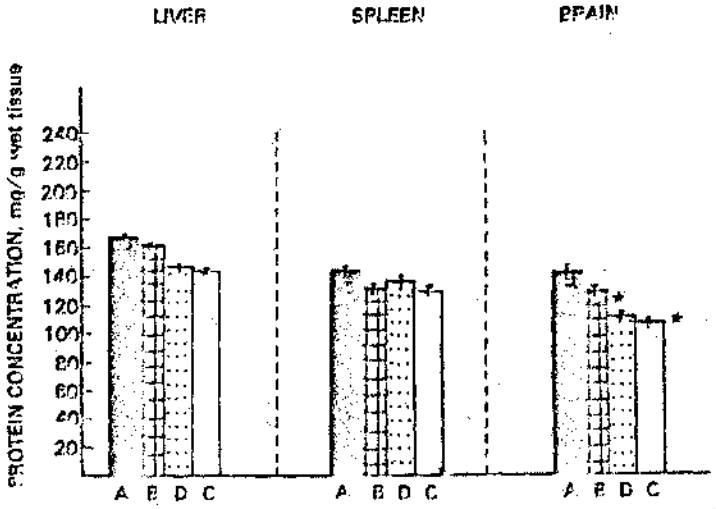

Fig. 2: Influence of Desferal on host (mouse) protein concentration in stress organs in *Plasmodium berghei* infection.
Liver, scale factor: original value only.
Spleen, scale factor: original value only.
Brain, scale factor: original value only.
A — Normal group.
B — Drug treated non-infected group.
D — Drug treated infected group.
C — Infected group.

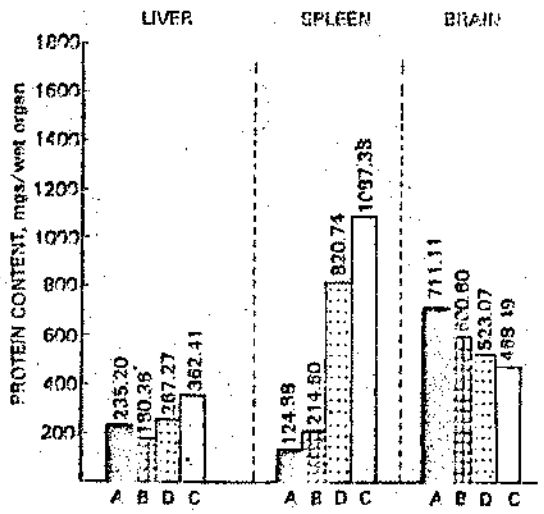

Fig. 3: Influence of Desferal on protein content in stress organs of host in *Plasmodium berghei* infection.
Liver, scale factor: original value only.
Spleen, scale factor: original values × 10.
Brain, scale factor: original values × 10.
A — Normal group.
B — Drug treated non-infected group.
D — Drug treated infected group.
C — Infected group.

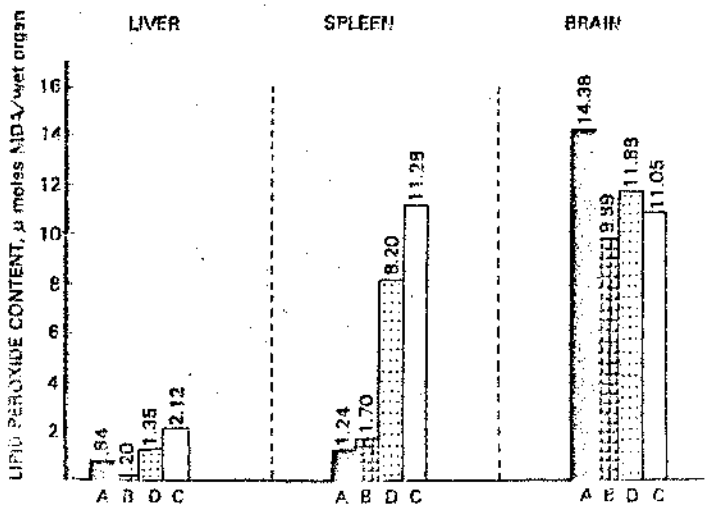

Fig. 4: Influence of Desferal on lipid peroxide content in stress organs of host in *Plasmodium berghei* infection.
Liver, scale factor: original value only.
Spleen, scale factor: original values × 100.
Brain, scale factor: original values × 100.
A — Normal group.
B — Drug treated non-infected group.
D — Drug treated infected group.
C — Infected group.

lowering effect on lipid peroxide in the non-infected mouse liver, spleen and brain, the liver being affected most as evident from the lowering effect on the concentration of lipid peroxides.

The pattern of lipid peroxide content in the three organs (Fig. 4) was different from that of the lipid peroxide concentration in the four experimental conditions. In infection, liver and spleen, each experienced a tremendous increase in lipid peroxide content to the extent of 250% and 950% respectively; the brain on the other hand exhibited a small fall of 24.0%. The observations imply that malarial infection has produced biochemical lesions in liver and spleen but not in brain (Sharma *et al.*, 1979a, 1979b). In case of liver and spleen this is understandable in view of the reported fall in the superoxide dismutase activity (Sharma *et al.*, 1978a) and an increase in the activity of xanthine oxidase (Sharma *et al.*, 1978a) in malarial infection. The above two conditions would obviously provoke lipid peroxidation in liver and spleen in malarial infection (Michelson, 1977; Sharma *et al.*, 1978a).

Desferal which is known as a protector against lipid peroxidation lowered the lipid peroxide content of the liver and brain of non-infected animals by 76% and 32.0% respectively. It exerted an opposite effect on the spleen. It greatly modulated the effect of infection in liver by lowering the lipid peroxide content from 260% to 160% in respect of the normal liver. The modulatory effect of Desferal in brain and spleen of infected mice was somewhat less marked.

Enzymes : The plasmodium infection, as expected, caused a significant fall in the activity of two drug metabolizing enzymes (Fig. 5). Though Desferal alone exerted a very small effect (decrease in the activities of the two enzymes) in association with malarial infection it caused a further fall in the activity of aminopyrine demethylase but a small rise in the activity of aniline hydroxylase. The effect of

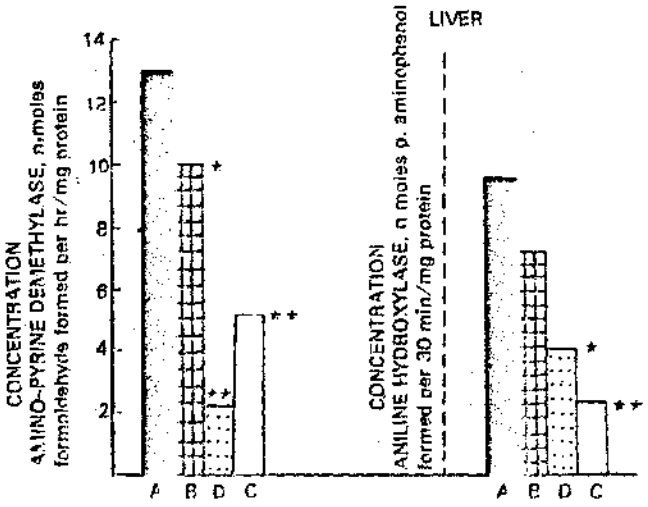

Fig. 5: Influence of Desferal on aminopyrine demethylase and aniline hydroxylase activities respectively of host (mouse) liver in *Plasmodium berghei* infection.
Liver aminopyrine demethylase concentration, scale factor: original values ÷ 10.
Liver aniline hydroxylase concentration.
A — Normal group.
B — Drug treated non-infected group.
D — Drug treated infected group.
C — Infected group.

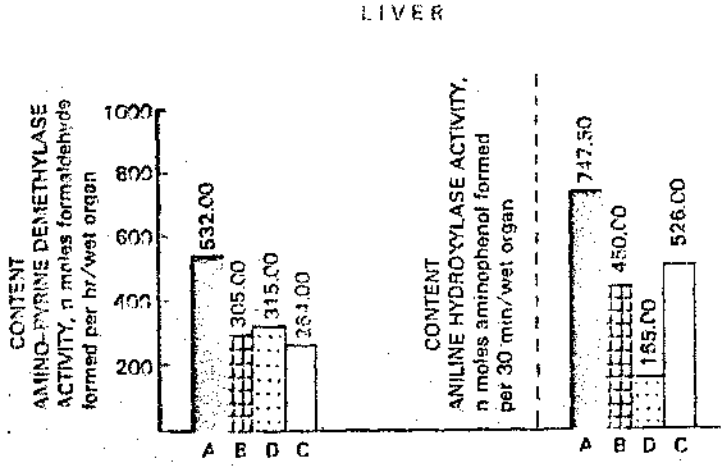

Fig. 6: Influence of Desferal on aminopyrine demethylase and aniline hydroxylase contents respectively of host (mouse) liver in *Plasmodium berghei* infection.
Liver aminopyrine demethylase content, scale factor: original values ÷ 10.
Liver aniline hydroxylase content.
A — Normal group.
B — Drug treated non-infected group.
D — Drug treated infected group.
C — Infected group.
* Implies that the difference is statistically significant, $p<0.05$.
** Implies that the difference is statistically highly significant, $p<0.01$.

Desferal on the two drug metabolizing enzymes may be partly understandable because Desferal is a Fe(ic) chelator and the drug metabolizing microsomal enzyme system operates in the animal host liver (microsomes) with both the forms of iron (ferrous and ferric) actually participating in its functioning (Lehninger, 1978). The altered drug metabolizing capacity of liver in infection may have bearing on the response of the infected host to antimalarials. As expected, the profile of the gross enzyme activity for each of the two enzymes in liver was different from that of enzyme activity expressed on unit weight basis (mg of wet weight of the tissue) i.e., enzyme concentration (Fig. 6).

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SHORT NOTES

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Mosquito Breeding in Water Meter Chambers

E. NARAYANAN[1] and V. MARUTHANAYAGAM[2]

In urban areas man-made structures may provide major breeding places for mosquitoes, thus resulting in disease transmission. A recent report of the overhead water tank as a major source for *Aedes aegypti* breeding during a dengue outbreak in Delhi is one such example (Uprety *et al.*, 1983). The following report deals with another such source — the water meter chamber — which proved to be the predominant breeding place for mosquitoes in a locality which was otherwise inhospitable for mosquito breeding.

A survey in 1981 of the 77 hectares of Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Pondicherry showed that the campus, being on a sloping absorbent land having an underground sewage system, was not favourable for mosquito breeding. The few open U-drains which sometimes harboured *Culex quinquefasciatus* larvae could be cleared effectively by routine cleaning. No significant breeding took place in cement and iron overhead tanks, the lids of many of which were open. The water meter chambers, constructed several years after the campus came into being, however, proved to be the predominant breeding place. At the time of survey the campus had 329 completed and occupied quarters and a total of 344 meter chambers. The meter chambers were constructed on the ground with bricks and cement. Top of the chamber which was on the ground level was closed & locked. The water pipe passing along the length inside the meter leaked through the joints and formed a pool inside, and some of the chambers were filled to full capacity. The lid when closed left sufficient gap for mosquitoes to enter, and even chambers having as little as 3 mm lid gap were found to breed mosquitoes, when water was present inside.

Results of a survey of chambers in a thickly populated area are given in Table 1. It was revealed that 52% of the meter chambers were breeding, and average density of immatures in infested chambers was 987. Mosquito breeding in some chambers was so heavy as to yield 100–200 larvae/pupae per 250 ml dipper. The most predominant species was *Aedes aegypti* followed by *Cx. quinquefasciatus, Armigeres subalbatus* and *Aedes albopictus*. Of the 116 chambers analysed for species specific breeding, 81 had *Aedes* (*Ae. aegypti* and *Ae. albopictus* together or singly), 4 had *Cx. quinquefasciatus* alone, 5 had *Ar. subalbatus* alone, 1 had *Ae. aegypti, Cx.*

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Table 1. *Mosquito breeding in water meter chambers*

Type of quarters	Total chambers	Chambers checked	Chambers with water	Chambers with mosquito larvae/pupae	Estimated immature stages (Nos.)
H	24	24	23	16	3896
G	100	83	57	46	43208
H	115	115	80	54	67410
Total	239	222	160	116	114514

quinquefasciatus and *Ar. subalbatus*, 4 had *Ae. aegypti* and *Cx. quinquefasciatus* and 21 had *Ae. aegypti* and *Ar. subalbatus*.

The meter chambers have introduced a serious mosquito problem in JIPMER campus. We strongly recommend to stop construction of underground meter chambers of this type. The meters could easily be installed above the ground to eliminate mosquito breeding.

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Instability of Resistance of *Plasmodium falciparum* to Chloroquine and Pyrimethamine *in vitro*

S. SINHA, A. GAJANANA and C.A. SIVARAMAN

In an earlier communication (Sinha *et al.*, 1984) we have reported a study on the comparative susceptibility of *Plasmodium falciparum* isolates to chloroquine before and after adaptation to continuous *in vitro* cultures. By the micro *in vitro* test (Pieckmann *et al.*, 1978), the isolates were resistant before adaptation, and after adaptation they were sensitive to the drug. One of them, FPN-1, was from a patient who showed *in vivo* resistance to chloroquine and Fansidar. This isolate was monitored for chloroquine and pyrimethamine susceptibility *in vitro* at various passage levels and in the aforesaid paper (Sinha *et al.*, 1984) only the results of studies on chloroquine susceptibility were presented. At the first passage level, during the process of adaptation a portion of the culture was cryopreserved by the method of Rowe *et al.* (1968) from which it was revived after 215 days and a separate culture line FPN-1/C-1 was established. Chloroquine and pyrimethamine sensitivity of FPN-1/C-1 was monitored during the course of adaptation by the method described before (Gajanana *et al.*, 1982). The results of studies on pyrimethamine sensitivity of both FPN-1 and FPN-1/C-1 as well as on chloroquine susceptibility of the latter have been summarized in this note.

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In Fig. 1a pyrimethamine sensitivity of FPN-1 at various passage levels has been depicted. It will be observed that the isolate was highly resistant to pyrimethamine when tested at 10th, 14th and 20th passage levels — the inhibitory concentrations were 8×10^{-5}M, 8×10^{-5}M and 8×10^{-6}M respectively (a strain was considered sensitive if it was inhibited by 10^{-8}M of pyrimethamine as per Smalley and Brown, 1982). At the 47th passage it was still resistant to the drug but at a much reduced level (8×10^{-8}M). When tested at 63rd passage level it was inhibited by 8×10^{-9}M of the drug indicating that it was sensitive. For comparison, the chloroquine requirements for inhibition at approximately the same passage levels have been reproduced in Fig. 1a from the earlier report (Sinha *et al.*, 1984). The above results showed that FPN-1 was resistant to chloroquine and pyrimethamine initially but became sensitive to the drugs following subsequent passages.

Fig. 1b indicates that FPN-1/C-1 at the 2nd passage level (after revival from cryopreservation) was sensitive to chloroquine but resistant to pyrimethamine — the inhibitory concentrations were 10^{-6}M and 10^{-5}M respectively. Subsequently, when tested at the 3rd and 10th passage levels, the line showed increased sensitivity to chloroquine being inhibited by 0.5×10^{-6}M and 0.25×10^{-6}M of the drug. However, it still retained its resistance to pyrimethamine at a

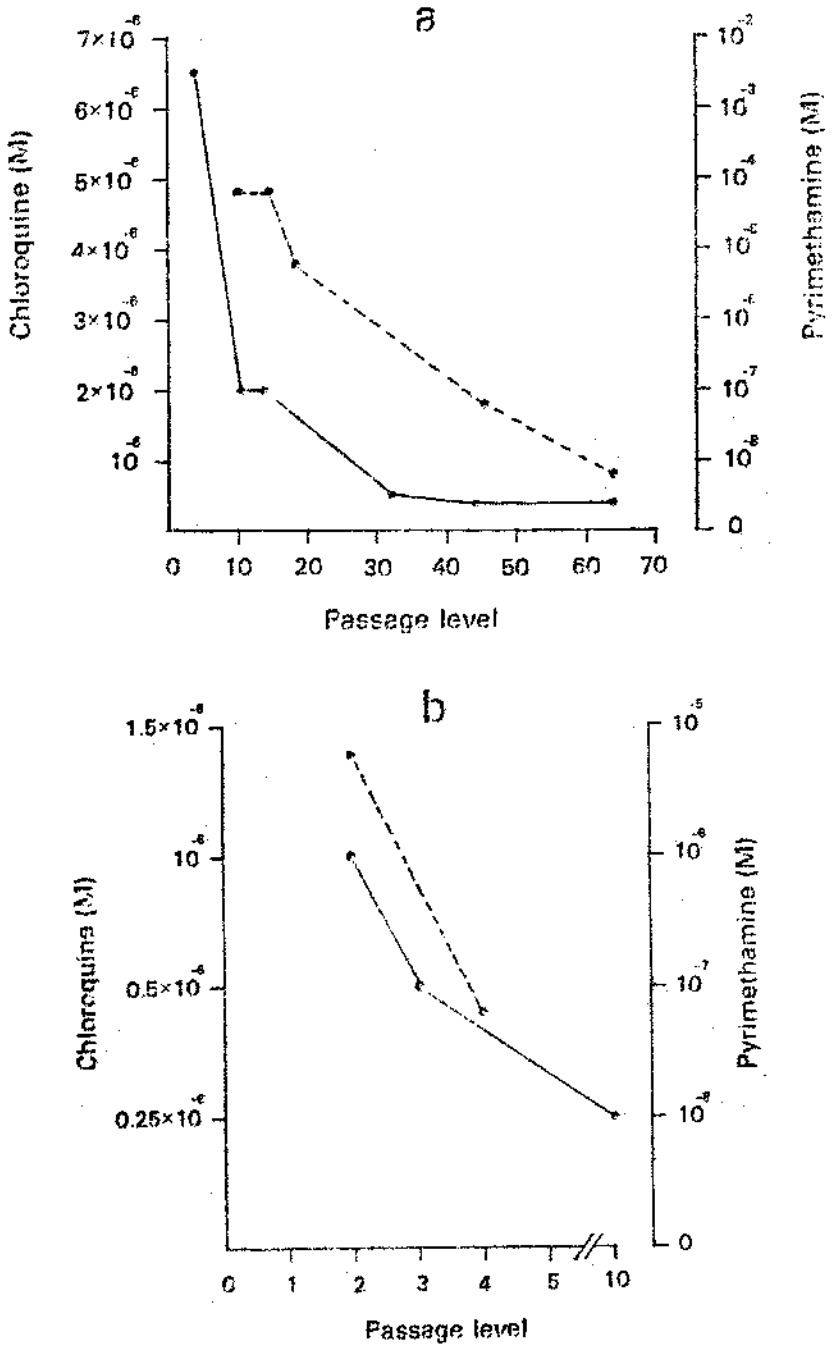

Fig. 1: Plasmodium falciparum in vitro: Effect of continuous culture on susceptibility to chloroquine ●—● and pyrimethamine ●--●.

a. FRN-1; b. FRN-1/C-1.

reduced level inhibitory concentration was 10^{-7}M). Therefore, the loss of resistance of this line to the drugs appeared to have followed a pattern similar to that of FRN-1. But interestingly, the loss was at much earlier passages. For example FRN-1/C-1 was sensitive to chloroquine from the first passage level itself whereas FRN-1 retained high level of resistance to the drug till about the 20th passage. This may be due to deletion of certain clones during revival after cryopreservation as suggested by Thaithong (1983). Further, it was observed that at certain passage levels both FRN-1 and FRN-1/C-1 exhibited resistant responses to pyrimethamine when they were sensitive to chloroquine. This indicated that pyrimethamine and chloroquine resistance are independent of each other, which is in conformity with the findings of Thaithong and Beale (1981), and Thaithong *et al.* (1984) on cloned *P. falciparum*.

It is now known that 'wild' *P. falciparum* isolates exhibit genetic diversity and uncloned parasites may change their characters (including susceptibility to drugs) during prolonged cultivation *in vitro*. So far the reported studies (Chin and Collins, 1980; Le Bras *et al.*, 1983; Jensen *et al.*, 1981) have shown that under culture conditions chloroquine resistant parasites are preferentially selected to sensitive ones. Therefore, our present and earlier finding that *in vitro* cultures may favour selection of chloroquine sensitive parasites as well, is at variance with the observation of others. It is as yet not clear what conditions favour selection of particular clones *in vitro*. Subtle differences in the micro environment may greatly influence the selection of particular clones. It is also possible that isolates from different geographical regions may behave differently under *in vitro* conditions.

ACKNOWLEDGEMENTS

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Incrimination of *Anopheles minimus* Theobald and *Anopheles balabacensis balabacensis* Baisas (*A. dirus*)* as Malaria Vectors in Mizoram

S.C. DAS[1] and I. BARUAH[1]

Anopheles minimus was the most important malaria vector in Assam during the forties, but for a decade or so, there have been very few reports of its presence. After the extensive use of insecticides under NMCP/NMEP, it was presumed that the species had disappeared from the region. Observers like Rao (1984), however, believed that the species though reduced in number, was still present and could be playing a role. This belief has been vindicated by the recent findings of Bhatnagar *et al.* (1982) in the foot hills of Nagaland and by Vareem *et al.* (1983) in Boko, Assam. Furthermore, *A.b. balabacensis* and *A. philippinensis* have also been incriminated as vectors of malaria in the northeastern region (Sen *et al.*, 1973; Rajagopal, 1976).

Mizoram appears to be an unexplored area, as no incrimination report about this region, has appeared so far in literature. This laboratory on receipt of the information that active malaria transmission is going on in certain pockets of Mizoram, undertook studies at Tuichang, a border town in South Mizoram during July, 1984. Civil and army medical authorities confirmed the resistance of *Plasmodium falciparum* to chloroquine in the same locality. To prevent malaria among the team members, human baits were avoided and CDC light traps were used for collection. However, adult mosquitoes were also collected from huts at night with aspirator tube for susceptibility test. The battery operated CDC light traps were hung in the GREF (General Reserve Engineer Force) barracks and makeshift labour camps at a height of about two metres from the ground. All the huts were newly constructed (less than one year old) and unsprayed. No record of insecticidal spray was available in that vicinity. On an average, twenty-five occupants were there in each unit or barracks. Traps were operated from dusk to dawn. The anophelines collected, were identified and dissected for their parity status and presence of sporozoites in the salivary glands. The trap collection of *Anopheles* mosquitoes are given in Table 1 and hourly collection of *A. minimus* and *A.b. balabacensis* in Table 2.

A total of 788 anophelines, represented by 8 species were captured on 14 trap nights. *A.b. balabacensis* and *A. minimus* were dominant, being 69% and 27.4% respectively. All the pin-

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* As per description of Peyton and Harrison (1979)

Table 1. Records of collection and dissection of mosquitoes in Tuichang — Mizoram, July '84

Sl. No.	Mosquito species	Per trap night	No. collected on 14 trap nights*	No. dissected**	No. of glands found positive	Sporozoite rate %	Parity %
1.	*A. aconitus*	0.714	10	—	—	—	—
2.	*A. b. balabacensis*	38.85	544	134	1	0.746	28.75
3.	*A. culicifacies*	0.071	1	—	—	—	—
4.	*A. maculatus*	0.928	13	—	—	—	—
5.	*A. maculatus* var. willmorei	0.142	2	—	—	—	—
6.	*A. majidi*	0.071	1	—	—	—	—
7.	*A. minimus*	15.43	216	58	2	3.44	64.1
8.	*A. nigerrimus*	0.071	1	—	—	—	—

* 56 mosquitoes (7 species) other than anophelines were also collected
** Only live mosquitoes were dissected.

Table 2. Hourly trap collection of mosquitoes (six trap nights)

Hours	*A. minimus*	*A.b. balabacensis*
1800–2000	3	4
2000–2200	27*	54*
2200–2400	25	50
0000–0200	31*	84*
0200–0400	8*	33*

* Level of significance $\geq$ 0.05

ned and preserved (positive for sporozoite) specimens of *A.b. balabacensis* brought to Tezpur laboratory were re-examined as suggested by Rao (1984) and were found to be *A. dirus* as per the key of Peyton and Harrison (1979). The hourly collections (two hour block) were analysed as per Fisher's t-test. It was found that 1800–2000 hrs collection of *A.b. balabacensis* differ significantly with 2000–2200 hrs (t=2.355), 0000–0200 hrs (t=2.767) and 0200–0400 hrs (t=2.723). The 1800–2000 hrs collection of *A. minimus* differ significantly with 0000–0200 hrs (t=3.166) and also 0000–0200 hrs collection with 0200–0400 hrs (t=2.518). However, high densities of these species were observed all through from 2000 to 0400 hrs with a peak between 0000–0200 hrs. Similar peak density of *A.b. balabacensis* from 2200–0200 hrs was observed during 1970 in Burnihat, Meghalaya (quoted by Rao, 1984) and in Nowgong, Assam by Rajagopal (1979).

The parity rate was as high as 64.1% for *A. minimus* and 28.75% for *A.b. balabacensis*. Ismail *et al.* (1982) observed 69.0% parity in the indoor CDC light trap catches of *A. minimus*. The sporozoite rate in *A. minimus* was 3.4% and *A.b. balabacensis* 0.75%. Both the species were highly susceptible to DDT (cent percent mortality was observed within 2 hours of holding/recovery period with 4% DDT).

From the present study, the role of *A. minimus* and *A.b. balabacensis* (*A. dirus*) in transmission of malaria in Mizoram is established. Detailed studies, however, are warranted to confirm the susceptibility status of vector species against DDT and HCH. The role of other vector species and their resting and breeding habits, host preferences etc. still remain to be investigated.

Present findings are in conformity with that of Ismail *et al.* (1982), who advocated that CDC light trap can be very effectively used in place of human baits to determine the relative density of malaria vectors, particularly where the baits are liable to contact malaria/resistant parasites.

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Studies on the Incidence of Malaria in Gadarpur town of Terai, Distt. Nainital, U.P.

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Terai region of district Nainital (U.P.) is well known for green revolution. Early attempts to colonize Terai were unsuccessful mainly because of high morbidity and mortality due to malaria. The situation took a dramatic turn with the availability of DDT in malaria control. Initially between 1947 to 1949 three colonization units were opened in Terai. These units *inter alia* made important contributions in determining the dosage of DDT and the frequency of its application. Subsequently, these units were merged and made NMEP unit. As a result of spraying under NMEP malaria was eradicated from Terai by the mid 1960's. Control of malaria resulted in the conversion of vast jungles into agricultural farms and simultaneous growth of agro-industries. To increase agricultural production, Govind Ballabh Pant University of Agriculture and Technology was established in 1960 and several dams were constructed for irrigation. Today the area is well irrigated by a network of canals and their tributaries and artesian wells.

In late 1960s malaria resurgence started throughout Terai, as it did in other parts of the country. To tackle this situation, the Government of India in 1977 implemented the Modified Plan of Operations (MPO). One objective of the MPO was to provide protection to the green revolution. In order to study the impact of the MPO in Terai region, a field station of the Malaria Research Centre was opened in 1979. Early studies revealed the occurrence of 29 mosquito species belonging to 8 genera, and that *A. minimus* was absent and *A. stephensi* was represented by a few specimens (Nagpal *et al.*, 1983). *A. culicifacies* was found throughout Terai in good densities. It is resistant to DDT and BHC and breeds in innumerable sites mostly associated with irrigation systems (Sharma *et al.*, 1983a). Studies in 1981-82 revealed that malaria in Terai was mainly due to *P. vivax* and there was a small focus of *P. falciparum* (Sharma *et al.*, 1983b). *A. culicifacies* and *A. fluviatilis* were incriminated as vectors of malaria (Choudhury, 1984).

Gadarpur town is situated in Terai. It has a population of about 6500. The town is an important centre of commerce and people from surrounding villages visit Gadarpur for shopping and medical help. It has a primary health centre (PHC) and several private practitioners. A one year sampling study (1982-83) revealed that Gadarpur PHC villages were endemic for malaria and that the incidence of falciparum malaria was extremely high (Choudhury *et al.*,

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Table 1. A comparison of MRC and NMEP data of malaria incidence in Gadarpur proper (town) by active surveillance during 1952-53

Months	M R C data							N M E P data						
	B.S. examined	Pv	Pf	Mixed	Total (+) ve	SPR	SFR	B.S. examined	Pv	Pf	Mixed	Total (+) ve	SPR	SFR
April	885	283	4	—	287	32.43	0.45	142	2	—	—	2	1.41	—
May	417	150	—	—	150	35.97	—	105	—	—	—	—	—	—
June	321	126	5	—	131	40.80	1.56	40	4	—	—	4	10.00	—
July	331	162	7	—	169	51.06	2.11	64	10	—	—	10	15.63	—
August	705	225	273	3	501	71.00	39.15	62	4	—	—	4	6.45	—
September	738	82	432	—	514	69.65	58.54	113	4	—	—	4	3.54	—
October	397	7	332	1	340	85.64	83.88	115	—	—	—	—	—	—
November	209	5	184	—	189	90.43	88.04	44	—	—	—	—	—	—
December	149	5	120	1	126	84.56	81.21	40	7	—	—	7	17.5	—
January	112	2	76	1	79	70.5	68.75	52	—	9	1	10	19.23	19.23
February	124	15	70	2	87	70.16	58.06	60	1	—	—	1	1.67	—
March	83	15	35	—	50	60.24	42.17	92	2	5	—	7	7.61	5.43
Total	4471	1077	1538	8	2623	58.66	34.58	929	34	14	1	49	5.27	1.61

Note—Gadarpur town population = 6475

1983a). *In vivo* tests showed decreased sensitivity of *P. falciparum* to chloroquine (Choudhury *et al.*, 1983b). During the same period, a study on the incidence of malaria in Gadarpur town was undertaken by continuously monitoring the same population for a one year period. Results of the study are reported in this paper.

Before the start of study, a census was made and all houses were numbered. Active surveillance was started in April, 1982 and continued till March, 1983. During a one year period, surveillance was intensified by deploying additional staff so as not to miss any fever case. Mass blood surveys were also done during April-August. All fever cases were given presumptive treatment (600 mg chloroquine or 4 tablets adult dose) followed by radical treatment as per the drug policy of the NMEP (Sharma, 1984). Blood smears were stained with JSB stain and at least 100 fields of thick film were examined before declaring the slide as negative. Parasite identification was confirmed by thin film examination.

Results of this study are given in Table 1. Active surveillance revealed extremely high incidence of malaria throughout the year, except for 2 months during the extreme winters. In April almost all infections were due to *P. vivax* followed by a drop to low numbers in October-March. There was a sudden increase in falciparum cases in August peaking in September followed by decline to low numbers during the winter months. A notable feature was the complete absence of *P. malariae* which used to occur in good proportions (about 17%) before the colonization of Terai (Srivastava and Chakrabarti, 1952). There were only 8 mixed (Pv+Pf) infections in one year. Slide positivity rate remained high throughout the study period and averaged 58.66 (range 32.43-90.43) and SFR averaged 34.58 reaching as high as 88.04 in November. During mass blood surveys, a total of 1798 blood smears were collected from April to August, 1982. Of these 299 were *P. vivax*, 12 *P. falciparum* and 2 mixed infections. The SPR was 17.38. There was therefore a high proportion of atypical malaria cases in the community. During the same period NMEP recorded a total of 49 malaria cases (34 Pv + 14 Pf and 1 mixed). The SPR and SFR was 5.27 and 1.61 respectively. It was therefore obvious that NMEP's surveillance was not adequate and a large number of parasite positive cases were being missed.

Improved surveillance and good spraying, targeted to achieve about 80% coverage, is recommended to contain malaria in Terai.

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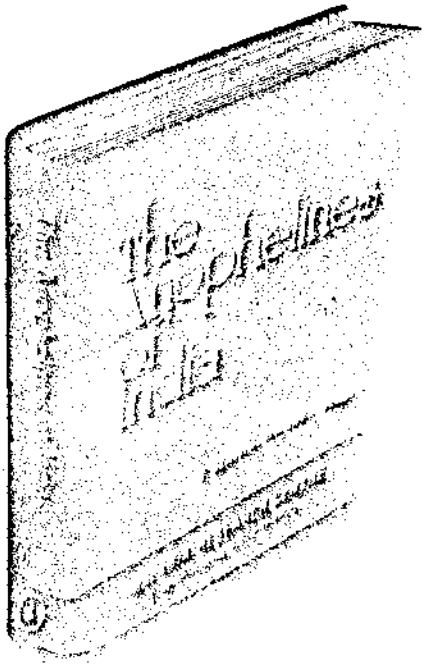

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Anopheles annularis as a Vector of Malaria in rural West Bengal

P.K. GHOSH[1], S. CHAKRABORTY[2], S. BHATTACHARYA[2], A. PALIT[2], NEELAM TANDON[2] and A.K. HATI[2]

Anopheles annularis was regarded as the secondary vector and *A.philippinensis* was the principal vector of malaria in rural West Bengal. *A.philippinensis* has now virtually disappeared, but *A.annularis* was caught in nature as well as off human baits. Human blood index of *A.annularis* varied from 8.84 to 19% according to different biotopes. When fed in the laboratory on patients with circulating gametocytes of *P.vivax*, 13.11% of *A.annularis* became infective. Out of 5428 wild caught *A.annularis* dissected, sporozoites were found in the salivary glands of one mosquito. In recent malarious situation in rural West Bengal *A.annularis* is found to be naturally infective and therefore may be responsible for malaria transmission in nature in the absence of other vector species.

INTRODUCTION

Resurgence of malaria has posed a serious health hazard in rural West Bengal since the seventies. The position has further been complicated owing to the fact that the principal vector responsible in transmitting malaria in the present situation is not definitely known. Previous studies showed that *Anopheles philippinensis* acted as the principal vector of malaria in rural West Bengal and *A.annularis* was regarded as the secondary vector in certain areas of India (Timber, 1935; Viswanathan *et al.*, 1941; Panigrahi, 1942; Senior White *et al.*, 1943). *A.philippinensis* has now virtually disappeared (NMEP, 1970) though Hati and Mukhopadhyay (1980) report the presence of *A.philippinensis* in certain pockets. The present study has been undertaken to investigate the role of *A.annularis* as the vector of malaria in rural West Bengal in the present situation.

MATERIAL AND METHODS

In village Gurap, of district Hooghly, an area endemic for malaria, situated about 70 km away from Calcutta, mosquitoes were caught off human baits placed indoors and outdoors throughout the night from 1800 to 0600 hours twice in a month (on each full moon and new moon) for one year from September 1979 to August 1980 following the method of Hati *et al.* (1981).

Mosquitoes were caught in nature from cowsheds and human habitations in the morning

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Table 1. Distribution of different species of mosquitoes which came in contact with man at night

Total hours spent	Species of mosquitoes	Number caught	Percentage	Per manhour density
576	*Culex quinquefasciatus*	3907	56.02	6.78
	Culex vishnui	1750	25.09	3.04
	Armigeres subalbatus	1023	14.67	1.78
	Mansonia annulifera	124	1.78	0.22
	Anopheles vagus	49	0.70	0.09
	Culex gelidus	45	0.64	0.08
	Anopheles annularis	31	0.44	0.05
	Anopheles subpictus	30	0.43	0.05
	Anopheles hyrcanus	7	0.10	0.01
	Aedes aegypti	4	0.06	0.01
	Aedes albopictus	4	0.06	0.01

hours spending 8 manhours per week from August 1979 to July 1983. Human blood index of *A.annularis* was performed by precipitin test employing the method of Ouchterlony and Nilson (1973).

Field collected larvae of *A.subpictus* and *A.vagus* were allowed to pupate and emerge as adults in the laboratory. In case of *A.annularis*, adults were obtained from the laboratory colony. The mosquitoes were fed on patients having gametocytes of *Plasmodium vivax* in the blood and these were dissected to note the laboratory infectivity rate.

A total of 5428 wild caught *A.annularis*, 5212 *A.subpictus* and 4820 *A.vagus* were dissected for the detection of parasites in the salivary glands.

In rural West Bengal (excluding Calcutta) the number of malaria cases were 17819, 24117 and 27452 in 1982, 1983 and 1984 respectively. In the district of Hooghly, 76, 135, 84, 113 and 105 cases of malaria were reported in 1980, 1981, 1982, 1983 and 1984 respectively, of which 1, 2, 6 and 1 were *P.falciparum* cases in 1980, 1982, 1983 and 1984 respectively. API in the district of Hooghly was 0.02 in 1980, 0.4 in 1981, 0.02 in 1982, 0.04 in 1983 and 0.005 in 1984. In the experimental village named Gurap, no malariometric data was maintained.

RESULTS

Table 1 shows the different species of mosquitoes which came in contact with man at night. *Anopheles* mosquitoes caught off human baits were *A.vagus* (0.70%), *A.annularis* (0.44%), *A.subpictus* (0.43%) and *A.hyrcanus* (0.10%).

Only 0.05 *A.annularis* came in contact with man per hour and per man hour contact indoors was 0.04. Though they were trapped in almost every hour of the night, the peak hours were between 2100 and 2400 hours. Maximum numbers of *A.annularis* were collected in the month of September. Human baits were placed from 0600 to 1800 hours on twenty-five occasions including the day on which the total solar eclipse occurred (16.2.80). No *A.annularis* was caught during the daytime.

All the *Anopheles* mosquitoes caught off human baits were dissected but no malarial parasites were detected in them.

During the period August 1979 to July 1983 altogether 5428 *A.annularis* collected both from cowsheds and human habitations were dissected, out of which the salivary glands of one *A.annularis* were infective with sporozoites of *P.vivax* malaria. The infectivity rate was 0.018%. In other species of wild caught mosquitoes no gut or gland infection was found (Table 2).

Table 2. Different species of *Anopheles* mosquitoes dissected during the period from 1980 to 1983 (upto July) for the detection of malarial parasites

Species of mosquitoes	Number dissected	Number positive for parasite		Percentage
		gut+	gland+	
A. annularis	5428	—	1	0.018
A. vagus	4820	—	—	0
A. subpictus	5212	—	—	0

Table 3. The average frequency of primate blood, compared with that of bovine and other blood in two different biotopes

Species	Spray status	Biotope class	No. of Positive smears	Positive for primate blood		No. of Positive bovine blood		No. of Positive other hosts	
				No.	%	No.	%	No	%
A. annularis	Nil	Human	200	38	19.00	129	64.5	10	5
		Cowshed	215	19	8.84	151	70.23	11	5.11

Table 4. Laboratory transmission of *P. vivax* by *A. annularis*, *A. subpictus* and *A. vagus*

Species of mosquitoes	Total no. of mosquitoes dissected	Sporozoites found	Percentage
A. annularis	61	8	13.11
A. subpictus	59	0	0
A. vagus	51	0	0

Table 3 depicts the average frequency of primate blood, compared with that of bovine and other blood in the different biotopes. The human blood index of *A.annularis* was 13.73%. But human blood index of *A.annularis* caught from human habitations was 19% which came down to 8.84% when human blood index of *A.annularis* caught from cowsheds was performed.

In Table 4 laboratory transmission of *P.vivax* by *A.annularis* and two other species of mosquitoes is shown. Altogether 61 *A.annularis* mosquitoes fed on patients with *P.vivax* gametocytes in blood, were dissected after 10 days and from 8, the sporozoites in the salivary glands were obtained. The percentage of infectivity was 13.11. A total of 59 *A.subpictus* and 51 *A.vagus* fed on the same patient and dissected after 10 days showed no stomach or gland infection.

DISCUSSION

While monitoring the activity of *A.annularis*, its role as a probable vector of malaria in rural West Bengal was brought into focus (Ghosh and Hati, 1982). No *A.philippinensis* was caught off human baits or from cowsheds and human habitations in the present study. Laboratory infected *A.vagus* and *A.subpictus* showed no stomach or gland infection. *A.vagus* and *A.subpictus* are generally not regarded as malaria vectors in India. However in Bitra

(Lakshadweep islands) *A.subpictus* and in Chetlet (Lakshadweep islands) either *A.subpictus* or *A.varuna* or both are stated to be the suspected vectors, though not incriminated by dissection (Roy *et al.*, 1978). In an outbreak of malaria in a few coastal villages near Pondicherry, similar circumstantial but inconclusive evidence has been cited in favour of *A.subpictus* (V.C.R.C., 1977), later of 6133 *A.subpictus* dissected 52 had oocysts (0.85%) and 4 had sporozoites (0.07%), (V.C.R.C., 1981).

There is a record that in the absence of the principal vector in Nepal, sporadic malaria transmission was caused by *A.annularis* (Parajuli *et al.*, 1981). In the recent outbreak of malaria epidemic in the district of Jalpaiguri, West Bengal, adult *A.annularis* were caught in large numbers. The expert team suspected that *A.annularis* may act as a secondary vector (Hati, 1982). Dash *et al.* (1982) detected sporozoites in the glands of one *A.annularis* out of 174 dissected, in Keonjhar district, Orissa which neighbours West Bengal.

Natural infection was found in one *A.annularis* in the present study. No infection was detected in *A.vagus* and *A.subpictus*. Covell in 1927 dissected 2511 *A.annularis* out of which 4(0.16%) were infective. Out of 34041 *A.annularis* dissected, Timber in 1935 found the gland infection in 8(0.02%) cases.

A. annularis has been previously reported to be highly zoophilic, with only 0-1.08% anthropophilic index (Rao, 1981), while here it was found to be relatively anthropophilic. In this village the density of *A.annularis* relative to man is low, sporozoite rate was low (about the same as reported by Timber in 1935) but it was the only anopheline species to be naturally and experimentally incriminated, and was therefore responsible for malaria transmission, even though previously believed to be only a secondary vector of lesser importance than *A.philippinensis*.

In view of natural infection, laboratory infectivity with *P.vivax* and man—vector contact of *A.annularis*, it may be concluded that this species acts as a vector of malaria in rural West Bengal, in the present situation.

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Evaluation of the Formulations of the Mosquito Larvicidal Agent BIOCID-S from *Bacillus sphaericus* 1593 M

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The efficacy of three experimental formulations of *Bacillus sphaericus* 1593M viz. HIL-8 (wettable powder), HIL-9 (dust formulation with 5% a.i.) and HIL-10 (dust formulation with 33% a.i.) were evaluated against *Anopheles* and *Culex* larvae in the laboratory and under field conditions. In the laboratory tests, 100% mortality was achieved against *Culex quinquefasciatus* II and IV instar larvae in 48 hours, with all the three formulations used at the levels of 5-25 mg a.i./sq. ft. With the formulation HIL-9 which was chosen for its efficacy against anophelines, as dust formulation, the larvicidal effect was demonstrated against *A. culicifacies* within 24 hours.

In field trials, the difference between the two formulations HIL-8 and 9 against anopheline larvae was clearly demonstrated. HIL-9 fared much better and in several instances high mortality (>90%) of *Anopheles* larvae was maintained up to one week. With *Culex*, the high organic pollutants of the habitats lowered the persistence of BIOCID-S.

INTRODUCTION

The commercial availability and wide application of *Bacillus thuringiensis* var. *israelensis* has opened a new approach in vector control and this has generated considerable interest to isolate and screen microbial agents pathogenic to mosquito vectors. *Bacillus sphaericus*, a spore-forming bacterium, is a potential larvicide of several mosquito species (Kellen *et al.*, 1965; Singer, 1980). It can germinate and multiply in the cadavers of larvae. It recycles and persists longer under natural conditions (Davidson and Sweeney, 1983; Hertlein *et al.*, 1979; Hornby *et al.*, 1981). It is highly effective against *Culex* and certain *Anopheles* species but much less insecticidal to *Aedes* species larvae (Davidson and Sweeney, 1983; Wraight *et al.*, 1982). The mortality of the larvae occurs after ingestion of bacteria toxin. Though several strains of *B. sphaericus* are known, only a few are insecticidal. About 30 mosquito larvicidal strains of *B. sphaericus* have so far been isolated (Singer, 1981). The strain 1593 from Indonesia can grow on inexpensive media and produces highly insecticidal spores, (Davidson and Sweeney, 1983; Singer, 1974). A more potent strain of *B.sphaericus* 1593 designated as 1593 M, was isolated in India

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(Subramanian *et al.*, 1980). Bioassay tests with lyophilized preparations of *B.sphaericus* 1593M showed higher efficacy against *Culex quinquefasciatus* than *Anopheles stephensi*. This paper reports the results of laboratory and field tests with three commercial formulations of *B.sphaericus* 1593M against the larvae of *Anopheles* and *Culex* species

MATERIAL AND METHODS

Formulations: Three biocide formulations produced by M/s. Hindustan Insecticides Limited were used in the present study.

HIL-8— wettable powder with 5% active ingredient.
HIL-9— dust formulation with 5% active ingredient.
HIL-10— dust formulations with 33% active ingredient.

Laboratory studies: Laboratory cultured *Anopheles culicifacies*, and *Culex quinquefasciatus* larvae were used for laboratory tests. These tests were carried out in enamel trays (20×15 cms) containing 500 ml stored tap water. Twenty-five larvae of a particular species in the same stage were introduced into each tray. Three larval instar stages (II, III and IV) were used in these studies and each instar was tested separately.

The dust formulations, HIL-9 and HIL-10 were weighed separately for each tray and sprinkled over the water surface. The wettable powder of HIL-8 was mixed with a known volume of water and sprayed over the trays.

The mortality of the larvae was recorded after 24 and 48 hours of exposure by counting the living and dead larvae. All the tests were conducted under controlled temperature (28°C ± 2°C) and each test was replicated at least three times.

Field trials: Small-scale field trials were conducted during July 1984 in certain rainwater pools and small ponds measuring 4 sq. ft. to 80 sq. ft. and supporting *Anopheles* or *Culex* breeding. Before application of the biocide formulation, larval density (per dip) of different pools was determined by taking ten dips, using a dipper of 500 cc capacity. All the formulations were tested at a dose of 0.5 gm/sq. ft. However lower doses were also tried with HIL-9 and HIL-10. Two pools were left untreated and served as control. The density of *Anopheles* and *Culex* larvae were monitored at 24 hours, 48 hours, and one week after the treatment. The collected larvae were counted and released back to the pond after each counting.

RESULTS

Laboratory trials: The formulations were tested at the higher concentrations of the a.i./sq. ft. than with pure biocide to cover any lapse that may have occurred during formulation. In addition the larvae were fed continuously during these trials to mimic the natural conditions. It can be seen from the results outlined in Table I that there were differences between the formulations, which were tested either for *Culex* species or *Anopheles* species. Some of the highlights of these results are:

1. With *Culex quinquefasciatus* larvae all formulations gave 100% mortality by 48 hours with the different stages of larvae at the dose of 5-25 mg a.i./sq. ft.

2. With *Anopheles culicifacies* larvae, the formulations exhibited diverse effect. The HIL-8 was less effective as expected, since it is a wettable powder and facilitates a uniform distribution throughout, rather than the surface flotation, most suited for *Anopheles*. HIL-9 fared well with *A. culicifacies* even at 5mg a.i./ sq. ft. within 24 hours. HIL-10, another dust formulation with higher a.i.% was also highly effective against *A.culicifacies*.

Field trials: There were considerable variations of the efficacy of these formulations under field conditions. With anopheline larvae HIL-9 and

Table 1. Laboratory evaluation of BIOCID-S formulations against *Anopheles culicifacies* and *Culex quinquefasciatus* larvae

Formulation	Dose gm/sq. ft.	Concentration of a.i. mg/sq. ft.	Larval instar	% Mortality			
				Anopheles culicifacies		*Culex quinquefasciatus*	
				24 hr	48 hr	24 hr	48 hr
HIL-8	0.1	5	II	24	39	98	100
			IV	7	36	90	100
	0.5	25	II	97	100	100	100
			IV	89	97	100	100
HIL-9	0.1	5	II	100	100	97	99
			III	97	100	89	100
			IV	93	97	74	100
	0.5	25	II	100	100	96	100
			III	100	100	—	—
			IV	92	100	97	100
HIL-10	0.05	16	II	97	100	76	100
			IV	80	100	100	100
	0.1	33	II	100	100	96	100
			IV	100	100	100	100

Table 2. Evaluation of BIOCID-S formulation against *Anopheles* species larvae in experimental ponds under field conditions

Formulation	Area covered sq.ft.	Dose gm/sq.ft.	Concentration of a.i. mg/sq.ft.	Initial larval count*	Percentage reduction in initial density days post-treatment		
					1	2	7
HIL-8	8	0.5	25	5.6	68	20	36
	50	0.5	25	13.4	78	63	81
HIL-9	80	0.2	10	11.8	32	100	91
	12	0.25	12.5	4.8	100	79	35
	4	0.5	25	7.3	55	71	55
	35	0.5	25	29.7	98	91	92
	12	0.5	25	5.6	100	100	99
HIL-10	20	0.1	33	3.0	30	—	—
	24	0.2	66	12.1	99	100	100
	44	0.25	82	8.2	87	—	58
	4	0.5	166	6.3	57	92	80

*No. of larvae/dip = average.

Table 3. Evaluation of BIOCID-S formulations against *Culex* species larvae under field conditions

Formulation	Area covered sq.ft.	Dose gm/sq.ft.	Concentration of a.i. mg / sq.ft.	Initial larval density*	Percentage reduction in initial density days post-treatment		
					1	2	7
HIL-8	8	0.5	25	296	97	99	96
	50	0.5	25	30	89	58	65
HIL-9	12	0.25	12.5	40	91	75	17
	20	0.5	25	17	0**	85	96
	4	0.5	25	118	79	92	70

*No. of larvae/dip = average.

**Very high density of freshly emerged I instar larvae.

(The ponds with *Culex* habitats were highly polluted with organic matter).

10 fared very well (more than 90% mortality), although in some cases the efficacy declined within a week, while in other instances there was retention of biocide action (Table 2).

With *Culex* larvae, the high pollution prevalent in these ponds made it difficult to demonstrate total efficacy. There were instances where once the formulation was effective its effect was retained for a week and in others there was emergence of fresh larvae (Table 3).

DISCUSSION

Bioassay with pure biocide from *Bacillus sphaericus* 1593M has shown *Culex quinquefasciatus* as the most susceptible mosquito species (Subramanian *et al.*, 1980). *Culex* species have also been shown to be most susceptible in various other studies with other isolates of *B.sphaericus* (Lacey and Singer, 1982; Singer, 1973). Since the larvicidal activity of the biocide occurs after ingestion of the toxin of the bacteria by mosquito larvae, it needs to be distributed in the econiche of the particular species. For surface feeding anopheline larvae various commercial formulations of the biocide, which spread better over the surface of water are more effective as seen in results with HIL-9 and 10, the dust formulations of *B.sphaericus* 1593M.

There were considerable variations in the efficacy of different biocide formulations under field conditions, probably because in the field the microbes which are a major part of the diet of newly hatched mosquito larvae and other environmental conditions could influence larval feeding and hence variable susceptibility to the same formulation of *B.sphaericus* in different pools was observed. Also the more normal bacteria there are present, the less is the chance for ingestion of a lethal dose of *B.sphaericus*.

It is evident from the present studies that the efficacy of the three biocide formulations against *Culex* and *Anopheles* larvae, though very high in the laboratory tests, varied in different field trials depending upon the type and availability of other food material. However, HIL-9 was found to be most effective of the three formulations against *Anopheles* larvae.

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Studies on Malaria Transmission in Hutments of Delhi

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Parasitological surveys in the itinerant labour camps of Delhi revealed that malaria was one of the most common causes of morbidity. *P. vivax* cases start early in spring and reach high numbers in August-September. During the same period, a sudden spurt in falciparum malaria results in high morbidity due to falciparum and vivax malaria. In later months, the incidence of *P.vivax* goes down but falciparum malaria remains at that level till the onset of extreme winter. *A. stephensi* was incriminated as the vector in September 1983. Studies brought out the importance of hutments in the epidemiology of malaria in Delhi.

INTRODUCTION

Delhi is endemic for malaria. During late 1960s, after the near eradication of malaria, resurgence started in Delhi as in other parts of the country. To tackle this situation, Delhi was included in the urban malaria scheme (UMS) introduced in the country in 1971–72 (Pattanayak *et al.*, 1981). The UMS had little impact and malaria cases increased to 11165 in 1975, 19204 in 1976, 111089 in 1977, peaking at 332683 in 1978. Although most of the malaria in Delhi was due to *P.vivax*, in the years that followed there was a considerable increase in falciparum malaria (Choudhury, 1984).

Most of the immigrant labourers that come to Delhi live in temporary hutments with extremely poor community facilities. As a result, the residents suffer from many diseases, malaria being the foremost. Since these hutments are found in the vicinity of well-planned authorized colonies all over Delhi, these hutment dwellers not only suffer themselves but also constitute an important source of infection to others. Therefore, a study of the epidemiology of malaria was undertaken among the hutment dwellers of Delhi during 1982–83. Results of this study are reported in this paper.

MATERIAL AND METHODS

Hutments or *jhuggi-jhopari* clusters are of two types viz., (i) split bamboos and tarpaulin, and (ii) brick and/or mud structure with thatched roof. The average area of each *jhuggi* was 1.5 to 2 sq. meters. *Jhuggis* were placed in rows with small lanes of 1 to 1.5 m. In the study sites, there was no drainage or piped water supply or lavatories. Residents depended on nearby community taps. There was no electricity and the average income of each family was about Rs.400/- p.m. Cooking was done in the open outside the *jhuggis*. About 10% children were

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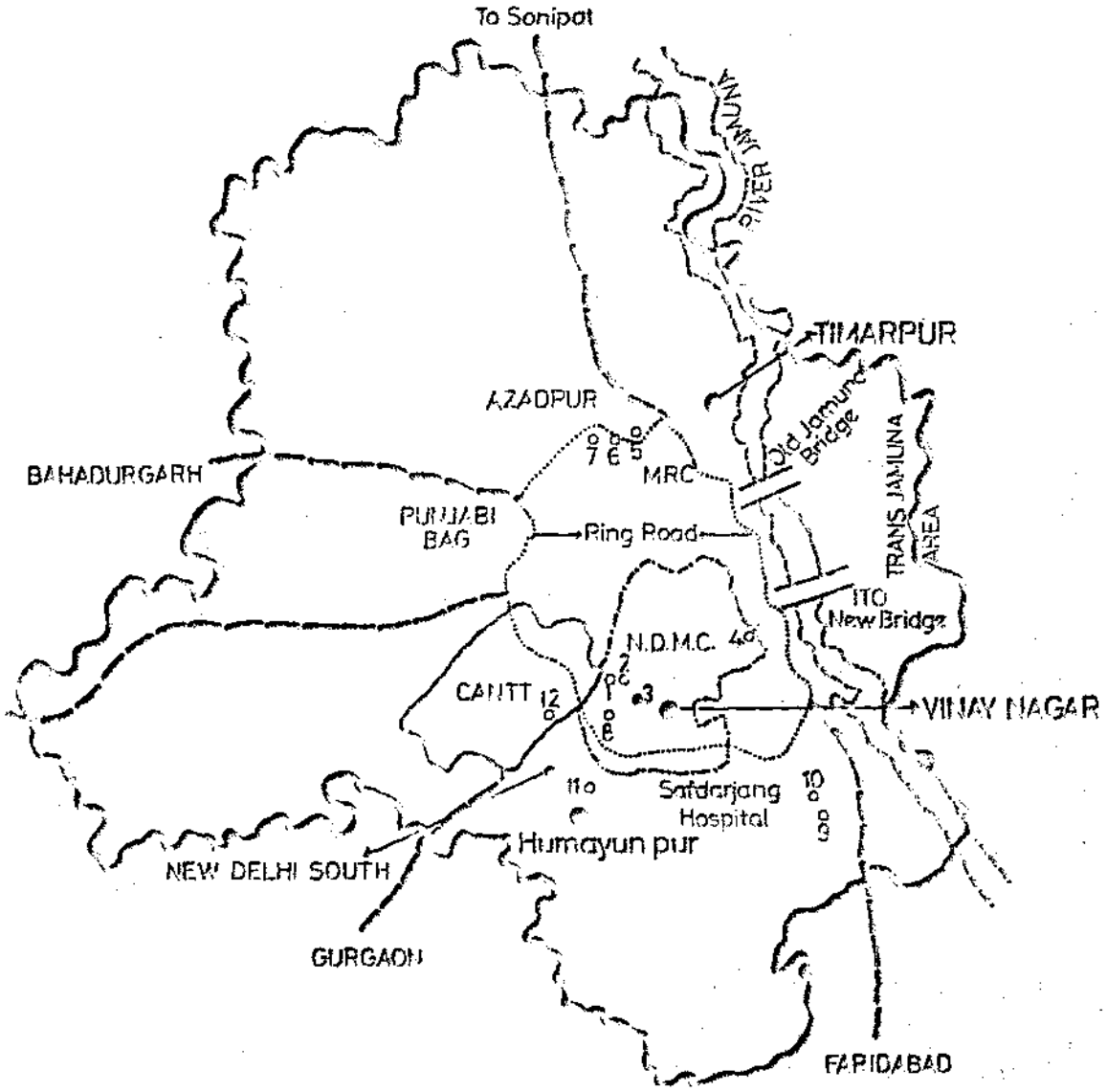

1. Indira Nagar Camp A, S.P. Marg;
2. Indira Nagar Camp B, S.P. Marg;
3. Sanjay Ekta Camp, Chanakyapuri;
4. Anna Nagar, Tilak Bridge;
5. Sukhdev Nagar, Wazirpur;
6. Udham Singh Park, Wazirpur;
7. Tool Room Camp, Wazirpur;
8. Nehru Camp, Chanakyapuri;
9. Tekhund Camp, Okhla;
10. Sanjay Camp, Okhla;
11. Ambedkar Basti, R.K. Puram;
12. Subroto Park, Dhaula Kuan.

attending schools. There were mosquito breeding sites in and around the hutments.

The following hutments in Delhi and New Delhi area were randomly selected for the study: (1) Indira Nagar Camp A, (2) Indira Nagar Camp B, (3) Sanjay Ekta Camp, (4) Anna Nagar, (5) Sukhdev Nagar, (6) Udham Singh Park, (7) Tool Room Camp, (8) Nehru Camp, (9)Tekhund Camp, (10) Sanjay Camp, (11)Ambedkar Basti and (12)Subroto Park (see Fig. for locations). During 1982 and 1983, one round of BHC was sprayed in the hutments and the coverage achieved was about 10-20%. Surveillance and follow-up treatment was also poor and residents depended on private/hospital treatment.

The study team carried out daily surveillance in all hutments and one fever treatment depot (FTD) was opened in each camp. Blood smears were collected from every fever case. Slides were stained with JSB and examined for the presence of malarial parasite. All parasite positive cases were given presumptive treatment of 600 mg chloroquine followed by the radical treatment i.e., (i) *P.vivax* : a single dose of 600 mg chloroquine base followed by 15 mg primaquine daily for 5 days, and (ii) *P.falciparum:* 600 mg chloroquine base on day 0 plus 45 mg primaquine followed by 300 mg chloroquine after 8 hours and 300 mg chloroquine each on the following two days. Children were given proportionate dosages.

No antilarval or antimosquito measures were undertaken in the study areas and no suppressive chemotherapy was given.

Spleen surveys were carried out in May 1982 and April 1983 in afebrile children of the 2-9 years age group. Spleen enlargement was determined by Hackett's method (Christophers *et al.*, 1958).

Mosquitoes were collected using a suction tube from fixed stations (*4 jhoparis* i.e., living rooms) from each camp at monthly intervals. Man hour density was calculated from the actual time spent in collections. Mosquitoes were brought to the Centre and dissected for vector incrimination.

RESULTS AND DISCUSSION

Initially 4 hutments (Indira Nagar, Sanjay Ekta Camp, Anna Nagar Camp and Nehru Camp) were taken up for parasitological investigations. Results of one year study (May 1982 to April 1983) are given in Table 1. Age and sexwise distribution of malaria cases is given in Table 2. As the work progressed more hutments were included for parasitological and entomological work. Results of 12 hutments (including 4 hutments taken initially) are given in Table 3. Observations revealed that there was no difference in the pattern of malaria in the 4 hutments or in all the hutments pooled together. Fever rate in the community was very high and showed great variation from month to month corresponding with the prevalence of malaria. During the summer months and until September, vivax malaria was dominant which gradually declined with simultaneous increase in falciparum malaria. *P. falciparum* cases peaked in September and October followed by decline during extreme winter. Observations also revealed low malaria incidence among infants (Table 2). This may be due to maternal immunity and also the fact that infants were generally kept well covered. Maximum incidence was observed in 9-24 years age group. This group was most active and children above 10 years generally accompanied their parents for work.

A few other observations made in the 4 hutments (population 5056) selected initially were that the body temperature in malaria patients ranged between 37.3° C to 41° C. Fever was more acute in falciparum than vivax malaria. Patients complained of severe gastric irritation (36 or 1.3%) and diarrhoea (44 or 1.5%) during malaria attacks. There were 15 pregnancies and of these, 3 had malaria. These three cases were given 300 mg chloroquine on weekly basis during the

Table 1. Epidemiological situation of malaria in hutments

Month/ Year	Blood Smears		Parasite Species			Cases/ 1000 pop.	SPR	SFR	Pf %	BER % (febrile)
	Examined	Positive	Pv	Pf	Mix					
May 82	587	220	218	2	0	43.5	37.1	0.3	0.9	11.6
Jun 82	682	274	273	1	0	54.2	40.1	0.1	0.3	13.5
Jul 82	682	267	265	2	0	52.8	39.1	0.3	0.7	13.5
Aug 82	1178	546	518	28	0	108.0	46.3	2.4	5.1	23.3
Sep 82	1431	610	504	105	1	120.6	42.6	7.3	17.2	28.3
Oct 82	749	341	204	137	0	67.4	45.5	18.3	40.2	14.8
Nov 82	357	170	104	63	3	33.5	47.5	17.6	37.0	7.0
Dec 82	268	83	34	47	2	16.4	30.9	17.5	56.6	5.3
Jan 83	280	42	16	25	1	8.3	15.0	8.9	59.5	5.5
Feb 83	311	62	47	14	1	12.3	19.9	4.5	22.6	6.1
Mar 83	334	78	64	13	1	15.4	23.3	3.9	16.6	6.6
Apr 83	332	158	139	17	2	31.2	47.6	5.7	10.8	6.6
Total	7191	2851	2386	454	11	563.9	39.6	6.4	16.31	142.2

Note : Population of 4 hutments 5056.

Table 2 : Age and sex-wise distribution of malaria cases

Age Group	Males			Females			Total		
	Pop.	Cases	%	Pop.	Cases	%	Pop.	Cases	%
0-11 months	100	11	11.00	117	6	5.12	217 (4.30%)	17 (0.33%)	7.83
1-4 years	283	99	41.42	276	82	29.71	559 (11.06%)	181 (3.58%)	32.38
5-8 years	298	175	58.72	249	134	53.81	547 (10.81%)	309 (6.11%)	56.49
9-14 years	248	221	89.11	199	150	75.37	447 (8.84%)	371 (7.34%)	82.99
15-24 years	572	697	121.85	419	239	56.32	991 (19.60%)	933 (18.45%)	94.14
25-34 years	864	488	56.48	503	195	38.76	1367 (27.04%)	683 (13.51%)	49.96
35-54 years	571	210	36.77	271	89	32.84	842 (16.65%)	299 (5.91%)	35.51
>55 years	52	38	73.07	34	20	58.82	86 (1.70%)	58 (1.15%)	67.44
Total	2988	1939	64.89	2068	912	44.10	5056	2851	56.39

Note : Population of 4 hutments 5056.

Table 3. Epidemiological situation of malaria in hutments

Month/ Year	Population covered	Blood Smears		Parasite Species			Cases/ 1000 pop.	SPR	SFR	Pf %	BER % febrile
		Examined	Positive	Pv	Pf	Mix					
Apr 82	3213	182	41	39	2	0	12.8	22.5	1.1	4.8	5.6
May 82	7700	648	235	233	2	0	30.5	36.2	0.3	0.8	8.4
Jun 82	13799	1685	556	553	3	0	40.3	32.9	0.2	0.5	12.2
Jul 82	15375	1631	481	479	2	0	31.3	29.4	0.1	0.4	10.6
Aug 82	23771	3196	1044	985	58	1	44.0	32.6	1.8	5.5	13.4
Sep 82	21127	3877	1314	1086	227	1	62.2	33.8	5.9	17.2	18.3
Oct 82	21127	1997	704	431	273	0	33.3	35.2	13.7	38.7	9.4
Nov 82	21127	826	295	153	139	3	13.9	35.7	16.8	47.1	3.9
Dec 82	21127	791	182	56	123	3	8.6	23.0	15.5	68.1	3.7
Jan 83	21127	753	107	41	61	5	5.0	14.2	8.1	57.0	3.6
Feb 83	21127	796	135	89	44	2	6.4	16.9	5.5	32.6	3.7
Mar 83	19491	714	126	104	20	2	6.4	17.6	2.8	15.9	3.6
Apr 83	19491	784	244	220	22	2	12.5	31.1	2.8	9.1	4.0
May 83	6388	605	338	327	10	1	52.9	55.9	1.8	3.2	9.5
Jun 83	4420	251	131	124	7	0	29.6	52.2	2.8	5.3	5.7
Jul 83	10848	1014	362	341	21	0	33.4	35.7	2.1	5.8	9.3
Aug 83	10848	1139	400	343	55	0	36.9	35.1	5.0	14.2	10.5
Sep 83	10848	2002	958	518	437	3	88.3	47.9	22.0	45.9	18.5
Oct 83	10848	1423	786	353	431	2	72.5	55.2	30.4	55.1	13.1
Nov 83	10848	691	332	73	259	0	30.6	48.0	37.5	78.0	6.4
Dec 83	10848	510	204	55	147	2	18.8	40.0	29.2	73.0	4.7

Note : Data of 12 hutments.

Table 4. Man hour density of anophelines in morning catches

Month/Year	*A.annularis*	*A.culicifacies*	*A.stephensi*	*A.subpictus*
May 82	1.50	3.00	4.00	7.00
Jun 82	0.50	2.00	2.00	7.00
Jul 82	3.00	4.00	10.50	6.00
Aug 82	3.50	5.00	24.50	16.50
Sep 82	1.50	3.50	33.50	21.00
Oct 82	2.50	3.50	40.50	43.00
Nov 82	0.50	2.50	19.50	28.00
Dec 82	0	0.50	9.50	4.50
Jan 83	6.00	1.75	0	0
Feb 83	7.00	2.25	0	0
Mar 83	3.25	7.00	0	0
Apr 83	2.50	4.00	1.00	7.00
May 83	1.00	4.00	0	6.75
Jun 83	0.75	0	0	42.00
Jul 83	2.50	2.00	5.00	132.50
Aug 83	0.25	0.50	13.75	47.00
Sep 83	4.50	0.75	17.50	37.50
Oct 83	0.75	0	8.25	20.00
Nov 83	0.50	0	0	2.25
Dec 83	0	0	0	0

Note: Per man hour density.
One specimen of *A stephensi* was found with sporozoites in September 1983.

antenatal period. All pregnancies delivered normal babies. Sixteen persons experienced severe vomiting after chloroquine administration. The drug was repeated in these cases, 2 hours after administration of antihistamines, without any subsequent complaint of vomiting. Measurements of malaria morbidity (duration of febrile illness after presumptive treatment of chloroquine at 600mg adult dose) was carried out from May to August 1982. It was revealed that duration of fever was upto 24 hours in 58 (4.44%) cases, 24 to 48 hours in 273 (20.89%) cases, 48 to 72 hours in 834 (63.81%) cases and over 72 hours in 142 (10.86%) cases. A 4 day afebrile period was taken as clinical cure from malaria.

Spleen survey was carried out in a total of 486 afebrile children of the 2–9 years age group. There were 149 (30.65%) children with enlarged spleen and of these children 71 (14.6%) were positive for the malarial parasite. As a result of prompt treatment of malaria positive cases, spleen enlargement and parasitaemia in afebrile children was considerably reduced. This was revealed by a second follow-up survey in April 1983 which showed that out of a total of 412 children examined, 27 (6.5%) had enlarged spleen and 7 (1.7%) had the malarial parasite.

Entomological observations in hutments revealed that *A.annularis, A. culicifacies, A.stephensi* and *A. subpictus* were the commonly encountered anophelines. The populations of *A. annularis* and *A. culicifacies* were extremely low and may not be responsible for any significant transmission. *A. subpictus* is not a vector of malaria in this part of the country but increase in its densities especially after the rains may be causing considerable nuisance. *A. stephensi* was the only vector species which could transmit malaria in the hutments. The densities of *A. stephensi* were reasonably high from July to December (see Table 4). A specimen of *A.stephensi* was found

infective in September 1983 in Sanjay Ekta Camp. In several other localities of Delhi, *A. stephensi* was incriminated as vector of malaria during the last few years (Choudhury, 1984). Dhir (1969) reported an outbreak of malaria casued by *A. stephensi* which was found breeding near the construction sites in Delhi. Pattanayak *et al.* (1977) also described the association of *A.stephensi* with local outbreaks of malaria in construction complexes. They also found one female *A. stephensi* positive in New Delhi South zone. *A. stephensi* was therefore the vector of malaria in Delhi including the hutments.

Rapid industrialization, large scale construction and many other opportunities induce people to move to Delhi in large numbers. Inter alia, many labour groups come from malaria endemic states, including regions with high *P.falciparum* incidence. They also come from regions known for chloroquine resistance in *P.falciparum*. In Delhi, they stay in temporary camps and settle down in clusters forming a hutment. These hutments are scattered all over Delhi, and have high malariogenic potential, sanitation facilities being almost non-existent. The anti-parasitic and anti-vector measures for malaria control are very poorly implemented. Thus the hutments constitute an important source of infection. The infection also spreads to other localities which have improved sanitation and higher standards of living. It is, therefore, important to provide better sanitation and water disposal facilities, and to intensify malaria control measures in hutments to eliminate a major source of malaria infection from Delhi.

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Studies on the Detection of Malaria at Primary Health Centres. Part I. Reliability of Parasitological Diagnosis by Decentralized Laboratories

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In a study spanning 39 Primary Health Centres of three districts in Orissa during July 1981-April 1983, technicians of the NMEP collected duplicate slides from 100-200 consecutive cases. One set was diagnosed at the PHC as per NMEP procedure and the duplicate set by the research laboratory following Giemsa staining. Parasite density (PD) was recorded species/form-wise in a gradation system: from grade 1 for less than one parasite per 10 fields to grade 5 for more than 100 parasites per field. Blind cross-checking of all positive and 20% of negative slides was carried out at NMEP Directorate.

Out of 4115 slides collected, paired results were available for only 3285 from 35 PHCs. The laboratories were categorized into four groups on the basis of *sensitivity* and *false positivity*. Four PHCs came under group I with very low sensitivity and results that seemed randomly assigned; seven under Group II with low sensitivity and low false positivity; eight under Group III with relatively high sensitivity but also very high false positivity and seven under Group IV with relatively high sensitivity and low false positivity.

Success in detection of parasites often depended on PD and some PHCs were more successful in detection of *P.vivax* rather than *P.falciparum*. Negative slides were declared *P.falciparum* in most cases of false positivity and *P.malariae* or mixed cases were never diagnosed as such. Despite low reliability of data in individual cases, the results of PHCs can be used for epidemiological analysis because of high correlation between true and reported SPR.

Low performance in many laboratories is due to high workload and poor supervision; proposed measures should improve the situation.

INTRODUCTION

During the malaria eradication effort in India, microscopic examinations of blood for malaria were centralized, this was done in relatively big laboratories located at the unit level, one unit usually covering a district or a part of it, with a population of about 1 to 1.5 million. Each laboratory employed about 10 to 12 microscopists.

In 1977, the Modified Plan of Operations was adopted, which meant a transition from a malaria eradication to a malaria control strategy (Pattanayak and Roy, 1980). At the same time, the process of integration of the antimalaria organization into the Primary Health Care system started, which by now is completed in most parts of the country. In the course of

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implementing the MPO, the laboratories were decentralized in almost all states.

Each Primary Health Centre (PHC) now has its own laboratory which is as a rule manned by one technician whose functions are to examine blood for malaria and, to a limited extent, sputum for tuberculosis. In some states there are provisions for two laboratory technicians. In such cases, the functions of the laboratory technicians are usually divided, one being responsible for malaria microscopy, and another for general pathological examinations. Sometimes, this second laboratory technician may help in blood slide examination if the number of slides increases. In some states there are also microscopists with a limited training background, who normally do some other job, but who can help to clear slide backlog, if required.

The decentralization of the laboratories had its advantages. It improved the access of the population to the laboratory services, and also created a nucleus of multipurpose laboratories on the block level. In other words, decentralization contributed to the development of Primary Health Care. On the other hand, decentralization created a problem of supervision because instead of centralized laboratories the PHC laboratories were functioning at nearly 6000 points, without adequate supervisory arrangements. As a result, the quality of blood examination declined in many cases.

To assess the accuracy of diagnosis of malaria by decentralized laboratories, a study was conducted in Orissa, as an applied research project under the Plasmodium falciparum Containment Programme (PfCP), objectives and organisation of which was described by Ray (1979).

MATERIAL AND METHODS

Orissa is known as a problem area for malaria, contributing to 30-49% of the total *Plasmodium falciparum* cases recorded in India in 1977-1984. PHCs were selected in three districts of Orissa state; Sambalpur, Mayurbhanj and Phulbani in which longitudinal entomological and epidemiological observations by PfCP were already under way. The selection was done in such a way as to represent different physiographic regions of Orissa, in which malariogenic conditions are known to vary considerably.

Almost all of the PHCs of the three districts were visited, objectives of the survey were explained to the PHC Medical Officers, and their cooperation was sought. Thirty-nine PHCs were enrolled in this way (Fig. 1).

Slides were collected in three phases; during the pilot survey in July-November 1981, the main survey in January-July 1982 and mopping up in September 1982-April 1983.

Laboratory technicians were asked to collect slides in duplicate from 100 consecutive cases who were referred for the blood examination for malaria. In areas with lower prevalence of malaria, 200 was the target. Brand new slides (manufactured by Superior, West Germany) were distributed to them, to be used for the slides to be examined by the project. However, ordinary slides were used for the matching blood films to be examined at the PHCs.

They were further instructed to perform the dehaemoglobination 72 hours after the collection, in their usual way, then to dry, wrap and keep the slides till a project worker collects them.

Staining and examination at the PHC was done following the NMEP guidelines (using JSB stain for staining and paraffin oil for examination). Nothing was done to change the established procedure or to improve the condition of the microscopes. It cannot be excluded, however, that being informed on the study design of the project, some of the laboratory technicians tried to examine the matching slides with more than usual care.

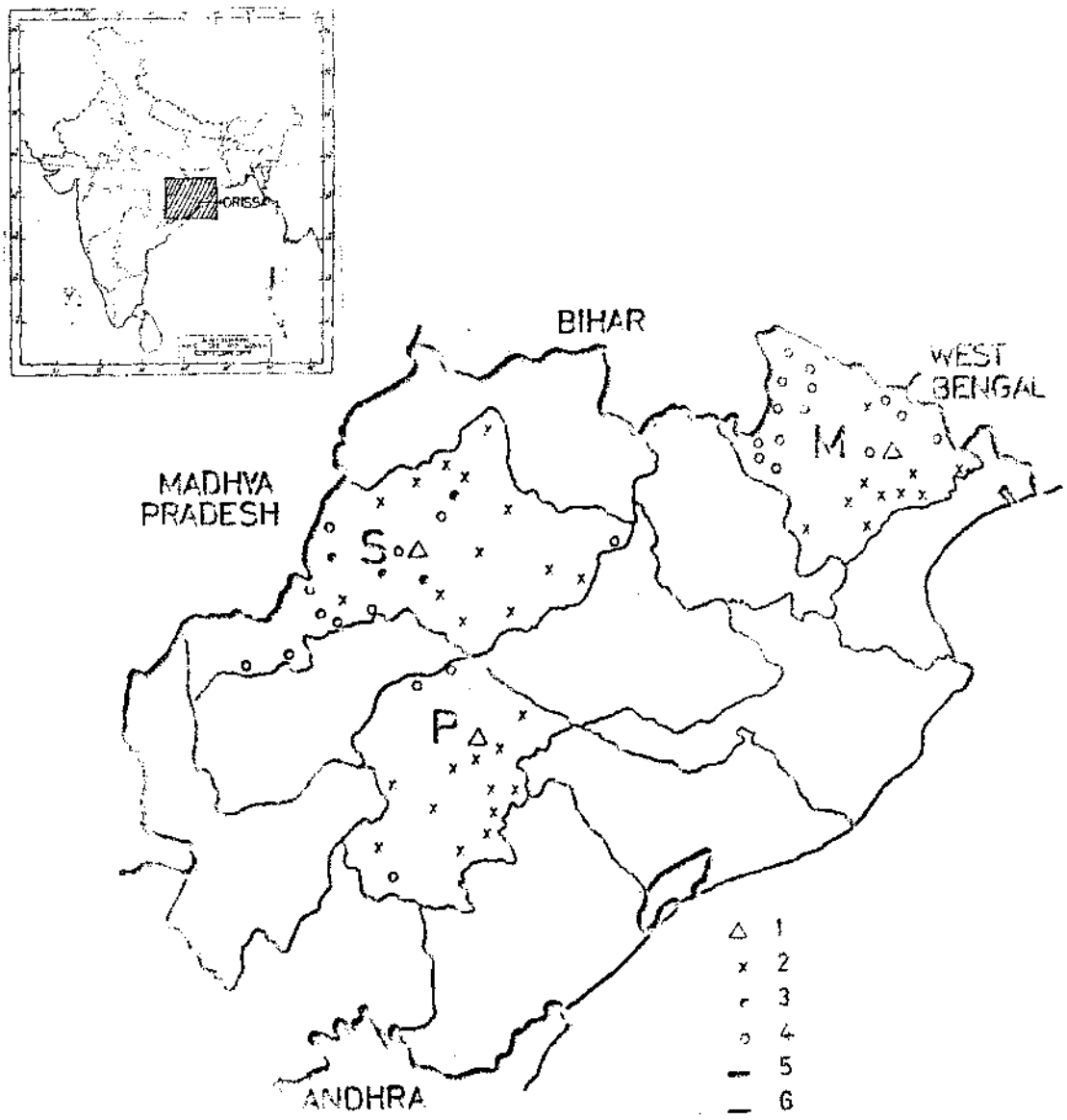


Fig. . Distribution of PHCs in three districts of Orissa.
LEGEND
1—District Headquarters;
2 and 3: PHCs covered by the Project;
2—readable slides obtained;
3—no readable slides obtained;
4—PHCs not covered by the Project;
5—State boundary;
6—District boundary.
Districts: M—Mayurbhanj; P—Phulbani; S—Sambalpur

Usually within a month after collection the slides were transported by a project worker to the PfCP research laboratory where they were stained by Giemsa stain (Merck) dissolved in phosphate buffer solution at pH 7.0. For examination new microscopes with built-in illuminators were utilized. Genuine immersion oil (Merck) was used.

For examination, an optical system of ×100 objective and ×7 eyepiece was utilized. Prior to this, areas suitable for examination were selected using ×10 lens. The criteria for selection were, presence of a noticeable background and sufficient number of leukocytes–10 to 20 per one microscopic field (×700).

Parasite densities (PD) were recorded separately for the following species/forms: *P. falciparum* (asexual), *P. falciparum* (gametocytes), *P. vivax* (all forms) and *P. malariae* (all forms). The density grades were recorded in the following way:

Grade	Number of parasites
5	more than 100 per field
4	10 to 100 per field
3	1 to 10 per field
2	1 to 10 per 10 fields
1	less than 1 per 10 fields.

If rare objects were seen, the laboratory technicians were instructed to mark their coordinates, so they could again be located if confirmation was needed. The correctness of the diagnosis was confirmed by one of the authors (K.C.S.). After that, all the positive and 20% of the negative slides were sent to NMEP Directorate in Delhi, where they were re-examined by a senior laboratory technician who did not know the results of the primary examination. Then the two sets of results were compared, and, in case of discrepancy the final diagnosis was established after a re-examination by one of the authors (A.E.B. or J.A.B. or both). After the results by the project laboratory had been finalised, the results from the field laboratories were obtained and compared.

The data was processed using a pocket computer Sharp PC 1251.

The flow-chart of the entire procedure is shown in Fig. 2.

RESULTS

Classification of the laboratories

From 39 PHCs, 4115 slides were collected. Quality of the blood films was, in general, low. The common defects were fixation of smear, insufficient amount of blood and presence of bacteria and miscellaneous dirt in the films. In some cases, probably due to the properties of the water used for dehaemoglobination, the films did not take up stain properly. Therefore, as a rule, it was more difficult to examine those slides, than the slides collected directly by the project workers elsewhere; 609 slides (15%) had to be discarded altogether as unfit for examination, including whole lots from 4 PHCs.

Out of 3506 slides examined at the research laboratory, the results could be compared with those produced by the PHC laboratories in 3285 cases. In the remaining cases, results were missing from the PHC.

In relation to the Slide Positivity Rate (SPR) as measured by the Project laboratory, two clear-cut groups of PHCs could be distinguished, a group of 7 PHCs with low SPR (1 to 3%) and a group of 26 PHCs with SPR ranging from 15 to 57%. Only two PHCs fell in between these two groups, with SPR 6 and 9%. In more detail, the parasitological findings are discussed elsewhere (Beljaev *et al.*, in press).

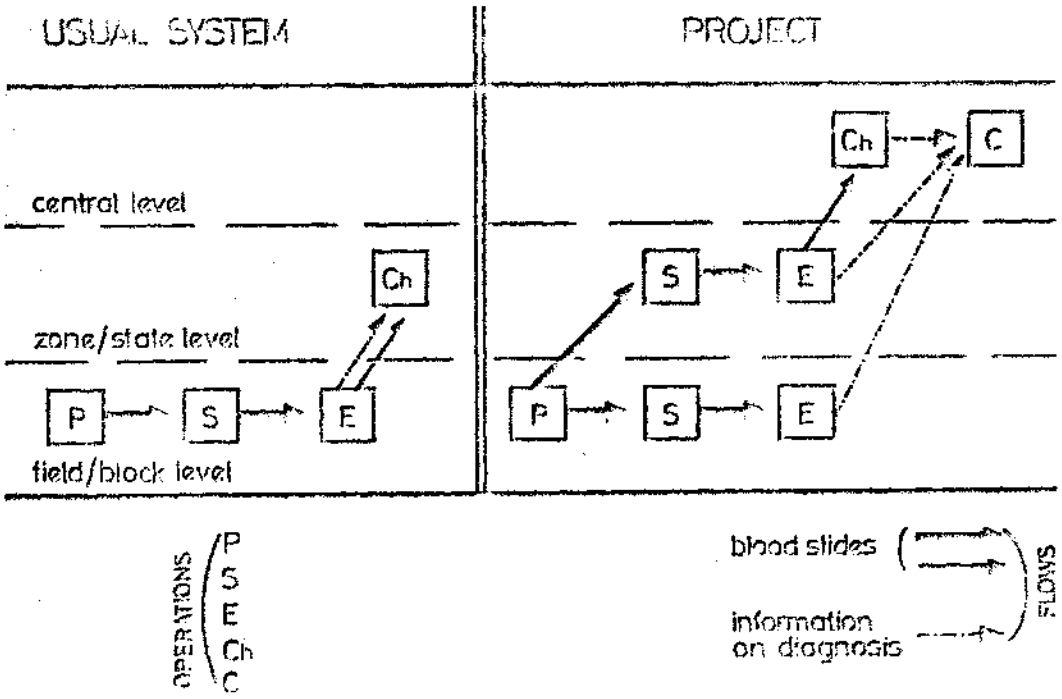


Fig. 2: Flow-chart showing the processing of slides by the Project compared with the usual cross-checking procedure.
Operations: P — preparation of the blood slides; S — staining; E — examination; Ch — cross-check; C — comparison.
Flows of slides are shown by solid arrows with their breadth corresponding to the amount of slides; flows of information are shown by broken arrows.

The accuracy of the diagnosis was measured by two parameters:

—*sensitivity* (percentage of slides declared positive by the PHC laboratory, out of the total number of positive slides detected by the reference laboratory) and

—*false positivity* (percentage of slides declared positive by the PHC laboratory, out of the total number of negative slides detected by the reference laboratory).

Data on 26 laboratories has been used for this analysis. The remaining have been excluded on the following grounds; in four of them all slides were unfit for examination and in nine the number of positive cases was too low to evaluate the sensitivity.

The distribution of the 26 laboratories in relation to the two parameters is shown on the scatter diagram (Fig. 3). On this kind of graph, the position of an ideal laboratory would be in the lower right corner (100% sensitivity and 0% false positivity). In fact, none of the laboratories reached this status, though some of them approached it closely enough.

It may be useful to assess the status of the laboratory in relation to a line drawn at 45° to the x-axis representing cases where sensitivity equals false positivity, their ratio being 1:1. The position of a laboratory would be on or near this line if the results are given at random. If, which is difficult to imagine, a positive slide is more likely to be declared negative and vice versa, the corresponding dot would be placed above that

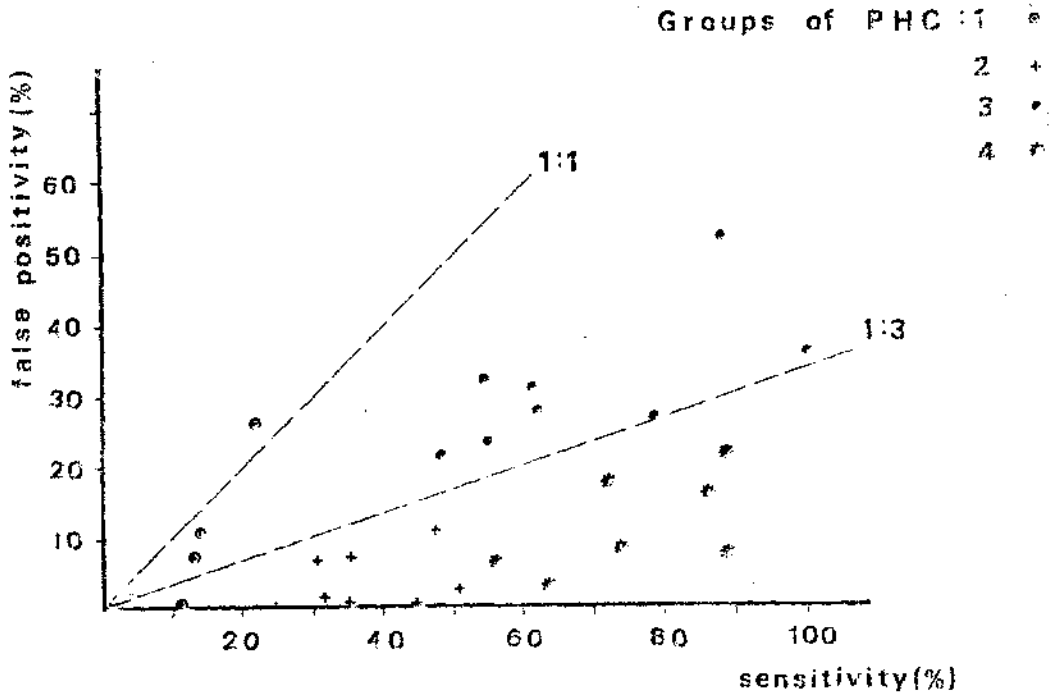


Fig. 3: Distribution of PHC laboratories in relation to their sensitivity and false positivity.
Each dot corresponds to one PHC (the definitions of the PHC categories are given in the text).
Lines (1:1 and 1:3) show the ratio of false positivity to sensitivity. With the ratio 1:1 the diagnosis is as if generated by a random process.

line. Another line, corresponding to the 1:3 ratio between the sensitivity and false positivity was also drawn. This line was used to distinguish between the laboratories with (relatively) high sensitivity and low false positivity (group IV below) and those with high sensitivity and high false positivity (group III).

In relation to those lines, four more or less well defined clusters of dots may be observed.

Group I consists of 4 laboratories located at the lower left corner of the graph, near the 45° line. In other words, the sensitivity is very low, and the results look as if they have been assigned to slides in a random manner.

Group II consists of 7 laboratories located to the right of the group I. The sensitivity is quite low, but the false positivity is low too. The probability of misdiagnosis is three or more times less for a positive slide than for a negative one.

Group III consists of 8 laboratories occupying an upper right position on the graph. The sensitivity is relatively high and may be as high as 100%, but the false positivity is also very high.

Group IV consists of 7 laboratories located in the lower right corner of the graph. The sensitivity is relatively high, and the false positivity is rather low.

Group (the non-categorized) consists of 7 laboratories in which the sensitivity could not be measured.

Influence of parasite species and density on the success of the detection

The success in the detection of parasites was analysed for each group of laboratories (Table 1). The classes of PD were combined to produce two broad classes, of low densities (classes 1 and 2 in which the parasites are not present in every field), and of high densities where, on the average, more than one parasite is present in each field. Comparisons between the groups were made using the t-test for two percentages. As a threshold value, $t = 1.645$ was selected which corresponds to a significance at 95% level in the one-tailed test.

It was found that only in the group (II) the success in detection of one species (*P.vivax* in this case) was significantly higher than of another (*P.falciparum*) and this is only in a case when the PD was low. Success in detection of parasites in cases with a higher PD as compared with cases of a lower PD (of the same species) was significantly higher only in groups II and IV of PHCs. In the PHCs which could not be categorized because of the low incidence, the success in detection of *P.vivax* was significantly higher than of *P.falciparum*.

In group I of the laboratories, where the sensitivity was unacceptably low, even cases of extremely severe parasitaemia (class 5) were missed as a matter of routine [as many as 9 out of 11 cases (82%)]. In the other three groups, this occurred, but only occasionally (two out of 21 cases of class 5 missed or 9.5%).

Table 1. Sensitivity in relation to the species and parasite densities

Number of cases detected by the PHC laboratories and confirmed/number of cases detected by the reference laboratory (sensitivity %)

Species	*P. falciparum*		*P.vivax*	
Parasite density classes	1 to 2 (FL)	3 to 5 (FH)	1 to 2 (VL)	3 to 4 (VH)
Groups of the PHC laboratories				
I	4/39 (10.3%)	15/84 (17.9%)	1/10 (10.0%)	3/13 (23.1%)
II	7/39 (18.0%)	25/49 (51.0%)	12/30 (40.0%)	23/36 (63.9%)
III	39/63 (61.9%)	48/69 (69.6%)	19/30 (63.3%)	24/38 (63.2%)
IV	28/41 (68.3%)	44/53 (83.0%)	15/24 (62.5%)	18/23 (78.3%)
(V)	0/ 3	1/ 4	4/ 7	3/ 6

FL = *P. falciparum* low
FH = *P.falciparum* high
VL = *P. vivax* low
VH = *P. vivax* high

Significant differences in the one-tailed t-test:

GROUP II
VL > FL
FH > FL
VH > VL
VH > FL

GROUP IV
FH > FL
FH > VL

NON—CATEGORIZED
All V > all F

A different approach may be based on the assumption that, if the success of detection depends on the parasite densities, they would be higher in detected cases than in the missed ones. This approach was tested and the conclusions were, by and large, the same as above (Table 2).

All the cases diagnosed as positives by the Project laboratory were subdivided into 8 sets (four PHC groups × two species). Each of these 8 sets were further subdivided into two: slides which were diagnosed as positive by the PHC (eventual discrepancies in species diagnosis were not taken into consideration) and those which were diagnosed as negative. For each of the resulting 16 groups, average parasite densities (APD) were calculated by summation of the density class numbers (for the few mixed cases present, the density of the predominant species was taken). For a comparison of the APD in individual groups, a one-tailed t-test on two means was used. To assess the trend in all the groups as a whole, a sign test was tried.

It could be observed that in every PHC group and for both species the APD was higher among the detected cases than among the missed ones, which is highly significant in the sign test. A comparison of individual groups using the t-test demonstrated the following:

(a) Among the missed *P.falciparum* cases, the APD was significantly higher in group I than in any of the other groups (in other words, more parasites were needed for a technician of group I to recognize them as parasites);

(b) Only in groups II and IV the APD in the detected cases of *P.falciparum* was significantly higher than in the missed ones, in these groups the success of the diagnosis depended on the parasite densities more than in others;

(c) Only in group IV was the APD in the detected cases of *P.vivax* significantly higher in the detected cases than in the missed ones. In other words, success in the detection of *P.vivax* is less influenced by the parasite density in the majority of the laboratories, except in the most successful ones which belong to the group IV.

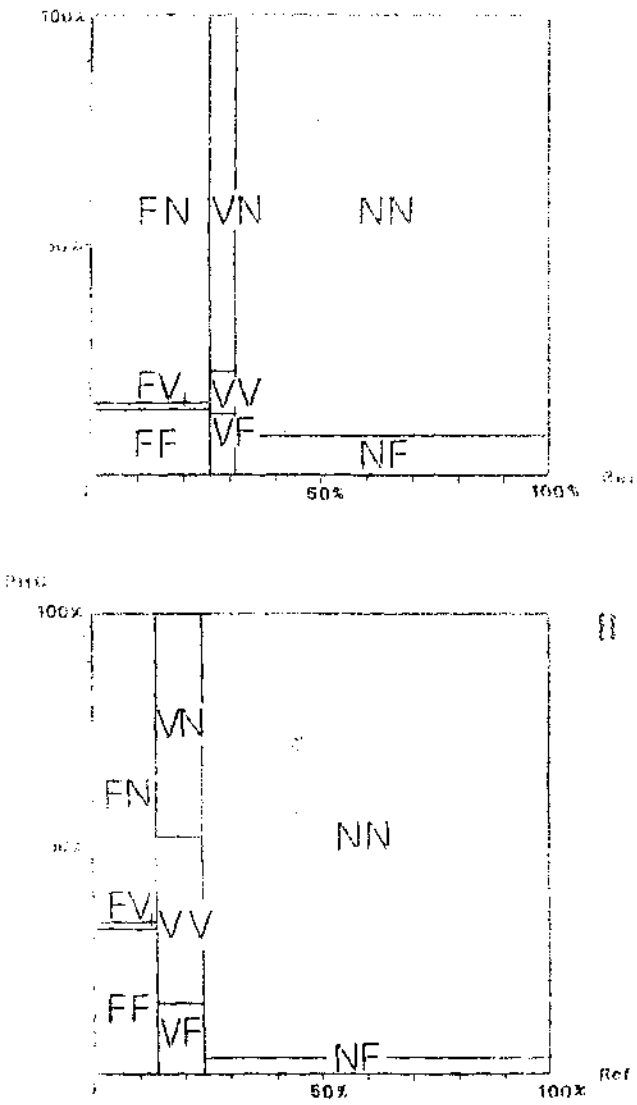
Comparison of various categories of the laboratories

The combined results of the comparison for each group of laboratories are given in Fig. 4. On the horizontal axis, the results by the Project laboratory are subdivided into three groups; (1) *P.falciparum;* (2) *P.vivax* (to which cases of *P.malariae* were also added because they were too few to be shown separately); (3) Negative. A few mixed infections were included under the

Table 2. The average parasite densities in the detected and missed cases

Groups of PHC laboratories and total cases, detected or missed	*P. falciparum*			*P.vivax* and *P.malariae*		
	NC	APD	SD	NC	APD	SD
I Detected	19	3.26	1.05	5	3.00	0.71
Missed	105	2.90	1.30	19	2.53	1.17
II Detected	33	3.33	1.41	34	2.68	0.84
Missed	66	2.00	1.34	32	2.44	0.84
III Detected	101	2.28	1.48	47	2.60	0.77
Missed	52	2.06	1.39	30	2.43	0.94
IV Detected	79	2.53	1.36	37	2.73	0.91
Missed	26	1.88	1.37	17	2.18	0.88

NC = Number of Cases
APD = Average Parasite Density
SD = Standard Deviation



species showing a higher density, which was, as a rule, *P. falciparum*.

The three groups which are represented by three vertical bars of varying width were further subdivided into three groups each according to the PHC diagnosis. For example, in group I of the PHCs which is represented in Fig. 4-I, 27% of the cases belonged to *P. falciparum* (which may be read on the horizontal axis), and out of them, 14% were diagnosed as such by the PHCs, 3% as *P. vivax*, and 85% as negative (which may be read on the vertical axis). As a result, the totality of the cases was subdivided into 9 groups which were labelled accordingly, e.g.:

FF — *P. falciparum* diagnosed as *P. falciparum;*
FV — *P. falciparum* diagnosed as *P. vivax;*
FN — *P. falciparum* diagnosed as negative, etc.

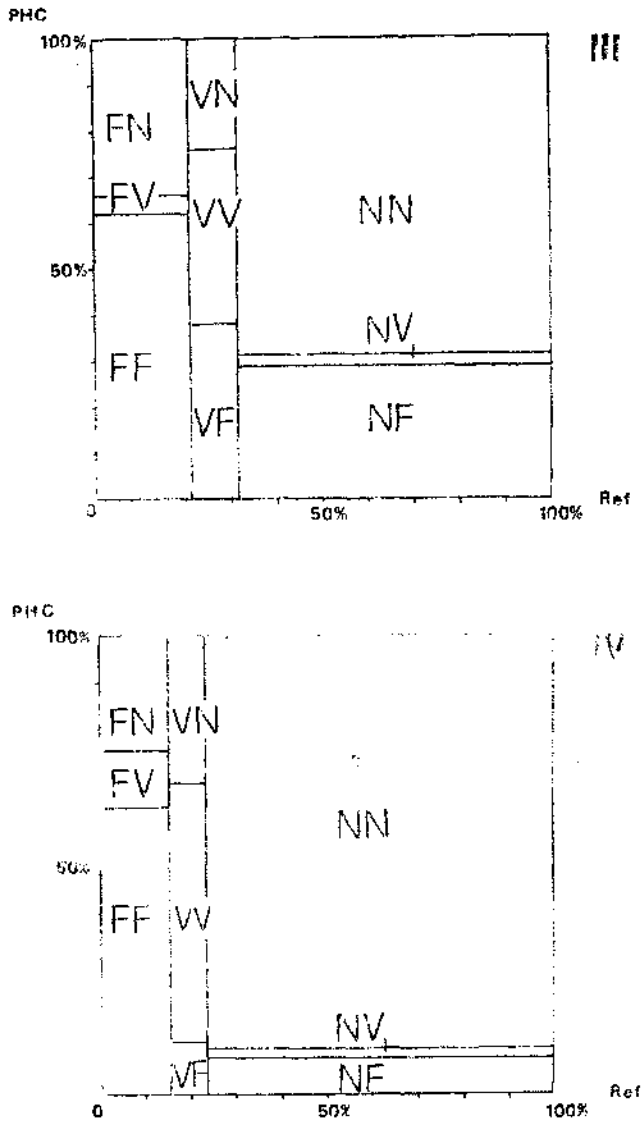


Fig. 4: Results of blood examination by the Project laboratory (x-axis) and PHC laboratories (y-axis).

Data are given separately for each of the four groups of laboratories (I to IV).

Symbols for the sets of slides:

FF — *P.falciparum* diagnosed as *P.falciparum;*
FV — *P.falciparum* diagnosed as *P.vivax;*
FN — *P.falciparum* diagnosed as negative;
VF — *P.vivax* diagnosed as *P.falciparum;*
VV — *P.vivax* diagnosed as *P.vivax;*
VN — *P.vivax* diagnosed as negative;
NF — Negative diagnosed as *P.falciparum;*
NV — Negative diagnosed as *P.vivax;*
NN — Negative diagnosed as negative;

FF+FV+VF+VV — All positive diagnosed as positive;
FN+VN — All positive diagnosed as negative;
NF+NV — All negative diagnosed as positive.

The advantage of this method is that the area of each rectangle is proportional to the absolute number of cases in each group. Combined groups are also easily seen: FF+FV+VF+VV representing all positive cases detected as positive, FN+VN representing all missed cases, and NF+NV representing all false positives.

Analysis of the data presented in Fig. 4 is given in Table 3. To quantify the species discrepancy rate, only true positives which were also recognized as positives by the PHC laboratories were taken, i.e., sets FF, FV, VF and VV. The error rate for *P.falciparum* is the proportion of *P. falciparum* wrongly diagnosed as *P.vivax* among the total number of *P.falciparum* cases recognized by both PHC and Project laboratories i.e., FV divided by the sum of FF and FV. The error rate for *P.vivax* is calculated in a similar way. It is obvious that the proportion of *P.vivax* cases diagnosed as *P.falciparum* far exceeds the proportion of *P.falciparum* cases diagnosed as *P.vivax*. The error of diagnosing *P.falciparum* as *P.vivax* occurred significantly more often in PHC group IV than in groups II or III. The error of diagnosing *P.vivax* as *P.falciparum* occurred more often in PHC groups I and III than in group IV, and in group III more than in group II.

To measure the false positivity rate, the proportion of false positives of each species (sets NF or NV) among the total number of negative cases (NF+NV+NN) was calculated. It may be observed here again that preference was given to *P.falciparum* over *P.vivax* in all the groups except NC. In other words, if a technician was coming across something which he thought might be a parasite, but in fact was not, he would much more often say that it was *P.falciparum* than *P.vivax*.

False *P.vivax* diagnosis occurred significantly more often in groups III, IV and NC than in groups I and II. False *P.falciparum* occurred more often in group III than in any other, and more often in groups I, III and IV than in groups II and NC. As for *P.malariae*, it was never correctly diagnosed, out of 29 cases seven were diagnosed as *P.falciparum*, seven as *P.vivax* and the rest as negative.

Mixed infections which were present in 15 cases (*P. falciparum* + *P vivax* in 13 cases, *P.falciparum* + *P.vivax* + *P.malariae* in one and *P.vivax* + *P.malariae* in one) have never been detected as such. In 3 cases *P.vivax* was diagnosed, in 3 *P.falciparum* and in 9 cases the result was declared negative.

Table 3. Errors in species diagnosis and false positivity

Diagnosis	*P.falciparum* called *P.vivax* (FV)/(FV + FF)	*P. vivax* and *P. malariae* called *P.falciparum* (VF)/(VF + VV)	Negative called *P.vivax* (NV)/(NV + NF + NN)	Negative called *P.falciparum* (NF)/(NV + NF + NN)*
Groups of PHC laboratories				
I	1/ 19(5.3%)	3/ 5(60.0%)	1/319(0.3%)	28/319(8.8%)
II	1/ 33(3.0%)	10/35(28.6%)	0/526(0.0%)	20/526(3.8%)
III	6/101(5.9%)	29/47(61.7%)	14/504(2.8%)	144/504(28.6%)
IV	12/ 79(15.2%)	7/38(18.4%)	11/543(2.0%)	46/543(8.5%)
Non-categorized	nil	nil	25/672(3.7%)	23/672(3.4%)

*For an explanation of the set symbols see Fig. 4.
Significant differences in the one-tailed t-test:

GROUP I	GROUP II	GROUP III	GROUP IV
VF > FV	VF > FV	VF > FV	VF > FV
NF > NV	NF > NV	NF > NV	NF > NV

Table 4. Frequency of *P.falciparum* gametocytes being missed

Number of cases detected by the PHC laboratories/total number of *P.falciparum* cases as detected by the reference laboratory (sensitivity in relation to gametocytes %)

Gametocyte density classes	1 (G1)	2 (G2)	3 (G3)
Groups of PHC laboratories			
I	0/ 19	0/ 3	nil
II	1/ 25 (4.0%)	0/ 9	1/3
III	2/ 40 (5.0%)	0/11	1/2
IV	1/ 26 (3.9%)	3/ 6	1/1
Total	6/110 (5.4%)	3/29 (10.4%)	3/6

Significant differences in the one-tailed t-test:
Group IV > Other groups combined;
G3 > G1; G3 > G2; G3 & G2 > G1.

P.falciparum gametocytes were under-reported, they were found by the PHC laboratory technicians only in 10 cases out of 145 (6.9%). True, the gametocyte densities were usually very low (Table 4). Success in detection of gametocytes was significantly higher (as assessed by a one-tailed t-test) in cases of higher gametocytaemia (PD class 2 and 3 combined vs. class 1) and in PHC group IV than in all other groups combined.

Correlation between the true and reported SPR

It was observed that false positive diagnoses are more likely in situations where malaria incidence is high (Fig. 5). To substantiate this observation, the correlation coefficients between the true SPR and false positivity were calculated. The least squares method was used, and linear, logarithmic, exponential and power models were tested. The best fitting curve was selected out of the four models, i.e., the curve with the highest correlation coefficient, *r*. In this case, the highest *r* was obtained with an exponential curve (*ab* in Fig. 5):

$$y = ae^{bx}$$ where:

x is true SPR
y is false positivity
e = 2.718...
Parameters: a = 3.10, b = 0.568, r = 0.48.

The exponential model cannot take into consideration points where y = 0 (there are five points like this). The best fitting line which takes into consideration all 35 points is the straight *cd*:

$$y = a+bx$$ with parameters
a = 5.19, b = 0.372, r = 0.40.

The true and the reported SPR were compared by the same method (Fig. 6). The best fitting curve was found to be a power curve:

$$y = ax^{b}$$ where:

x is the true SPR
y is the SPR by PHC laboratories
Parameters: a = 3.10, b = 0.568, r = 0.64.

If the laboratories belonging to group I with absolutely unreliable results are excluded, the parameters change in the following way: a = 3.44, b = 0.577, r = 0.70.

DISCUSSION

Inference from the results

The results indicate that (i) many cases of malaria are missed or misdiagnosed; (ii) many negative cases are wrongly identified as positive; (iii) the performance of the PHC laboratories is strikingly variable.

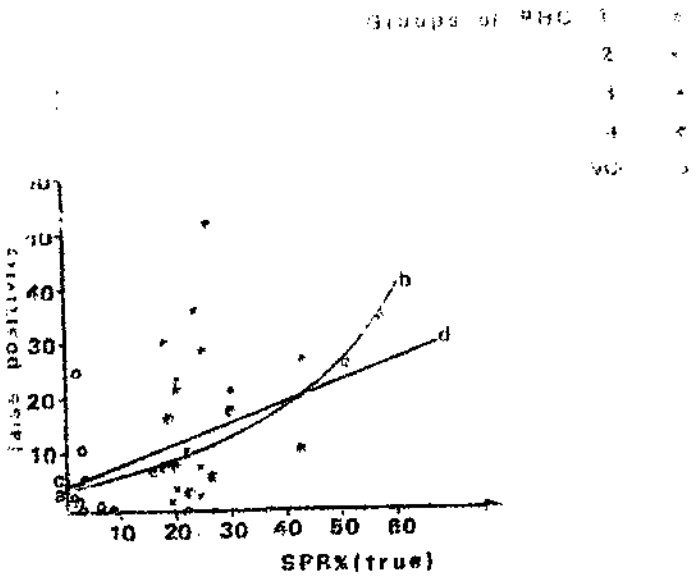


Fig.5: Correlation of "true" SPR and false positivity.
Each dot corresponds to one PHC (the definitions of the PHC categories are given in the text).
The highest correlation coefficient r is obtained with the exponential curve *ab* but the best fitting line taking all points into consideration is the straight *cd*.

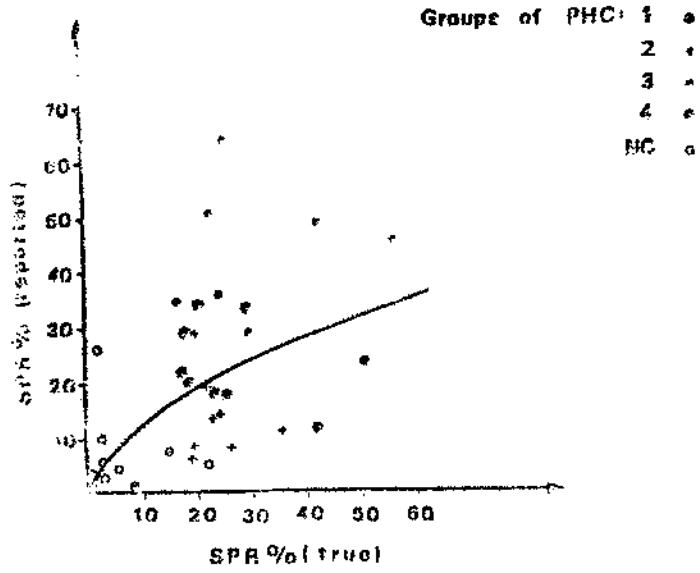


Fig.6: Comparison of SPR obtained from the results of the Project laboratory ("true") and PHC laboratories ("reported").
Each dot corresponds to one PHC (the definitions of the PHC categories are given in the text).
The best fitting curve is drawn (which is a power function; see text).

Among the various types of errors, the most important are cases in which the parasites are not recognized at all. Still, even in these cases, low-density infections, which are more likely to be missed, are to some degree controlled by the presumptive treatment which is given to everybody at the time of blood collection. The real problem is the high density infections that are missed, which occurs quite often, even in the laboratories showing better performance (Table 1).

Incorrect species diagnosis is a less important problem from the therapeutic point of view, since:

(a) the treatment prescribed for *P.vivax* infections (presumptive treatment with 600 mg of chloroquine followed by a radical treatment with one dose of 600 mg of chloroquine plus 15 mg of primaquine daily for 5 days) is usually sufficient for the clearance of rings and gametocytes of chloroquine sensitive *P.falciparum;*

(b) the standard treatment for *P.falciparum* which differs in the dose of primaquine in the radical treatment (45 mg are given in one dose) will not prevent late manifestations of *P.vivax*. This is probably not very important in a control programme.

The study showed that the latter type of therapeutic error is bound to be prevalent, because of the tendency to misdiagnose in favour of *P.falciparum* rather than *P.vivax* in all Groups of PHCs, except the PHCs with low incidence (NC) (Table 3).

False positivity is probably the least important type of error. True, some wastage of efforts and resources is involved, but this is not very substantial. In the group of patients covered by this particular study, chloroquine was presumably given as part of the presumptive treatment to all 3282 persons examined. In addition, chloroquine should have been given as a part of radical treatment to 362 true positives (3644 doses in total). The treatment of 312 false positive cases added only 9% to the amount of chloroquine spent.

The PHC laboratories can be categorized into 4 groups.

The results given by the technicians of group I were absolutely unreliable. The reasons were personality problems (some of the technicians in question were known for years as habitual defaulters, but no administrative action could be taken against them) and/or poor condition of the equipment.

Technicians of groups II and III have difficulty in recognizing parasites. Technicians of group II are cautious and not so easy to mistake an artifact for a parasite so easily. They are more confident in the diagnosis of *P.vivax* and probably need to see many parasites before becoming convinced that parasites are there indeed. On the contrary, technicians of group III are quite liberal in declaring a slide to be positive. That is why the results depend on the PD in group II and do not do so in group III and why false positive results are much more prevalent in group III.

Group IV was the best, though blunders occur even there.

The PHCs operating in areas with a low incidence of malaria could not be categorised. Nevertheless, it seems that they share some common features. False positive results were rather common, but unlike other groups, no preference was given to *P.falciparum* in this case. *P.falciparum* was missed much more often than *P.vivax* in this group.

Reasons for inadequacies in performance

One of the reasons for cases being missed is probably insufficient examination time. This is

demonstrated by the fact that the success in the diagnosis depended on PD at least in some categories of cases and also by the fact that *P.falciparum* gametocytes, which are quite conspicuous though usually few in numbers (Table 4), are often missed. Another reason may be that the technicians usually defy the rule to start examination with a low power lens, to select the portions of slide which are good for examination. They usually start (maybe because of the lack of time) with the immersion lens, and, as a result, quite often examine unproductive fields. This may explain the blunders which occur even with relatively good technicians.

Work with a microscope requires specific mental and physical abilities. Most obvious is the ability to distinguish colours. In some states a colour vision test is mandatory in the course of recruiting microscopists. Unfortunately, the physical ability to work with a microscope is never checked in Orissa. Some of the gross inadequacies which we observed in this study were probably due to that factor.

The factors mentioned above could be relatively easily checked before decentralization, when the laboratory technicians worked in a big group under close supervision of more experienced senior technicians and within easy reach of a specialist, such as a Unit Malaria Officer. Now, the technicians work in isolation. The supervision over them is entrusted to PHC Medical Officers who usually have little technical knowledge of malaria microscopy or running the laboratory. Such supervision is often inadequate.

The psychological effects of isolation are important. A technician has nobody to discuss technical matters with nor can he obtain a second opinion in case there is doubt about the diagnosis. The identification of laboratory errors and malfunctions of the microscopes has become more difficult for many of the technicians. They often have become used to the bad quality of their staining or the poor functioning of their only microscope, because they do not have any standard for comparison.

With decentralization, repairs have become more difficult. Occasional breakdowns of the microscopes in a small laboratory have more serious consequences, since no stand-by microscope is usually available. Logistic problems have become more numerous and not so easy to solve.

Before decentralization, the work of collecting and consolidating epidemiological information was usually done by specially designated workers. Now, the laboratory technician has to do everything himself, he not only has to process blood slides, but also must maintain a number of registers and record forms which have nothing to do with the laboratory work proper, but are for primary epidemiological analysis. The laboratory technician has been overloaded by this paper work.

Under the prevailing conditions, the deterioration of the blood films begins during collection, transportation and dehaemoglobination, even before they are stained.

For staining of blood slides, the National Malaria Eradication Programme (NMEP) uses the JSB method (Jaswant Singh and Bhattacharji, 1944), in a modification described by Jaswant Singh *et al.* (1953). The main merits of the JSB method as Jaswant Singh (1956) pointed out, is its low cost, easy preparation and very fast staining. This method gives excellent results when conducted properly. However, it is better suited for a clinical laboratory where fast staining is essential, than to epidemiological work. In the latter case, the high speed of staining becomes a disadvantage as per the procedure laid down under the NMEP, the slides have to be stained one by one, which may be very time consuming when a mass staining is required. In such circumstances Giemsa staining which may

go unattended for most of the time is much less time-consuming. Because of the high speed of staining, very careful timing is necessary to obtain stable results in JSB staining. An under — or over-exposure of, say, ten seconds, which is of no consequence in a slow method, may be disastrous where JSB is concerned. It seems that standard results may be obtained much easier with Giemsa than with JSB.

Because of the lack of supervision, individual technicians do not follow the technique exactly. The most serious deviation is that the time required for staining in JSB-I solution is seldom measured accurately. Laboratory technicians never adjust their timing according to the diminishing concentration of stain during the course of the working day. Sometimes the same amount of diluted stain is used for several days without filtration. Another common deviation is a failure to use the phosphate buffer solution. Instead, water from any source, often contaminated, is used, without any treatment, as a rule. As a result, the chromatin of the malaria parasites is seldom properly differentiated, and the microscopic field contains a lot of micro-organisms and miscellaneous dirt.

Though normally staining should be done within one to two weeks after collection, cases are common where the transportation of the slides from the field is delayed and/or they are not processed immediately after their having arrived at the laboratory. In such cases the films often become fixed or are deteriorated by fungi and bacteria.

As per the NMEP guidelines, 100 microscopic fields of each slide should be examined. Rarely is it being done. Usually, after coming across the first few parasites the technicians declare the slide positive and decide which species is involved. They are compelled to act in this way because of a tremendous workload. It is not unusual that a laboratory technician examines 70 and more slides a day, besides doing staining and paperwork. On the other hand, if the slide is too bad to be examined, say, because the blood has been washed away or otherwise destroyed, and no parasites are in sight, the slide is always declared negative.

Shortcomings of the cross-checking system

Our study showed much higher discrepancy rates than the usual cross-checking does. This is because the cross-checking has its own limitations. The primary one is probably the bad quality of the slides which makes their examination very difficult even for an expert. Further deterioration occurs during storage (dust and fading, to which JSB stained slides are prone—Jaswant Singh, 1956) which may continue for many months before the slides are sent for cross-checking.

Another factor is that the primary diagnosis is always known to the cross-checking technician and he tends to agree with it, especially taking in view the bad quality of the slides.

The procedure prescribes that slides for cross-checking are selected on the basis of the last digit of their serial number, as communicated from the cross-checking agency. Not uncommonly, clever technicians re-examine slides before sending them, or just weed out slides which are very bad or in which the technician is not sure about the diagnosis.

Finally, in some places, some of the factors mentioned in relation to the PHC laboratory are operating on the level of the cross-checking laboratories, such as the poor condition of microscopes, heavy workload or poor super-vision.

This study was organized in a quite different way, compared with the ordinary cross-checking (Fig. 2), and many of the factors listed above could be neutralised. For example, the cross-checking in our study was done in a blind way.

Due to the cross-checking on different levels, some of the slides have been thoroughly examined more than once which increased the chances to detect low-level parasitaemia. On the other hand, a probability of under-detection could not be completely excluded because a standard quality of staining could not be attained in every case, due to the faulty preparation of films and their deterioration during dehaemoglobination and storing.

Reliability of the epidemiological indices produced on the basis of laboratory data

This study indicates that only a few laboratories produce absolutely irrelevant results. In principle, such laboratories may be easily detected, since their results would often disagree with those of the neighbouring laboratories. Often, the reasons for producing irrelevant results are self-evident. For example, in one of the laboratories of Group I the ×100 lens was broken, and the technician was using ×40 lens plus his 'clinical' judgement, when possible.

It seems, however, that the effect of under-detection of positive cases is counterbalanced by the tendency of producing false positive results, which, as shown in Fig. 5 is stronger in situations with higher incidence. As a result, a strong correlation exists between the true and recorded SPR (Fig. 6) which means that, by and large, the data of PHC laboratories reflect the trends of malaria and may be utilized, with some reservations. The highest correlation coefficient was obtained using the power curve, which means that logarithms, rather than the actual values of the true and reported SPR are correlated.

In individual cases, however, the probability of very serious diagnostic errors is very high, even in relatively good laboratories. Therefore, it would be wise to insist on administration of the presumptive treatment to all fever cases including those patients who agree to wait at the institution till the examination is completed.

Conclusions and recommendations

In Orissa, due to a number of administrative and financial problems, the efficiency of antimalaria services is low, compared with the majority of the states of India. The poor efficiency of the PHC laboratories revealed by this study is one of the many symptoms of unsatisfactory development of Primary Health Care in the state.

Only three laboratories are located in the area of sensitivity above 80% and false positivity below 20% (Fig. 3) which may be considered as a tolerable level of errors. Three more laboratories are showing a sufficient level of sensitivity, but also a high level of false positivity.

The question arises whether the decentralized laboratories in such multi-problem areas as Orissa are serving any useful purpose or that they have completely failed. Our strong belief is that decentralization of laboratories is the only way to make comprehensive laboratory services available to everybody. This component of the Primary Health Care should not be abandoned, but, on the contrary, it should be strengthened and its functions should be diversified.

The factors which are responsible for decreasing efficiency of the laboratories are mostly poor staining and poor maintenance of technical equipment, which are usually rooted in poor supervision. It seems that these failures are relatively easy to overcome, providing the problems are identified and proper supervision is established.

To improve the work of the PHC laboratories, more consistent rapport should be maintained between the technical people on the district level and those in the field. It may be recommended that a team consisting of a few experienced laboratory technicians be provided in the district to tour the PHCs spending a few days in each and doing the following:

(i) identifying problems concerning the

equipment, specially microscope and the lighting system;

(ii) if necessary, implementing simple improvements which may be done on the spot, such as tightening of the joints or cleaning of lenses;

(iii) identifying problems regarding staining and rectifying them;

(iv) checking on the diagnostic skills of the technician using teaching slides with a known diagnosis;

(v) consulting on difficult slides.

In many cases, besides the technical equipment, the physical facilities in the laboratories will need improvement, like provision of a separate room, furniture, or electric lighting. Regular supplies of reagents and stationery are also essential. From the zonal/state level the supervision over the DMO laboratory should include control of the quality of the reagents which are sent by them to the field.

Evidence was obtained that cases of low parasitaemia are very often missed because of an insufficient examination time. In view of the changed pattern of jobs performed by a technician, a revision of the existing workload normatives may be recommended. It is essential also that the vacancies be filled and leave reserve be arranged. In some areas, the number of posts may be revised taking into consideration the quantity of slides which are collected. As a result, the number of technicians may be decreased in non-endemic areas, but increased in highly malarious blocks.

Fitness of an incumbent to work as a microscopist should be checked, and an alternative job offered in cases of colour blindness or similar disorders.

To improve the quality of the cross-checking laboratories the following may be recommended:

(i) primary diagnosis should not be known to the cross-checking technician;

(ii) slides which are unfit for examination should be rejected;

(iii) comments on the quality of the films should be sent, and not only diagnosis, as is being done now;

(iv) slides for cross-checking should not be stored for long periods (to overcome the eventual fading of the JSB stained slides);

(v) feedbacks should be prompt.

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SHORT NOTES

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Field Trials on the Application of Expanded Polystyrene (EPS) Beads in Mosquito Control

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In laboratory experiments expanded polystyrene (EPS) beads were found very effective in the control of mosquito breeding and they prevented gravid females from laying eggs on the EPS treated water surface (Reiter, 1978; Sharma, 1984). This is a report of the results of preliminary field trials on the control of mosquito breeding in their natural habitats using EPS beads.

Trials were carried out in Nadiad taluka villages, district Kheda, Gujarat. The following sites were selected: (i) a biogas plant, in the slurry around the drum there was heavy breeding of *Culex quinquefasciatus* mosquitoes, (ii) three abandoned wells in which there was heavy breeding of *Culex* sp. mosquitoes, and (iii) two septic tanks in which there was heavy breeding of *Culex* mosquitoes. Before the start of the experiment, anti-malaria staff of the Municipal department treated the septic tanks with larvicidal oil (MLO). All immature stages of mosquitoes died. The EPS beads were applied 1 week after MLO treatment to study whether the septic tanks would become positive for mosquito breeding in presence of EPS beads. No MLO was applied to the slurry of biogas plant and the wells.

EPS beads were weighed and applied manually by hand at the rate of 50gm/1000cm^2 in the biogas plant (5 to 6 layers of about 2.6 cm thickness), 20 and 85gm/1000cm^2 (i.e., 2 to 3 layers of 1.0 cm and 9 to 10 layers of 4.4 cm thickness) in wells and 70gm/1000cm^2 in septic tanks (6 to 7 layers of 3.6 cm thickness). The density of immatures was measured in the biogas plant and septic tanks by a 9.5 cm diameter dipper and in wells by a 25 cm diameter well net by taking an average of 5 samples each time. Observations on the density of immatures were made on day 0 followed by weekly intervals upto six weeks.

Results in biogas plant and wells are given in Table 1. Breeding in the slurry of biogas plant and well-I declined gradually and reached zero level in the 4th week in biogas plant and in 5th week in well-I. Whereas in well-II and well-III, larval density reached zero in the second and first week respectively and remained at that level upto the 6th week post-treatment period, it may be noted that breeding continued upto four and five weeks post-treatment in slurry and in one of the wells. This was unusual as the breeding generally terminates in the first one or two

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Table 1. Impact of EPS beads on the control of mosquito breeding

Observation Interval (one week)	Density of Immatures											
	Biogas plant			Well-I			Well-II			Well-III		
	IV instar	Pupae	Larval density	IV instar	Pupae	Larval density	IV instar	Pupae	Larval density	IV instar	Pupae	Larval density
Day 0	14.4	4.8	65.0	Very high density			2.5	0	239.5	7.5	0	31.
Week I	31.0	5.0	56.6	Very high density			5	0	14	0	0	0
Week II	25.2	9.2	25.2	11.0	0	334.5	0	0	0	0	0	0
Week III	5.6	2.0	5.6	4.5	0	46	0	0	0	0	0	0
Week IV	0	0	0	6.5	0	15.5	0	0	0	0	0	0
Week V	0	0	0	0	0	0	0	0	0	0	0	0
Week VI	0	0	0	0	0	0	0	0	0	0	0	0

Notes: 1. Rate of application of EPS beads was 50gm/1000cm² (i.e., 5 to 6 layers of 2.6 cm thickness) in biogas plant, 85gm/1000cm² (i.e., 9 to 10 layers of 4.4 cm thickness) in well-I and III, and 20gm/1000cm² (i.e., 2 to 3 layers of 1.0 cm thickness) in well-II.

2. Larval density includes the density of I, II, III and IV instars.

weeks. The mud in slurry develops the tendency to stick together and these were being disturbed by children. As a result breeding persisted for a long time, however, educating the children helped in keeping the layer intact on the slurry and consequently the breeding was terminated. In the case of well-I, some floating objects and a dead dog created a few spots where mosquito breeding persisted. In other wells there was no such disturbance. In the two septic tanks treated with EPS beads one week after the application of MLO, no egg rafts were laid by the mosquitoes. These septic tanks remained negative for mosquito breeding and for egg rafts throughout the six week period of observation.

Wells are the main source of mosquito production in many parts of India. In Delhi rural areas, profuse breeding of *Culex quinquefasciatus* occurs in wells while occasionally *Anopheles culicifacies* and *A.stephensi* are also encountered breeding in wells (Sharma, 1985; Rao, 1984). In other parts of the country such as in Salem and Hyderabad *A.stephensi* breeds mainly in wells and maintains malaria transmission throughout the year (Seetharaman *et al.*, 1975; Batra and Reuben, 1979). Since EPS beads are safe and one application lasts for a very long time, application of EPS beads in wells can greatly reduce mosquito breeding in wells and, it is hoped, would also reduce or eliminate disease transmission. Similar application of EPS beads in septic tanks can eliminate an important source of mosquito breeding on a semi-permanent to permanent basis. Recently the Government of India has placed considerable importance on alternative energy sources, and installation of biogas plants is one of the major areas receiving emphasis by the Department of Non-conventional Energy Sources. However, biogas plants are becoming another source of mosquito production in rural areas because the slurry of these plants supports heavy breeding of *Culex quinquefasciatus* which are vectors of filariasis and the major nuisance mosquito. The application of EPS beads on slurry of the biogas plants would eliminate this important source of mosquito production, thus improving the environment and reducing disease transmission. In a recent study, Curtis and Minjas (1985) have shown that the application of beads in soakage pits and pit latrines is an effective method of mosquito control.

In view of the usefulness of EPS beads in the control of mosquito breeding, more trials and integration of beads in the non-insecticidal methods of vector control are envisaged in the near future.

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Mosquitoes of Mandla District, M. P.

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The opening of the Regional Medical Research Centre in 1985 in Jabalpur by the Indian Council of Medical Research provided an opportunity to study the entomological and epidemiological aspects of malaria transmission in tribal populations of this region. Mandla district was selected for field work as it has about 60% tribal population. Personal discussions with the District Malaria Officer and the villagers revealed that malaria was one of the major health problems among the tribals. The area has been under DDT spray for several years now, and in many tribal villages the incidence of malaria was extremely high. There was no information on the mosquito fauna of the region, and insecticide susceptibility tests were not done. It was, therefore, decided to study the mosquito fauna of Mandla in the first instance. Results of this study are reported in this paper.

Mandla district has about 1 million population. It has an area of 13257 sq. km and its adjoining districts are Jabalpur, Bilaspur, Shahdol and Seoni. Physical features of the district are mainly rocky hills, dense forest with some plain areas and innumerable streams. Average rainfall varies from [illegible] Primary Health Centres (PHCs) divided into 351 sections. Malaria incidence in 177 sections is more than 2 API and there were sections with as high as 60 API.

Mosquito fauna surveys were carried out from 8 to 17 April 1985. Mosquito collections were made from 53 villages of 13 PHCs. Mosquitoes were collected with the help of suction tube from cattlesheds, human dwellings, mixed dwellings, shrubs, other man-made structures and huts in forest areas. All collections were made either in the morning (0400-0700 hours) or in the evening (1800-2000 hours). At least 3 cattlesheds and 5 human dwellings were searched thoroughly in each village for adult collections. Mosquitoes biting man and cattle were also collected in the evening hours. In these villages larval surveys were also done. Immatures were collected from ponds, rivers, streams, pools, pits and wells etc. and held in cages until adult emergence. All mosquitoes were killed in ether and packed in cellophane paper. Mosquitoes were identified at the RMRC, Jabalpur and identification was confirmed at MRC, Delhi using the keys of Christophers (1933) and Barraud (1934) and the catalog of Knight and Stone (1977).

Table 1 gives details of all mosquito collection. A total of 11614 mosquitoes were collected from the villages either as adults or larvae/pupae. Identification of mosquitoes revealed that the

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Table 1. Results of mosquito fauna survey of Mandla district (M.P.)

Species collected	Primary Health Centres													Total
	Amarpur	Bazag	Bichiya	Bramni	Dhindori	Ghugari	Karanjia	Narian-ganj	Nainpur	Mawai	Mehad-wani	Moha-gaon	Samna-pur	
A.annularis	85	745	201	134	281	—	16	61	57	67	7	25	10	1689
A.culicifacies	1081	980	233	402	2418	625	266	1190	252	435	33	532	506	8953
A.fluviatilis	—	19	—	14	—	2	6	—	—	—	—	—	4	45
A.jeyporiensis	—	4	—	4	—	—	2	—	—	—	—	—	6	16
A.maculatus	2	—	—	—	—	—	—	—	—	—	—	—	—	2
A.nigerrimus	1	—	—	—	—	—	—	—	—	—	—	—	—	1
A.philippinensis	—	3	—	4	—	4	9	1	—	—	1	—	—	22
A.pallidus	2	—	—	7	—	—	—	—	—	—	—	—	—	9
A.splendidus	1	7	—	—	2	—	48	2	—	—	—	—	48	108
A.subpictus	11	74	78	57	81	6	11	25	—	41	4	21	7	416
A.turkhudi	14	19	—	2	24	4	—	—	2	—	—	—	—	65
A.vagus	—	—		2	—	—	—	—	—	—	—	—	—	2
Ae.albopictus	—	7	—	—	—	—	—	—	—	—	—	—	—	7
Ae.aegypti	—	15	—	—	—	—	—	—	—	—	—	—	—	15
Cx.tritaeniorhynchus	2	15	4	1	2	2	4	—	2	4	2	1	—	39
Cx.quinquefasciatus	14	40	27	2	6	13	8	4	16	22	12	14	2	180
Ma.annulifera	5	—	—	2	2	1	2	—	—	2	1	—	4	20
Ma.longipalpis	3	—	—	—	1	—	4	—	1	—	—	1	—	10
Ar.kuchingensis	—	—	—	2	—	12	—	—	—	—	—	—	1	15
Total	1222	1928	543	633	2817	669	376	1283	330	571	60	594	588	11614

fauna of Mandla comprises of 5 genera viz., (i) 11328 specimens of genus *Anopheles* consisting of 12 species, (ii) 22 specimens of genus *Aedes* comprised of 2 species, (iii) 15 specimens of genus *Armigeres* represented by one species, (iv) 219 specimens of genus *Culex* composed of 2 species, and (v) 30 specimens of genus *Mansonia* consisting of 2 species. It may be noted that among anophelines, the most prevalent species was *Anopheles culicifacies* comprising 79% collections followed by *A.annularis* and *A.subpictus* with 14.9% and 3.7% specimens respectively. Among culicines the most common mosquito was *Culex quinquefasciatus* accounting for about 63% specimens.

Larval surveys revealed heavy breeding of anophelines in Chakra river, pits and intradomestic containers. These breeding sites were commonly encountered in all the villages surveyed. It is noteworthy to mention that adults of *A.culicifacies* and *A.fluviatilis* and larvae of *A.culicifacies* were found in tree holes in Orissa for the first time (Nagpal and Sharma, 1985). Tree hole breeding was not checked which may be another important source of mosquitoes. Because of the forest in Mandla district, breeding of malaria vectors in tree holes if present may contribute significantly to the mosquito population in villages. The surveys also revealed that in some areas the density of immatures in the river/stream was very high i.e., several hundred larvae per dip. Most of the mosquito larvae were *A.culicifacies*. The two anopheline species viz., *A.stephensi* and *A.varuna* reported by Subramanian and Dixit (1948) from Nimar district (M.P.) were not found in this survey.

The mosquito fauna of Mandla district comprises of known malaria vectors viz., *A.culicifacies, A.fluviatilis* and *A.philippinensis*. Although no vector incrimination studies were done, the most likely vector was *A.culicifacies*. The densities of *A.fluviatilis* and *A.philippinensis* were very low during the present survey. These vectors may also play an important role in malaria transmission if their densities become significant. It is, therefore, important to monitor the densities of the vector species in different structures, their breeding sites and feeding preferences throughout the year to provide base line data for studies on the epidemiology of malaria. These studies are being initiated in tribal villages (Baiga tribe) with a view to evolve the strategy of the bio-environmental control of malaria in Mandla district.

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Tree hole Breeding and Resting of Mosquitoes in Orissa

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In India only 6 anopheline species viz., *A.culiciformis, A.barianensis, A.asiaticus, A.annandalei, A.annandalei* var. *djajasanensis* and *A.sintoni* were known to breed in tree holes (Iyengar, 1929; Puri, 1931; Rao, 1984). But in subfamily Culicinae and Toxorhynchitinae the mosquitoes of the following genera; *Aedes, Armigeres, Culex, Ficalbia, Harpagomyia, Herzmannia, Orthopodomyia, Tripteroides, Uranotaenia* and *Toxorhynchites* were also reported to breed in tree holes in India (Barraud, 1934; Christophers, 1960; Rao *et al.*, 1970; Shetty and Geevarghese, 1977; Panicker and Rajagopalan, 1978). Apart from these mosquitoes, no other mosquito species was reported from India as breeding in tree holes. During a recent survey of mosquito fauna in Orissa, tree hole breeding and resting of adult mosquitoes was observed in a few villages of Koraput and Phulbani districts. Results of this observation are summarised below.

The mosquito survey was conducted in the forest, plains and coastal areas of Orissa between December 1984 and March 1985. The forests in Orissa are mainly in Koraput, Phulbani, Kalahandi and Balangir districts. During the routine surveys, search for the tree hole breeding and resting of mosquitoes was conducted in Koraput and Phulbani districts. Adults resting in tree holes were collected by suction tube, and larvae with the help of a dipper or a pasteur pipette. The immatures collected from tree holes were held in the field laboratory, given larval food and allowed to emerge. Adult mosquitoes were identified with the help of the keys of Christophers (1933) and Barraud (1934) and the catalog of Knight and Stone (1977).

A total of 167 tree holes of Teak *(Tectona grandis)*, Saal *(Shorea* spp.*)*, Mango *(Mangifera indica)*, Peepal *(Ficus religiosa)* and Banyan *(Ficus benghalensis)* were searched for breeding and resting of mosquitoes in 7 villages of Koraput and Phulbani districts. Out of these tree hole breeding was found in Dalimba and Subai villages of Koraput district, and Sahupada and Trazakia villages of Phulbani district. A total of 120 tree holes were searched for mosquito breeding and resting. Of these 42 tree holes were found positive for adult mosquitoes and 6 for the larvae. No tree hole was found with adults and larvae together. Adult collection yielded 235 resting mosquitoes. These mosquitoes belonged to genus *Anopheles* (5 mosquito species in 167 specimens), genus *Culex* (2 mosquito species in 47 specimens) and genus *Aedes* (21 specimens of one species). In addition, 78 larvae of *Anopheles culicifacies* and 17 larvae of *Aedes aegypti* were collected from 6 tree holes, and these were the only two species found breeding in tree holes.

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Table 1. Mosquito collection from tree holes in Orissa

Villages surveyed	Dalimba*		Subai*		Trazakia**		Sahupada**	
Mosquitoes collected	Adult	Larva	Adult	Larva	Adult	Larva	Adult	Larva
A.barbirostris	0	0	0	0	3	0	0	0
A.culicifacies	65	68	22	10	2	0	7	0
A.fluviatilis	4	0	1	0	4	0	0	0
A.jeyporiensis	27	0	8	0	15	0	3	0
A.nigerrimus	0	0	6	0	3	0	0	0
C.tritaeniorhynchus	16	0	4	0	4	0	4	0
C.vishnui	11	0	3	0	0	0	5	0
A.aegypti	12	17	2	0	0	0	7	0
Total	135	85	46	10	28	0	26	0
Tree holes checked	39	14	21	11	10	1	17	7
Tree holes positive	21	5	11	1	5	0	5	0

Dates: *20-21 Dec. 1984
**11-13 Jan. 1985.

Larvae of *A.culicifacies* were collected from 4 tree holes in Dalimba village and one tree hole in Subai village. In Dalimba village, one tree had two holes, one each was breeding for *A.culicifacies* and *A.aegypti*. Details of the larval and adult collections are given in Table I.

Tree holes with mosquito breeding were either perpendicular or parallel to the ground and their size generally ranged from 10 to 25 cms in diameter. However, some of the trees (with diameter 0.5 to 1 meter) were rendered hollow by natural circumstances. These hollow spaces were also found to shelter adult mosquitoes. *A.culicifacies* was the most common mosquito resting in tree holes in three villages and *A.jeyporiensis* in the fourth village (see Table I). Other anophelines collected were 9 *A.fluviatilis*, 6 *A.nigerrimus* and 3 *A.barbirostris*.

This is the first report of the resting and/or breeding of malaria vectors (viz., *A.culicifacies* and *A.fluviatilis*) in tree holes in India. However, collections of resting adults of *A.culicifacies* in tree holes in Anuradhapur (Sri Lanka) has been reported by Butticker (1958). Studies are, therefore, indicated to relate the epidemiology of malaria with the exophilic populations of *A.culicifacies* and *A.fluviatilis* in Orissa.

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Follow up Studies of Malaria Epidemic in Villages of Shahjahanpur District, U.P.

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During 1983 deaths occurred due to malaria in 38 villages of Shahjahanpur district (Negoyi and Tilhar PHCs). Within three months (August to October) there were 249 deaths in Negoyi PHC and 96 deaths in Tilhar PHC from all causes. Soon after the report of deaths, Malaria Research Centre (MRC) undertook epidemiological studies in eight affected villages of Negoyi PHC. Studies revealed that despite intensive antimalaria measures undertaken by the NMEP, the slide positivity rate (SPR) in the fever survey was 75.3% and Pf% was 96.1. Results of blood smear examination of the family members of the deceased in 5 villages showed that out of 396 blood smears collected, 214 were positive for *P.falciparum* and 9 for *P. vivax*. Therefore, the likely cause of most of the deaths was falciparum malaria (Chandrahas and Sharma, 1983). This was the situation of malaria when the villages were sprayed with 3 rounds of BHC at 200 mg/m² in 1983. To prevent further deaths, the National Malaria Eradication Programme (NMEP) undertook additional malaria control measures in the affected villages by giving fever radical treatment (FRT) and mass radical treatment (MRT) in the affected villages during 1983 and 1984.

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During 1984 NMEP further intensified antimalaria work in the affected villages. This was done by improving the spraying, surveillance and drug distribution system. During 1984 a special round of BHC was sprayed in March followed by three other rounds of BHC. A similar spraying schedule is being followed in 1985. Antimalarial drugs were easily available in villages through the drug distribution centres and the fever treatment depots. Radical treatment of the parasite positive cases was administered more effectively. The impact of these measures was checked by the MRC during 1984 and 1985. Results of this study are briefly summarized below.

Parasitological surveys were carried out in six villages in September-October 1984 and in eight villages in May 1985 (Table 1.). During 1984, 247 blood smears were collected from the fever cases. Of these, 114 slides were positive for the malaria parasite viz., 27 *P. vivax* and 87 *P. falciparum*. The slide positivity rate (SPR) averaged 46.2 (range 31.5-65.4%). Mass blood survey was carried out in 3590 population during the same period. A total of 1317 blood smears were collected. Of these, 88 blood smears were positive for *P.vivax* and 179 for *P. falciparum*. The SPR was 20.3 and Pf% was 67.0. During May 1985 before the commencement of the transmission season, 145 blood smears were collected from fever cases. Of these 57 were

Table 1. Results of parasitological surveys in Shahjahanpur villages

Period	Villages Surveyed	Blood smears collected	Blood smears positive for the malarial parasite				
			Total	Pv	Pf	SPF	SFR
(i) *Fever Survey*							
October 1983*	10	377	284	11	273	75.3	72.4
September–October 1984	6	247	114	27	87	46.1	
May 1985	8	145	57	54	3	39.3	2.1
ii) *Atas Blood Survey*							
October 1985*	5	396	214	9	204	54.0	51.5
September–October 1984	6	1317	267	88	179	20.3	13.6

*Chandrahas and Sharma (1983), *Indian J. Malariol.*, 20: 163-166.

positive for the malarial parasite i.e., 54 for *P.vivax* and 3 for *P.falciparum* and the SPR was 39.3. Following the 1983 epidemic, observations during 1984 revealed that intensive antimalaria measures were not effective in interrupting transmission and observations during May 1985 confirmed that the parasite load in the community was high with moderate to high vector densities, and that any significant change in the disease incidence or its pattern is unlikely to occur. Under the circumstances a change in the malaria control strategy was warranted.

The indoor resting collection conducted between 26 September and 3 October 1984 yielded 955 anophelines, comprising of four species i.e., *Anopheles culicifacies, A. annularis, A. subpictus* and *A. aconitus*. The per man hour density of *A. culicifacies* ranged from 2.5 to 16.3. Pyrethrum space spray collection yielded 1691 anophelines and 15 *Mansonia* mosquitoes. *A. subpictus* (52.4%) and *A. culicifacies* (31.35%) formed bulk of the collections. Of the 232 *A. culicifacies* dissected in the fourth week of September 1984, natural infection was found in one specimen. No infection was found in 67 *A. annularis* and 13. *A. aconitus* dissected.

Cytological examination of *A.culicifacies* revealed the presence of sibling species A and species B. The proportion of sibling species B ranged from 30.0% to 93.0% in different villages. It is noteworthy to mention that in villages around Delhi during September-October the proportion of species B was about 40.0% during the same period (Subbarao, 1984). Implications of these observations are under investigation.

Insecticide susceptibility tests were carried out in May 1985 with the field collected *A.culicifacies*. Adults were exposed to DDT (4.0%) and dieldrin (0.4% and 4.0%) impregnated papers as recommended by WHO. Results revealed that *A. culicifacies* was resistant to DDT and dieldrin, but the level of resistance to dieldrin was much greater (Table 2). Based on the insecticide susceptibility test results it was clear that DDT spraying would have produced much greater impact on malaria transmission than BHC spraying.

The study revealed that malaria transmission continued uninterrupted in study villages even in the presence of antimalaria measures. Although there was considerable drop in the SPF and SfR

Table 2. Susceptibility of *A. culicifacies* to insecticides

Village	Impregnated papers	Exposure period	Mosquitoes exposed (Nos.)	Mosquitoes dead (Nos.)	Corrected mortality
Garha	DDT (4%)	1 hour	20 × 2	23	52.77
	Dieldrin (0.4%)	1 hour	20 × 2	9	13.88
	Dieldrin (4.0%)	2 hours	20 × 2	14	27.77
Jahanpur	DDT (4%)	1 hour	20 × 1	15	73.68
	Dieldrin (0.4%)	1 hour	20 × 1	4	15.78
	Dieldrin (4.0%)	2 hours	20 × 1	8	36.84

Note : Susceptibility tests were done in May 1985.

in 1984 as compared to 1983 (i.e., SPR declined from 75.3 to 46.1 and SfR from 72.4 to 35.2), the parasite load in the community remained very high. BHC spraying during 1984 may have reduced vector densities, but the remaining vector populations were high enough to restart transmission and maintain it at high levels. It may be noted that malaria incidence remained high in the community even after the administration of fever and mass radical treatments. There was however reluctance on the part of some villagers in taking the antimalarials. The magnitude of this problem was not ascertained. This may partly explain the prevalence of high parasite rate in the community. The Shahjahanpur episode is of great importance in the present day context of malaria control. Malaria situation could have been tackled better by spraying DDT instead of BHC. Complete interruption of transmission was only possible with the use of replacement insecticides like malathion, as was done in Haryana villages (Subbarao *et al.*, 1984).A more lasting solution to the problem of malaria control would be to implement the integrated disease vector control methodology on Nadiad pattern (MRC Annual Report, 1983-84)

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A Three Year Report of the Malaria Clinic in Haldwani, District Nainital, U.P.

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In late 1981, a malaria clinic was opened at the field station of the Malaria Research Centre in Haldwani. This clinic started receiving a large number of fever cases for malaria test. A three year (1982-84) report of the malaria incidence as recorded at the malaria clinic is given below.

Fever cases for malaria test came from: (i) Haldwani and Kathgodam which are in one municipal limit comprising of about 80,000 population; (ii) Fatehpur area, a group of 20 villages situated about 10 kms from Haldwani; (iii) Gola Par area, comprising of 20 villages settled along the river in about 25 kms; (iv) referred cases from civil and army hospital; and (v) miscellaneous cases which includes visitors, guests and people from far-off villages of Bhabar and Terai.

Fever cases were first registered and information on age, sex, name of the village, history of malaria, complaints of illness and drugs already taken etc. were noted. Finger prick blood was collected from patients and a thick and thin blood smear prepared. The blood smear was stained with JSB and examined under the microscope. As a routine, thin blood films (200 fields) were examined first. The negative slides were confirmed by thick film (50 fields) examination.

During 1982 and 1983 all malaria cases were given 600 mg chloroquine adult dose as presumptive treatment and patients were advised to take radical treatment from civil hospital. During 1984 presumptive and radical treatment was given by the staff of NMEP deputed to the clinic by the District Malaria Officer.

Table 1 gives month-wise data of malaria incidence from January 1982 to December 1984. During 1982, 752 fever cases reported to the clinic, and of these 337 were positive for the malarial parasite. There were 276 cases of *P.vivax*, 54 of *P.falciparum* and 7 mixed (Pv+Pf) infections. The slide positivity rate (SPR) and slide falciparum rate (SfR) was 44.8 and 8.1 respectively. During 1983, a total of 6456 fever cases reported to the clinic. Blood smear examination revealed that 3023 cases were positive for malaria i.e., 1632 for *P. vivax*, 1293 for *P.falciparum* and 98 mixed (Pv+Pf) infections. The SPR and SfR was 46.8 and 21.6 respectively. During 1984, 14507 fever cases reported for malaria test, and of these 7885 cases were positive for the malarial parasite i.e., 5493 *P. vivax*, 2333 *P.falciparum* and 59 mixed (Pv+Pf) infections. The SPR and SfR was 54.4

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Table 1. Malaria cases detected at malaria clinic

1982	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
B.S.	2	3	4	2	20	23	61	87	168	207	123	52	752
Pv	1	1	1	1	10	13	30	31	75	73	34	6	276
Pf	0	0	0	0	0	0	0	2	11	11	22	8	54
Mix (Pv+Pf)	0	0	0	0	0	1	1	0	0	4	1	0	7
Total Positive	1	1	1	1	10	14	31	33	86	88	57	14	337
SPR	50.00	33.33	25.00	50.00	50.00	60.86	50.81	37.93	51.19	42.51	46.34	26.92	44.81
SFR	0	0	0	0	0	4.34	1.63	2.29	6.54	7.24	18.69	15.38	8.11
1983													
B.S.	39	22	58	62	108	244	446	914	1025	1819	1263	456	6456
Pv	6	1	7	23	60	142	245	403	368	237	91	49	1632
Pf	4	2	1	0	0	3	5	26	153	521	400	178	1293
Mix (Pv+Pf)	0	2	3	0	0	2	0	1	26	36	21	7	98
Total Positive	10	5	11	23	60	147	250	430	547	794	512	234	3023
SPR	25.64	22.73	18.97	37.10	55.55	60.25	56.05	47.05	53.37	43.65	40.54	51.32	46.82
SFR	10.26	18.18	6.90	0	0	2.05	1.12	2.95	17.46	30.62	33.33	40.57	21.55
1984													
B.S.	263	266	504	637	1226	1698	1974	3313	2277	1360	588	401	14507
Pv	49	63	142	293	682	902	928	1435	607	194	110	88	5493
Pf	68	25	14	4	9	5	33	512	654	577	266	166	2333
Mix (Pv+Pf)	7	2	2	0	0	0	4	18	8	7	6	5	59
Total Positive	124	90	158	297	691	907	965	1965	1269	778	382	259	7885
SPR	47.15	33.83	31.35	46.60	56.36	53.42	48.89	59.31	55.73	57.20	64.97	64.59	54.35
SFR	28.52	10.15	3.17	0.60	0.73	0.29	1.87	15.99	29.07	42.94	46.26	42.64	16.49

and 16.5, respectively. There was a considerable increase in malaria cases during the 3 year period. One reason for the increase in malaria cases from 1982 to 1984 could be the popularity of malaria clinic because of the fact that the clinic provides prompt and correct diagnosis of malaria. Also there was a considerable increase in malaria in Bhabar and Terai as substantiated by other studies (Sharma *et al.*, 1983; Choudhury *et al.*, 1983). This was also reflected in the increase in SPR and SfR from 1982 to 1984. The incidence of malaria is extremely high from the onse of rains till the onset of extreme winter in January (see Table 1). A notable feature of the positive cases was that vivax malaria started increasing from January and gradually reached peak numbers in August followed by a gradual decline in the later months of the year. Falciparum malaria suddenly peaked in August and remained at about the same level till November-December and then declined to low numbers in January-February.

Table 2 gives sex and agewise split of all malaria positive cases for the 3 year period. It was revealed that children upto 1 year age had less malaria as compared to other groups. In children of 1-5 age group there was considerable increase in the incidence of malaria, but it was relatively less than the older age groups. In all other age groups, the incidence of malaria remained at about the same level. There was also no difference in the incidence of malaria in males and females. Study also revealed that 70% of all malaria cases came from Haldwani and Kathgodam, 15% from Gola Par area, 7% from Fatehpur area, 4% referred cases and 4% cases of miscellaneous origin.

Haldwani is situated in Bhabar which is between hills in the north and Terai in the south. Before the eradication of malaria Bhabar was described as the death trap of malaria (Phillips, 1924) and surveys by Issaris *et al.*(1953) revealed extremely high incidence of malaria in this region. Malaria was nearly eradicated from Bhabar and Terai in early 1960's as a result of the spraying of insecticides under the National Malaria Control/ Eradication Programme (NMCP/ NMEP). As a result there were vast developmental changes, particularly in Terai and this region became an important green revolution area of the country. This followed a period of malaria resurgence and the situation in Bhabar and Terai has gradually assumed serious proportions. It is also noteworthy to mention that initially most of the malaria cases were due to *P. vivax* but during the last few years *P.falciparum* has increased tremendously (Sharma *et al.*, 1983; 1984; Choudhury *et al.*, 1983; Malhotra *et al.*, 1985). This increase in falciparum malaria was also substantiated by the records of the malaria clinic. There is, therefore, an urgent need to re-organize malaria control operations in this region to protect green revolution areas from the ravages of malaria.

Table 2. Age and sex-wise malaria cases detected at the malaria clinic.

Age (Yr.)	B.S. Collected	B.S. Positive		SPR		SFR	
		Male	Female	Male	Female	Male	Female
<1	126	14	15	18.18	30.61	2.60	14.29
1-5	1360	297	271	40.14	43.71	10.41	9.84
>5-10	2183	630	511	51.43	53.34	15.51	16.70
>10-15	2458	845	587	57.60	59.23	20.59	19.37
>15-25	7281	2755	993	50.21	55.35	18.55	18.23
>25-50	7331	2559	1195	50.32	53.21	17.78	18.43
>50	976	399	174	61.29	53.54	22.12	14.15

Note: Three year data (Jan. 1982 to Dec. 1984) as shown in Table 1.

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BOOK REVIEW

Essential Malariology by Leonard Jan Bruce Chwatt

V.P. SHARMA[1]

This comprehensive treatise on malaria is authored by a renowned expert on malariology. Written in simple language, the book offers a wealth of information compiled from a large number of journals, monographs and text books. It would be of great value in teaching of malariology, and malariologists would benefit immensely from its reading.

The contents of the book are presented in 11 chapters, apart from a selected bibliography and 7 useful appendices. Each chapter provides a historical account and an update. Chapter 1 is devoted to historical outline of malaria, important dates in the history of malaria and its control. Chapter 2 deals with the classification of Plasmodia, the life cycle, biology, biochemistry and the *in vitro* cultivation of the malarial parasite. Chapter 3 deals with clinical course of malaria, primary attack and relapses, clinical pattern of the disease, falciparum malaria and its complications, vivax malaria and the pattern of short and long term relapses, description of malaria in children and pregnancy, imported malaria, transfusion malaria and therapeutic malaria. Chapter 4 provides a detailed account of the pathological picture in various systems and organs. Black water fever, an important complication of falciparum malaria is described in detail including its epidemiology and clinical picture.

Chapter 5 deals with the immunology of human malaria. It provides comprehensive background information on immunology, immunization against malaria, and immunopathology. Also included are the human genetic factors responsible for innate resistance, and the spleen and its role in immune response. Chapter 6 provides a detailed account of the technique of slide preparation for malaria test, malaria parasite identification in thick and thin films, an elegant colour plate for the identification of the species of the malaria parasite. The chapter also includes serological tests with discussion on their value as diagnostic methods. The anopheline vectors of malaria are described in Chapter 7. This chapter is very useful as it describes many entomological techniques used in day to day research. The epidemiology of malaria is discussed in chapter 8, including methods of malariometric surveys, seroepidemiology of malaria, factors responsible for transmission and quantitative epidemiology of malaria. It also briefly summarises the epidemiological characteristics of malaria in different regions of the world. Chapter 9 on chemotherapy and chemoprophylaxis provides a very useful update on various aspects of malaria treatment, including antimalarial compounds in relation to their selective action on different stages of the parasite's life cycle, action of their structure relationship, dosage, treatment of acute malaria, drug resistance and its monitoring methods, drug protection and adverse effects of the antimalarials. The rationale and treatment of malaria control are dealt in chapter 10. Besides the principles of malaria prevention and control, all methods of vector control are briefly discussed with emphasis on environmental and biological control. It describes application

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equipments and techniques of application, insecticide resistance and status of resistance in the world and safe use of insecticides. The contents of chapter 11 range from malaria eradication to malaria control, phasing of programme to achieve eradication, the trends and situation of malaria in different regions of the world and the future prospects of malaria control and research.

The present book is the second edition which reveals considerable improvement over the first edition. However, an obvious lacuna in chapter 6, relates to the JSB stain which is found to be very useful in India as it produces results comparable to those of Giemsa. Moreover, in JSB the staining time is much shorter and the stain is being used extensively in the National Malaria Eradication Programme in India. On page 146, Figs. 7-14, the map of malaria vectors given is incorrect and not updated. *A.stephensi* is the vector of urban malaria and at present 132 towns in India are under urban malaria scheme. *A.balabacensis (=dirus)* is not shown, *A.fluviatilis* has much wider distribution and *A.sundaicus* is now limited to Sunderbans and A and N Islands (also on page 202 para 3 last 3 lines). Further, the description of *A.culicifacies* sibling species complex is an important omission, while describing *Anopheles* species complexes.

This edition has been published by William Heinemann Medical Books Ltd., London, it consists of 462 pages and is priced at £ 22.50.

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Historical notes on the INDIAN JOURNAL OF MALARIOLOGY

In August 1999, the Government of India pointed out the necessity of a permanent organization that would deal with problems connected with malaria. Consequent to this recommendation, a committee was constituted for the study of malaria in India. The committee decided to establish a Central Malaria Bureau at Kasauli. The publication of a journal or bulletin was also undertaken and the title *Paludism* was approved by the committee.

Paludism was the first attempt at disseminating information on malaria in India. The first number came out in July 1910. It was initially edited by Major S.P. James and later by S.P. James and R.Christophers. *Paludism* was published from 1910-1912 and a total of five issues covered that period. The scope of the journal covered publication of routine reports on the work of the committee which included enquiries on epidemic malaria, the conditions associated with endemic malaria, the bionomics of anophelines and studies on quinine. *Paludism* may be considered to be the original precursor of the *Indian Journal of Malariology*.

The year 1927 saw the constitution of the Malaria Survey of India under the auspices of the Indian Research Fund Association (now Indian Council of Medical Research). The first issue of the *Records of the Malaria Survey of India* was published in October 1929. The issue consisted of a 200 page bibliography of malaria in India by Major J.A.Sinton, Director of the Malaria Survey of India. This monumental compilation included 2,200 papers—the record of thirty years of research in malaria, spanning the period which elapsed since Sir Ronald Ross made his famous discovery in 1898, till 1927. Many of the papers indexed in this bibliography were valuable notes unnoticed or obscured because they were published in local or provincial publications. The bibliography is a chronological listing of these references which have been indexed in four ways i.e., journal index, author index, subject index and geographical index.

The scope of this journal was wider than that of *Paludism*, the authors were predominantly Englishmen with a few contributions by Indians. This journal continued till 1938.

The Government of India in December 1937 announced the take-over of the Malaria Survey of India as regards its public health and advisory functions, and the name of the organization was changed to the Malaria Institute of India.

In March 1938 the name of the journal was changed from the *Records of the Malaria Survey of India* to the *Journal of the Malaria Institute of India*. This was published under the editorship of Lt. Col. Covell (later Maj. Gen. Sir Gordon Covell). At this stage the journal was gradually evolving into a publication of ever greater significance and widening audience, also, the circle of contributors had expanded considerably to include several sources outside the Institute.

In 1943, the headquarters of the institute was transferred from Kasauli to Delhi. The activities, staff and buildings of the institute were also greatly expanded.

Therefore, in 1947, the journal was given the new name of the *Indian Journal of Malariology*, an appellation that was more in keeping with its wider scope and status. The editor of this journal was Col. Jaswant Singh who became the first Indian Director of the Malaria Institute of India.

The *Indian Journal of Malariology* was renowned for its high standards at home and abroad. For several years the journal served as the main organ for dissemination of information on malaria and its control.

As a result of the spectacular success of malaria control under the National Malaria Eradication Programme, malaria was nearly eradicated from the country, and there were only about 1,00,000 cases and no deaths due to malaria in 1965. At that time there was a natural decline in research on malaria, as a result of which Col. Jaswant Singh announced the retirement of the *Indian Journal of Malariology*. The last number of the journal was published in December 1963 (Vol. 17, No. 4).

In the wake of widespread resurgence of malaria in India, the Indian Council of Medical Research opened a new Institute in 1977 and named it Malaria Research Centre. By this time research in malariology was re-started at many centres in the country. There was therefore a felt-need of a journal in malariology. Malaria Research Centre revived the *Indian Journal of Malariology*, as a bi-annual publication. The journal which is being published under the editorship of Dr. V.P. Sharma, Director of the Malaria Research Centre has now entered its fifth year of publication.

—P. SHARMA

INDIAN JOURNAL OF MALARIOLOGY

Instructions to Authors

(Revised June 1985)

Editorial Policy

The Indian Journal of Malariology is devoted to the publication of original research which contributes significantly to the knowledge of any field of malariology. Papers of routine and repetitive nature dealing with gross observations may not be included. Articles will be published at the editor's discretion in the order accepted. Date of acceptance will be that date on which copy is accepted in final form for publication. Manuscripts should be submitted in triplicate to:

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The matter should be arranged in the following order: Title, Name(s) of the author(s) with address of the Institute/University (as footnotes and numbered serially in superscript), Abstract, Introduction, Material and Methods, Results, Discussion, Acknowledgement and References. Authors should provide a short title to be used as running title, of not more than 5 words in case the title of the paper is long.

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Paper presented at Symposium/Conference

Subbarao, S.K. (1981). *Cytoplasmic incompatibility in mosquitoes.* Paper presented at the International symposium on recent developments in the genetics of insect disease vectors, Bellapio, Italy, 20–24 April.

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AUTHOR'S CORRECTION

Volume 22 No. 1 June 1985 issue, pp. 49-51.

Please read chloroquine concentrations in text and in Fig. 1a, b as $10^{-7}M$ instead of $10^{-6}M$, viz., 10^{-6} as $10^{-7}M$, $0.5 \times 10^{-6}M$ as $0.5 \times 10^{-7}M$, $0.25 \times 10^{-6}M$ as $0.25 \times 10^{-7}M$ and so on.

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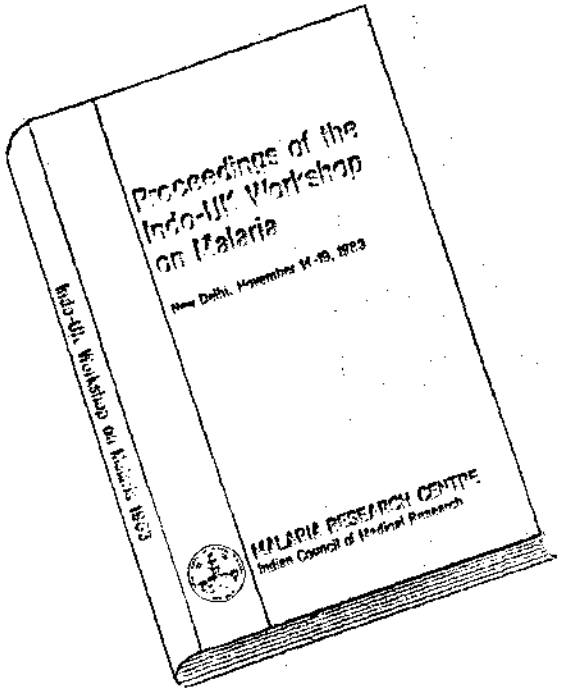

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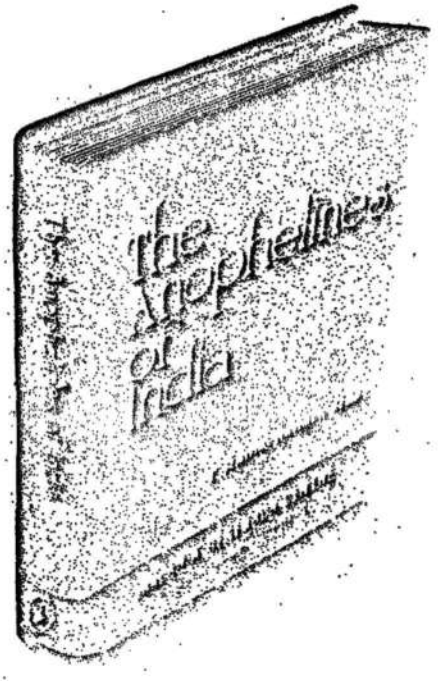

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