A STUDY OF THE GENETICS OF THE SUSCEPTIBILITY OF *ANOPHELES GAMBIAE* SPECIES A TO MALARIA INFECTION

by

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ABSTRACT

The purpose of this research was to study the genetics of susceptibility and refractoriness of Anopheles gambiae Species A to Plasmodium species. Nine generations of selection resulted in a susceptible line (PB) showing 100% and a refractory line (LD) showing 0% susceptibility to Plasmodium berghei berghei (a rodent malaria). It was found that the parasite degenerated part way through the sporogonic cycle in the LD line. The F_1 progeny from reciprocal crosses between the lines differed in their susceptibility to the parasite. Backcrosses to the parent lines did not produce proportions of susceptible and refractory individuals consistent with single gene inheritance or with cytoplasmic inheritance.

Tests of the lines with two other species of rodent malaria, P. yoelii and P.y.nigeriensis gave similar results to those with P. b. berghei.

In an attempt to check whether the genetic mechanism controlling susceptibility to P. b. berghei has an influence on human malaria infection, the two lines were fed on a chimpanzee infected with P. vivax. The PB line was fully susceptible to the human parasite while the LD line was partially susceptible. Feeding the two lines of mosquitoes on human volunteers suffering from P. falciparum showed a difference between the lines in their rate of susceptibility to the infection which was statistically significant in two out of four replicates.

The results will be discussed in relation to the possible replacement of disease vectors by harmless strains.

INTRODUCTION

That there is a genetic element in the ability of hosts to support infections with parasites seems now beyond doubt. The possibility of selecting refractory hosts therefore exists and their introduction into natural eco-systems could and can be desirable. Food crops resistant to numerous types of pests have long been used in many parts of the world. The same principle could in theory be used to substitute harmless populations for vector populations of disease.

It was with this idea in mind as it applies to mosquitoes and malaria that the present investigation was undertaken and a species of the Anopheles gambiae complex was chosen on the grounds that this is one of the most important malaria carriers in the world. The species chosen was species A. Until 1956, A. gambiae was considered a single species. However, the discovery of the mode of inheritance of dieldrin-resistance (Davidson, 1956) and the numerous crosses from all over the African continent and its surrounding areas have all confirmed that A. gambiae is a complex of at least six species (Davidson, 1958, 1962, 1964a, 1964b, 1974; Davidson et al, 1967; Davidson and Hunt, 1973).

The malaria parasites chosen initially were those infecting rodents but as will be seen some cross-refractoriness was evident to human malarias. Curtis (1968, 1975) and Davidson (1974) postulated that a translocation bearing strain could be used as a transport mechanism replacing an existing population by one whose genotype is susceptible to an insecticide or refractory to disease transmission or carry conditional lethal genes. Although a fully refractory line

has been successfully established to mouse malaria in the present study, it is only partially refractory to human malaria. It is however hoped that the information put forward in the present work will provide the means for more fruitful efforts for selecting a refractory strain to human malaria in the near future.

The objectives of this work is to select a highly susceptible and highly refractory line of two strains, PALA and LSW, respectively, which belong to A. gambiae species A. After the establishment of these two lines, the mode and pattern of inheritance will be investigated.

LITERATURE REVIEW

Susceptibility of Mosquito to Infection

One means of measurement to study the correlation between any host organism and an infection agent is the susceptibility. Susceptibility is a term applied to individuals who can support the full development of a parasite as defined by Macdonald (1967). The measurement of the susceptibility of mosquito vectors to malaria and filarial infections is an extremely difficult one because of the very great complexity of the inter-relationship and the many factors which might interfere with the relationship. Thus it would be advisable to review these factors under two different sub-headings -

1. Non-Genetic Factors and Susceptibility
2. Genetic Factors and Susceptibility.

1. Non-Genetic Factors and Susceptibility

The factors affecting susceptibility are numerous (see Boyd, 1949). The more important non-genetic ones are considered to be:-

Gametocytes

The production of viable gametocytes is a character dependent on many things - the method of maintenance of the strain, the species of vertebrate host and the humoral factors in the host (Lumsden and Bertram, 1940; Boyd, 1942; Cantrell and Jordan, 1946; Bishop and McConnachie, 1956; Huff and Marchbank, 1955; Wery, 1968; Bafort, 1971). It was found that the continuous blood passaging of a malaria parasite from mouse to mouse would be harmful to the production of healthy gametocytes

(Rodhain and Vincke, 1951; Sergent and Poncet, 1956; Jadin et al, 1959; Yoeli et al, 1963a, 1966b, Bafort et al, 1965; Wery, 1968). It has also been shown that the vertebrate host plays an important role in influencing the infection in mosquito vectors. James (1931) and Boyd (1942) have classified the patients into good and poor infectors according to their ability to infect mosquitoes. Huff (1948) demonstrated that in the case of chicken malaria, P. relictum was readily transmissible from an infected canary to Culex pipiens while no infection was accomplished when these mosquitoes were fed on a pigeon infected with the same parasite. Humoral factors also play an important part in the viability and infectivity of the gametocytes. It has been found that the gametocytes are always viable during the early stages of infection in the peripheral blood of the vertebrate host (Lumsden and Bertram, 1940; Cantrell and Jordan, 1946; Box et al, 1953; Vincke, 1954; Huff and Marchbank, 1955; Celaya et al, 1956; Yoeli et al, 1964) but may not be later. This is due to the build up of the host immunity in the later stages of infection and the toxic products of the fulminating infection which both affect tremendously the viability of the gametocytes (Garnham, 1966).

The relationship between gametocyte number and the resulting infection in the mosquito has been extensively studied. In general the higher the number of gametocytes the higher the number of oocysts on the midgut of the infected mosquitoes (Boyd and Stratman-Thomas, 1932; Eyles 1951; Shute, 1951). In spite of some evidence to the contrary (Green, 1929; Barber and Olinger, 1931; Kligler and Mer, 1937; Knowles and Basu, 1943; Robertson, 1945; Shute, 1951; Draper, 1953) there is not always a direct relation between gametocyte number and the proportion of mosquitoes becoming infected, provided other variables are held constant (Boyd and Stratman-Thomas, 1932; Young et al, 1948; Micks, 1949; Jeffery et al, 1956

Burgess, 1960). This of course is only to be expected if susceptibility is controlled by genetic factors. It has also been found that the depletion of an essential nutrient in the blood of the host resulting from high parasitaemia had, in turn, lowered the threshold of infectability of gametocytes to mosquitoes (Cantrell and Jordan, 1946; Eyles, 1951, 1952a, 1952b).

Host-Parasite Specificity

A species of mosquito or strains within it may be more susceptible to infection with one species of parasite than with another. Boyd and Stratman-Thomas (1934) found that A. crucians was more susceptible to P. falciparum than to P. vivax. Sometimes the specificity of the mosquito extends to strains of parasite as well as species. It was found that A. maculipennis atroparvus is refractory to infection with Indian and African strains of P. falciparum while it is susceptible to Roman, Sardinian and Rumanian strains (James et al, 1932; Shute, 1940, 1951). Sometimes one species of mosquito is susceptible to different species of plasmodia. For instance, the main vector of human malaria in the United States, A. quadrimaculatus, is extremely susceptible to monkey plasmodium and partially susceptible to an avian malaria parasite (Coggeshall, 1941).

Tables 1 and 2 summarise some of the experimental attempts to infect different species of mosquitoes with malarial (Table 1) and filarial (Table 2) parasites. Exclusively laboratory-induced infections with the different species of malaria in Anopheles such as studied by Bray and Garnham (1964) and Bafort (1970,1971) are not included. Such figures again illustrate that there is not always a direct correlation between gametocyte density and infectivity and that some species do not support some parasites. In all cases where A. gambiae is referred to it cannot be categorically

stated which species was involved as the observations were made before the complex was recognised. They are all referred to as A. gambiae s.l. (sensu lato) except in the case of salt-water A. gambiae reference Pringle (1962). This was undoubtedly A. merus as the observations were made in East Africa.

TABLE 1

LABORATORY EXPERIMENTAL FEEDING ON MALARIA INFECTION

SPECIES OF PLASMODIUM	VECTOR SPECIES	VERTEBRATE HOST	GAMETOCYTE DENSITY per C.mm.	INFECTION RATE % STOMACH* GLAND*	AUTHORITY
falciparum	A. gambiae s.l.	man	11 - 100	20 (45)	Barber and Olinger (1931)
			101 - 200	48.1 (27)	"
			201 - 500	100 (7)	"
			501 - 1,000	56.6 (30)	"
			1,000 +	90 (11)	"
falciparum Mexican strain	A. quadrimaculatus Tallahasse strain	man	340♂ + 3600♀	72.2 (36)	Boyd (1941)
	Tennessee Valley strain		" ♂ " " ♀	70.97(31)	"
falciparum Long strain	Tallahasse strain		600 - 96000♂ & 750-96000♀	85.94(64)	"
	Tennessee Valley strain		" " ♂ " " ♀	93.75(48)	"
vivax McCoy strain	Tallahassee strain		60 - 15000♂ & 0 - 15000♀	58.35(79)	"
	Tennessee Valley strain		" " ♂ " " ♀	60.87(92)	"
vivax	A. quadrimaculatus	man	No gametocytes seen	49.2 (230)	Boyd (1942)
			" " "	58.2 (153)	"
			" " "	61.8 (269)	"
			1 - 100 ♂	81.7 (554)	"
			" ♀	71.2 (427)	"
			101 - 200 ♂	66.8 (527)	"
			" ♀	70.0 (202)	"
			201 - 300 ♂	67.6 (253)	"
			" ♀	75.2 (424)	"
			301 - 600 ♂	77.6 (644)	"

* Figures in parenthesis represent the number of total dissected mosquitoes

TABLE 1: continued

SPECIES OF PLASMODIUM	VECTOR SPECIES	VERTEBRATE HOST	GAMETOCYTE DENSITY per C.mm.	INFECTION RATE % STOMACH* GLAND*	AUTHORITY
vivax	A. quadrimaculatus	man	301 - 600 ♀	71.8 (618)	Boyd (1942)
falciparum (Mexican strain)	A. quadrimaculatus	man	0 - 910	78.6Δ(42)	Boyd and Earle (1939)
	A. pseudopunctipennis		0 - 910	4.1 (24)	"
falciparum (Florida strain)	A. quadrimaculatus	man	150 - 1550	43.2Φ(44)	"
	A. pseudopunctipennis		150 - 1550	7.1 (28)	"
vivax	A. quadrimaculatus	man		47.4 (849)	Boyd & Stratman-Thomas (1932)
			0	46.1 (13)	"
			1** ♂	69.0 (45)	"
			2** ♂	51.3 (163)	"
			3 ♂	55 (20)	"
			5 ♂	67.3 (49)	"
			6 - 10 ♂	57.7 (225)	"
			11 - 20 ♂	62.2 (93)	"
			Total	58.1 (608)	"
			0	69.3 (85)	"
			1** ♀	49.3 (178)	"
			2 ♀	65.2 (121)	"
			3 ♀	57.5 (54)	"
			4 ♀	88.8 (9)	"
			5 ♀	10 (10)	"

* Figures in parenthesis represent the number of total dissected mosquitoes

** Gametocytes (macrogametocytes or microgametocytes) per 100 leucocytes

Δ An average of 4 feedings

Φ An average of 5 feedings

TABLE 1: continued

SPECIES OF PLASMODIUM	VECTOR SPECIES	VERTEBRATE HOST	GAMETOCYTE DENSITY per C.mm.	INFECTION RATE % STOMACH*	% GLAND*	AUTHORITY
vivax	A. quadrimaculatus	man	6 - 10 ♀	59.6 (89)		Boyd & Stratman-Thomas(1932)
			11 - 20 ♀	55.7 (45)		"
			50 + ♀	52.8 (17)		"
			Total	58.1 (608)		"
falciparum	A. quadrimaculatus	man	2 - 28***	63.7Ⓓ (135)		Boyd & Stratman-Thomas(1934)
	A. crucians		2 - 28	14.7 (34)		"
falciparum	A. gambiae s.l.	man	69	96.9	100.0	Burgess (1960)
			133	65.2	46.7	"
			140	96.3	88.9	"
			181	100.0	71.4	"
			253	100.0	No dissection	"
			257	86.2	0.0	"
			429	91.7	95.2	"
			482	100.0	93.8	"
			708	78.6	91.7	"
			1166	61.9	80.0	"
			1431	31.6	36.4	"
			2310	92.7	85.7	"
	A. melas		69	100.0	94.4	"
			133	46.1	0.0	"
			140	100.0	No. dissection	"
			181	100.0	79.1	"
			253	83.3	No. dissection	"

* Figures in parenthesis represent the number of total dissected mosquitoes
*** per 100 leucocytes
Ⓓ an average of 6 feedings

TABLE 1: continued

SPECIES OF PLASMODIUM	VECTOR SPECIES	VERTEBRATE HOST	GAMETOCYTE DENSITY per C.mm.	INFECTION RATE % STOMACH*	GLAND*	AUTHORITY
falciparum	A. melas		257	80.0	No. dissection	Burgess (1960)
			429	82.6	75.0	"
			482	16.7	84.5	"
			708	33.3	100.0	"
			1166	36.3	No. dissection	"
			1431	33.3	"	"
			2310	69.2	"	"
cynomolgi	A. quadrimaculatus	monkey	Numerous	30 (250)		Cogeshall (1941)
knowlesi	A. quadrimaculatus	monkey	"	0 (505)		"
		man	"	0 (206)		"
	C. pipiens	man	"	0 (25)		"
	A. punctipennis	man	"	0 (102)		"
		monkey	"	0 (32)		"
	C. pipiens	monkey	"	0 (40)		"
inui	A. quadrimaculatus	monkey	"	0 (98)		"
lophurae	A. quadrimaculatus	chicken	"	46 (56)		"
	C. pipiens	chicken	"	0.1 (167)		"
	Ae. aegypti	chicken	"	2.8 (318)		"
inui (L strain)	A. stephensi	monkey	Gametocytes were seen in the peripheral blood	67		Collins et al (1966)
	A. freeborni			93		"
	A. quadrimaculatus			79		"
	A. albimanus			22		"
	A. maculatus			88		"

* Figures in parenthesis represent the number of total dissected mosquitoes

TABLE 1: continued

SPECIES OF PLASMODIUM	VECTOR SPECIES	VERTEBRATE HOST	GAMETOCYTE DENSITY per C.mm.	INFECTION RATE % STOMACH*	GLAND*	AUTHORITY
inui (OS strain)	A. stephensi			60		Collins et al (1966)
	A. freeborni			45		"
	A. quadrimaculatus			50		"
	A. albimanus			0		"
	A. maculatus			45		"
brasilianum	A. quadrimaculatus	monkey	male and female gametocytes were seen	23 (209)		Collins et al (1969)
	A. albimanus			0 (201)		"
	A. stephensi			27 (22)		"
	A. sundaicus			11 (37)		"
	A. maculatus			9 (79)		"
	A. freeborni			70.2 (442)		"
falciparum	A. gambiae s.l.	man	1 - 10	1.1 (92)		Draper (1953)
			11 - 100	4.4 (91)	1.1 (91)	"
			101 - 200	13.3 (30)	30 (30)	"
gallinaceum	Ae. aegypti	chicken		90.3 (1424)		Eyles (1951)
gallinaceum	Ae. aegypti)Grp.			99.2 (252)		Eyles (1952c)
	A. quadrimaculatus) 1			59.0 (327)		"
	Ae. aegypti) Grp.			87.5 (23)		"
	A. freeborni) 2			14.5 (42)		"
malariae	A. labranchiae atroparvus	man	±	3.0 (65)		Garnham et al (1966)
			±	9.8 (67)		"

* Figures in parenthesis represent the number of total dissected mosquitoes
± 1 male in 3 thick drops

TABLE 1: continued

SPECIES OF PLASMODIUM	VECTOR SPECIES	VERTEBRATE HOST	GAMETOCYTE DENSITY per C.mm.	INFECTION RATE % STOMACH*	GLAND*	AUTHORITY
malariae	A. labranchiae atroparvus		+	0	(70)	Garnham et al (1966)
			+	20.2	(74)	"
			25	6.7	(74)	"
			+	1.0	(98)	"
			+	5.9	(68)	"
			+	19.6	(51)	"
			+	13.5	(59)	"
			+	18.0	(77)	"
			+	27.9	(68)	"
			+	12.2	(49)	"
			+	41.6	(84)	"
			‡	33.3	(36)	"
			‡	0	(74)	"
			‡	0	(72)	"
ovale	A. quadrimaculatus	man	male and female gametocytes were seen	47.9	(314)	Jeffery et al (1955)
				50.1	(198)	"
	A. albimanus (Florida strain)			37.8	(301)	"
				37.7	(195)	"
	A. albimanus (Panama strain)			19.5	(185)	"
				19.5	(185)	"

* Figures in parenthesis represent the number of total dissected mosquitoes
\+ 1 - 3 males in thick drop
‡ 3, 5, 7, males in 1 thick drop

TABLE 1: continued

SPECIES OF PLASMODIUM	VECTOR SPECIES	VERTEBRATE HOST	GAMETOCYTE DENSITY per C.mm.	INFECTION RATE % STOMACH*	GLAND*	AUTHORITY
falciparum (S.Carolina strain)	A. quadrimaculatus	man	< 10	27.2 (217)		Jeffery and Eyles (1955)
			10 - 99	56.3 (240)		"
			100 - 999	77.7 (121)		"
			1000 - 9999	99.0 (97)		"
			10000 +	100.0 (7)		"
	A. albimanus (Panama strain)		< 10	12.3 (65)		"
			10 - 99	43.8 (137)		"
			100 - 999	88.7 (309)		"
			1000 - 9999	99.3 (152)		"
			10000 +	100.0 (11)		"
elongatum	C. pipiens	canary	Ø	1.5 (723)		Micks (1949)
	C. quinquefasciatus	"		0 (78)		"
	C. restuans	"		2.2 (182)		"
	Ae. aegypti	"		0 (168)		"
	Ae. triseriatus	"		2.4 (82)		"
	Ae. vexans	"		0 (10)		"
	A. quadrimaculatus	"		0 (47)		"
	C. pipiens	sparrow		1.4 (72)		"
	Ae. aegypti	"		0 (89)		"
	A. quadrimaculatus	"		0 (20)		"
	C. pipiens	duck		5.2 (398)		"
	C. quinquefasciatus	"		0 (47)		"
	Ae. aegypti	"		0 (65)		"
	Ae. atropalpus	"		0 (52)		"
	Ae. triseriatus	"		3.7 (27)		"
	A. quadrimaculatus	"		0 (21)		"

* Figures in parenthesis represent the number of total dissected mosquitoes
Ø 100 - 800 gametocytes/10,000 red cells

TABLE 1: continued

SPECIES OF PLASMODIUM	VECTOR SPECIES	VERTEBRATE HOST	GAMETOCYTE DENSITY per C.mm.	INFECTION RATE % STOMACH*	GLAND*	AUTHORITY
falciparum	A. gambiae (fresh water)	man	8	12	(60)	Pringle (1962)
			9	16	(25)	"
			12	4	(23)	"
			12	0	(24)	"
			12	0	(49)	"
			15	Not exam'd		"
			15	"	"	"
			15	"	"	"
			18	13	(15)	"
			24	0	(19)	"
			24	Not exam'd		"
			85	85	(47)	"
			130	66	(9)	"
			140	70	(44)	"
	A. gambiae (salt water)	man	8	13	(52)	"
			9	20	(25)	"
			12	9	(23)	"
			12	0	(24)	"
			12	0	(49)	"
			15	50	(6)	"
			15	20	(10)	"
			15	0	(13)	"
			18	13	(15)	"
			24	26	(19)	"
			24	18	(34)	"
			85	60	(50)	"
			130	80	(15)	"
			140	60	(48)	"

* Figures in parenthesis represent the number of total dissected mosquitoes

TABLE 1: continued

SPECIES OF PLASMODIUM	VECTOR SPECIES	VERTEBRATE HOST	GAMETOCYTE DENSITY per C.mm.	INFECTION RATE % STOMACH*	GLAND*	AUTHORITY
falciparum	A. gambiae s.l.	man	1 - 10	8 (13)		Robertson (1945)
			11 - 100	51.5 (27)		"
			201 - 500	92 (13)		"
berghei	C. salinarius Ae. aegypti A. quadrimaculatus } Grp. 1	hamster	Numerous gametocytes	0 (64)	0 (39)	Yoeli (1973)
				0 (124)	0 (46)	"
				36.7 (322)	8.6 (70)	"
	A. quadrimaculatus A. aztecus A. stephensi } Grp. 2	"		25.6 (820)	6.9 (72)	"
				31.2 (314)	13.0 (54)	"
				40.6 (960)	21.1 (76)	"
	A. stephensi A. dureni } Grp. 3	"		24.2 (182)	30.6 (72)	"
				75.0 (32)	71.4 (28)	"
falciparum	A. quadrimaculatus	man	< 1	0.1 (5269)		Young et al (1948)
			1 - 5	6.6 (256)		"
			6 - 10	26.2 (164)		"
			11 - 20	2.7 (376)		"
			21 - 30	1.7 (59)		"
			31 - 40	28.9 (204)		"
			41 - 50	1.6 (62)		"
			81 - 90	58.1 (43)		"
			> 100	0.0 (67)		"

* Figures in parenthesis represent the number of total dissected mosquitoes

TABLE 2

LABORATORY EXPERIMENTAL FEEDING ON FILARIA INFECTION

SPECIES OF FILARIA	VECTOR SPECIES	VERTEBRATE HOST	AVERAGE MICROFILARIA COUNTS per 20 C.mm. OF PERIPHERAL BLOOD	INFECTION RATE % WITH INFECTIVE LARVAE*	AUTHORITY
D. uniformis	A. quadrimaculatus	rabbit	95	64 (39)	Duxbury et al (1961)
			253	49 (37)	"
			310	43 (53)	"
			3395	4 (12C)	
W. bancrofti	A. gambiae s.l.	man	20	25.0 (36)	Gelfand (1955)
	A. melas		20	24.4 (41)	"
	C. thallasius		13	42.6 (54)	"
D. immitis	A. punctipennis	dog	Microfilariæwere seen in the peripheral blood	100 (35)	Hu (1931)
	Ae. vexans			80.3 (56)	"
	C. pipiens			27.4 (182)	"
	C. territans			21.9 (41)	"
D. immitis	Ae. aegypti	dog	16000 - 18500 mf. per cc of blood	7.4 (229)	Kartman (1953)
	Ae. albopictus			91.5 (201)	"
	C. quinquefasciatus			30.0 (210)	"
	C. pipiens			18.4 (201)	"
	A. quadrimaculatus			91.4 (210)	"
	A. freeborni			69.0 (200)	"
	C. hybrid I +			18.6 (210)	"
	C. hybrid II ++			29.9 (244)	

* Figures in parenthesis represent the number of total dissected mosquitoes

\+ Hybrid I represents progeny of C. pipiens ♀♀ X C. quinquefasciatus ♂♂

++ Hybrid II represents progeny of C. quinquefasciatus ♀♀ X C. pipiens ♂♂

TABLE 2

LABORATORY EXPERIMENTAL FEEDING ON FILARIA INFECTION

SPECIES OF FILARIA	VECTOR SPECIES	VERTEBRATE HOST	AVERAGE MICROFILARIA COUNTS per 20 C.mm. OF PERIPHERAL BLOOD	INFECTION RATE % WITH INFECTIVE LARVAE*	AUTHORITY
D. uniformis	A. quadrimaculatus	rabbit	95	64 (39)	Duxbury et al (1961)
			253	49 (37)	"
			310	43 (53)	"
			3395	4 (120)	"
W. bancrofti	A. gambiae s.l.	man	20	25.0 (36)	Gelfand (1955)
	A. melas		20	24.4 (41)	"
	C. thallasius		13	42.6 (54)	"
D. immitis	A. punctipennis	dog	Microfilariæwere seen in the peripheral blood	100 (35)	Hu (1931)
	Ae. vexans			80.3 (56)	"
	C. pipiens			27.4 (182)	"
	C. territans			21.9 (41)	"
D. immitis	Ae. aegypti	dog	16000 - 18500 mf. per cc of blood	7.4 (229)	Kartman (1953)
	Ae. albopictus			91.5 (201)	"
	C. quinquefasciatus			30.0 (210)	"
	C. pipiens			18.4 (201)	"
	A. quadrimaculatus			91.4 (210)	"
	A. freeborni			69.0 (200)	"
	C. hybrid I +			18.6 (210)	"
	C. hybrid II ++			29.9 (244)	

* Figures in parenthesis represent the number of total dissected mosquitoes

\+ Hybrid I represents progeny of C. pipiens ♀♀ X C. quinquefasciatus ♂♂

++ Hybrid II represents progeny of C. quinquefasciatus ♀♀ X C. pipiens ♂♂

TABLE 2: continued

SPECIES OF FILARIA	VECTOR SPECIES	VERTEBRATE HOST	AVERAGE MICROFILARIA COUNTS per 20 C.mm. OF PERIPHERAL BLOOD	INFECTION RATE % WITH INFECTIVE LARVAE*	AUTHORITY
W. bancrofti	C. fatigans	man	0	20.0 (10)	Krishnaswami et al (1959)
			1 - 10	21.9 (82)	"
			11 - 50	82.2 (143)	"
			51 - 100	96.8 (118)	"
			101 - 150	98.8 (83)	"
			210 - 220	85.7 (28)	"
D. immitis	Ae. aegypti	dog	146 - 241		
	strains:- Bwamba			0 (50)	McGreevy et al (1974)
	GSC			0 (36)	"
	Ocala			0 (42)	"
	fmFt *			0 (65)	"
	Lanfiera			1 (101)	"
	New Meridian			8 (59)	"
	Chacito			9 (33)	"
	Pugu			86 (44)	"
	fmft *			89 (85)	"

* Figures in parenthesis represent the number of total dissected mosquitoes
* Selected from fm which is a laboratory stock of Ae. aegypti constructed by Macdonald and Sheppard (1965)

TABLE 2: continued

SPECIES OF FILARIA	VECTOR SPECIES	VERTEBRATE HOST	AVERAGE MICROFILARIA COUNTS per 20 C.mm. OF PERIPHERAL BLOOD	INFECTION RATE % WITH INFECTIVE LARVAE*	AUTHORITY
B. pahangi	C.p. fatigans Malayan strain	cat+++		0 (135)	Ogunba (1969)
	C.p. molestus			36.3 (134)	"
B. pahangi	Ae. aegypti	jird	20		
	Geographic strains:-				
	Bejuma-Dry			5 (103)	Paige and Craig (1975)
	Bejuma-Wet			35 (84)	"
	Bongoyo			8 (50)	"
	Buguruni			26 (101)	"
	Bundibugyo			0 (104)	"
	Bwamba			59 (73)	"
	Cz			23 (112)	"
	Dhow			0 (101)	"
	Ganda			0 (68)	"

* Figures in parenthesis represent the number of total dissected mosquitoes
+++ Microfilarial count = 20.4 microfilariae per C.mm. of cat's blood

TABLE 2: continued

SPECIES OF FILARIA	VECTOR SPECIES	VERTEBRATE HOST	AVERAGE MICROFILARIA COUNTS per 20 C.mm. OF PERIPHERAL BLOOD	INFECTION RATE % WITH INFECTIVE LARVAE*	AUTHORITY
B. pahangi	Ae. aegypti	jird	20		
	Geographic strains:-				
	Kampala			0 (95)	Paige and Craig (1975)
	Kombeni-Ovit			1 (65)	"
	Lyatonde			6 (110)	"
	Masaka			9 (83)	"
	Mazas-In			0 (100)	"
	Mbaa			3 (104)	"
	Mbarara			4 (123)	"
	Mbudya			54 (102)	"
	Mkwaja			0 (146)	"
	Momsabu-Dry			0 (102)	"
	Momsabu-Wet			0 (56)	"
	Moyo-In			0 (71)	"
	Masani			17 (100)	"
	Pugu			27 (104)	"
	Rabai			0 (103)	"
	Shauri-Moyo			0 (52)	"
	Shimba			4 (77)	"
	Sofi			37 (105)	"
	Wasani-Out			50 (88)	"
	Wasini-Yellow			0 (116)	"
	Control (Black-Eye)Ø			94.3 (2460)	"
B. malayi sub-periodic form	Ae. aegypti	cat	155 - 282 mf per 60 Cmm of blood		
	Liverpool strain			31.0 (316)	Ramachandran et al (1960)
	Cooper strain			19.6 (41)	"
	Malayan strain			0.5 (195	"

* Figures in parenthesis represent the number of total dissected mosquitoes

Ø Black-eye strain of Ae. aegypti, originating from the Liverpool School of Tropical Medicine (W.W. Macdonald). This strain had been selected for uniformly high susceptibility.

TABLE 2: continued

SPECIES OF FILARIA	VECTOR SPECIES	VERTEBRATE HOST	AVERAGE MICROFILARIA COUNTS per 20 C.mm. OF PERIPHERAL BLOOD	INFECTION RATE % WITH INFECTIVE LARVAE*	AUTHORITY
B. malayi Periodic form	Ae. aegypti	cat	48 - 85 mf per 60 Cmm of blood		
	Liverpool strain			29.4 (85)	Ramachandran et al (1960)
	Cooper strain			26.4 (34)	"
	Malayan strain			4.9 (82)	"
B. pahangi	Ae. aegypti	jird	10 - 392		
	American strains:-				
	Bahama			0 (94)	Rodriguez and Craig (1973)
	Cardizal			0 (76)	"
	Chacito			2 (66)	"
	Curacao			0 (68)	"
	GSC			6 (80)	"
	Isla-Verde			0 (97)	"
	Ocala			0 (85)	"
	Puerto-Rico			0 (87)	"
	New-Meridian			0 (141)	"
	Rock			0 (128)	"
	Trinidad			0 (98)	"
	African strains:-				
	Amani			0 (74)	"
	Brazza			0 (78)	"
	Buguruni			1 (84)	"
	Bwamba			53 (79)	"
	Cz			30 (63)	"
	Dahomey			0 (74)	"
	Dhow			0 (111)	"
	Dodoma			10 (84)	"
	Ganda			0 (73)	"

* Figures in parenthesis represent the number of total dissected mosquitoes

TABLE 2: continued

SPECIES OF FILARIA	VECTOR SPECIES	VERTEBRATE HOST	AVERAGE MICROFILARIA COUNTS per 20 C.mm. OF PERIPHERAL BLOOD	INFECTION RATE % WITH INFECTIVE LARVAE*	AUTHORITY
B. pahangi	Ae. aegypti				
	African strains:-	jird	10 - 392		Rodriguez and Craig (1973)
	Gongoni			0 (86)	"
	Kumba			0 (73)	"
	Lanfiera			0 (100)	"
	Maziwi			0 (47)	"
	Mbudya			21 (75)	"
	Mkwaja			13 (64)	"
	Moyo			0 (83)	"
	Masasani			1 (92)	"
	Muheza			4 (78)	"
	Newala-Bamboo			1 (85)	"
	Newala-House			0 (89)	"
	New-Gkep			0 (138)	"
	Poura			0 (83)	"
	Pugu			37 (70)	"
	Sofi			33 (82)	"
	Ssisa			0 (91)	"
	Tiassale			0 (80)	"
	Asian strains:-				"
	Amphur			0 (90)	"
	Bangkok			0 (97)	"
	Cebu			0 (92)	"
	Koh-Samui			0 (71)	"
	Tahiti			0 (77)	"
	Viet-nam			0 (73)	"

* Figures in parenthesis represent the number of total dissected mosquitoes

TABLE 2: continued

SPECIES OF FILARIA	VECTOR SPECIES	VERTEBRATE HOST	AVERAGE MICROFILARIA COUNTS per 20 C.mm. OF PERIPHERAL BLOOD	INFECTION RATE % WITH INFECTIVE LARVAE*	AUTHORITY
W. bancrofti	Ae. pseudoscutellaris	man	3 - 681	81 (836)	Symes (1960)
	C. fatigans		7 - 169	45 (592)	"
	Ae. fijiensis		5 - 681	76 (298)	"
	Ae. polynesiensis		4 - 192	82 (85)	"
	Ae. aegypti		3 - 158	2.0 (290)	"
	Ae. vexans		8 - 161	0 (507)	"
	C. annulirostris		11 - 134	1.1 (339)	"
	C. sitiens		27 - 134	0 (110)	"
W. bancrofti (periodic strain)	A. donaldi	man	1.9 - 16.7 mf. per C.mm. of blood	17 (6)	Wharton et al (1963)
	A. letifer			82 (61)	"
	A. maculatus			75 (8)	"
	A. roperi			100 (1)	"
	A. separatus			100 (1)	"
	A. umbrosus			0 (25)	"
	C. pipiens fatigans			30 (493)	"
	C. pseudovishnui			0 (2)	"
	C. tritaeniorhynchus			100 (1)	"
	Ae. poecilus			38 (8)	"
	M. annulata			0 (5)	"
	M. dives			0 (15)	"
B. malayi (periodic strain)	A. barbirostris	man	1.2 mf. per C.mm. of blood	51 (49)	"
	A. donaldi			64 (14)	"
	A. crawfordi			100 (2)	"
	A. peditaeniatus			86 (14)	"
	A. sinensis			0 (6)	"
	A. kochi			0 (3)	"
	A. maculatus			2 (58)	"

* Figures in parenthesis represent the number of total dissected mosquitoes

TABLE 2: continued

SPECIES OF FILARIA	VECTOR SPECIES	VERTEBRATE HOST	AVERAGE MICROFILARIA COUNTS per 20 C.mm. OF PERIPHERAL BLOOD	INFECTION RATE % WITH INFECTIVE LARVAE*	AUTHORITY
B. malayi	A. philippinensis	man	1.2 mf per C.mm. of blood	0 (26)	Wharton et al (1963)
	M. annulata			5 (19)	"
	M. bonneae			16 (56)	"
	M. dives			39 (36)	"
	M. uniformis			91 (11)	"
	Ae. aegypti			6 (262)	"

* Figures in parenthesis represent the number of total dissected mosquitoes

Age of Mosquitoes

Duxbury et al (1961) tried to study the relationship between the age of the mosquito and the intensity of the infection. They fed different age groups of mosquitoes of A. quadrimaculatus on a carrier infected with Dirofilaria uniformis. They concluded that the best age groups of mosquitoes for securing the best susceptibility to filarial infection were 9 - 10 days and 12 - 13 days. They postulated that the susceptibility may be related to senility though it should be pointed out that there was much more mortality among older A. quadrimaculatus. Terzian et al (1956) working on the same aspects, came to different conclusions. Mosquitoes of Aedes aegypti became more resistant to infection with P. gallinaceum with advancing age Thus susceptibility in groups of ageing mosquitoes, 2 - 4 weeks old, were significantly lower than that of newly emerged control groups. However, a normal blood meal nine days prior to the infective feed restored the susceptibility to those ageing mosquitoes. Similarly, feeding the mosquitoes on raisin infusion for four weeks before offering an infective meal turned them susceptible. They also found that old mosquitoes when fed solutions of chick or human plasma, haemoglobin and lysed red cells combined, had increased their refractoriness to infection. However, they related the whole variation in the susceptibility to the addition or depletion of specific physiological or metabolic factors which operate in low concentration.

Desowitz and Chellappah (1962) in an attempt to study the affects of age and prior non-infective blood meals on the infection rate of Brugia species in C. p. fatigans found that the average range of infections in five groups of mosquitoes ranged from 8.5% to 25.4%. the rates being higher and significant in older groups of mosquitoes (20 - 22 days old) whether they had been maintained on raisin alone or were given two non-

infective blood meals prior to the infective one. Wharton et al (1963) found that young (5 - 8 days) or old (18 - 25 days) mosquitoes of C. p. pipiens which had no blood meal prior to their infective one were found less susceptible to infection with Wuchereria bancrofti than old mosquitoes (18 - 25 days) which had a blood meal prior to their infective one.

Superinfections

The effect a primary infection might have on another infective meal and the final outcome on the susceptibility of a vector was investigated by many workers.

Huff (1930) demonstrated the susceptibility of C. pipiens to three species of avian malaria, P. cathemerium, P. elongatum and P. relictum by means of double infectious feeding upon different species of malarial parasite at a few days interval. He found no correlation existed between the susceptibility of individual females to one species of parasite and the susceptibility to another species. Furthermore, in the case of two separate feedings on the same parasite he found that each individual mosquito either became infected both times or failed entirely to become infected. Thus he concluded that the susceptibility of a species of mosquito to a given parasite is firmly fixed in the case of the individual. On the same line, Ward (1966) working with Ae. aegypti and P. gallinaceum found that all mosquitoes which were infected during the first meal also developed oocysts after the second meal while those which were first resistant remained refractory to infection. Boyd and Stratman-Thomas (1932) reported that the refractory characteristic cannot be overcome by repeatedly feeding the mosquitoes on a patient. They further added that its nature is unknown. Repeated feedings increase the intensity of infection in susceptible mosquitoes only quantitatively. It does not appear, however, that successive feedings of the mosquitoes

on the patient increase the proportion of those becoming infected. This is contrary to the finding of Shute (1951) who reported that if a batch of mosquitoes is fed three or four times on blood containing P. vivax gametocytes 100% do become infected. He further added that this suggests that no individual mosquitoes are completely refractory to infection and that the number of feeds required to produce 100% infection represent the degree of susceptibility. It seems that Shute was dealing with a 100% susceptible population of mosquito with P. vivax infection, but it appears that the mosquitoes were not all having the chance to pick up the right number of gametocytes which could establish the infection. However, these mosquitoes were infected and showed 100% susceptibility when tried 2 - 3 times on gametocyte carriers on several occasions. Kartman (1953) in double infective feedings of Ae. aegypti on two species of filaria, D. immitis and Foleyella brachyoptera (frog filaria), found that the mosquitoes reacted independently to each species of filaria whether ingested simultaneously or at spaced intervals. Duxbury et al (1961) working with A. quadrimaculatus and D. uniformis found that in one experiment the results suggested the possibility that the first exposure might bring about a reduction in the normal growth of the larvae of microfilariae of the subsequent one, while the results were reversed in the other experiment. (Their results were not decisive). Bertram et al (1964) in a study to investigate the double infection of both Semliki Forest virus and P. gallinaceum in Ae. aegypti found that suppression of malarial infection was not induced by the infection with the virus. Furthermore, they showed that a doubly-infected Aedes can transmit both Semliki Forest virus and P. gallinaceum simultaneously. Also McGreevy et al (1974) found that the ft gene which controls development of D. immitis in Malpighian tubules, and fm which controls the development of Wuchereria and Brugia in the thoracic muscles, are

distinct because there was no relationship between the development of D. immitis and B. pahangi in the double infective meals in Ae. aegypti from the selected stocks. Similarly, ft had no influence on the development of D. corynodes in fat body. This is because filarial susceptibility alleles directly affect the organ lodging the parasite rather than the parasite itself.

Irradiation

Terzian (1953) in investigating the effect of irradiation on the host-parasite relationship of Ae. aegypti and P. gallinaceum found that X-irradiation in doses ranging from 5,000 to 30,000r increased significantly the refractoriness of the mosquitoes to malarial infection. On the other hand, X-irradiated, antibintic fed mosquitoes prior to the infective blood meal were found as susceptible as the control. However, mortality among irradiated mosquitoes was twice as much as that of the control. Ward et al (1960) reported great fluctuations in the susceptibility of the mosquitoes. They found that Ae. aegypti became more resitant to infection with P. gallinaceum when the larvae were irradiated with a dose of 500 - 1500r. On the other hand, the F_1 of these mosquitoes showed an increased susceptibility of 2 - 6 times that of the control, but the susceptibility returned to normal in the F_3.

Artificial Diets

Micks et al (1948) found no correlation between exflagellation of P. elongatum and pH in the stomachs of C. pipiens, C. quinquefasciatus, Ae. aegypti and A. quadrimaculatus. Furthermore, Micks and [illegible] (1961) working with C. fatigans and P. relictum found that in general the susceptibility increased after these mosquitoes had been treated with antibiotics. They gave two explanations for the results obtained. First, the competition between the micro-organism already found in the midgut

and the malarial parasites for essential nutrients, in which case antibiotics would work in favour of the parasite and second, that it may be that certain strains of these micro-organisms supply factors needed by the host for metabolic processes and defence mechanisms and that killing the organisms increases host susceptibility to malaria. However, the criterion for judging the susceptibility was based entirely on the presence or absence of the oocysts and its intensity; no consideration was given to checking the infectivity of the sporozoites.

Terzian (1950, 1955) and Terzian et al (1949, 1952) and Terzian and Stahler (1960) were able to change the susceptibility measured by the number of oocysts of P. gallinaceum in Ae. aegypti by feeding the experimental mosquitoes various metabolites, antibiotics, hormones, vitamins, acids, bases and salts. These substances have affected the susceptibility by either increasing or decreasing it. Sometimes the effect on susceptibility is so critical that it depends mainly on the concentration of the substance being used. However, they reported that they had not been able to determine and design an experiment that could show unequivocally whether the effects of these compounds on the host-parasite relationship are directly on the host or on the parasite. No transmission experiments had been conducted to confirm the infectivity of the sporozoites.

On the other hand, Ghosh and Ray (1957) accelerated the development of oocysts by providing infected Ae. aegypti with supplementary blood meals from uninfected hosts during the mosquito incubation period. Noblet and Weathersby (1973) showed that a number of metabolites such as para-aminobenzoic acid (PABA), NaOH, HCl, KOH, $MgCl_2$, and vitamins will

affect the innate susceptibility of Ae. aegypti and C. p. pipiens to P. gallinaceum. Furthermore, they succeeded in increasing the number of occysts in the refractory C. p. pipiens. However, no malaria transmission by this refractory mosquito was accomplished.

Ward (1965) and Kilama (1972) working with the same species of mosquito and malaria parasite reached different results. The former was able to cause some change in the susceptibility of Ae. aegypti to infection with P. gallinaceum by orally administering extracts from refractory mosquitoes before feeding on a gametocyte carrier. Mosquitoes fed by this method showed fewer oocysts compared with the control ones. On the other hand, Kilama found that injection of extract from susceptible into refractory mosquitoes caused the refractory to turn relatively susceptible to infection with P. gallinaceum. The extracts from the refractory had no effect on the susceptible mosquitoes. Later on, Kilama found these experiments were non-repeatable.

It has been suggested that mosquito species, strains and individuals respond to parasitic infection in various ways and probably several mechanisms may be involved in such responses to invasion by the parasites. The first of these which would be encountered by the invading parasite is the stomach wall. It was found that Murray Valley encephalitis would develop in the refractory mosquitoes when injected into the haemocoels of A. annulipes, and eventually invade the salivary glands but not the stomach epithelium. The same virus successfully invaded the stomach epithelium and the salivary glands when injected into the haemocoels of susceptible Ae. queenslandis and C. annulirostris (McLean, 1953, 1955). Also, Gubler and Rosen (1976) working with dengue viruses in different geographic populations of Ae. aegypti found that the mosquito midgut was the actual barrier to infection, but once this barrier has been

overcome further virus growth is the same in all mosquito populations.

It was not until 1952 that Weathersby obtained complete development of exogenous stages of P. gallinaceum in the haemocoel of susceptible Ae. aegypti and viable sporozoites were recovered from the salivary glands of unfed mosquitoes which received haemocoel inoculations of gametocytes, all ages of oocysts or mature sporozoites instead. Transmission infections in chicks were not successful in the case of those mosquitoes injected into the haemocoel with sporozoites, but were successful in both cases of mosquitoes injected into the haemocoel with different ages of oocysts and with blood having a high gametocyte count. On the other hand, all the above mentioned attempts were repeated this time with the refractory C. pipiens using P. gallinaceum and failed to establish malarial infection in the haemocoel in spite of the fact that the classical barrier, stomach walls, was bypassed. Thus he concluded that the association between the parasite and the stomach wall is not as important as it had been thought to be and the factors that are responsible for killing the parasite in the refractory C. pipiens are systemic and not localized in the stomach wall only but are distributed all over the body. Later on this work was substantiated by further studies (1954, 1960a, 1960b, 1965; Weathersby and McCall, 1968) and immunity was indicated by most to be due to the action of toxic agents (Weathersby, 1963, 1965, 1967; Weathersby et al, 1971). Finally Weathersby and Noblet (1973) found that the metabolites and other dietary components can greatly influence mosquito susceptibility to P. gallinaceum in both susceptible and refractory species of mosquitoes.

However, Stohler (1957, 1961) reported that the rate of formation of the peritrophic membrane might influence the degree of susceptibility

of mosquitoes to malarial infection. He found that the peritrophic membrane becomes thick thirty hours after the infective blood meal was taken. Thus he concluded that the rigid membrane might restrict the movement of the ookinetes of P. gallinaceum even in the susceptible Ae. aegypti. Kilama (1972) working with Ae. aegypti and P. gallinaceum found that neither the coagulation of the blood meal, nor the rate of formation of the peritrophic membrane influences refractoriness. Furthermore, Kilama concluded that "gut barrier" is not involved.

Temperature

When all other factors are held constant, infection may fail to become established in the mosquito if the environmental temperature is not favourable. In the case of rodent malaria it is decisive especially in P. berghei mosquito infections.

Since the discovery of P. berghei in 1948 by Vincke and Lips, many attempts have been tried to infect different species of anopheline mosquitoes experimentally with P. berghei and have failed (Yoeli and Wall, 1951, 1952a, 1952b, Perez-Reyes, 1953; Ramakrishnan et al, 1953; Bray, 1954; Vincke, 1954; Rodhain et al, 1955 and Celaya et al, 1956). The main reason for their failure was simply because they kept the mosquitoes at 23 - 27^{o}C after they had been fed on the gametocyte carriers. Yoeli and Most (1964) succeeded in obtaining light sporozoite infections in the salivary glands of A. quadrimaculatus which had previously fed on a mouse infected with P. berghei and when the mosquitoes subsequently were kept at 22^{o}C. Moreover they succeeded in transmitting the infection to two tree rats (Thamnomys surdaster) by allowing the

infected mosquitoes to feed on them. It was not until 1965 that Yoeli discovered the optimum temperature of 21°C for the sporogonic cycle of P. berghei in the natural host mosquito, A. dureni, by studying the natural climatic conditions in the forest galleries of Katanga. Furthermore, in a controlled experiment he obtained the best rate of infection when the mosquitoes of A. quadrimaculatus fed on a P. berghei gametocyte carrier were kept at 18 - 21°C. The rate was 66% for midgut and 43% for salivary gland infections. Mosquitoes kept at both 14°C and 28°C were absolutely negative, while the mosquitoes kept at 18°C showed 83% midgut infection and 23% gland infection. On the other hand, mosquitoes kept at 21°C gave 49% oocyst and 22% gland infections while those mosquitoes maintained at 24°C were only 36% positive for oocysts and were negative for salivary gland infection. Later on, Yoeli et al (1965), Vanderberg and Yoeli (1965, 1966) and Yoeli and Bona (1967) found that the sporogonic cycle of P. berghei though similar in the time required in its maturation to P. vivax or P. cynomolgi is more strictly temperature dependent within narrow limits of 4°C (18 - 22°C).

Vincke et al (1966) reported on the optimum temperature which allows normal development of P. berghei in A. quadrimaculatus and A. stephensi. It affects in the same way the sporogonic cycle of the same parasite in A. gambiae and A. maculipennis var atroparvus.

Yoeli and Upmanis (1968) reported on the important role of temperature on the sporogonic phase of malarial infection in the mosquitoes. They found that the temperature may not act directly on the parasite but on the mosquitoes harbouring the parasite by increasing or decreasing the rate of biological activities which eventually might affect gametocyte fertilization, ookinete formation and further oocyst development.

Undoubtedly these factors are important but it seems that the temperature affects directly the parasite of P. berghei in which the biological activities are adjusted to a very narrow range of 4°C (18 - 22°C). This was proved not only under laboratory conditions where the infected mosquitoes were kept (Yoeli, 1964, 1965; Wery, 1968) but also in the natural habitat of the parasite (Yoeli, 1965).

2. Genetic Factors and Susceptibility

On genetic control possibilities in mosquitoes there have been several extensive reviews (Davidson et al, 1967; Macdonald, 1967, 1976; Davidson, 1974; Pal and La Chance, 1974; Pal and Whitten, 1974). Genetic factors in malaria parasites and their effect on host-parasite relationships have been discussed by Walliker, 1976. Within one species of mosquito there may be strains which are more susceptible than others, and they may be genetically distinct. This was the hypothesis put forward by Huff (1927) who was the first to show natural refractoriness of certain individuals of C. pipiens against infection of the avian malarial parasites, P. cathemerium and P elongatum. Later, Huff (1929, 1931) demonstrated conclusively that susceptibility or refractoriness is a hereditary character. By selective breeding, Huff succeeded in three generations to increase the rate of susceptibility to infection with P. cathemerium to 65% by selecting from infected females and he decreased the rate of susceptibility down to 8% by selecting from non-infected mothers. Huff (1930) proved convincingly that the susceptibility of C. pipiens to P. cathemerium, P. elongatum and P. relictum by means of double infectious feedings upon the same or different species of avian malaria, had no correlation between susceptibility

of individual females to one species of parasite and susceptibility to another species, and the existence of both susceptible and refractory individual mosquitoes within the same species. He proved that the ability of a susceptible mosquito to support the infection is inherited. Later, Huff (1935, 1941) concluded that the refractoriness of C. pipiens to avian malaria, P. cathemerium, was a hereditary phenotype behaving as a simple Mendelian dominant. This conclusion was based on F_2 progeny which gave 3:1 ratio for refractory (100 negative females) and susceptible (33 stomach positive females) mosquitoes. However, the data obtained from backcrosses showed a significant departure from the expected ratio of 1:1. The finding of Huff that the susceptibility of C. pipiens to infection with P. cathemerium is controlled by a single recessive gene was later confirmed (Dennhofer, 1971).

Trager (1942) succeeded in increasing the level of susceptibility of Ae. aegypti to P. lophurae through six generations of selective breeding. Complete cessation of selective pressure for more than one year did not affect the rate of susceptibility in the selected line. Neither Boyd and Russell (1943), Jeffery (1944) nor Hovanitz (1947) were able to select refractory or susceptible strains using P. vivax in A. quadrimaculatus, P. lophurae in Ae. aegypti, and P. gallinaceum in Ae. aegypti respectively, although selective breeding continued for several generations. But it has been found at the Rockefeller Foundation (1948, 1950) that two strains of A. quadrimaculatus were established, one fully susceptible while the other was only partially

susceptible to P. gallinaceum infection. From the results of crossing the two lines, it was found that the F_1 progeny was intermediate in susceptibility. It was concluded that although there was evidence of a genetic basis controlling relative susceptibility, this was probably dependent on multiple interacting genes. Micks (1949) succeeded in elevating the susceptibility of C. pipiens to P. elongatum. Within six generations of selective breeding the rate of susceptibility was increased from 13% to 49%. Also there was some indication that those females with the greatest number of oocysts per stomach gave rise to the largest number of susceptible offspring.

Ward (1963) studied the genetic mechanism of the susceptibility of Ae. aegypti to infection with P. gallinaceum. Over a long course of selective breeding and after 26 generations he succeeded in reducing the susceptibility in the selected refractory line which showed a 98% decrease in oocyst numbers. Cessation of selective pressure for thirteen generations did not produce a significant change in the susceptibility. Reciprocal crosses between the selected refractory and the susceptible parental strains gave F_1 and F_2 hybrids which were intermediate in their susceptibility. Results obtained from backcrosses showed a tendency toward bimodality. Accordingly he concluded that a single pair of genes or a block of closely related genes was involved which lacked dominance. The very drawn out selection method (26 generations) might have been due to the technique Ward adopted in selecting from the females which showed low count oocysts but not from absolutely negative females.

Kilama and Craig (1969) reported a wide variation in susceptibility to P. gallinaceum in nineteen strains of Ae. aegypti. Within one generation of selective breeding they succeeded in establishing two refractory lines. They defined the refractory mosquitoes as having up to six oocysts on the wall of their midgut. From the results of crosses, they concluded that the refractory character is controlled by a simple autosomal recessive factor, which they designated pls (plasmodium susceptibility).

Frizzi et al (1975), by selective breeding from A. stephensi to infection with P. gallinaceum, succeeded in establishing two strains. One was highly susceptible, the females of which showed in their midgut an average of thirty oocysts seven days after having the infective blood meal. The other strain was highly refractory in which only 3% of the females showed oocysts. From the results of crossing the two parent strains both ways and backcrossing the F_1 offspring to the parents, they concluded that their data supported the hypothesis that resistance of A. stephensi to P. gallinaceum is a dominant phenotypic expression at a single locus or possibly the result of coordinated action of a group of closely linked loci.

Van der Kaay (personal communication) succeeded in establishing a highly susceptible line of A. atroparvus to infection with P. b. berghei. The susceptibility rate was 98.7% (75 mosquitoes dissected) with a mean oocyst number of 189, after 14 generations. Selection for the refractory line was not as easy as that for the susceptible one; it showed a lot of variations. After fourteen generations the susceptibility in the refractory line was 6.9% (91 mosquitoes

dissected) with a mean oocyst count of 5.

Gubler and Rosen (1976) in an attempt to study the comparative susceptibility of thirteen geographic strains of Ae. albopictus to the four serotypes of dengue virus infection, found that when one strain was susceptible to one dengue serotype it also was susceptible to the other three serotypes. The hybrid resulting from crossing the susceptible and the refractory mosquito strains was found to be intermediate in its susceptibility. Selective breeding of Ae. albopictus was successful in decreasing the susceptibility to infection from 74% to 13% in two generations. However, they could not decrease further the rate of susceptibility in spite of the effort to produce a completely refractory line. Tesh et al (1976) found differential susceptibility of sixteen different geographic strains of Ae. albopictus to oral infection with chikungunya (CHIK) virus. Genetic selection for 3 - 6 generations was unsuccessful in establishing resistant and susceptible lines. Crosses between the resistant and the susceptible lines gave an F_1 which was intermediate in susceptibility.

In conclusion it should be pointed that differences in infectiveness of parasites can and do occur as well as differences in susceptibility of hosts (both definitive and intermediate). Coradette et al (1970) were able to infect selected refractory A. stephensi by changing strains of P. gallinaceum for example. Walliker et al (1971, 1973) and Walliker (1976) were in fact able to demonstrate genetic recombination in malaria parasites themselves. Strains of P. berghei differing in certain enzymes and drug tolerance were crossed by feeding mosquitoes (A. stephensi) on mice infected with the two strains. The resulting ookinetes were

allowed to develop into sporozoites, and these sporozoites were then used to infect rodents. The presence of recombinants were identified initially and qualitatively by merely giving the animal a discriminating dose of drug sufficient to kill the susceptible parasites and then screening the surviving parasites for enzyme variants. The presence of any new variant would indicate cross fertilization between the gametes of the two parental lines.

Filarial Infection

Excellent reviews on the subject of susceptibility to filarial infections are given by Lavoipierre (1958), Hawking and Worms (1961), Macdonald (1967, 1976).

Roubaud et al (1936) and Roubaud (1937) were the first to record that the susceptibility of Ae. aegypti to filaria infection is under genetic influence. Kartman (1953) investigated the host-parasite relationship of D. immitis in several species of Aedes, Culex and Anopheles mosquitoes (Table 2). After eight generations of selective breeding from Ae. aegypti to infection with D. immitis, he found that the total average percentages of the females with filarial development was 7.7 in the refractory line, 41.4 in the susceptible line, 25.0 in the parent and 28.0 in the controls. However, he failed to establish refractory and susceptible lines to D. immitis from a C. pipiens ♀♀ x C. quinquefasciatus ♂♂ hybrid.

Macdonald (1962a) succeeded in selecting a strain of Ae. aegypti

highly susceptible to sub-periodic B. malayi. He increased the rate of susceptibility from 17.1% to 93.8% in one generation. The mean susceptibility rate of 84.8% was maintained over fifteen subsequent generations. Furthermore, he established a homozygous refractory strain and maintained it for five generations. Later, Macdonald (1962b) concluded from results obtained from crossing the refractory and the susceptible lines and from backcrosses and testing the offspring against infection with sub-periodic B. malayi, that the susceptibility of Ae. aegypti is sex-linked and recessive and he designated the gene as fm (filarial susceptibility, Brugia malayi). Macdonald (1963a) as a continuation to the previous work of the susceptibility of Ae. aegypti to B. malayi, concluded that the penetrance of the fm factor is not complete and its expressivity is variable. Later on, it was found that the recombination of the fm with the sex locus in male mosquitoes was 3.4 ± 1.1% (Macdonald, 1963b; Macdonald and Sheppard, 1965). Macdonald and Ramachandran (1965) showed that fm controlled the susceptibility of Ae. aegypti to infections of all filariae which develop in the thoracic muscles, such as B. pahangi, both forms of periodic and sub-periodic B. malayi and periodic W. bancrofti (Malayan rural strain) and sub-periodic W. bancrofti (Fijian strain). But fm had no influence on those filarial nematodes which develop in the Malpighian tubules, such as D. immitis and D. repens.

Raghavan et al (1967) selected two lines of Ae. aegypti for susceptibility and refractoriness to Dirofilaria infection. At the end of three generations a 100% susceptible line was established and it almost maintained its susceptibility over 21 subsequent generations. On the other hand, selection for the refractory line showed great fluctuations over a period of 22 generations, at the end of which a line with 82.0% refractoriness was achieved. However, selection for the refractory line

was affected by alternate feeding on donors infected with D. immitis sometimes and at other times on D. repens. This probably delayed selection.

Partono and Oemijati (1970) and Singh and Curtis (1974) failed to select strains of C. p. fatigans refractory to infection with W. bancrofti after three and five generations of selective breeding respectively. However, they have attributed their negative results to the absence of the gene(s) for refractoriness in the original stocks.

Susceptibility to malaria and filarial infections is a character which manifests itself only in the female and the male, therefore, always remains an unknown factor in genetic studies. However, it seems that this problem has been solved at least in filarial infection by the successful inoculation of exsheathed microfilariae into males of a susceptible strain of Ae. aegypti which were found fully susceptible (Terwedow and Rodriguez, 1973; Townson, 1974, 1975). However, the problem of development of filariae in some of the inoculated males in stocks where the females are refractory has been observed in both Ae. aegypti and Ae. malayensis on inoculation with B. pahangi and remains to be solved.

As a result of a series of crosses and backcrosses between strains of C. pipiens, susceptible and refractory, it was found that the susceptibility of C. pipiens to B. pahangi was controlled by a sex-linked recessive gene which was designated as sb (Obiamiwe and Macdonald, 1973). The same above-mentioned conclusion was reached by Zielke (1973) and McGreevy et al (1974) working independently with D. immitis in Ae. aegypti. However, the latter have designated the sex-linked

recessive gene as ft (filarial susceptibility, Malpigian tubules).

Crossing Ae. malayensis which is refractory to B. pahangi and W. bancrofti with Ae. polynesiensis, which is susceptible to these two filariae, the hybrid females are all refractory. Furthermore, backcrosses of both hybrid males and females to Ae. malayensis produce nothing but refractory offspring. However, the more informative reciprocal backcrosses remain to be done (Macdonald, 1976).

MATERIALS AND METHODS

Mosquitoes

The mosquito strains used were :-

LSW:-

This mutant was discovered in A. gambiae species A in a population from Tungan Buzu, Western Sokoto, Nigeria after it had been selected for three generations with DDT. About forty white-eyed males were found among the offspring of the selected stock. These mutants were crossed with normal females of another strain collected at Lagos, Nigeria, 1951. Then, by inbreeding, white-eyed males and females were obtained. Since that time this stock of mosquitoes was maintained in the Ross Institute.

Mason (1967) described the mutant LSW as the white-eyed gene controlling the production of pigment in the eyes of the larva, pupa and adult. It manifests itself by the complete lack of pigment in the larva and developing imago eyes. This condition persists through the pupal and adult stages where the eyes are a light cream colour. The mutant is readily distinguishable from the wild-type larva, pupa and adult. The above mentioned author proved that the mutation was a single recessive sex-linked gene. This finding was confirmed in the present work by reciprocal crossing and backcrossing to both the normal and white-eyed individuals as mentioned elsewhere.

The stock of LSW was chosen as the original source for selecting the refractory line because -

1) the marker, being very easily recognised, would show

immediately if any contamination had occurred.

2) this strain (LSW) showed low susceptibility to infection with P. berghei compared with PALA in the first pilot experiments which were done by Dr. G. Davidson, Dr. C.C. Draper and Miss J. Dorell from the Ross Institute in the late 1960's.

3) on the assumption that the genetic mechanism controlling the refractoriness turned out to be sex linked this marker (the gene controlling the colour of the eye) would serve for linkage studies.

PALA:-

This is a wild strain of A. gambiae species A in which the mosquitoes are normal eyed. It was collected at Pala near Bobo Dioulasso, Upper Volta and has been maintained in the insectary of the Ross Institute since 1967.

BEECH:-

This is a strain of A. stephensi originally derived from Delhi, India in about 1947. This strain was used throughout as a control to check the infectivity of the malarial parasites in all experiments. Yoeli (1965), Landau and Killick-Kendrick (1966), Vanderberg and Yoeli (1966), Vincke et al (1966) and Wery (1968) reported that A. stephensi was found to be highly susceptible to laboratory infections with P. berghei.

P. b. berghei was chosen in the experiments for selecting the refractory and susceptible lines of mosquitoes because -

a) it is a mammalian Plasmodium easy to handle, more dependable, easy to transmit to different small laboratory animals and which could have kinship in its clinical course, pathology and immune response to the human plasmodia (Yoeli et al, 1965).

b) the characteristic low temperature (18 - 21°C) which governs and affects the sporogonic development of this malaria parasite enables the research workers to adapt alien mosquitoes to serve as experimental vectors (Yoeli et al, 1965).

c) it is a useful model for malarial investigation (Vanderberg et al 1968).

Rearing of Mosquitoes

The eggs of the two strains, PALA and LSW, of A. gambiae species A used in the selection experiments and BEECH of A. stephensi (used as a control for all experiments) were washed with 0.01% formalin solution for 30 - 40 minutes to try to eliminate possible microsporidian infections which might interfere with the development of subsequent malarial infection. The damaging effect of microsporidia on mosquitoes has been confirmed by many authors (Garnham, 1956; Bray, 1958; Reynolds, 1966; Yoeli and Bone,1967; Alger and Undeen, 1970; Hulls 1971 Ward and Savage, 1972 and Macrae, personal communication).

After hatching, first instar larvae were distributed in several large bowls, 12" X 5". Care was taken to prevent overcrowding. These bowls

were autoclaved each time they were used for rearing larvae. Each bowl contained 500ml of tapwater and a small chunk of grass with some earth to encourage the growth of micro-organisms which might be used as another source of food for the first stages of the larvae. Moreover, daily sprinkling of finely ground Farex as routine larval food was given twice a day. During the later stages of development of the larvae food was offered three or four times a day. In experiments requiring virgin males and females, pupae were isolated individually in 1" X 3" vials containing a minimal amount of water and allowed to emerge.

After emerging, mosquitoes were kept in 12" X 12" X 12" cages. These cages were either autoclaved or bleached before being used. Adult mosquito colonies were kept in a separate room where the temperature was 20 ± 1°C and a relative humidity of 80 - 90% and the lighting system was automatically regulated to give 12 hours light and 12 hours of darkness. The mosquitoes received only 20% glucose solution for five to seven days before feeding them on infective gametocyte carriers. Care was taken to minimize bacterial, yeast and fungal contamination of the sugar solutions on which the mosquitoes fed by sterilizing all bottles, solutions and glass tubes, and using clean lint and changing them every two days. Before feeding on a gametocyte carrier, mosquitoes were starved by removing the glucose solutions from the cages for twelve hours.

Malarial Parasites

a) Rodent Malarias:-

P. b. berghei (ANKA strain) was used throughout the present work.

This strain was maintained by three methods -

1) Blood passages: A few drops (0.1ml) of blood was taken from the tail of an infected mouse and mixed with 0.4ml sterile normal saline. The amount of 0.5ml was injected intraperitoneally into two mice. Vincke (1954) reported that all routes of infection appeared good, but the peritoneal route is the most convenient for the best results. These mice of blood induced infection were ready for feeding the mosquitoes after three days (72 hours).

2) Cyclical transmission: An infective batch of mosquitoes were allowed to feed on 2 - 3 mice left over night in the cage. These mice of sporozoite-induced infection developed a high rate of parasitaemia with 5 - 7 days. This method of transmission was used whenever a fresh stock of infected blood was required.

3) Preservation of the malarial parasites in liquid nitrogen: It is a well known fact in rodent malarias that continuous blood passages from mouse to mouse have resulted in the loss of their ability to produce gametocytes (Vincke, 1954; Sergent and Poncet, 1956; Yoeli et al, 1963b and 1966b Bafort et al, 1965; Vanderberg et al, 1968; Hawking, 1972).
To avoid this phenomenon and to

standardize the infectivity of the gametocytes, a large number of mice of sporozoite induced infection were killed, and their blood was pooled. Each 5ml of the blood was mixed with 0.4ml of glycerol and sequestrene anticoagulant. After thoroughly mixing, the glycerolized blood was divided into 8 - 10 plastic vials. These vials were then labelled and kept at low temperature (-196°C) in the liquid nitrogen for possible future use. It was found during the present work that the liquid nitrogen kept the infectivity of the parasites satisfactory for well over two years. Molinari(1961), Jeffery (1957, 1962) and Wery (1968) found that the addition of glycerine (4.2 and 8.4%) to blood containing P. berghei and P. gallinaceum helps in the viable preservation at the freezing temperature of -20°C or -196 °C for two years.

Other rodent malarias used in the present study were P. yoelii, P. chabaudi and P.y.nigeriensis.

b) Simian Malaria:-

Only one species of monkey malaria, P. knowlesi, was used in the present work.

c) Human Malarias:-

The species of human malaria used were P. vivax (Chesson strain), P. falciparum and P. malariae, the latter two being used in The Gambia.

Gametocyte Carriers

a) Mice Gametocyte Carriers:-

All the rodent malarias were maintained in a strain of white mice (Theiler's original mice). This strain is highly susceptible to all the mouse malarias used in this work. Yoeli (1965) and Wery (1968) reported that in trophozoite-induced infections of P. berghei the white mouse had been the most widely used experimental host, with susceptibility of nearly 100%. Male mice of four to six weeks old, weighing between 12 - 20 grammes were found to be susceptible to the four species of mouse malarias, i.e. P.b. berghei, P. yoelii, P. chabaudi and P.v.nigeriensis The taxonomic nomenclature of the rodent malarias used in this study is that proposed by Killick-Kendrick (1974). It was found during the present work that the infection of P. b. berghei is fatal to this strain of mice, and always terminated in death after eight to ten days of being infected with the parasite. P. yoelii, P. chabaudi and P.v.nigeriensis were found to run similar courses of infection in the white mice and were seldom fatal.

For each of the rodent malaria infection experiments, a group of two to four white mice were inoculated with deep frozen blood. Thin blood smears were made daily, stained in Giemsa and examined for the presence or absence of gametocytes. The number of gametocytes and exflagellation were also observed. The rising of parasitaemia takes three to six days in this group of mice injected with deep frozen blood.

Another group of two to four mice were sub-inoculated. The building up of parasitaemia is of a very quick and explosive nature. After three days the mouse with a good gametocyte count was used as the gametocyte carrier to infect the mosquitoes.

b) Simian Gametocyte Carriers:-

A rhesus monkey infected with simian malaria, P. knowlesi, and a chimpanzee infected with human malaria, P. vivax Chesson strain, were used to feed the mosquitoes.

c) Human Gametocyte Carriers:-

In The Gambia, the opportunity was given to feed the mosquitoes on human volunteers suffering from P. falciparum and P. malariae.

All the infected mosquitoes were kept under controlled conditions of temperature and humidity. The temperature in the insectary was always changed to match the optimum temperature for completion of the sporogonic cycles of P. b. berghei, P. yoelii, P.y.nigeriensis, P. chabaudi, P. knowlesi and P. vivax, i.e. 20, 24, 26, 26, 27 and 27°C respectively. The mosquitoes fed on human volunteers suffering from P. falciparum and P. malariae were kept in an insectary where the temperature was 28 - 30°C.

Infection of Mosquitoes

To standardize the method, the mouse of the third blood passage was used in each experiment. After three days (72 hours) and after being sub-inoculated, thin blood films were made from the tail of the mouse at the time of feeding. These films were fixed with methyl alcohol and stained with Giemsa for 15 - 20 minutes. The number of male and female gametocytes were counted under oil immersion lens. Parasitaemia ranged from 12 - 16 per 100 red blood cells and 2 - 5% of the parasites found were gametocytes.

In the case of the mouse infected with P. chabaudi, the mouse was introduced into the cages on the eighth day after blood sub-inoculation. This was because no gametocytes were seen before that date. Yoeli et al (1966a) working with P. chabaudi, reported that parasitaemias rise progressively to reach a first peak at about the eighth day descending to low levels and passing into latency with an occasional exacerbation in sub. equent months.

The infected mouse was introduced into the mosquitoes' cage lying on its back on a wooden board, immobilised by means of adhesive plaster and drawing pins. The abdomen was closely shaved.

The mosquitoes were always fed overnight from late afternoon until the next morning, the cages being fed in sequence on a single infected mouse for one hour each. Ward (1963) showed that no significant difference in mean oocyst counts were found among replicate lots despite a lapse of up to three hours between feedings. All the males and unfed or partially fed female mosquitoes were discarded. The fully engorged females were left undisturbed for five days or tubed immediately after feeding when keeping them in a constant temperature cabinet.

On several occasions for unknown reasons the mosquitoes were very reluctant to feed on the immobilized mouse. Switching the light off, and putting the cage under an electrical bulb all failed to stimulate the mosquitoes into feeding. In some cases the mouse was left for more than two hours in the cage but the mosquitoes would still not feed on it. Under such circumstances, all the unfed mosquitoes were sucked out and 15 - 20 females were placed in small glass tubes of

1" X 3" covered with netting. Two to four tubes at a time were put against the sides of the abdomen of the mouse and within five to ten minutes nearly all the mosquitoes were fed by this method.

Another one or two feeds was normally offered after egg laying. These extra blood meals were done on clean mice or on human arm.

In some experiments and before dissection, infected mosquitoes were allowed to feed on clean mice to ascertain the infectivity of their sporozoites.

Thirteen to fourteen days after having the infective blood meal, (Yoeli, 1965, and Wery, 1968, found that the sporogonic development of P. berghei in A. quadrimaculatus takes 13 - 14 days when the infected mosquitoes were maintained at 21°C) all the surviving mosquitoes were killed and dissected in one or two drops of normal saline according to the method recommended by Shute and Maryon (1966). The presence or absence of the parasite, the number of oocysts on the wall of the stomach and the density of the sporozoites in the salivary glands were recorded.

RESULTS

EXPERIMENT 1

SELECTION FOR SUSCEPTIBILITY AND REFRACTORINESS

Three cages of PALA, LSW and BEECH, each containing more than 200 mosquitoes, males and females, were first allowed 5 - 7 days for copulation and were then fed on a mouse infected with P. b. berghei. Fully engorged females were separated and placed singly in test tubes, each provided with a covering net to prevent the mosquitoes escaping.

A specific serial number, i.e. P_1, P_2, P_3 and L_1, L_2, L_3 etc., was given and written on the tubes. Control mosquitoes were left without numbering. The tubes, containing the fully fed mosquitoes, were kept in wire racks, forty tubes in each. All the racks were kept in a room where the temperature was kept constant at $20^0 \pm 1^0C$ with a relative humidity of 80%. Late in the afternoon on the fourth day after feeding on the infected mouse, a few drops of water were added to all tubes to allow for egg laying. The following morning, eggs were usually deposited. The hungry females were transferred to another set of tubes with the same serial numbers and another blood meal was offered, this time on a clean mouse or on a human arm. Two or three egg batches were collected by this same method. No food of any sort was offered between the blood meals.

The mosquitoes which died within the first eight days after feeding on the infected mouse were discarded and their egg batches were eliminated; those which died between the ninth and thirteenth days were dissected and their results recorded. On the fourteenth day, the period of two weeks which is required for the completion of the sporogonic cycle of P. b. berghei in the invertebrate host, all the survivors were killed and dissected for both stomach and salivary glands. The number

of oocysts on the wall of the stomach were counted and the presence or absence of sporozoites in the salivary glands recorded.

In the first few experiments, death among the tubed females was encountered, but later on it was learnt that the gentle handling of the fragile fully fed females, and feeding them as soon as the blood disappeared from the stomach were the two major contributors to their survival.

Selection

The type of selection used was by pooling the emerging adults resulting from two to three egg batches obtained from a single positive female for the selection of the susceptible line, and pooling two to three egg batches from a single female, negative for infection of the stomach and salivary gland, in the case of the selection for the refractory line. After emerging, adult mosquitoes were left for one week to allow for brother-sister mating. This kind of mating was followed throughout the selection experiment. Mass mating of brothers and sisters was necessary because single pairs of A. gambiae species A will not mate in captivity. This ruled out the method used by Macdonald (1962) in selecting for susceptibility of Ae. aegypti to B. malayi in which he was able to deduce the genotype of individual males by single pair mating them to a group of females and observing the progeny from each. In the present experiments there was no way of testing the genotype of males and it was necessary to assume that since the males used were the sons of selected females, the male genotypes would gradually change in the desired direction during the course of selection. Another problem was encountered by Huff (1929) using C. pipiens and P. cathemerium and Macdonald (1962a) in their works for selection of a

highly susceptible strain of Ae. aegypti for infection with sub-periodic B. malayi, where the close inbreeding resulting from repeated full sib mating affected the pupal stage in which mortality was high. However, in the present work with Anopheles, the adverse effects of inbreeding were not severe and did not prevent nine generations of full sib mating.

There is a variety of intrinsic factors which can affect oocyst development in a susceptible mosquito host. Among these are temperature and humidity (Chao and Ball, 1962; Ward, 1963; Wery, 1968; Yoeli and Upmanis, 1968) age and nutrition of the mosquito (Terzian et al, 1956) and relative size of the infective meal (Hovanitz, 1947). These factors have been held constant under the conditions described under the heading of Materials and Methods.

The normal susceptibility rate of the parental stock of PALA to P. b. berghei infection ranged from 60 to 70% while the rate of susceptibility of the parental LSW to the same parasite fluctuated from 30 to 63%. These rates were used as baseline values to evaluate any progress of the selection studies.

As mentioned previously, selection proceeded steadily on both lines, except during the time when there was a big fluctuation in temperature during June and August, 1973, when it rose to 25°C in the insectary and down to 16°C in the constant temperature cabinet respectively. In actual fact, this fluctuation did not affect the process of selection for the higher susceptibility but it did in the case of selection for the refractory line because here the author could not differentiate between the negative females of the line LD, whether the female was negative

because of the selection or because of the change in the optimum temperature required for the developing parasite. This might explain the noticeable fluctuation in the rate of susceptibility in the F_4 and F_6 in selecting for the refractory line, as is shown in Table 3. Although selection was not effective on two occasions in F_5 and F_7 because of the big fluctuation in temperature, selection for the high susceptibility was steadily increasing even on those two occasions, while selection for refractory line showed a considerable decrease in rate of susceptibility from 13.6% in F_6 to 0% in F_8. Hicks (1949) commenting on the problem he encountered in the course of selection, reported that although fifth generation females of C. pipiens were fed on an uninfected duck, a marked rise in the incidence of infection occurred in the following generation to infection with P. elongatum. It has been found in the present work that 2 - 3°C above or below 20°C can affect resulting infectivity. It is worth mentioning here that while high temperature does not necessarily kill the parasite of P. b. berghei in the mosquitoes, low temperature really does.

Table 3 shows a consistent trend towards increased susceptibility of the females of PALA line, and a steady decrease in the rate of susceptibility of LSW line, while the control exhibited no trend of changes in the parasite infectivity.

The rate of infection in PALA increased from 52.4% to 95.7% in one generation of selection. Continuation of selection for five more generations resulted in a 100% susceptibility rate. Moreover, that level of susceptibility was maintained in subsequent generations with no fluctuations. On the other hand, a decrease in the rate of

TABLE 3

THE RESULTS OF FEEDING 9 GENERATIONS OF SELECTED SUSCEPTIBLE (PALA) AND REFRACTORY (LSW) STRAINS OF *A. GAMBIAE* ON MICE INFECTED WITH *PLASMODIUM BERGHEI BERGHEI*

GENERATION	DATE OF FEEDING 1973	STRAINS					
		PALA (Parental stock)		LSW (Parental stock)		*A. STEPHENSI* (CONTROL)	
		Dissected Mosquitoes	% Susceptible	Dissected Mosquitoes	% Susceptible	Dissected Mosquitoes	% Susceptible
F_1	1/2	21	57.1	24	62.5		
F_2	10/3	21	52.4	33	27.3		
F_3	17/4	23	95.7	50	28.0	10	100
F_4	17/5	60	93.3	74	9.5	13	100
F_5	16/6						
F_6	17/7	43	95.4	59	13.6	9	100
F_7	17/8						
F_8	18/9	13	100	48	4.2*	10	100
F_9	18/10	43	100	60	5.0*	13	100

* Degenerated oocysts but no sporozoites were found in the salivary glands of these mosquitoes

susceptibility in selection for the refractory line was from 62.5% to 27.3% in one generation only. As in the case of selection for higher susceptibility, 100% refractoriness was established after only five more generations. In addition, females in the refractory line of LSW showed a continuous drop in the numbers of oocysts and sporozoites. Two and three females with abortive infection were seen in F_8 and F_9 respectively, with one to two completely degenerated oocysts on the wall of the stomachs. The salivary glands of those mosquitoes were absolutely negative for the presence of sporozoites.

By generation F_8, two lines of mosquitoes homozygous for susceptibility and refractoriness to P. b. berghei were considered established.

At the end of F_9, the offspring of ten highly susceptible females were reared together and maintained thereafter as a separate susceptible colony designated as PB or selected susceptible stock. The offspring of another ten negative females of the line LSW at the end of F_9 were pooled together and designated as LD or selected refractory stock.

EXPERIMENT 2

STUDY OF THE MODE AND PATTERN OF INHERITANCE OF SUSCEPTIBILITY AND REFRACTORINESS

Crosses were made between the selected susceptible and selected refractory lines of mosquitoes. Reciprocal crosses were made between the two selected strains and the F_1 offspring resulting from both crosses were selfed and backcrossed to the parent strains and the F_2 offspring and the offspring of each of the eight possible backcrosses were tested for their ability to support infections of P. b. berghei. The results are summarised in Tables 4a, 4b, 4c, 4d and 5.

Tables 4a and 4d show that from the cross of LD♀♀ X PB♂♂ almost all the progeny (104 out of 109) were found to be susceptible to the infection with P. b. berghei. In three successive experiments much the same results were obtained and the average rate of susceptibility for three replicates was 95.67%. From these results it would appear that the susceptibility to infection with mouse malaria is dominant and refractoriness is recessive.

However, from the reciprocal cross PB♀♀ X LD♂♂ a much larger proportion of the progeny were refractory when tested for infection with P. b. berghei. The overall rate of susceptibility was 63.54%. A similar difference between reciprocal crosses was seen in the "sporozoite index" (Table 4a) which is a measure designed to take account not only of the proportion of mosquitoes positive for infection, but also the intensity of the infection in positive mosquitoes.

The unexpected difference between the progenies of the reciprocal F_1's

TABLE 4a

DATA ON SPOROZOITE INFECTION FOR EACH REPLICATE OF THE LD AND PB SELECTED LINES AND THE F_1 FROM THE TWO RECIPROCAL CROSSES BETWEEN THEM

PARENTS	REPLICATE NO.	PROGENY								
		NO. OF MOSQUITOES						% POSITIVE	TOTAL SPOROZOITE SCORE	SPOROZOITE INDEX
		TOTAL	-	+	++	+++	TOTAL POSITIVE			
LD	0*	101	101	0	0	0	0	0	0	0
	i	2	2	0	0	0	0	0	0	0
	ii	20	19	1	0	0	1	5.0	1	0.05
	iii	20	20	0	0	0	0	0	0	0
TOTAL		143	142	1	0	0	1	.7	1	0.01
PB	0*	114	0	14	9	91	114	100	305	2.68
	i	15	0	4	1	10	15	100	36	2.40
	ii	19	1	2	2	14	18	94.74	48	2.53
	iii	20	0	0	0	20	20	100	60	3.00
TOTAL		168	1	20	12	135	167	99.4	449	2.67
F_1 PB♀♀ X LD♂♂	i	20	8	5	3	4	12	60.00	23	1.15
	ii	61	14	12	19	16	47	77.05	98	1.61
	iii	28	13	6	1	8	15	53.57	32	1.14
TOTAL		109	35	23	23	28	74	67.89	153	1.40
F_1 LD♀♀ X PB♂♂	i	35	1	6	2	26	34	97.14	88	2.51
	ii	45	3	5	6	31	42	93.33	110	2.44
	iii	29	1	6	4	18	28	96.55	68	2.34
TOTAL		109	5	17	12	75	104	95.41	266	2.44

NOTES on abbreviations used in Tables 4a, 4b, 4c and 4d

1. Mosquitoes with up to approximately 10 sporozoites are classified as +
   " " " " " 100 " " " " ++
   " " over 100 sporozoites are classified as +++

2. Total sporozoite score = the sum of: (1 X number of mosquitoes classified as +)
   (2 X " " " " " ++)
   (3 X " " " " " +++)

3. Sporozoite index = total sporozoite score ÷ total number of mosquitoes dissected.

* The end product of the selection of the two parental lines LD and PB.

TABLE 4b

DATA ON SPOROZOITE INFECTION FOR EACH REPLICATE OF THE BACKCROSSES TO THE LD (REFRACTORY) STRAIN

PARENTS ♀♀	PARENTS ♂♂	REPLICATE NO.	PROGENY								
			NO. OF MOSQUITOES						% POSITIVE	TOTAL SPOROZOITE SCORE	SPOROZOITE INDEX
			TOTAL	-	+	++	+++	TOTAL POSITIVE			
*F_1(PB♀♀ X LD♂♂)	X LD	i	20	19	0	1	0	1	5.0	2	0.10
		ii	14	12	0	2	0	2	14.29	4	0.29
		iii	64	60	3	0	1	4	6.25	6	0.09
TOTAL			98	91	3	3	1	7	7.14	12	0.12
LD	X F_1(PB♀♀ X LD♂♂)	i	-	-	-	-	-	-	-	-	-
		ii	24	17	4	0	3	7	29.17	13	0.54
		iii	35	28	5	2	0	7	20.00	9	0.26
TOTAL			59	45	9	2	3	14	23.73	22	0.37
*F_1(LD♀♀ X PB♂♂)	X LD	i	14	8	0	5	1	6	42.86	13	0.93
		ii	23	18	4	1	0	5	21.74	6	0.26
		iii	68	54	13	0	1	14	20.59	16	0.24
TOTAL			105	80	17	6	2	25	23.81	35	0.33
LD	X F_1(LD♀♀ X PB♂♂)	i	7	7	0	0	0	0	0	0	0
		ii	21	18	2	1	0	3	14.29	4	0.19
		iii	31	31	0	0	0	0	0	0	0
TOTAL			59	56	2	1	0	3	5.08	4	0.07

* These females proved susceptible when tested by oral infection with P. b. berghei

TABLE 4c

DATA ON SPOROZOITE INFECTION FOR EACH REPLICATE OF THE BACKCROSSES TO THE PB (SUSCEPTIBLE) STRAIN

PARENTS ♀♀ ♂♂	REPLICATE NO.	PROGENY								
		NO. OF MOSQUITOES						% POSITIVE	TOTAL SPOROZOITE	SPOROZOITE INDEX
		TOTAL	-	+	++	+++	TOTAL POSITIVE			
*F_1(PB♀♀ X LD♂♂) X PB	i	7	0	1	0	6	7	100	19	2.71
	ii	15	15	0	0	0	0	0	0	0
	iii	62	52	8	1	1	10	16.13	13	0.21
TOTAL		84	67	9	1	7	17	20.24	32	0.38
PB X F_1(PB♀♀ X LD♂♂)	i	24	4	6	3	11	20	83.33	45	1.87
	ii	17	13	3	0	1	4	23.53	6	0.35
	iii	20	13	4	1	2	7	35.00	12	0.60
TOTAL		61	30	13	4	14	31	50.82	63	1.03
*F_1(LD♀♀ X PB♂♂) X PB	i	2	0	0	0	2	2	100	6	3.00
	ii	28	18	5	2	3	10	35.71	18	0.64
	iii	33	0	1	2	30	33	100	95	2.88
TOTAL		63	18	6	4	35	45	71.43	119	1.89
PB X F_1(LD♀♀ X PB♂♂)	i	12	0	0	1	11	12	100	35	2.92
	ii	21	15	2	2	2	6	28.57	12	0.57
	iii	40	5	17	8	10	35	87.50	63	1.58
TOTAL		73	20	19	11	23	53	72.60	110	1.51

* These females proved susceptible when tested to the infection with P b berghei

TABLE 4d

SUMMARY TABLE OF DATA OF SPOROZOITE INFECTIONS OF P. B. BERGHEI OF THE TWO SELECTED LINES LD AND PB, THE F_1's FROM THE TWO RECIPROCAL CROSSES AND THE PROGENY OF ALL POSSIBLE BACKCROSSES

CATEGORY		PARENTS ♀♀	PARENTS ♂♂	PROGENY NO. OF MOSQUITOES TOTAL	-	+	++	+++	% POSITIVE	SPOROZOITE INDEX
Selected lines	1	LD	LD	143	142	1	0	0	.7	.01
	2	PB	PB	168	1	20	12	135	99.4	2.67
F_1 hybrids	3	PB	LD	109	35	23	23	28	67.89	1.40
	4	LD	PB	109	5	17	12	75	95.41	2.44
Backcrosses to LD stock:	5	F_1(PB♀♀ X LD♂♂)	LD	98	91	3	3	1	7.14	.12
	6	F_1(LD♀♀ X PB♂♂)	LD	105	80	17	6	2	23.81	.33
	7	LD	F_1(PB♀♀ X LD♂♂)	59	45	9	2	3	23.73	.37
	8	LD	F_1(LD♀♀ X PB♂♂)	59	56	2	1	0	5.08	.07
Backcrosses to PB stock:	9	F_1(PB♀♀ X LD♂♂)	PB	84	67	9	1	7	20.24	.38
	10	F_1(LD♀♀ X PB♂♂)	PB	63	18	6	4	35	71.43	1.89
	11	PB	F_1(PB♀♀ X LD♂♂)	61	30	13	4	14	50.82	1.03
	12	PB	F_1(LD♀♀ X PB♂♂)	73	20	19	11	23	72.60	1.51

TABLE 5

SHOWING THE SUSCEPTIBILITY OF THE PARENTAL REFRACTORY AND SUSCEPTIBLE LINES AND F_1 AND F_2 OFFSPRING TO P. B. BERGHEI

CATEGORY		PARENTS ♀♀	PARENTS ♂♂	NUMBER OF MOSQUITOES DISSECTED +	NUMBER OF MOSQUITOES DISSECTED -	PERCENTAGE OF SUSCEPTIBILITY
Selected lines:	1	LD	LD	-	60	0.0
	2	PB	PB	43	-	100
F_1 hybrids:	3	PB	LD	41	26	61.19
	4	LD	PB	43	5	89.58
F_2 offspring:	5	F_1(PB♀♀ X LD♂♂)	F_1(PB♀♀ X LD♂♂)	95	32	74.80
	6	F_1(LD♀♀ X PB♂♂)	F_1(LD♀♀ X PB♂♂)	38	70	35.19

was shown to be statistically significant by the method detailed in the Appendix. The existence of a difference between reciprocal crosses might suggest involvement of cytoplasmic factors, but the direction of the difference is not the expected for maternally inherited cytoplasmic particles. On the contrary it is the male parent which appears to have a predominant effect on the susceptibility status of the progeny. (Table 4d).

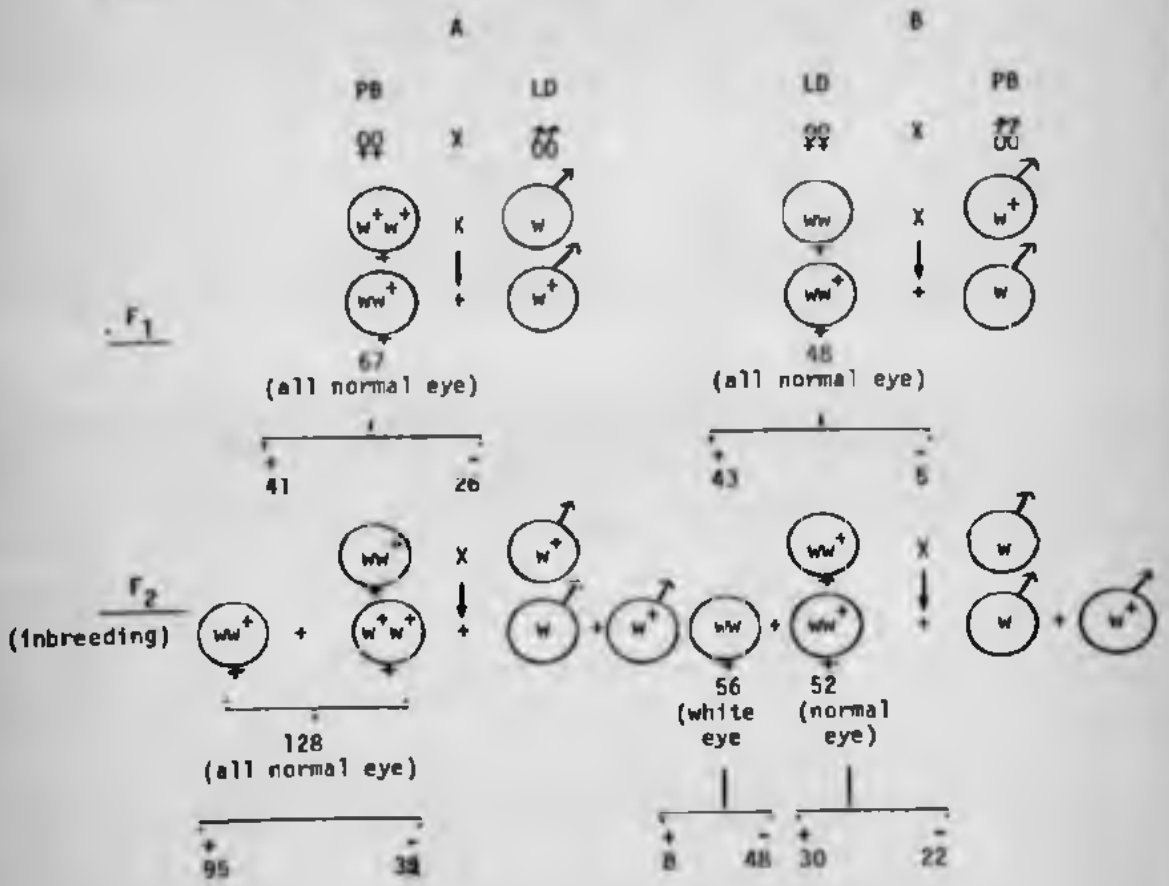
In the progeny of the backcrosses of the F_1's to the parent strains, the two characters (susceptibility and refractoriness) re-appeared but not in any known Mendelian ratio.

Table 5 gives the results of further reciprocal crosses between the LD and PB lines and the results of selfing the F_1's and the produce F_2's. Once again the F_1's showed a difference between the reciprocal matings in the same direction as that noted previously. It can also be seen that while the F_2 generation derived from the parental mating between the LD males and the PB females gave a near perfect 3:1 ratiothe other F_2 generation did not.

An attempt was made to correlate the inheritance of the two characters of susceptibility and refractoriness with that of the colour of the eye. As noted above, the susceptible population has normal wild type (black) eye colour while the refractory line has white eye since it was derived from the LSW stock which carries the sex linked recessive mutant white eye (Mason, 1967). Figure 1 shows in cross A that from the cross of normal-eyed PB$\varphi\varphi$ X white-eyed LD$\sigma\sigma$ an F_1 generation was obtained in which all the progeny had normal eyes. When the F_1 progeny was inbred to produce F_2 progeny, again, all the female offspring were normal-eyed, as would be expected. Figure 1 also shows cross B

FIGURE 1

Relationship between the colour of the eye and the infectability of mosquitoes with P. b. berghei in F_2 offspring resulting from crossing the LD and PB lines



w^+ - Dominant gene for normal eye

w - Sex linked recessive gene for white eye

where white-eyed LD♀♀ X normal-eyed PB♂♂ gave an F_1 generation in which all the females were normal-eyed and all the males where white-eyed. When the F_1 progeny was inbred to produce the F_2, 108 females survived until the time of dissection. Fifty-two were normal-eyed and fifty-six were white-eyed which agrees well with the expected 1:1 ratio. Dissection revealed that thirty out of fifty-two normal-eyed females were found positive for infections with P. b. berghei, while only eight out of fifty-six white-eyed females were found positive for the same parasite. The chi-square values for these data proved highly significant (x^2 = 22.28 P<0.001).

In only two out of the eight backcrosses of the F_1's to the parental strains was there segregation of white-eyed and normal-eyed females. Table 6 shows that in the progeny of these two backcrosses the overall number of normal-eyed females at the time of dissection was 100 out of which 25 females were found positive for the infection of P. b. berghei, while among the 103 white-eyed females, only 7 were found positive. A significance test on these results also proved significant (x^2 = 12.48 P<0.001).

If refractoriness is dependent on a single recessive autosomal gene (Fig. 2) we would expect the following results from both the mating of LD♀♀ X PB♂♂ and the reciprocal. The F_1 would be 100% susceptible while the F_1 backcrossed to the susceptible would give 100% susceptible progeny and it would give 50% susceptible and 50% refractory in the case of backcrossing to the refractory parent, as shown in Figure 2. Comparing the expectations from this with the observed results for the LD♀♀ X PB♂♂ cross the F_1 progeny showed almost complete recessiveness of refractoriness but in the case of the offspring resulting from backcrossing the F_1 to the susceptible and to the refractory parents,

TABLE 6

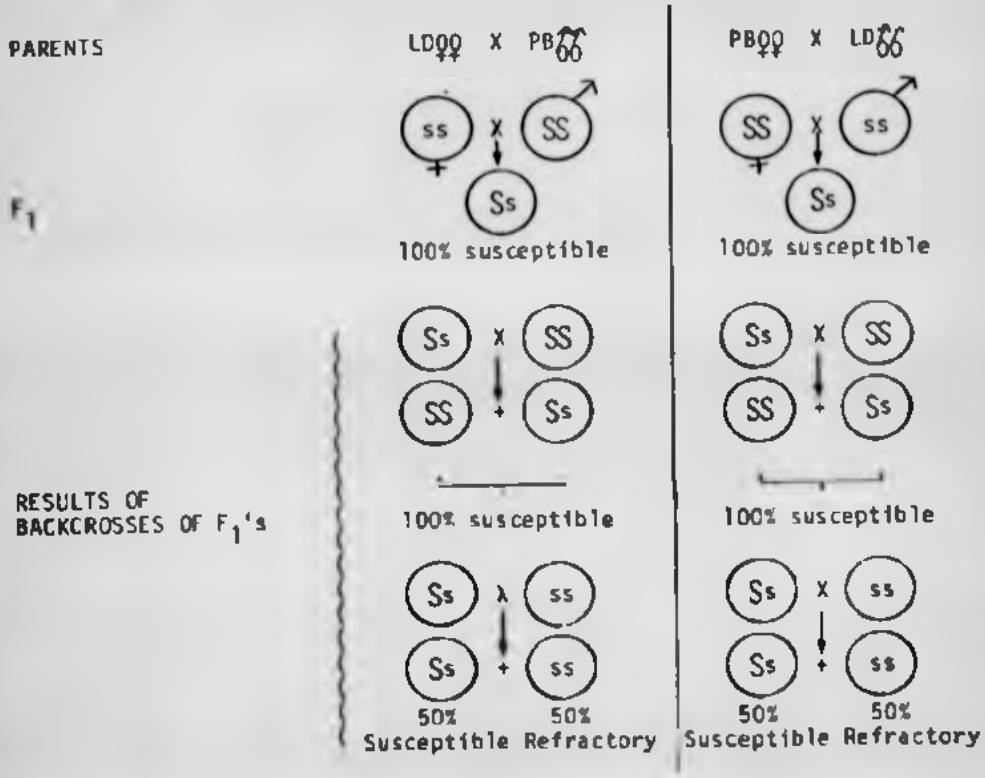
RELATIONSHIP BETWEEN THE COLOUR OF THE EYE AND THE INFECTABILITY OF MOSQUITOES WITH P. B. BERGHEI IN THE OFFSPRING OF BACKCROSSES [illegible] THE LD AND PB LINES

COLOUR OF THE EYE	REPLICATE NUMBER	DISSECTED MOSQUITOES	
		+	-
		F_1(LD♀♀ X PB♂♂) X LD♂♂	
NORMAL	1	4	2
	11	5	8
	111	12	34
Total		21	44
WHITE	1	2	6
	11	0	10
	111	2	20
Total		4	36
		F_1(PB♀♀ X LD♂♂) X LD♂♂	
NORMAL	1	0	9
	11	2	4
	111	2	18
Total		4	31
WHITE	1	1	10
	11	0	8
	111	2	42
Total		3	60
	GRAND TOTAL		
NORMAL		25*	75*
WHITE		7*	96*

* Heterogeneity χ^2 = 12.48 P<0.001

FIGURE 2

Hypothesis that the refractoriness is due to a single autosomal recessive gene: the expected results of reciprocal crosses between the susceptible and refractory lines and the backcrosses



S = dominant allele for susceptibility to the infection by P.b. berghei

s = recessive allele for refractoriness to the infection by P.b. berghei

the expected 100% and 50% proportion of susceptibility were not found (Table 4d).

From the reciprocal mating, as already noted, a much lower degree of dominance of the susceptible type was observed in the F_1 and once again the backcross progenies did not approximate to the expected 100% and 50% susceptibility respectively (Table 4d).

It is concluded that the inheritance of susceptibility cannot be explained by the action of a single recessive autosomal gene for refractoriness. In view of the partial correlation of white eye with refractoriness noted above, the hypothesis that refractoriness is due to a single sex linked recessive gene appeared an important one to test. If refractoriness is due to a single sex linked recessive gene (Table 7, Fig.3), we would expect the following results from the mating of LD♀♀ X PB♂♂. F_1 females would be 100% susceptible. Backcrosses of F_1 females or males to the susceptible parent would give 100% susceptible female progeny. Backcrosses of F_1 males to the female refractory parent would give 100% refractory female progeny, while F_1 females backcrossed to male refractory parent would give 50% susceptible and 50% refractory female progeny.

Comparing these expected results from the supposed model with observed results in Tables 4a, 4b, 4c and 4d, it is found that the F_1 females almost fit the hypothesis of full dominance of susceptibility since the overall rate of susceptibility was 95.67% (Table 4a) of F_1 progeny resulting from the mating of LD♀♀ X PB♂♂. The observed results of backcrosses of the F_1 to the PB parent showed that although most of the dissected females were positive for infection, they were nowhere

TABLE 7

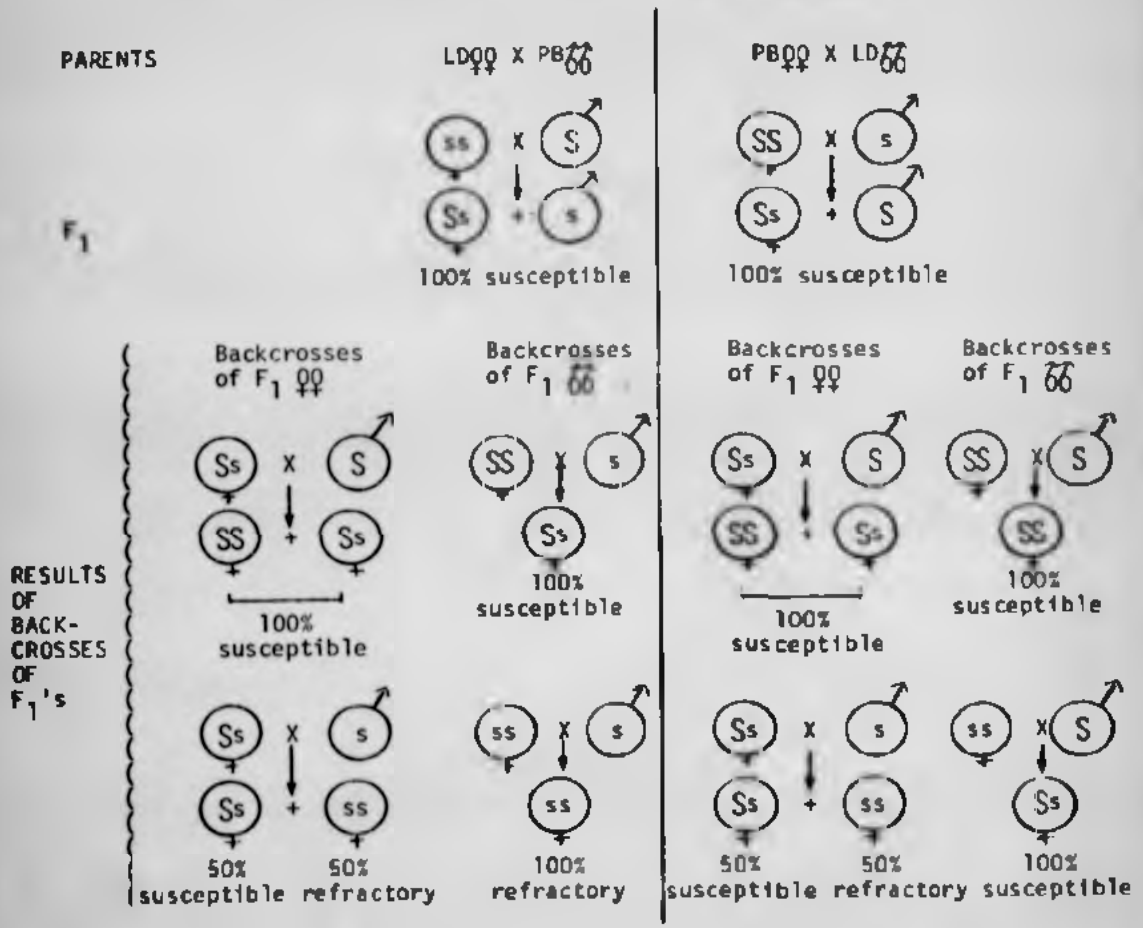
SUSCEPTIBILITIES WHERE REFRACTORINESS IS DEPENDENT ON A SINGLE RECESSIVE GENETIC FACTOR

CROSSES	ACTUAL (%)	IF SEX-LINKED (%)	IF AUTOSOMAL (%)
F_1 generations			
LD♀♀ X PB♂♂	95	100	100
PB♀♀ X LD♂♂	68	100	100
Backcrosses to refractory			
F_1♀♀(PB♀♀ X LD♂♂) X LD♂♂	7	50	50
F_1♀♀(LD♀♀ X PB♂♂) X LD♂♂	24	50	50
LD♀♀ X F_1♂♂(PB♀♀ X LD♂♂)	24	100	50
LD♀♀ X F_1♂♂(LD♀♀ X PB♂♂)	5	0	50
Backcrosses to susceptible			
F_1♀♀(PB♀♀ X LD♂♂) X PB♂♂	20	100	100
F_1♀♀(LD♀♀ X PB♂♂) X PB♂♂	71	100	100
PB♀♀ X F_1♂♂(PB♀♀ X LD♂♂)	51	100	100
PB♀♀ X F_1♂♂(LD♀♀ X PB♂♂)	73	100	100

LD = refractory PB = susceptible

FIGURE 3

Hypothesis that the refractoriness is due to a single sex-linked recessive gene



S = dominant allele for susceptibility to infection by P. b. berghei

s = recessive allele for refractoriness to infection by P. b. berghei

near 100% susceptible. The range of susceptibility of three replicates was 71.43 - 72.60%(Table 4c). The progeny of backcrosses of F_1 males to the refractory parent showed a lower susceptibility (5.08%) than the corresponding backcrosses to F_1 females (23.81%) (Table 4b). A difference in this direction is expected on the sex linked hypothesis (Fig.3) but the rates of susceptibility were far lower than the expected 50% and 100%. The difference between the reciprocal crosses appears to be the result of the same genetic factor detected in investigating the effect of white eye. The difference between reciprocal crosses is in the comparison of the F_1's from LD♀♀ X PB♂♂ and from PB♀♀ X LD♂♂. That is, in these backcrosses it was the matings when the LD stock was the female which showed the lower susceptibility.

In the reciprocal mating PB♀♀ X LD♂♂ the F_1 progeny would be expected to be 100% susceptible and the backcrosses of the F_1's to the PB would also yield 100% susceptible progeny. In the case of backcrossing to the refractory parental male, the results would be 50% susceptible females and 50% refractory ones, while in the case of backcrossing to the refractory parental female, the outcome would be 100% susceptible females. Now, if we compare the expected results with those observed (Table 4d) we find that the model does not accommodate the results neither in F_1 nor in F_1 backcrosses progeny.

Wherever the F_1 offspring was backcrossed to the refractory parent, the

resulting progeny showed a tendency to be negative to infection with P. b. berghei and to show a low sporozoite index. This was true in four cases out of four and irrespective of the original parental matings. (The F_1 backcrosses to LD were 7.14%, 23.73%, 23.81% and 5.08%; the sporozoite indices were 0.12, 0.37, 0.33 and 0.07 respectively. (Tables 4b and 4d)). Conversely in the case of backcrossing F_1 offspring to the susceptible parent where there was a tendency among the resultant progeny to be susceptible to the same infection and to show a high sporozoite index, this was true in three cases out of four. (F_1 backcrosses to PB were 20.24%, 50.82%, 71.43% and 72.60%; the sporozoite indices were 0.38, 1.03, 1.89, and 1.51 respectively. (Tables 4c and 4d)).

As shown in the Appendix there is evidence for a statistically significantly greater sporozoite index in the progeny of the four backcrosses to PB than in the four backcrosses to LD.

EXPERIMENT 3

INFECTIVE HEREDITY

There are many examples in the literature on infective heredity transmitted through the cytoplasm.

A good example of infective heredity was found in protozoa. Beale (1954) reported inheritance of the killer trait (kappa) in Paramecium aurelia. When no cytoplasm is transferred during conjugation, the killer trait is transmitted to those progeny receiving their cytoplasm from the killer-type parent, whereas in the event of cytoplasmic exchange, all progeny become killers. Thus the inheritance of the killer progeny strictly follows transmission of cytoplasm, and is independent of gene segregation provided the genotype will support the cytoplasmic factor, Kappa. Kappa is a cytoplasmic determinant responsible for the killer trait. It is a large bodysome 0.2μ in diameter, containing DNA and protein, and it can be isolated from killer cells and introduced into sensitive cells converting them to killers; thus kappa is an infectious agent. Two single pairs of genes have been identified which influence kappa; the better known of these, K, is necessary for maintenance of kappa. Cells containing only k lose their kappa particles and become sensitive.

Another example of the cytoplasmic inheritance was found when Laven (1957), from backcrossing experiments, showed that the factors responsible for incompatibility between populations of the C. pipiens complex are transmitted through the maternal cytoplasm and that they persist through generations of backcrossing with no dilution of effect. Wolbachia pipientis, a small rickettsia-like symbiote, exists

in close association with the cytoplasm of the germ cells in both males and females and is carried from one generation to the next through the eggs. It is an extrachromosomal self-replicating unit which causes little pathology in the female but some cell death in males. Because of the symbiotic residency in the germ cells, it fits very well the cytoplasmic inheritance model developed by Laven. However, Wolbachia has been found only in certain members of the C. pipiens complex (Irving-Bell, 1974).

A sample experiment was carried out of many LD and PB both in the larval and adult stages to see if susceptibility or refractoriness were infective.

Two hundred larvae of the selected susceptible line PB were mixed with another two hundred larvae from the selected refractory line LD. After leaving them mixed for three to four hours in a small bowl, four inches wide with 100ml. tap water, the contents of the small bowl was divided into four big bowls. One hundred larvae were picked out at random and placed in the big bowl with 500ml. tap water and a clump of grass and mud. All four bowls were kept in a room where the temperature was 25°C.

After two weeks, the emerging adult mosquitoes were pooled into a single cage where they were fed on 20% glucose solution, and kept in a room where the temperature was 20°C. After another week, a good gametocyte carrier was introduced in the control cage of A. stephensi for thirty minutes and after that period the same infected mouse was introduced into the cage of mixed population of PB and LD mosquitoes. After another thirty minutes the mouse was taken out and the males, unfed females and partially fed females were discarded.

All the females were killed on the fourteenth day after their infective meal. Before dissection, the mixed females were sorted out according to the colour of the eye, i.e. if it was normal it was PB♀♀ and if it was white it was LD♀♀. Table 8 shows the results of dissection which indicate that the susceptibility to infection with P. b. berghei is not transmittable to the individuals of the other line under these test conditions.

TABLE 8

THE INFECTIVITIES OF A MIXED POPULATION OF THE SUSCEPTIBLE LINE (PB) AND THE REFRACTORY LINE (LD) AFTER BEING FED ON A MOUSE INFECTED WITH P. B. BERGHEI

STRAINS	DISSECTED MOSQUITOES			
	(+)	(-)	TOTAL	% SUSCEPTIBILITY
PB	83	-	83	100
LD	-	74	74	0
CONTROL *A. stephensi*	30	2	32	94

EXPERIMENT 4

CYTOPLASMIC INHERITANCE

The variable results obtained in Experiment 2 led to the suspicion that they might have been influenced by cytoplasmic inheritance. To confirm or deny this hypothesis, the following experiment was carried out.

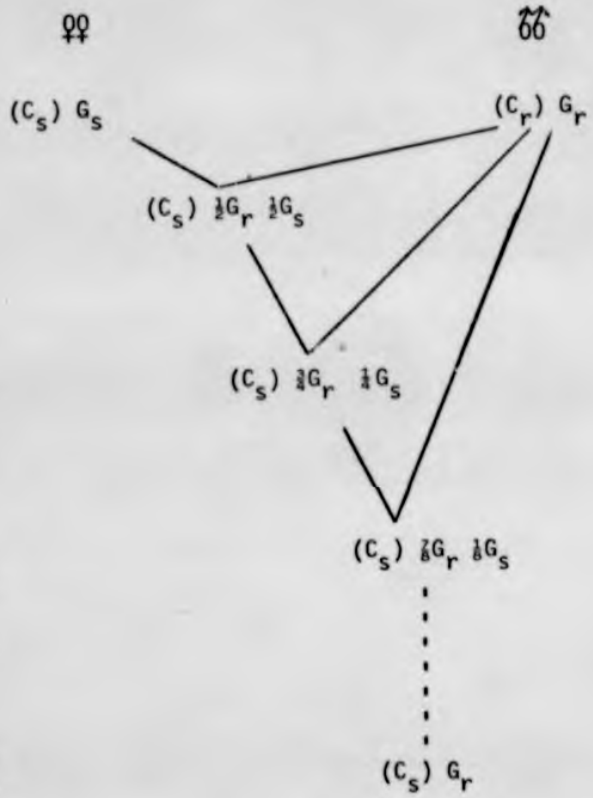
Fifty males of the selected refractory line (LD) were released in a cage where another fifty virgin females of the selected susceptible line (PB) were. After feeding them two to three times on guinea pigs over a period of five to seven days, eggs were collected and after hatching, larvae were reared under 25 - 27°C. Emerging female mosquitoes were carefully separated from males. The females were released into a new cage while their brothers were discarded. Newly emerged males from the parental refractory stock (LD) were mixed with F_1 virgin females. This procedure was repeated until F_{10} females were obtained from the continuous mating with the refractory males.

It was thought that after ten generations the susceptibility to *P. b. berghei* should be completely diluted if it was due to any genetic factor(s) rather than cytoplasmic inheritance (Fig. 4).

More than 150 females, all white-eyed, of F_{10} and more than fifty females of BEECH (control) were fed on a mouse infected with *P. b. berghei*. After two weeks, all surviving mosquitoes of F_{10} (56) were found negative for both stomachs and salivary glands, while the mosquitoes of the control were all positive (20).

FIGURE 4

Expectation of repeated backcrossing to LD males
on the hypotheses of either cytoplasmic or chromosomal inheritance
of susceptibility/refractoriness



C_s = hypothetical cytoplasmic particle for susceptibility

C_r = " " " " refractoriness

G_s = " chromosomal genes for susceptibility

G_r = " " " " refractoriness

EXPERIMENT 5

THE RATE OF SUSCEPTIBILITY OF THE TWO SELECTED LINES TO DIFFERENT SPECIES OF RODENT MALARIA

Each experiment with any of the rodent malarias took one month - two weeks for rearing healthy mosquitoes and two weeks for maturation of the parasite in the mosquito at 20°C. At the beginning of each experiment five cages of mosquitoes were ready for feeding on the infected mouse. These cages were selected susceptible line PB, PALA (the original parent stock from which PB was derived), selected refractory line LD, LSW (the original parent stock from which LD was established), and A. stephensi as control.

As is clear from Table 9, all the species of rodent malaria established their infection in the mosquitoes except P. chabaudi. This may be due to the system of introducing the infected mouse of the third blood passage on the third day after being injected with the infected blood; it appears that this does not work with this species of mouse malaria. Another attempt was made to introduce the infected mouse with P. chabaudi on the tenth day but this also did not establish infection in any of the five cages mentioned above.

Table 9summarises the results of feeding five cages of mosquitoes of PB, PALA, LD, LSW and the BEECH (A. stephensi) on mice infected with P. b. berghei (Fig.5), P. yoelii (Fig.6) and P.y.nigeriensis (Fig.7). The LD population maintained its status of being absolutely negative for stomach (oocysts) and salivary glands (sporozoites) when fed on mice with P. b. berghei and P.y.nigeriensis, while in the case of feeding on a mouse infected with P. yoelii, the rate of susceptibility was still negative for the presence of sporozoites while on the wall

TABLE 9

MOSQUITO TRANSMISSION OF P. B. BERGHEI, P. YOELII AND P.Y. NIGERIENSIS

PLASMODIUM	TEMP. °C	DISSECTED MOSQUITOES											
		Selected Susceptible Line PB		% Susceptibility	Parental Population (PALA)		% Susceptibility	Selected Refractory Line LD		% Susceptibility	Parental Population (LSW)		% Susceptibility	A.stephensi (CONTROL)		% Susceptibility
		(+)	(-)		(+)	(-)		(+)	(-)		(+)	(-)		(+)	(-)	
P.b. berghei	20	43	0	100	12	9	57.1	0	57	0.0	15	9	62.5	13	0	100
P.yoelii	24	18	1	94.7	12	6	66.7	1*	13	7.1	2	12	14.3	14	2	87.5
P.y.nigeriensis	26	10	8	55.6	10	12	45.5	0	21	0.0	2	16	11.1	8	10	44.4

* No sporozoites were seen in the dissected salivary glands nor in the fluid surrounding the stomach but 2 degenerated oocysts were seen.

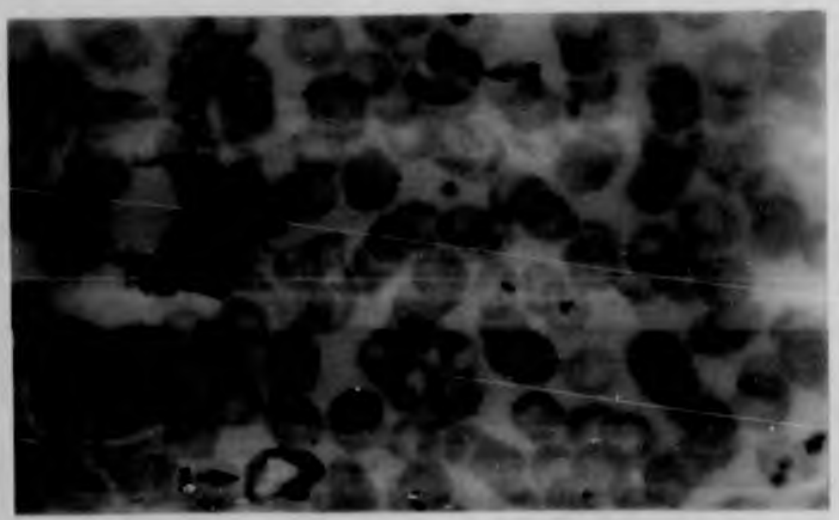


FIGURE 5

Macrogametocyte(1) and microgametocyte (2) of P. b. berghei in thin film of the peripheral blood of white mouse.
Giemsa stain (oil immersion)

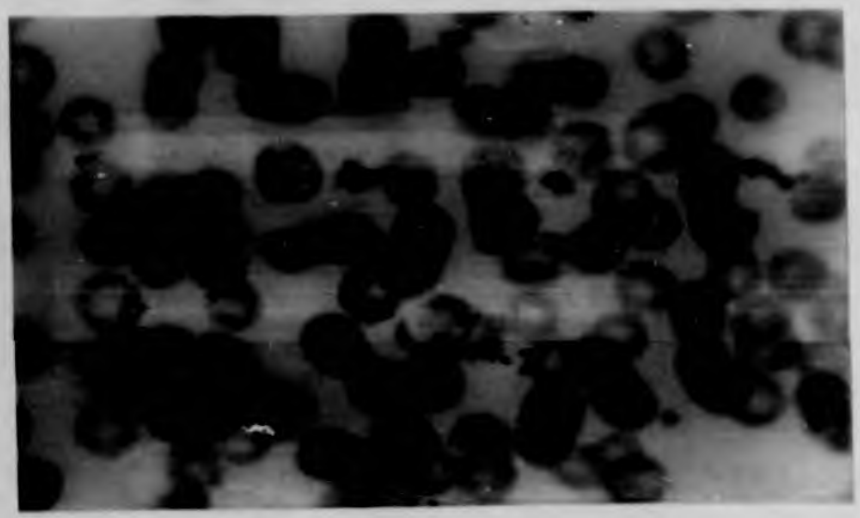


FIGURE 6

Macrogametocyte(1) and microgametocyte(2) of P. yoelii within blood film of white mouse. Giemsa stain (oil immersion)

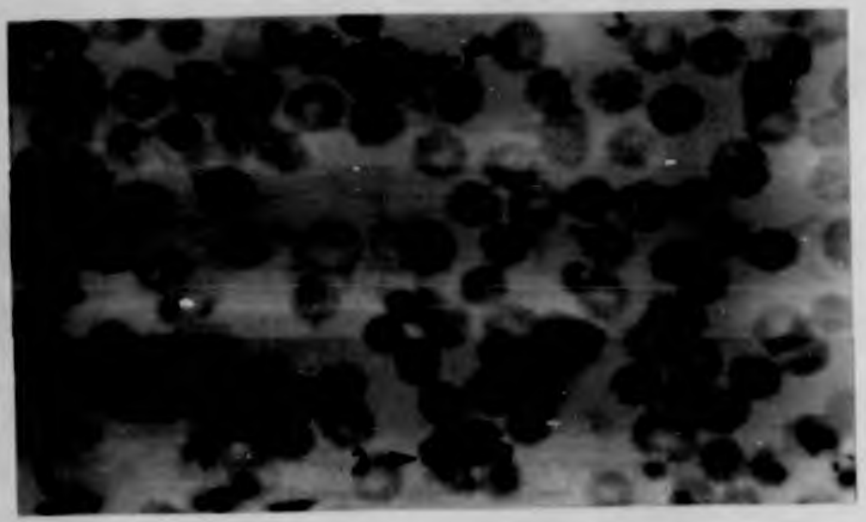


FIGURE 7

Macrogametocyte(1) and microgametocyte(2)
of P. y. nigeriensis in thin blood film of white mouse.
Giemsa stain (oil immersion)

of the stomach, two degenerated oocysts were seen.

The selected susceptible line, PB, proved 100% and 95% susceptible to infection with P. b. berghei and P. yoelii respectively. The P. y. nigeriensis strain used was not fully infective to either susceptible PB or to the control mosquitoes.

EXPERIMENT 6

TESTING THE SUSCEPTIBILITY OF THE TWO SELECTED LINES (PB AND LD) TO HUMAN AND MONKEY MALARIAS

To see if the genetic mechanism controlling infectivity to mouse malaria in the two selected lines of mosquito of A. gambiae species A also affected their susceptibility to monkey and human malarias, the following experiments were carried out:-

A. Feeding on monkeys infected with P. knowlesi and P. vivax.

B. Feeding on human patients infected with P. falciparum and P. malariae.

A. FEEDING ON MONKEYS

1) Rhesus Monkey infected with P. knowlesi

The opportunity to feed the mosquitoes on a rhesus monkey infected with P. knowlesi was provided by Dr. Richards of Wellcome Research. When the gametocyte count of a monkey malaria was thought high enough to feed the mosquitoes, three cages, PB, LD and BEECH, were taken to the Wellcome Research Laboratories where they were fed immediately on the monkey. After almost two hours, all the cages were back in the insectary where the temperature was 27°C and the humidity was 80%. After two weeks, dissection revealed no sign of infection in all three cages. To explain this pheromenon, I should say that the gametocytes of P. knowlesi were immature thus not being able to establish the infection in the invertebrate hosts.

ii) Chimpanzee infected with P. vivax Chesson strain

The opportunity was taken of the existence in the Institute of a chimpanzee infected with the Chesson strain of P. vivax. Infection of the chimpanzee was achieved by the intravenous inoculation of infected blood. Daily blood films, thick and thin, were done, starting one week after the inoculation of the infected blood. Two months before infecting the chimpanzee, its spleen had been removed to lower the defence mechanism in order to obtain a good level of parasitaemia and eventually a high count of gametocytes.

Three weeks after the inoculation, the level of parasitaemia was rising steadily. A blood count was done to assess the number of gametocytes which was considered to be high enough for feeding the mosquitoes. A fresh blood test was done to check the maturity of the gametocytes. Exflagellation was observed under both microscope and in humid petri dishes as described by Shute and Maryon (1966).

On the same day, late in the afternoon, the chimpanzee was given 5ml 'serylaw' injection in the buttock to sedate it. The five cages of PB, PALA, LD, LSW and BEECH were fed simultaneously by resting them against the abdomen and arms of the monkey. The cages were kept on for 40 - 45 minutes and the feeding of the mosquitoes was assessed visually. At the end of that period a good number of fully engorged females were secured in all five cages. All five cages were kept in a room where the temperature was 27^{o}C and 80% humidity.

On the twelfth day after feeding on the monkey, each of these five cages were brought up to the laboratory where the mosquitoes were killed and dissected for both the stomach and the salivary glands.

Table 10 shows the results of dissection of the five groups of mosquitoes, LD, LSW, PB, PALA and BEECH, and the successful establisnment of the infection in all cages except in that of LD which represents the selected line of refractory mosquitoes. As is clear from the table, the rate of susceptibility to infection with P. vivax in LSW, the parental population, was twice as high as that in LD.

Although the rate of infection in both PB and PALA was very high, it was still higher in PB.

The high rate of infection among the mosquitoes of the control (BEECH) is a clear evidence that the malaria parasite used in this experiment was of full infectivity.

P. vivax Chesson strain used in this experiment proved not only infective to the invertebrate host used, but also infective to the chimpanzee through the biting of infective mosquitoes. This was done when a few mosquitoes of the susceptible line, which two weeks earlier had been fed on that chimpanzee which was infected with the P. vivax, fed on another chimpanzee. Dissection of the salivary glands showed that these mosquitoes were heavily infected with viable sporozoites. One month later the second chimpanzee was found infected with P. vivax.

B. FEEDING ON HUMAN PATIENTS

In October 1975, the two lines, PB and LD, were taken to The Gambia in the form of egg batches, wrapped in wet filter paper and kept in a

TABLE 10

SUSCEPTIBILITY OF ANOPHELES GAMBIAE SPECIES A AND A. STEPHENSI TO PLASMODIUM VIVAX CHESSON STRAIN

PLASMODIUM	TEMP. °C	DISSECTED MOSQUITOES									
		Selected Susceptible Line PB	% Susceptibility	Parental Population (PALA)	% Susceptibility	Selected Refractory Line LD	% Susceptibility	Parental Population (LSW)	% Susceptibility	A. stephensi (CONTROL)	% Susceptibility
		(+) (-)		(+) (-)		(+) (-)		(+) (-)		(+) (-)	
P. vivax	26	60 0	100	50 6	89.3	30 36	45.5	59 7	89.4	28 3	90.3

small self-sealing plastic envelope. In the insectary of the Medical Research Council Laboratories at Fajara, the colonies of mosquitoes were established.

Gametocyte Carriers

There are two sources for securing patients with high counts of gametocytes:

1. Through the Outpatient Clinic of the Medical Research Council

   Five days a week, 30 - 40 patients visit the outpatient clinic of the MRC seeking treatment mainly for malaria. P. falciparum was found to be the most widely distributed malaria among the patients, reaching 90% or more, followed by P. malariae and to a lesser extent P. ovale. Double infections of P. falciparum and P. malariae are not uncommon.

2. Extensive Survey of School Children

   Four to five days before the mosquitoes were ready to be fed, an extensive survey was started until a patient with a good gametocyte count was found. This survey was done by choosing a school at Sukuta. Sukuta is one of the big villages in St. Mary District of The Gambia. It is connected with Fajara by a good road, only half an hour's drive from the MRC. The village is considered as holoendemic for malaria.

   Marsden (1964) described Sukuta as a semi-rural village

and he has chosen this village because of the goodwill and co-operation already present in the village and its proximity to available laboratory and ward facilities. There are some social services in the form of the school, infant welfare clinic and dispensary. Moreover, he reported regarding malaria that P. falciparum trophozoites were found in the blood of every baby studied and it was responsible for all the clinically severe attacks. P. malariae infections were detected in 15 babies out of 95, but always in conjunction with falciparum infections and one P. ovale infection was noted.

The blood films were taken back to the MRC malaria laboratory where they were stained by Field stain technique. All the slides of the stained thick films were examined under the oil immersion lens. Gametocytes of all three species of malaria were counted against the number of leucocytes. A rough estimate of the density of the gametocytes was done by counting up to 1,000 leucocytes. The slide which showed more than 100 gametocytes against 1,000 leucocytes was regarded as a promising gametocyte carrier (Fig.8). Counting was performed by having two hand numerators, the one in the right hand for counting the leucocytes while the other in the left counted the gametocytes.

Prior to feeding the mosquitoes on the patient, an exact blood count of the white cells, thin and thick blood films were done on each patient. The

FIGURE 8

Macrogametocyte (1) and microgametocyte (2) of P. falciparum in thin blood film of a 7 year old boy.
Giemsa stain (oil immersion)

precise number of gametocytes was found by enumerating them against the number of leucocytes. Since the exact number of the white cells was very easy to find in a given amount of blood in the patient by using the haemocytometer, the exact number of gametocytes per cubic mm. of the blood of the patient was easily obtained. Mosquitoes of LD and PB were usually put in 5" X 5" glass pots opened at both ends which were covered with mosquito net. After 15 - 20 minutes a good number of fully fed mosquitoes would be obtained.

Having finished feeding on the volunteer, the mosquito pots were taken back to the insectary where the temperature was maintained at 28 - 30°C and 80% humidity and the mosquitoes were sorted out. After an incubation period of ten to eleven days (Macdonald (1952) reported ten days for the completion of the sporogonic cycle of P. falciparum at 29°C), all survivors were killed and dissection and search for the oocysts on the stomach wall and sporozoites in the salivary glands were performed in one or two drops of normal saline. As late as this date after feeding on an infective gametocyte carrier one should bear in mind that most of the oocysts should have ruptured and very few oocysts should be expected on the wall of the dissected stomachs. In the same time one should expect a high load of sporozoites in the salivary glands. These two phenomena were clearly encountered among the dissected mosquitoes. Although dissection was basically aiming at the infective stage of the parasite, i.e. the sporozoites, the counting of the oocysts was not neglected. The stomach and the salivary glands of each mosquito were dissected and were searched for oocysts and sporozoites.

Feeding on human volunteers was in actual fact repeated seven times.

six times on patients suffering from P. falciparum and once on a child suffering from P. malariae. Three of these feeds (two on malignant tertian and one on quartan malaria) produced no mosquito infections in either line. This is perhaps because of the very low gametocyte count in the two patients with malignant tertian malaria, while the failure to achieve infection in the child with quartan malaria may have been due to the immaturity of the gametocytes since exflagellation could not be seen on the slides on that occasion. Table 11 summarises the results of four successful feeds. In all four experiments the mosquitoes of the selected refractory line (LD) showed a lower rate of infection with P. falciparum than that of the selected susceptible line (PB). However, only in two out of four experiments was the difference in susceptibility between LD and PB mosquitoes proved statistically significant.

I could not find any difference in the morphology of the oocysts or in the movement of the sporozoites associated with chloroquine treatment in experiments 1 and 2 where the same volunteer was used to infect the mosquitoes in both of the experiments.

TABLE 11

SUSCEPTIBILITY OF ANOPHELES GAMBIAE SPECIES A -TO PLASMODIUM FALCIPARUM FROM THE GAMBIA, WEST AFRICA

EXPERIMENT NUMBER	MOSQUITOES						PATIENTS		
	Selected Susceptible Line (PB)			Selected Refractory Line (LD)			Age	Gametocyte Count per cubic mm	Remarks
	+	-	% susceptibility	+	-	% susceptibility			
1	30	20	60.0	12	45	21.1	4 months	482	Before being treated with chloroquine $\chi^2 = 16.95$ $P<0.001$
2	44	5	89.8	50	15	76.9	4 months	3198	Same patient as above, after being treated with chloroquine $\chi^2 = 3.2$ $P>0.05$
3	42	2	95.5	27	12	69.2	6 years	2415	Before treatment $\chi^2 = 10.14$ $P<0.01$
4	40	2	95.2	49	5	90.7	9 years	2500	Before treatment $\chi^2 = .74$ $P>0.05$

EXPERIMENT 7

TESTING TO CHECK a) THE SUSCEPTIBILITY STATUS and b) THE INFECTIVITY OF THE SPOROZOITES IN THE FIVE LINES THREE YEARS AFTER CESSATION OF SELECTION

This experiment was designed to fulfil two purposes -

A. To check the susceptibility status not only in the two selected lines, PB and LD, for susceptibility and refractoriness, but also to check that of the original stocks PALA, LSW and the BEECH. Thus five cages of these lines of mosquitoes were used for feeding on a mouse suitably infected with *P. b. berghei*. Each cage contained more than four hundred mosquitoes, males and females. A single infected mouse of the third blood passage was introduced into each of the five cages for thirty minutes in the following sequence - BEECH, LD, PB, LSW and PALA. Fully fed females separated from the males, and the unfed and partially fed females were maintained in a room where the temperature was 20°C until the day of dissection. Under the previously described conditions, sporozoites usually reach maturity in about 13 - 14 days and accumulate in enormous numbers in the salivary glands on the fourteenth day or one or two days later. Table 12 summarizes the result of dissection. It is clear from the table that PB, the selected susceptible line, is 100% susceptible while LD, the selected refractory line is 0% susceptible to the infection with *P. b. berghei* and after more than three years of complete cessation of selactive pressure. However, slight fluctuations in the rate of infection of the two parental populations were noticed in

TABLE 12

MASS FEEDING OF THE FIVE LINES OF MOSQUITOES ON INFECTED MOUSE WITH PLASMODIUM BERGHEI BERGHEI TO CHECK THE RATE OF SUSCEPTIBILITY

PLASMODIUM	TEMP. °C	DISSECTED MOSQUITOES									
		Selected Susceptible Line PB	% Susceptibility	Parental Population (PALA)	% Susceptibility	Selected Refractory Line LD	% Susceptibility	Parental Population (LSW)	% Susceptibility	A.stephensi (CONTROL)	% Susceptibility
		(+) (-)		(+) (-)		(+) (-)		(+) (-)		(+) (-)	
P.b. berghei	20	88 0	100	29 29	50	0 95	0	17 57	23.0	41 0	100

comparison to three years earlier in infection with the same parasite of P. b. berghei (Table 3).

The viability of P. b. berghei did not show any change over more than three years and the parasite is fully infective to A. stephensi, the mosquitoes of the control.

B. To check the infectivity of the sporozoites in the five cages. To explore this, the following experiment was carried out: Thirty 4 - 6 week old uninfected white mice (Theiler original) were made ready to be fed upon. At this time the infection of P. b. berghei had matured enough to produce sporozoites in the infected mosquitoes. For experimental infections of the laboratory animals, ten mice were immobilized, each on a separate cork board, and introduced into the five cages, two mice in each cage, and left overnight. This was repeated once more on the following night. Thus four mice were fed upon by the mosquitoes of each of the five cages on the two consecutive nights. Each of these four mice were labelled on the tail and were kept in five separate cages in the animal house for further observation.

Another parallel experiment was done by feeding single infected mosquitoes on a single clean uninfected mouse. This was done as follows. One mosquito was sucked from the cage PB and put into a 3" X 1" tube covered with mosquito net and placed against the shaved abdomen of the mouse. After a few minutes, if the mosquito became fully engorged, it was dissected immediately to see the presence or absence of sporozoites. If this mosquito was positive the mouse was labelled and kept in the corresponding cage; if it was negative, the process of feeding was repeated by feeding another mosquito on the

same mouse. This process was continued until the feeding of two confirmed infected mosquitoes on two mice from each of the four cages was achieved. Although this process of feeding single infected mosquitoes on a single mouse was successfully done in the case of PB, PALA, LSW and the BEECH, it could not be done in the case of the mosquitoes of cage LD. In actual fact this attempt was repeated with more than twenty mosquitoes but all these attempts failed to find a single infected mosquito among them.

Two cages of mice for each of PB, PALA, LSW, BEECH and one cage for LD were maintained for each cage of mosquitoes. Daily examination of thin blood films was started after the third day of the mice being fed upon by the mosquitoes. All these slides were stained with Giemsa stain and examined with oil immersion. Though the normal period between infective feed and appearance of parasites in the peripheral blood is between three and seven days, to make absolute certain a follow-up to search for the infection in the mice was extended to three weeks in the case of the mice fed upon by mosquitoes from cage LD.

Table 13 summarizes the results of sporozoite induced P. b. berghei infections. Twenty-eight white mice, exposed to mass and single feeding upon by different infected populations of mosquitoes, were examined. As is clear from Table 13 all the mosquitoes of the line LD had failed completely to infect the exposed mice which had been left in the cage overnight on two consecutive nights.

On the other hand, a successful transmission of mouse malaria was achieved by exposing twenty-four mice to all the other lines of

TABLE 13

FREQUENCY DISTRIBUTION OF PREPATENT PERIOD OF SPOROZOITE-INDUCED P.B. BERGHEI INFECTIONS IN WHITE MICE

STRAIN of MOSQUITOES	NUMBER OF POSITIVE ANIMALS EXHIBITING PREPATENT PERIOD LASTING (DAYS)									REMARKS
	4	5	6	7	8	9	10	11 — 21	TOTAL	
PB		2	2		1*	1*			6	Eventually all died of high parasitaemia
PALA	1	3			2*				6	Eventually all died of high parasitaemia
LD										All remained alive and were parasite free until termination of the experiment
LSW			2	2	1*		1*		6	Eventually all died of high parasitaemia
BEECH	1	2	1			2*			6	Eventually all died of high parasitaemia

* Mouse (Mice) on the abdomen of which single infected mosquitoes were allowed to bite and engorge

mosquitoes (PB, PALA, LSW and BEECH). These mice had contracted the infection of P. b. berghei irrespective of whether they were subjected to mass feeding by large numbers of mosquitoes in the cages or to a single feeding by a single infected individual mosquito. Although some differences were noticed in the prepatent periods of infection in the different groups of mice, this may be attributed to the individual mouse immunity and to the load of sporozoites injected by the infected mosquito.

EXPERIMENT 8

THE FATE OF PLASMODIUM BERGHEI BERGHEI IN THE REFRACTORY AND IN THE SUSCEPTIBLE LINES OF MOSQUITOES

To study the day to day changes of P. b. berghei in the two selected populations of mosquitoes, the following experiment was made. Two cages were prepared each with two hundred female mosquitoes, one cage representing PB (selected susceptible line) and the other representing LD (selected refractory line).

An infected mouse with P. b. berghei was ready to be fed upon at 3.00.pm. After leaving the mouse for half an hour in each of the two cages, all unfed and partially fed females were discarded. The two cages were transferred to a big polythene cage specially fitted into a constant-temperature cabinet (Fig. 9a and 9b), where the temperature and the humidity were kept at 20°C and 80 - 90% respectively. Mosquito dissections were carried out regularly during the course of the sporogonic cycle. Exflagellation, ookinete formation and oocyst development in each group of mosquitoes was noted.

Exflagellation of Microgametocytes

To confirm the maturity of the gametocytes seen in the blood films of the mouse, a few drops of the blood were taken by nicking the tip of the tail. The blood was covered by a cover slip and left in a room where the temperature was 27°C and 80% relative humidity. After 10 - 15 minutes, the fresh blood film preparations were examined under the high dry objective. A large number of microgametes were seen with the characteristic lashing movements of their flagella (Fig. 10).

FIGURE 9a

Inside of constant-temperature cabinet showing polythene bag with two zip openings for access to the cages.

FIGURE 9b

Opened polythene bag inside cabinet showing cages at 20°C and 90% R.H.

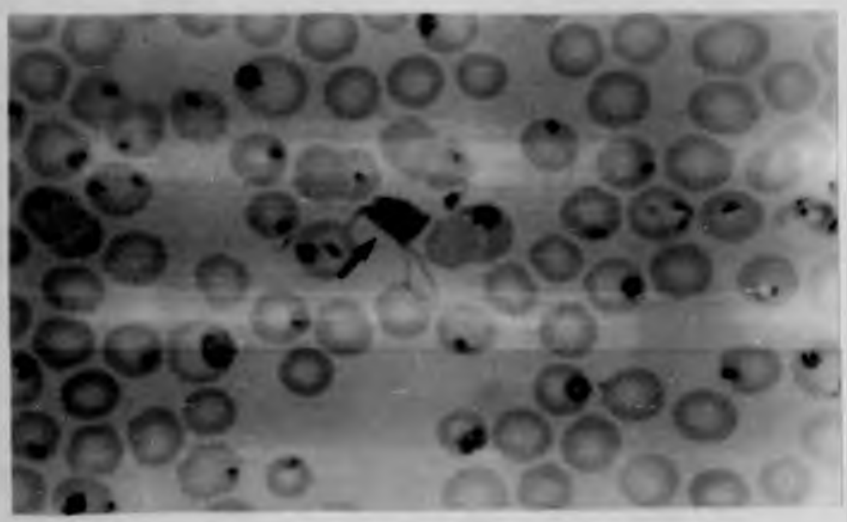


FIGURE 10

Exflagellation
Giemsa stain (Oil immersion)

To make permanent slides of the exflagellating microgametes, five mosquitoes from each of the two cages were dissected separately six hours after being fed on an infected mouse. Their blood was pooled together with a few drops of normal saline, mixing thoroughly. From this pooled blood, several smears were made, dried at 37°C in an incubator for a few minutes and were thereafter fixed in methanol for a few seconds and stained in Giemsa stain for a few minutes.

No proper exflagellation was seen in the stained gut blood smears of both cages of mosquitoes. Instead, a lot of changes in the shape of the gametocytes and the arrangements of the pigment in the cytoplasm around the nucleus were seen. Zygotes and transitional forms between the zygote and the proper ookinete were also seen. These transitional forms assumed a retort shape (Figs. 11, 12, 13).

Ookinete Formation

Ten mosquitoes of each of the two cages were dissected separately. Stained stomach smears were done six, twenty-four and forty-eight hours after the infected blood was offered. A search to look for any change in the parasite in both lines of mosquitoes was followed up daily by measuring and photographing the whole sporogonic cycle of the parasite in the two groups of mosquitoes.

After the first six hours, no proper ookinete could be seen in either group, but a lot of changes were noticed within the gametocytes, particularly in the microgametocyte. Each one showed changes of the pigments in the form of filamentous arrangements which would indicate the initial stages of exflagellation. These activities of the male gametocytes were seen within the red blood cell.

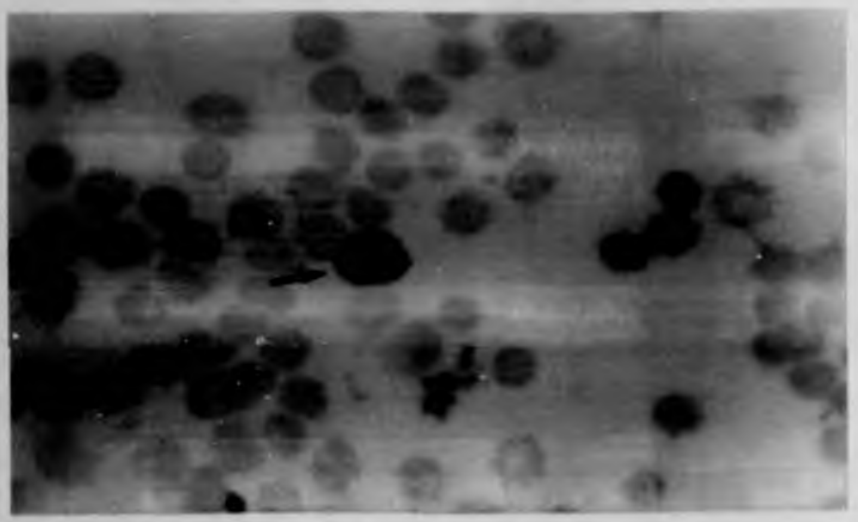
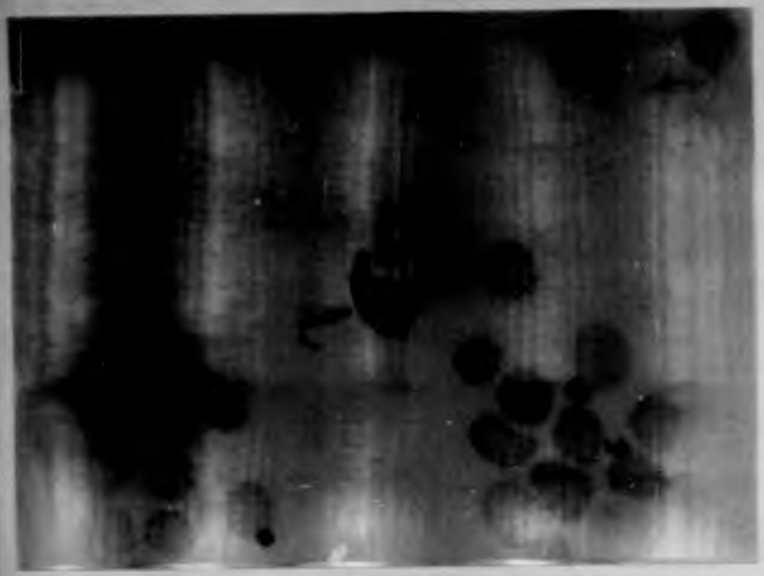


FIGURE 11

Showing zygote formation of P. b. berghei from a stained stomach smear of the selected susceptible mosquito six hours after being fed on an infected mouse.



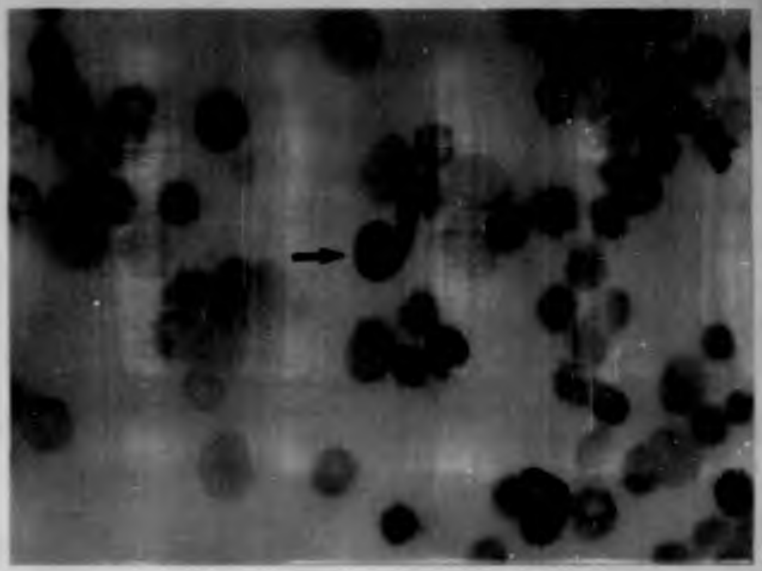


FIGURE 12

First stages of the ookinete formation of P. b. berghei from stained stomach smears of the selected refractory mosquitoes (LD) 6 hours after being offered an infective blood meal, showing retort forms of the ookinetes.

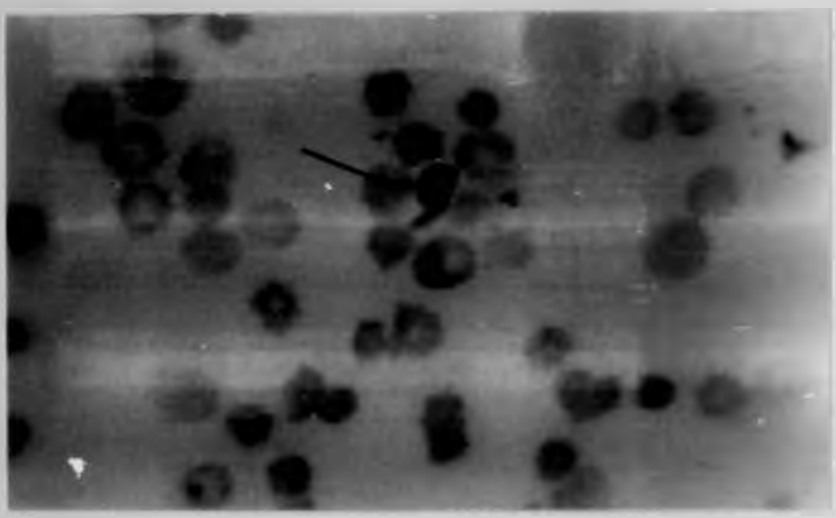


FIGURE 13

First stage of the ookinete formation of P. b. berghei from stained stomach of the selected susceptible mosquitoes(PB) six hours after being fed on infected mouse.

After twenty-four hours a large number of normal ookinetes were seen in stained gut smear preparations in both groups of mosquitoes. However, no difference could be noticed between the refractory and the susceptible mosquitoes neither in the vermicular shape of the ookinete nor in the number. Each ookinete was characterized by having a spindle shape with clearly differentiated thin (anterior) and a thicker (posterior) end in which lies a big nucleus which stains solid dark red, while the whole cytoplasm stains light blue in Giemsa stain. These worm-like ookinetes did not seem to undergo any signs of degeneration in both lines of mosquitoes at this stage of time (Figs.14 and 15). Even the measurement of the ookinetes in both groups seemed to be comparable (Tables 14 and 15). The average length and width of the ookinetes in the refractory and susceptible mosquitoes were 10.4μ X 2.2μ and 11.5μ X 2.2μ respectively.

Forty-eight hours after taking the infective blood meal, a large number of degenerated ookinetes were seen in both groups of mosquitoes, and normal ones could still be found but not as often as on the previous day. The measurement of the length and width of the ookinetes were 10.7μ X 1.8μ and 10.0μ X 2.0μ in the LD and PB mosquitoes respectively (Figs. 16 and 17).

Seventy-two hours after being fed on an infected mouse, the number of ookinetes in the stained stomach smear preparations started dwindling rapidly in both groups, and no proper ookinetes were seen among these quickly degenerating forms. By this time the blood meal was almost completely disappearing. Tables 14 and 15 show the measurement of the ookinetes up to forty-eight hours after feeding on a gametocyte carrier.

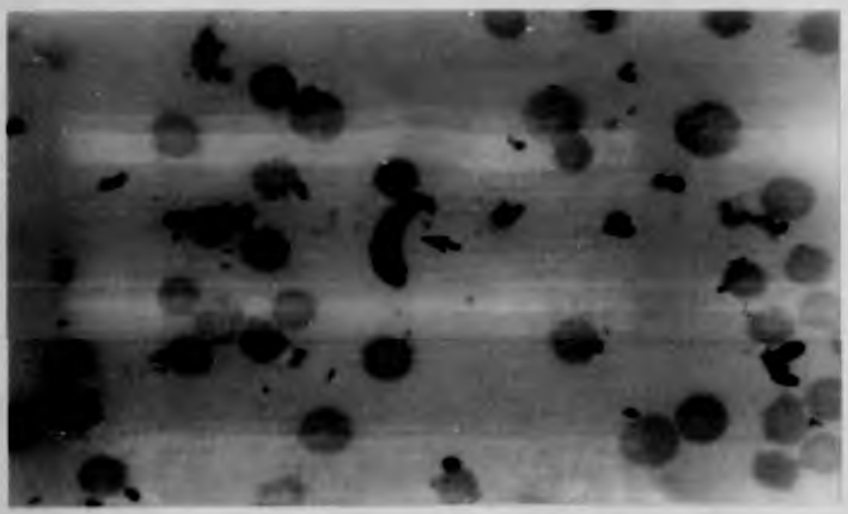
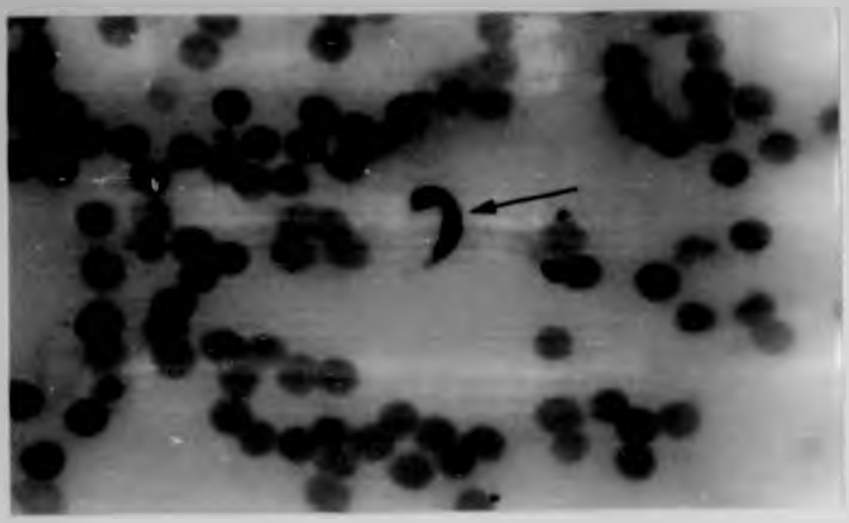


FIGURE 14

Showing normal ookinete of P. b. berghei from stained stomach smear of susceptible mosquitoes (PB) twenty-four hours after being fed on infected mouse.



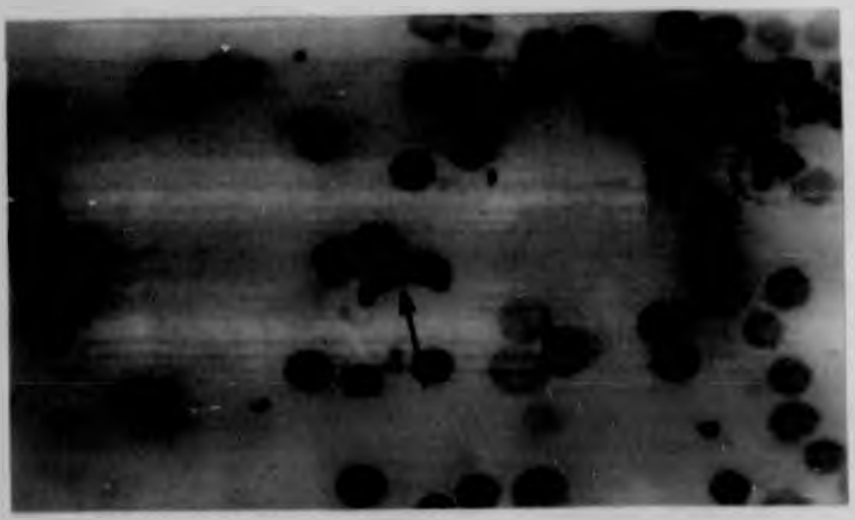


FIGURE 15

Showing mature ookinetes of P. b. berghei from stained stomach smears of the selected refractory mosquitoes (LD) 24 hours after being offered an infective blood meal.

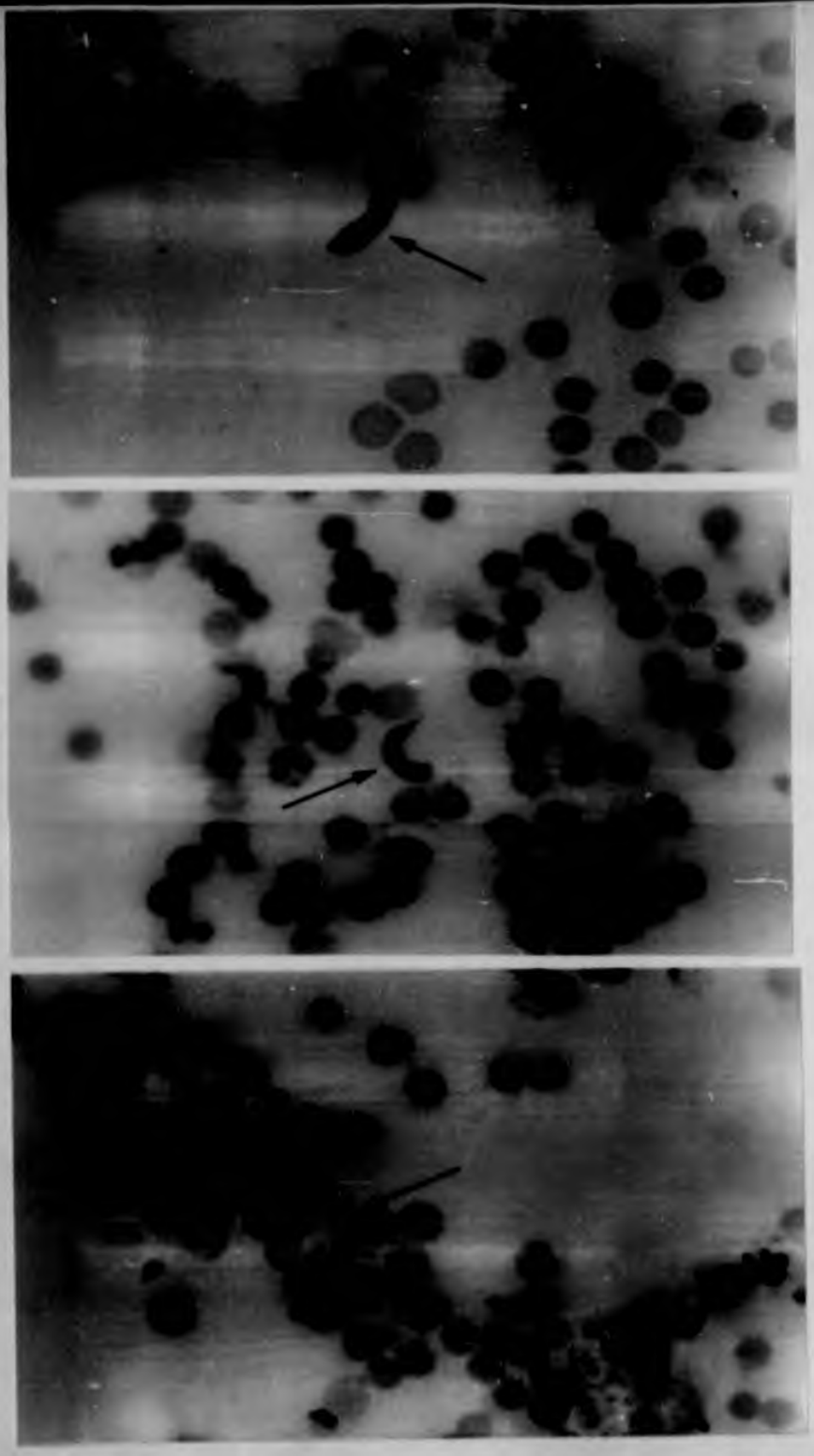


FIGURE 15 continued

TABLE 14

DEVELOPMENT OF THE OOKINETES OF PLASMODIUM BERGHEI BERGHEI IN THE SELECTED REFRACTORY LINE OF MOSQUITOES (LD) FROM SAMPLES OF 10 OOKINETES FOR EACH DAY

Age of Ookinete (Hours)	Average length in μ (range μ) ± standard error of the mean	Average width in μ (range μ) ± standard error of the mean	Remarks
6	-	-	After 6 hours of infected blood meals dissection revealed no ookinetes
24	10.4 (8.0 - 12.0) .5	2.2 (2.0 - 2.4) .07	Large numbers of normal ookinetes were seen
48	10.7 (8.0 - 18.0) 1.0	1.8 (1.6 - 2.0) .07	Large number of ookinetes were degenerated. Some looked fatter and shorter than those of previous day

TABLE 15

DEVELOPMENT OF THE OOKINETES OF PLASMODIUM BERGHEI BERGHEI IN THE SELECTED SUSCEPTIBLE LINE OF MOSQUITOES (PB) FROM SAMPLES OF 10 OOKINETES FOR EACH DAY

Age of Ookinete (Hours)	Average length in μ (range μ) ± standard error of the mean	Average width in μ (range μ) ± standard error of the mean	Remarks
6	-	-	Mosquitoes were offered infective blood meal at 3.00.pm. and dissected at 9.00.pm. on the same day. No ookinete was seen. Internal changes in microgametocytes were evident
24	11.5 (10.0 - 13.6) .46	2.2 (2.0 - 2.8) .11	Large numbers of normal ookinetes were seen
48	10.0 (8.4 - 12.0) .48	2.0 (2.0 - 2.4) .05	The number of healthy ookinetes decreased. Degenerated ones were not uncommon

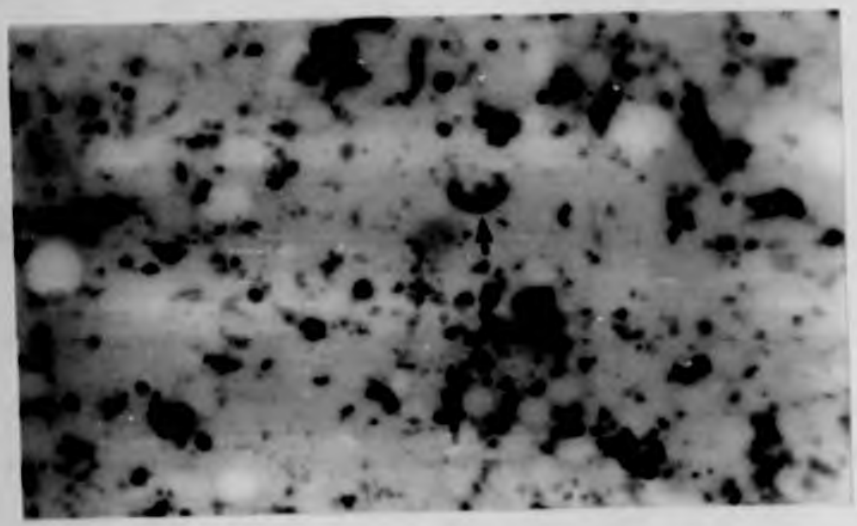


FIGURE 16

Showing degenerated ookinete of P. b. berghei from stained stomach smear of susceptible mosquitoes (PB) after 48 hours

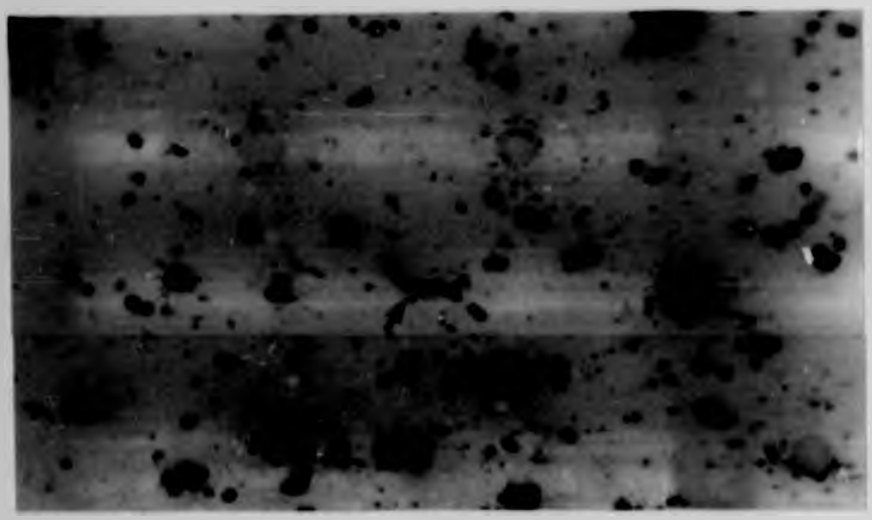


FIGURE 17

Showing degenerated ookinete of P. b. berghei from stained stomach smear of the selected refractory mosquitoes (LD) 48 hours after being offered an infected blood meal.

The Oocysts Formation

The process of oocyst development was followed up until the fourteenth day in both groups of mosquitoes after being offered an infective blood meal. Because of the low temperature, 20°C, which is the optimal temperature for the maturation of the sporogonic phase of P. b. berghei in the invertebrate host, digestion of the blood is slow. Thus the search for oocysts started after three days of having that meal.

All the dissected guts were preserved in a fixative for half an hour, then stained with Harris haematoxylin for a few minutes, blued in alkaline water, passed through a series of upgraded concentrations of 50%, 70% 80%, 90% and 100% alcohol for dehydration. All the slides were counter-stained in Eosin solution followed by a quick one or two dips in xylol and then mounted in Euparal for making permanent slides. (Shute and Maryon, 1966). All the forms of the parasite stained deeper red than the surrounding tissues of the stomach.

After four days of feeding on the vertebrate host, no oocysts were seen in ten dissected mosquitoes of LD, while all the susceptible mosquitoes of PB showed oocyst development in different intensities. The measurement of the average diameter of the oocyst was 7.2μ and the range in diameter was 6.3 - 8.1μ. Uniform growth of oocysts was noticed, and no parasite in the form of ookinete was seen in the tissue of the stomach at this stage.

On the fifth day, two out of ten mosquitoes of the refractory line were found positive - one gut with two ookinetes and two oocysts while the other showed one ookinete (Fig.18). This was the only occasion

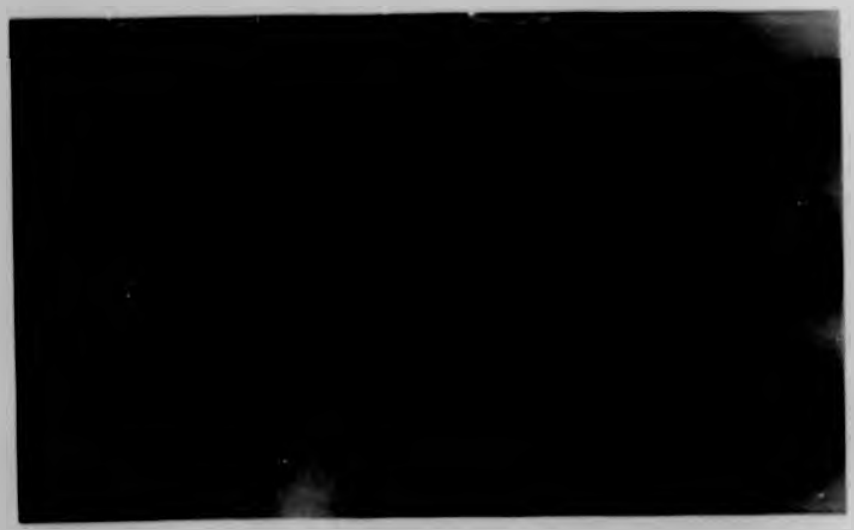


FIGURE 18

Permanent whole mount slides of the stomachs of two infected mosquitoes of the refractory line (LD) stained with Harris haematoxylin 120 hours after being offered an infective blood meal (oil immersion).

when some mosquitoes of the refractory line revealed some sort of infection during the search for the oocysts. However, such oocysts as were found were only one-fifth the diameter of their counterparts in the susceptible mosquitoes. (Fig. 19).

In the susceptible mosquitoes, oocysts measured 9.43μ (6.9 - 11.5μ) and stained prominent red against the tissue of the stomach (Table 16).

Six days after being fed on the infective mouse, oocysts could easily be seen in both fresh midgut preparations as well as in stained guts of the dissected mosquitoes of PB. In fresh preparations they looked round and transparent with fine pigment assuming one or two dotted lines.

On the eighth day some oocysts showed sporozoite formation. On the ninth day almost fully mature sporozoites were seen radiating from the centre of the oocyst.

On the tenth day fully mature oocysts were seen in abundance, and they were easily rupturing under the pressure of the cover slip, releasing a large number of sporozoites which were seen swimming freely in the fluid surrounding the dissected stomachs.

On the eleventh day (Fig. 20) the growth of oocysts were not uniform in some mosquitoes; very small oocysts were seen alongside very large ones on the same gut. Large numbers of sporozoites were seen around the stomach, and a few were seen in the dissected salivary glands of some mosquitoes.

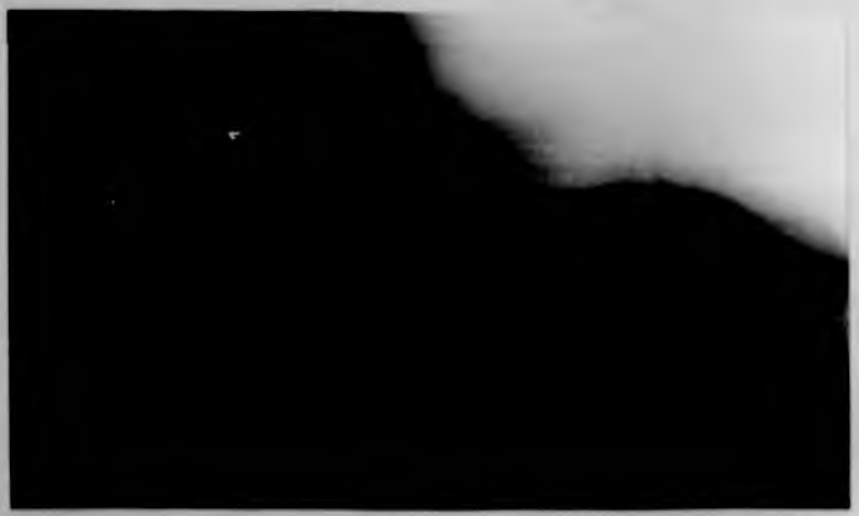


FIGURE 19

Stained stomachs of susceptible mosquitoes (PB) showing oocysts development 120 hours after infective blood meal.
Harris haematoxylin stain (oil immersion)

TABLE 16

SHOWING DAY BY DAY GROWTH OF OOCYSTS OF *PLASMODIUM BERGHEI BERGHEI* IN THE SELECTED SUSCEPTIBLE LINE OF MOSQUITOES (PB)

AGE OF OOCYSTS IN DAYS	AVERAGE DIAMETER in μ	± STANDARD ERROR OF THE MEAN	RANGE OF DIAMETER in μ
4	7.2*	.13	6.3 - 8.1
5	9.43	.25	6.9 - 11.5
6	11.88	.36	9.2 - 14.9
7	12.54	.28	9.2 - 13.8
8	17.1	.46	11.5 - 20.7
9	22.23	.74	16.1 - 27.6
10	21.01	.71	16.1 - 27.6
11	25.15	1.36	13.8 - 39.1
12	26.99	1.16	16.1 - 39.1
13	25.45	.65	16.1 - 29.9
14	26.05	1.34	16.1 - 43.7

* The measurement of oocysts were based on 30 oocysts for each day.

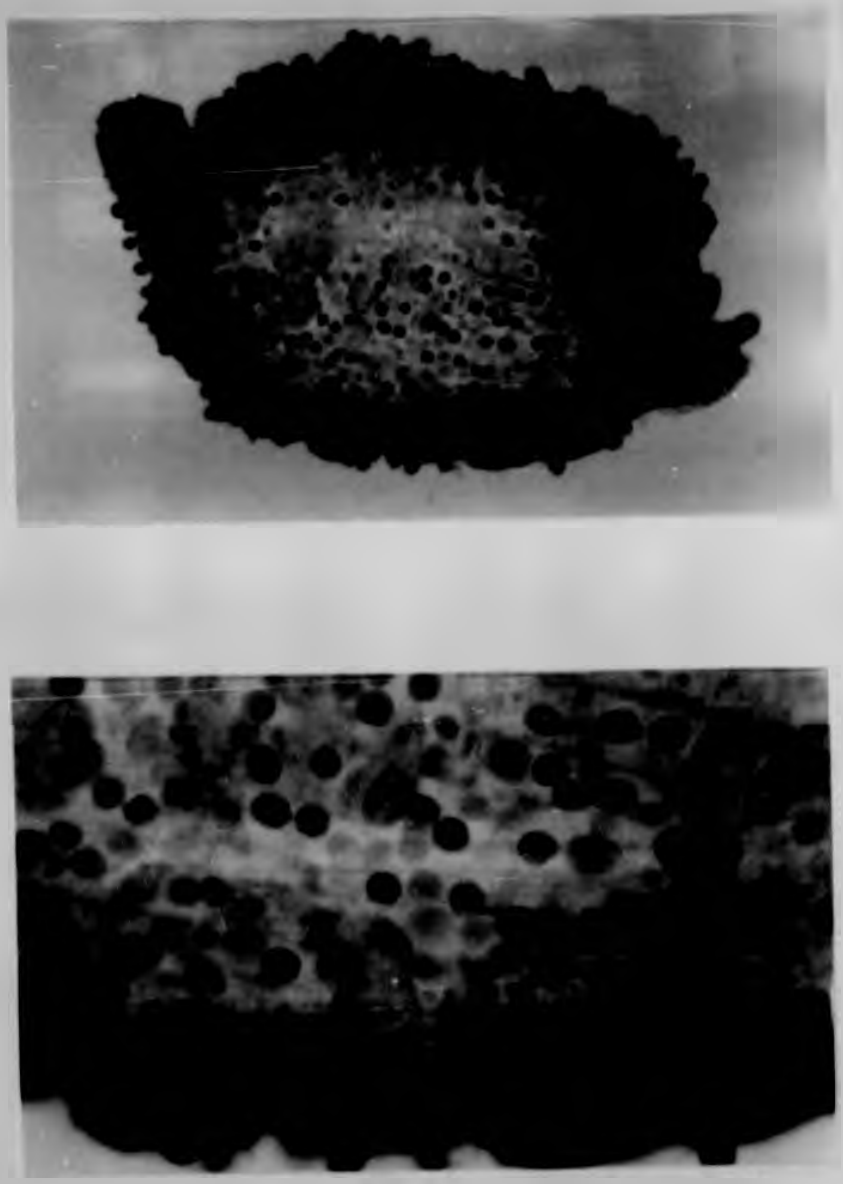


FIGURE 20

Stained stomach of the susceptible mosquitoes (PB) showing very heavy oocyst development 11 days after infective blood meal (low power)

On the twelfth day all the mosquitoes proved positive for the sporozoites in their salivary glands.

On the thirteenth day the number of oocysts was getting smaller and smaller, while the number of sporozoites reaching the salivary glands was getting larger and larger. Some retarded oocysts and a few degenerated ones were seen side by side. Collapsed empty oocysts were also seen (Fig.21).

On the fourteenth day the picture looked the same as on the previous day as far as the dissected stomachs were concerned, but the salivary glands showed enormous numbers of fully mature sporozoites. They came out of the salivary glands in big bundles after exerting a little pressure on the cover slip. Sporozoites were fixed with methanol alcohol and stained with Giemsa stain. Out of twenty, the average length was 10.1u (standard error of the mean was ±1.02) (Fig.22).

FIGURE 21

Showing two oocysts, one fully mature with sporozoites(1) while the other is empty with collapsed wall(2) 13 days after feeding the PB line on infected mouse

Harris haematoxylin stain (oil immersion)

FIGURE 22

Sporozoites from a crushed salivary gland of susceptible mosquito (PB) 14 days after being offered an infective blood meal.
Giemsa stain (oil immersion)

DISCUSSION

Selection

Many attempts have been made to select homozygous lines for susceptibility or refractoriness to malarial and/or filarial infections but in most cases they have resulted in populations impure for the characters selected, that is to say in refractory lines there are always some susceptible and in susceptible lines always some refractory individuals (Huff, 1929, 1931; Trager, 1942; Micks, 1949; Rockefeller Foundation, 1948, 1950; Macdonald, 1961, 1962a, 1962b, 1963a, 1963b; Ward, 1963, Kilama and Craig, 1969; McGreevy et al, 1974; Frizzi et al, 1975; Van der Kaay, personal communication).

A consistent trend toward increased susceptibility of the females was noted in selecting for high susceptibility in PALA in this investigation. Also, a consistent trend toward increased refractoriness of the females was noted in selecting for the refractory line in LSW. The control group of mosquitoes (in this case *A. stephensi* and the unselected parent lines) showed practically no change in the parasite infectivity. The successful establishment of two homozygous susceptible and refractory lines to infection with *P. b. berghei* is the most important result of the present work. Although F_8 and F_9 generations of LD had shown 1 - 2 degenerated oocysts, the dissected females revealed no sporozoites whatsoever, neither in the fluid surrounding the dissected gut nor in the salivary glands.

Changes in the infectivity of the gametocytes of *P. berghei* was noticed by many investigators (Yoeli, 1966; Wery, 1968). To avoid such variation,

infected mouse of the third blood passage and after three days of being inoculated was used as gametocyte carrier throughout the present work. Moreover, all feeding experiments involving the selected mosquitoes were coupled with feeds on a control group of mosquitoes. Thus any change in the infectivity of the parasite would have been reflected in both the experimental as well as the control groups of mosquitoes.

The rate of susceptibility in the original parent stock of LSW to P. b. berghei infection fluctuated from 30 - 63% over a period of one year. From this stock a homozygous selected refractory line (LD) was established by selective breeding in nine generations. Fluctuations in susceptibility of laboratory stocks of mosquitoes to malarial, filarial and virus infections are not uncommon. Huff (1940) found a big fluctuation of 30 - 63% in susceptibility of a laboratory strain of C. pipiens to infection with P. cathemerium over one year. Rutledge et al (1970) demonstrated the instability in the susceptibility of laboratory populations of anopheline mosquitoes to infection with the malarial parasite. Furthermore they concluded that intraspecific variation in the susceptibility of anopheline to infection with malarial parasites is substantial, extending under laboratory conditions even to separate colonies from the same stock. The susceptibility of a given laboratory population is itself variable over the course of a period of time.

The homozygosity of the two selected lines, the 100% refractoriness of LD and the 100% susceptibility of PB to infection with P. b. berghei was maintained over a period of more than three years after the complete halt of selective pressure. Other workers have also shown

stability but not over such a long period. Trager (1942) maintained his susceptible line of Ae. aegypti to infection with P. lophurae for more than one year after cessation of selective pressure. The mean susceptibility rate of 84.81% was maintained over fifteen generations in Ae. aegypti to infection with sub-periodic B. malayi after a complete cessation in selective pressure (Macdonald, 1962a). Ward (1963) found that cessation of selective pressure for thirteen generations did not produce significant change in the susceptibility of Ae. aegypti to P. gallinaceum, while Raghavan et al (1967) found their susceptible line of Ae. aegypti kept its susceptibility over twenty-one subsequent generations.

Selection for the refractory line in the present work was aimed from the start at retaining the egg batches only from the females which were negative for both oocysts on the midgut and sporozoites in the salivary glands. Dissection of mosquitoes should never be performed before the end of the required period for the completion of the sporogonic cycle in order to take into consideration the presence and absence of sporozoites and their infectivity to the vertebrate host. This may explain the reason behind establishing homozygous lines of susceptible and refractory mosquitoes not only fairly quickly but also pure enough to maintain their stability over a period of more than three years. Also, it may explain the failure of some investigators in achieving pure refractory lines of mosquitoes because they selected from females which showed a low count of oocysts instead of being absolutely devoid of oocysts when they dissected the mosquitoes halfway through the sporogonic cycle. Furthermore, as was evident in the present work, some oocysts may look fairly normal after 6 - 7 days post feeding on infective gametocyte carrier but subsequently undergo

degenerative processes towards the end of the sporogonic cycle. Thus therefore, one or more of these factors may offer an explanation for the many failures in attempting to establish 100% refractory line of mosquito to malaria parasites (Ward, 1963; Kilama and Craig, 1969; Frizzi et al, 1975 and Van der Kaay, personal communication). Shute and Maryon (1952) reported that in order to obtain a clear picture of the rate of infection, dissection for both the gut and salivary glands for each mosquito should be performed.

It is not advisable to consider a mosquito as refractory when it has a single oocyst. However, Kilama and Craig (1969) considered their selected line of mosquitoes as refractory when the females showed up to six oocysts on their midguts. Huff (1934) in his definition of a susceptible mosquito stated that a mosquito must allow at least one oocyst to grow to maturity following the ingestion of sufficient gametocytes. Huff (1954) found that it is possible for transmission to be effected by a mosquito infected with a single oocyst. In wild caught mosquitoes, Pringle (1965) found that a mature oocyst of P. falciparum contained 9,555 sporozoites. Furthermore, Pringle (1966) concluded from the data obtained during dissection of naturally infected A. gambiae and A. funestus in East Africa that the mosquitoes were infective even when having only 1 - 2 fully mature oocysts. In laboratory infected mosquitoes, transmission of P. berghei was successfully established in mice by injecting only ten sporozoites (Vanderberg et al, 1968), thirty sporozoites (Hulls, 1971) and fifty sporozoites (Yoeli and Most, 1965a).

Microsporidia can have two different effects on the course of infections with malarial parasites in mosquitoes. Firstly they can interfere with

the normal development of the oocysts and sporozoites and secondly, they can weaken the mosquito, shorten its longevity and reduce its capability for egg-laying and subsequently eliminate laboratory colonies. Many authors have had bad experience with this pathogen (Garnham, 1956, 1964, 1966; Bray, 1958; Jadin et al, 1966; Reynolds, 1966; Yoeli and Bone, 1967; Alger and Undeen, 1970; Hulls, 1971; Ward and Savage, 1972; Hilton, 1974; Macrae, personal communication).

Throughout this investigation precautions were taken to minimise the possible effects of microsporidia and other possible pathogens. The methods adopted are referred to under the materials and methods section. In addition, mosquitoes of LD, LSW, PB, PALA and BEECH were closely examined on 3 - 4 occasions over a period of four years by electron microscopy to make sure that they were devoid of pathogenic viruses which might interfere with the result of the susceptibility to malarial infections and in particular in the two selected lines of mosquitoes PB and LD. Examination by negative staining and sectioning of the stomachs and ovaries were performed and in each case the results showed that the mosquitoes were apparently devoid of pathogenic viruses. Many investigators have reported the presence of adverse effects of pathogenic viruses on malaria parasite. (Bertram et al, 1964; Terzakis, 1969; Davies et al, 1971; Bird et al, 1972).

In the case of selection for the susceptible line only those mosquitoes which showed a high oocyst count on the midgut and a high number of sporozoites in the salivary gland had their egg batches retained for the subsequent generation. Micks (1949) found that a more highly infected female mosquito would produce more progeny which would be susceptible to the same parasite than a mosquito with an extremely light stomach infection.

Mode and Pattern of Inheritance

The susceptibility to infection with P. b. berghei was found to be largely dominant to the refractoriness. In three replicates the F_1 resulting from the parental cross of LD♀♀ X PB♂♂ were capable of supporting normal infection of malarial parasite showed rates of 97.14%, 93.33% and 96.55% of infection with P. b. berghei respectively. (Table 4a). The total number of positive females was 104 out of 109. This finding was further substantiated by another replicate (Table 5) in which the susceptibility to the same parasite was 89.58% (43 positive out of 48). Although a much larger proportion of the F_1 resulting from the parental cross PB♀♀ X LD♂♂ were refractory to the infection with P. b. berghei, the overall rate of susceptibility was 63.54% for the three replicates from Table 4a, while in the fourth replicate the rate of susceptibility was 61.19%.

The finding in the present work that the susceptibility of A. gambiae species A to infection with P. b. berghei was dominant and refractoriness was recessive is in complete harmony with that of Kilama and Craig (1969) who found that the susceptibility of Ae. aegypti was dominant to refractoriness with P. gallinaceum and designated the gene as pls (Plasmodium - susceptibility). On the other hand, it was found that the F_1 progenies resulting from crossing the susceptible and refractory lines of mosquitoes were resistant to infection with P. gallinaceum (Huff, 1935; Frizzi et al, 1975). Others found F_1 offspring were intermediate in their susceptibility to malaria infection (Rockefeller Foundation, 1948, 1950, Ward, 1963). Tesh et al (1976) found that in crosses between the resistant and the susceptible lines of Ae. albopictus to chikungunya virus gave an F_1 progeny which was intermediate in susceptibility.

In the case of filarial infection, Macdonald (1962a, 1962b, 1963a, 1963b) and Macdonald and Ramachandran (1965) established that the susceptibility of Ae. aegypti to infection with sub-periodic B. malayi is sex-linked and recessive. McGreevy et al (1974) also reached the same conclusion working with the same species of mosquito but with a different filarial nematode, D. immitis.

In the present work the possibility was considered that the existence of differences in the rate of susceptibility between progenies of the reciprocal crosses and also in the sporozoite index were due to cytoplasmic inheritance. However this possibility was ruled out completely by two pieces of evidence. Firstly, the direction of the difference between reciprocal crosses is not that expected from maternally inherited cytoplasmic particles. In other words, it was not the female which strongly influenced the susceptibility of her offspring in the parental mating of PB♀♀ X LD♂♂, but the male who induced refractoriness among his daughters. Secondly, the result obtained from Experiment 4 (page 85) which showed a repeated backcrossing of the hybrid females to the refractory males succeeded in rendering the progenies of the parental mating of PB♀♀ X LD♂♂ completely refractory over a period of only ten generations. The ease and swiftness in reaching the absolute status of refractoriness indicates beyond doubt that cytoplasmic inheritance was not involved.

These results contrast with the findings of Laven (1959) from crosses between incompatible populations of the C. pipiens complex. Laven showed that the cross ♀ Og* X ♂ Ha** was incompatible but ♀ Ha X ♂ Og was compatible. Females from the latter cross

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* Og = C. pipiens strain Oggelshausen, Germany
** Ha = C. pipiens strain Hamburg, Germany

were backcrossed to Og ♂♂, and this backcross was repeated over fifty generations. At the end, both the males and females behaved in crosses exactly like the original Ha strain despite the fact that the backcrossing had replaced the entire genome of Ha stock by that of Og. Thus he concluded that the phenomenon of incompatibility in Culex is controlled by the cytoplasm and not by the chromosomes, and the inheritance of compatibility or incompatibility is dependent on the nature of the parental mother used in the mating. If the determination of the susceptibility or refractoriness to infection with P. b. berghei of PB and LD respectively resides in the cytoplasm, the replacement of the original PB genome by the LD genome in the hybrid females of the second, third and up to the tenth generations of continuous backcrossing to the refractory males (PB♀♀ X LD♂♂) X LD♂♂ should remain always susceptible. As is clear from Figure 4 the factor (C_s) would persist from generation to another generation without any dilution. Dissection of all hybrid females of the tenth generation were all negative for both oocyst and sporozoites.

Table 5 gives the results of another attempt at reciprocal crosses between the LD and PB lines and the results of inbreeding F_1's and the resulting F_2's. Once again, the F_1's showed a difference between the reciprocal matings in the same direction as that noted in Table 4a. The ratio of 3:1 of susceptible individuals to refractory ones which would be expected from single gene inheritance was obtained in the F_2 generation derived from the parental mating of PB♀♀ X LD♂♂. This might have been due to pure chance, however, as the other F_2 generation

derived from LD♀♀ X PB♂♂ did not give the same ratio.

The finding of Mason (1967) regarding the inheritance of the colour of the eye in the mutant population of LSW that the white phenotype is inherited as a single, recessive, sex-linked gene has been confirmed in the present work. (Fig. 1A). Some correlation between white eye and refractoriness to P. b. berghei infection indicated in these observations and in fact proved significant in four replicates. Three replicates were treated together (Table 6); chi-square values proved significant, 12.48 $p<0.001$. The finding of the fourth replicate was also found highly significant ($\chi^2 = 22.28$ $p<0.001$), (Fig. 1B). This indicates either that the white eye gene has a pleiotropic effect on refractoriness or that the white eye locus is partially linked to another gene with an influence on refractoriness. It is clear that a pleiotropic effect of the white eye gene is not the whole explanation of the refractoriness of the LD stock because the original LSW stock was homozygous for w but not fully refractory.

It was discovered that the white-eye gene has pleiotropic effects on other parts of the larva, pupa and adult of the mutant (Mason, 1967). He reported that there is no pigment in the body of white-eyed larva. In the adults the gene for white-eyed exerted its multiple effects on the testes sheath and accessory glands of the male and on the fecundity in the female. The mutant female is so affected by the gene that she produces about one third fewer eggs than is normal. Furthermore, the white-eyed gene also shortens longevity.

It was assumed that refractoriness to infection with P. b. berghei

is dependent on a single recessive autosomal gene (Fig. 2), but the expected results were not in agreement with observed results which were obtained from both the mating of LD♀♀ X PB♂♂ and the reciprocal. Thus the susceptibility of F_1 offspring and that of F_1 backcrossed to the susceptible and refractory parents does not conform with the expected results of the assumed model. It is concluded that the inheritance of susceptibility cannot be explained by the model of a single recessive autosomal gene for refractoriness. In the same way, the suggestion that refractoriness is due to a single sex-linked recessive gene was ruled out for the same reasons that the observed results were not conforming with the expectations (Table 7) and the assumed model does not accommodate fully the results neither in F_1 nor in F_1 backcrosses progeny. However, there is a slight resemblance to the predicted results in that the susceptibility from backcrossing male F_1's to LD was somewhat higher than that from backcrossing female F_1's to LD. This, once again, seems to be due to the factor at or close to the w locus already referred to.

It is concluded that no single locus model will explain all the facts. Because there is evidence for a statistically significantly greater sporozoite index in the progeny of four backcrosses to PB than in the four backcrosses to LD, and there is a greater tendency for refractoriness in the females resulting from F_1 backcrossing to the refractory parent, and furthermore, there is a greater tendency for susceptibility in the females resulting from F_1 backcrossing to the susceptible parent, the involvement of additive polygenes in influencing susceptibility is suggested. On this view the factor identified at or close to the w locus would constitute one of a series of polygenes with an effect on susceptibility to infection with P. b. berghei. However, this polygenic model does not explain the

difference between the F_1's from reciprocal crosses. It also does not explain why the progeny of the backcrosses to PB had no higher susceptibility than the F_1's. Susceptibility to malaria infection in mosquitoes controlled by polygenes is not uncommon and is reported by many authors, (Boyd and Russell, 1943; Research workers at Rockefeller Foundation, 1948, 1950; Ward, 1963, and Frizzi et al, 1975).

The Rate of Susceptibility of the Two Selected Lines to Different Species of Rodent Malaria.

As mentioned previously, all three species of rodent malaria, P. b. berghei, P. yoelii and P.y.nigeriensis, established their infections in all lines of mosquitoes except that of LD which maintained its condition of being absolutely negative for stomach (oocysts) and salivary glands (sporozoites) infections when fed on mice infected with P. b. berghei and P.y.nigeriensis. Although one mosquito of the refractory line showed two oocysts of P. yoelii on the midgut, these were abortive infections and the oocysts were completely degenerated and showed no development of sporozoites. Furthermore, this fact was confirmed by dissecting the salivary glands which were also negative. This substantiated the fact that the selected refractory line of LD was not only refractory to the infection with P. b. berghei against which it was selected but also refractory to other species of rodent malarias such as P. yoelii and P.y.nigeriensis.

The selected susceptible line, PB, proved fully susceptible to infection with P. b. berghei and almost so with P. yoelii. The P.y. nigeriensis strain used was not fully infective to either susceptible PB or to the control mosquitoes but there is no

doubt that the refractory line LD is refractory to this species. Since the rate of infection of P.y.nigeriensis was low in the control group of mosquitoes, it must be a clear indication that the infectivity of the gametocytes in the peripheral blood of the mouse which was used as a gametocyte carrier must have been low at the time when feeding was performed. An identitical finding was reported by Ramachandran et al (1960); Macdonald and Ramachandran (1965) and McGreevy et al (1974) as mentioned in the literature review.

The complete failure to establish the infection in any of the five lines of mosquitoes with P. chabaudi on two attempts may have been due to the fact that the parasite of P. chabaudi had lost its capability to produce infective gametocytes over a long period of storage in deep frozen state in liquid nitrogen, before being used in the present work. However, quite a few workers have had difficulties in handling this species of rodent malaria (Landau and Killick-Kendrick, 1966; Wery, 1968; Hilton, 1974).

Finally, the following conclusions could be drawn with certainty that both lines selected for susceptibility (PB) and refractoriness (LD) for P. b. berghei proved to be so to the other rodent malarias, namely P. yoelii and P.y.nigeriensis if viewed against their own wild populations. Thus it appears that the line selected for refractoriness to P. b. berghei is cross-refractory to both P. yoelii and P.y.nigeriensis though the latter species failed to fully infect the susceptible and control lines.

Testing the Susceptibility of the Two Selected Lines (PB and LD) to Monkey and Human Malarias.

The failure to establish the infection of the monkey malaria, P. knowlesi, in the three lines of mosquitoes of PB, LD of A. gambiae species A and A. stephensi, which was run as a control, might have been due to the inability of gametocytes to infect the above mentioned two species of mosquitoes, or that the above mentioned species of mosquitoes, A. gambiae species A and A. stephensi, were not good vectors for P. knowlesi. Coggeshall (1941) was also unable to establish any mosquito infection with P. knowlesi, although several attempts had been made to infect A. punctipennis, A. quadrimaculatus and C. pipiens when fed on a monkey infected with P. knowlesi. However, Jaswant Singh et al (1949) reported that A. stephensi mosquitoes were experimentally infected with P. knowlesi but they regarded this species of mosquito as not being a good vector for P. knowlesi.

The Result of Feeding a Chimpanzee Infected with P. vivax:-

Though all five groups of mosquitoes fed on a chimpanzee infected with P. vivax showed some infection, the rate of infection in LD was considerably lower than in the susceptible line and, what is more, less than in the parental strain from which the refractory line was selected. This in itself implies that the selection for refractoriness to mouse malaria has also had an effect on the susceptibility to human malaria, P. vivax. The selected susceptible line PB proved fully susceptible to the infection with P. vivax. Furthermore, the successful malarial transmission by the bite of infective mosquitoes was a clear indication that the P. vivax Chesson strain used in the present work was not only infective to the mosquitoes, but also to the chimpanzee. The feeding of infective mosquitoes on a malaria free

chimpanzee produced a heavy parasitaemia after only one month.

The Results of Feeding on Human Volunteers Infected with P. falciparum:-
Feeding of the two lines LD and PB mosquitoes were made on three human patients showing gametocytes of P. falciparum in the peripheral blood. The age of the volunteers ranged between four months and nine years. Draper (1953) working with A. gambiae and P. falciparum chose children under the age of ten years for his investigations, this being the section of the population showing the greatest frequency and density of gametocytes. Four feedings on gametocyte carriers of P. falciparum were successfully performed. Although the mosquites of the refractory line LD showed a lower rate of susceptibility than that of the selected susceptible line (PB) in all four experiments, the difference in the rate of infection was statistically significant in only two feedings.

Testing the Susceptibility Status in the Five Lines of Mosquitoes Three Years After Cessation of Selection.

For well over three years after the selection of the two lines ceased, it was found that the selected susceptible line PB was still fully susceptible when tested to the infection with P. b. berghei and the LD selected refractory line was fully refractory to the same parasite.

The Infectivity of Sporozoites in the Five Lines of Mosquitoes.

Notable results of these experiments were first the complete failure to transmit the infection by the selected refractory line of mosquitoes when they were allowed to feed on clean mice on two occasions either individually or in mass feeding; this is clear proof that LD mosquitoes were no longer capable of transmitting rodent malaria, P. b. berghei. Secondly, it has been successfully proved that malarial transmission is not only possible by mass feeding of infected mosquitoes on clean mice, but also by feeding single infected mosquitoes of PB, PALA, LSW and the control (BEECH).

In the present study it was found that in white mice P. b. berghei produces a fatal infection in all cases within 1 - 2 weeks after inoculation with infected blood or after being bitten by infective mosquitoes. This finding was also noticed by many investigators (Vincke and Van den Bulcke, 1949; Ramakrishnan and Prakash, 1950; Rodhain, 1954; Celaya et al, 1956; Yoeli, 1965; Yoeli et al, 1966a; Vanderberg et al, 1968). None of the mice which were left overnight in the cages of LD mosquitoes on two nights developed malarial infection even after a follow-up of three weeks. On the other hand, 100% infection was obtained among the white mice exposed to the bite of PB, PALA, LSW and BEECH. Furthermore, all the infected mice died over a period of 7 - 14 days after the appearance of parasitaemia in the peripheral blood of the mice. Vincke and Van den Bulcke (1949) obtained fifty infections among 92 blood-inoculated tree rats with P. b. berghei and the mortality among the infected animals was 24%. The difference in the rates of infection and in the mortality in this experiment and that of the present work may have been due to the fact that the tree rat is a natural host for P. b. berghei and thus it is

better adapted to infection with this parasite than the white mice.

The difference in the prepatent periods noticed among the infected mice in the present work might have been due to the individual differences in the mice rather than to the size of the inoculum of sporozoites injected by the infected mosquitoes. Yoeli (1965) reported that the route of inoculation and the number of sporozoites inoculated did not affect the length of the prepatent period. He found inoculation of 360 and 72,000 sporozoites produced parasitaemias within a six days prepatent period. Later, Yoeli and Most (1965b) reported that the majority of the 94 sporozoite-induced infection of laboratory animals (white mice, golden hamsters, young albino rats and tree rats) showed a prepatent period of 3 - 6 days. A larger prepatent period of 8 - 10 days was also noticed. Also, Vanderberg et al (1968) found a range of 3 - 8 days in the prepatent period in different groups of sporozoite induced mice.

The Fate of the Parasite of P. b. berghei in the Selected Refractory (LD) and Selected Susceptible (PB) Mosquitoes.

It was thought that a day to day analysis of the comparative development of the parasite, P. b. berghei, in the two selected lines, LD and PB, after being fed on the same gametocyte carrier would give a clear picture of the course of the sporogonic cycle of the parasite in the refractory and susceptible mosquitoes. To check the infectivity of the gametocytes, exflagellation was successfully observed on slides from blood taken directly from an infected mouse without passing through the stomach of the mosquitoes. The actual site or the stomach of the insect is not

essential for such development. Weathersby (1954) found that the sporogonic development of plasmodia will readily take place in the haemocoel of a susceptible mosquito or in other parts of their bodies when blood containing ripe gametocytes is introduced therein. Yoeli et al (1963b) reported that exflagellation of microgametocytes occurs readily in vitro. In the present study it is of particular interest that in the two lines of mosquitoes, LD and PB, the survival of the parasites both in the lumen of the midgut and in the initial penetration of the gut wall was very different.

It was found that exflagellation, fertilization and ookinete formation occurred with a resulting oocyst infection in the stomach of a susceptible species, C. pipiens, while in the refractory, C. quinquefasciatus, exflagellation and fertilization took place but penetrations of the stomach wall by the ookinete or its development into the oocyst was prevented by some factors. (Huff, 1927, 1932, 1934, 1941, and Micks et al, 1948) Furthermore, Micks (1949) showed that refractory C. pipiens, C. restuans and Ae. triseriatus were found to be susceptible to P. elongatum up to the point of partial oocyst development. Eyles (1952a) found that the ookinetes of P. gallinaceum in the refractory species of A. quadrimaculatus and A. freeborni may penetrate the gut then cease development soon after becoming established upon the gut wall. Bennet et al (1966) showed resistance against ookinetes, oocysts and sporozoites with P. cynomolgi in different species of anopheline mosquitoes. Garnham (1966) found that in refractory mosquitoes, exflagellation, fertilization, formation of ookinetes and oocysts took place but oocysts degenerated at an early stage of development. Kilama (1969, 1970, 1972) and Kilama and Craig (1969) showed that in refractory mosquitoes the ookinete moved through the gut wall but the newly formed oocyst dies

within 40 - 60 hours after feeding on the infective gametocyte carrier. Yoeli (1973) found normal ookinetes of P. berghei in the midguts of refractory Ae. aegypti. Furthermore, he noticed that in A. quadrimaculatus infected with P. berghei, ten to eleven days following the infective blood meal, many oocysts were found stunted in their growth, lacking in their normal internal structure and degenerated. He called the phenomenon of abnormality and degeneration seen in the oocysts as a phenomenon of rejection; however, he considered A. quadrimaculatus as a semi-susceptible experimental host and inadequate for regular cyclical transmissions of P. berghei.

In the case of filarial infections the phenomenon of encapsulation and arrest of the parasites in the refractory mosquito hosts was observed. (Kartman, 1953; McGreevy et al, 1974). Weathersby (1952, 1954) demonstrated conclusively that the factors responsible for refractoriness in mosquitoes were systemic in nature and not restricted to the stomach wall. Later investigations (Weathersby, 1960a, 1960b, 1963, 1965, 1967; Weathersby and McCall, 1968; Weathersby et al, 1971) found that the antagonistic forces in the refractory mosquitoes were responsible for the killing and elimination of the parasite. The nature of the antagonistic forces is thought to be of a biochemical nature rather than physical (Huff, 1934; Micks et al, 1948; Warren et al, 1963; Ward, 1965; Bennet et al, 1966; Bafort, 1968; Wery, 1968; Hilton, 1974).

Dissected mosquitoes of PB steadily continued to show oocysts development and the presence of oocysts on the wall of the midguts was a daily finding throughout the sporogonic cycle. Oocyst development normally involved a steady increase in the average diameter. However a few retarded small sized oocysts were seen among 200 - 300 oocysts on the wall of a single mosquito of the PB line. This phenomenon is apparently of

normal occurrence in P. berghei infections. Vanderberg and Yoeli (1965), Yoeli and Bone (1967) and Yoeli (1973) found heterogeneity of oocyst growth of P. berghei not only in infected A. stephensi, A. quadrimaculatus and A. aztecus but also in the natural host A. dureni following a single infective blood meal. Furthermore, they added that the heterogeneity of oocyst growth, a characteristic of P. berghei infection, differs from monomorphic development observed in experimental infections of P. gallinaceum in Ae. aegypti and P. cynomolgi and P. vivax in A. stephensi or in A. sacharovi.

In the present work most of the oocysts reached the mature stages where they released the infective stage of the parasite. Enormous numbers of sporozoites were seen in the dissected salivary glands. The average diameter of the fully mature fixed and stained oocyst of P. b. berghei was 26.99μ (16.1 - 39.1μ) and the average length of fixed and stained sporozoite was 10.1μ. This is in agreement with the measurement recorded by other investigators for the same parasite. (Rodhain et al, 1955; Yoeli and Most, 1960; Vanderberg et al, 1967).

The Potentiality of Refractoriness as a Genetic Control Measure

The introduction of a pathogen refractory genotype into mosquito populations appears to offer a means of genetic control of vector populations with a more stable end result than methods aimed at eradication. The introduction of refractory genotypes would not leave any empty ecological niche available for occupation by the progeny of the survivors of an incompletely successful eradication programme, immigrants of the same species or even another potentially dangerous species.

The most economical way of replacing a vector population by a pathogen refractory genotype undoubtedly would be by linking it with a genetic system capable of "driving" the process of replacement by the principles of either negative heterosis or meiotic drive. Unfortunately such systems are not yet available in Anopheles. However, simple theoretical simulations suggest that "dilution" of pathogen susceptible genotypes by the release at several generations of males carrying refractory genotypes would have reasonable prospects of success provided that the fitness of the refractory type was close to that of the wild type.

A logical sequel to this work is the further selection of the selected lines using human malarial parasites rather than mouse or monkey ones. Ideally the two selected strains should be taken to the field and further selection carried out with human volunteers infected with the various species of malarial parasites. Also, eventually selected lines should be tested in different parts of Africa to see if they are cross-refractory to different populations of the same species of malaria parasite.

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APPENDIX*

A. To test the statistical significance of the unexpected difference between reciprocal crosses three procedures were used. In each case data from the three replicate experiments are treated separately and tests are made for a significant difference between crosses within replicates.

1) The proportions positive for infection were compared after logistic transformation. The mean differences of the transformed proportions were calculated for each replicate; the average of these differences for all replicates was compared with its standard error (Cox 1970, Ch.3.).

The results are shown in Table A1 and they indicate a highly significant difference between the reciprocal crosses.

2) In addition to the difference in proportion positive, it appeared that among the positives there was a higher rate of infection among the LDQ X PBG progeny than among those of the reciprocal cross. An arbitrary scoring system was adopted to score the rate of infection and this is defined in the footnote to Table 2a. An analysis of variance was made for an unbalanced 2 X 3 design, as described by Armitage (1971, pp 264,265). The three grades of sporozoite infection (+, ++, +++) were assumed to be on an equal-interval scale in this analysis.

As indicated in Table A2, the difference between the reciprocal

crosses was highly significant.

3) Data on positivity and negativity was amalgamated with that on rate of infection among the positives by adopting a scoring system as follows - (negative for infection), +, ++ and +++. An analysis of variance was carried out as in the previous section. The 4 categories (-, +, ++ and +++) are assumed to be on a equal-interval scale. The results are shown in Table A3. Once again the F_1's proved to be highly significantly different.

B. To test the significance of the difference between the sporozoite indices for all the backcrosses to LD and all the backcrosses to PB, an analysis of variance was carried out as in section 3 above. The results are shown in Table A4. A significant difference between the two sets of backcrosses was found but there was also a significant interaction, i.e. the extent of the difference between the sets of backcrosses varied between the three experimental replicates and the interpretation was doubtful. Therefore a separate comparison of the sporozoite indices from each of the experimental replicates was made by a series of t tests as follows:-

	d.f.	t (difference between backcrosses to LD and to PB)	P
Replicate (i)	84	10.32	<0.001
(ii)	161	0.92	N.S.
(iii)	351	7.85	<0.001

This indicates that there is a significant difference in the susceptibility to the infection with P. b. berghei in the progeny resulting from backcrossing the F_1's to the susceptible parent and those resulting from backcrossing to the refractory parent, except in replicate (ii) where the

difference was statistically insignificant.

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* Statistical analyses carried out with the assistance of Drs. T. Marshall and C.F. Curtis.

TABLE A1

COMPARISON OF LOGISTIC SCORES OF TEST OF SIGNIFICANCE OF DIFFERENCES IN THE POSITIVITY RATE BETWEEN THE F_1's FOR THE TWO RECIPROCAL CROSSES WITH S.E.

	Replicate 1		Replicate 2		Replicate 3		Weighted average	
	z	SE	z	SE	z	SE	z	SE
PBQ ♀ X LD♂	0.4055	0.4564	1.2111	0.3045	0.1431	0.3789		
LDQ ♀ X PB♂	3.5264	1.0146	2.6391	0.5976	3.3322	1.0177		
Difference	3.1209	1.1125	1.4280	0.6707	3.1891	1.0859	2.1656	0.507

χ^2 for difference same in each replicate

= 2.835 with 2 df; not significant

approximate SND for test of average difference = $\frac{2.1656}{0.5077}$ = 4.27

P<0.001

logistic difference = 2.1656

Ratio of odds = 8.7

Average proportions for comparison only:- PBQ ♀ X LD♂ 69%

LDQ ♀ X PB♂ 95%

TABLE A2

ANALYSIS OF VARIANCE OF MEAN SCORES AMONG POSITIVES ONLY

	1		2		3		Total	
	n	Σx	n	Σx	n	Σx	n	Σx
F_1(PB♀ X LD♂)	12	23	47	98	15	32	74	153
F_1(LD♀ X PB♂)	34	88	42	110	28	68	104	266
Difference in means	0.6716		0.5339		0.2952		178	419

	d.f.	SS	MS	VR	
Replicates	2	0.221	0.110	<1	N.S.
Reciprocal cross	1	10.480	10.480	16.4	P<0.001
Reps and Crosse	2	0.695	0.347	<1	N.S.
Residual	172	109.528	0.6368		
Total	177	120.707			

Weighted [illegible]rence = 0.5067; SE = 0.1249

SS = sums of squares

MS = mean squares

VR = variance ratio

TABLE 4.1

ANALYSES OF VARIANCE OF DIFFERENCES BETWEEN RECIPROCAL F_1's

CLASSIFIED ON THE SCALE 0, +, ++ and +++

	d.f.	SS	MS	VR	
Replicates (unadj)	2	2.282	1.141	1.02	N.S.
Crosses	1	59.708	59.708	53.4	P<0.001
Reps. and Crosses	2	2.743	1.371	1.23	N.S.
Residual	212	236.942	1.1176		
Total	217	301.674			

Mean difference = 1.063; SE = 0.145

TABLE A4

ANALYSES OF VARIANCE OF THE DIFFERENCE BETWEEN ALL THE BACKCROSSES TO PB AND ALL THE BACKCROSSES TO LD

	d.f.	SS	MS	VR
Replicates (unadj)	2	98.873	49.436	61.0
Backcrosses to PB vs backcrosses to LD	1	99.107	99.107	122
Reps. and Crosses	2	34.381	17.190	21.2
Residual	576	466.555	0.8100	
Total	581	698.916		

Weighted mean difference = 0.835; SE = 0.0755

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Selections of *Anopheles gambiae* species A for susceptibility and refractoriness to malaria parasites

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The purpose of this research is to select highly susceptible and highly refractory lines of *Anopheles gambiae* (strain PALA and strain LSW respectively). After the establishment of these two lines, the mode and pattern of inheritance will be investigated.

The eggs of the two strains of *A. gambiae* used in the selection experiments and *A. stephensi* (used as a control for all experiments) were washed with 0·1% formalin solution for 1–2 hours to try to eliminate possible microsporidian infections which might interfere with the development of subsequent malarial infections.

Larvae were reared at a temperature of 21–25° C. but after emerging the adult mosquitoes were maintained at a temperature of 21° C. and a relative humidity of 70–80% and received only 20% glucose solution for 6–10 days before feeding on an infected mouse. Before exposing the infected vertebrate host, mosquitoes were starved, by removing the glucose solution from the cages, for 12 hours.

Plasmodium berghei berghei (ANKA strain) was used in all experiments, and maintained by blood passages in a strain of white mice (Theiler's original mice). To standardize the method the mouse of the second blood passage was used in each experiment. Blood films were made from the tail of the infected mouse at the time of feeding. These films were stained with Giemsa and the number of male and female gametocytes were counted: parasitaemia ranged from 12–16 per 100 red blood cells and 2–5% of parasites found were gametocytes.

The infected mouse was introduced into the mosquitoes' cage lying on its back on a wooden board, immobilized by means of adhesive plaster and drawing pins; the abdomen was shaved.

The results of feeding 9 generations of selected susceptible and refractory strains of *A. gambiae* (PALA and LSW) on mice infected with *Plasmodium berghei berghei*

Generation	Date of feeding 1973	Strains								
		PALA (susceptible line)			LSW (refractory line)			*A. stephensi* (control)		
		Dissected mosquitoes		% susceptible	Dissected mosquitoes		% susceptible	Dissected mosquitoes		% susceptible
		+	−		+	−		+	−	
F_1	1/2	12	9	57·1	15	9	62·5			
F_2	10/3	11	10	52·3	9	24	27·2			
F_3	17/4	22	1	95·7	14	36	28·0	10	0	100
F_4	17/5	56	4	92·2	7	67	9·5	13	0	100
F_5	16/6									
F_6	17/7	41	2	95·3	8	51	13·6	9	0	100
F_7	17/8									
F_8	18/9	13	0	100	2	46	4·2	10	0	100
F_9	18/10	43	0	100	3	57	5·0	13	0	100

The mosquitoes were always fed overnight, from late afternoon until the next morning, cages of mosquitoes being fed in sequence of a single infected mouse for one hour per cage. All the unfed or partially engorged mosquitoes were discarded. The fully engorged females were placed in another cage for 5 days, until complete digestion of the blood meal had taken place, and then were tubed individually in small vials for oviposition.

Those mosquitoes that died within the first 8 days after feeding on the infected mouse were discarded: those that died between the 9th and 13th days were dissected and the results recorded; on the 14th day all the survivors were killed and dissected for both stomach and salivary glands. The number of oocysts on the wall of the stomach was counted and the presence or absence of sporozoites in the salivary glands recorded. The results of 9 experiments or 9 selections are shown in the accompanying Table. The susceptible line is now completely susceptible while the refractory line shows only 5% susceptible individuals.

Crossings between the two lines are in the process of being carried out to determine the mode of inheritance.

In an attempt to check whether the genetic mechanism controlling susceptibility to *P. b. berghei* has an influence on human malaria infection, the two lines were fed on a chimpanzee infected with *P. vivax*. The susceptible line was fully susceptible to the human parasite while the refractory line was only partially so.