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FURTHER OBSERVATIONS ON THE TECHNIQUE FOR QUANTITATIVE ESTIMATION OF SUSCEPTIBILITY OF BED-BUGS TO CONTACT INSECTICIDES.

BY

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[January 17, 1961.]

INTRODUCTION.

SHARMA AND SINGH (1960) explored the possibility of using the World Health Organization's test-kit for body-lice for assessing the insecticide susceptibility of bed-bugs. In their laboratory studies, they found that this kit gave better performance than the World Health Organization's prototype kit for bed-bugs. The former kit was found time-saving and with it the insect mortality in control tests was much lower than with the latter kit. With the body-lice kit, it was considered possible to provide wider variety of insecticides in powder form and wider range of insecticidal concentrations which might give critical range of mortality in a resistant strain. This is not possible with the World Health Organization's prototype test-kit where impregnated filter papers are used and studies can only be carried out with insecticides soluble in Risella oil. The highest concentration of insecticide to be used is limited to the extent of its solubility in oil. High control mortality, following five days exposure to the Risella oil impregnated filter papers, has also been observed by other investigators (Mofidi, 1958; Burden, 1958). This finding which was more pronounced in the field tests, but was also observed in a large number of laboratory studies, has resulted in reconsidering the entire procedure involving use of impregnated filter papers in insecticide susceptibility tests on bed-bugs (Fay, 1960). Therefore, the need for further studies on the utility of the body-lice kit in estimating the insecticide susceptibility of bed-bugs was considered urgent. Hence laboratory and field studies were carried out on the different aspects of the test procedure so that it is well standardized before its adoption as a standard technique for wide-scale use.

(*A*) LABORATORY INVESTIGATIONS.

Laboratory investigations on the different aspects of the test technique were carried out with bed-bugs of the species, *Cimex hemipterus*. The results obtained are discussed below:-

(I) EFFECT OF DILUENT POWDER ON BED-BUGS.

In the earlier experiments on the susceptibility of bed-bugs to contact insecticides using the World Health Organization's body-lice test-kit, insects were exposed to clean cloth pieces as the inert diluent powder was not supplied with the kit. However, it was considered desirable that for proper comparison, control tests are carried out on cloth pieces treated with the diluent powder without any insecticide (Sharma and Singh, 1960 *loc. cit.*). Therefore, tests were carried out with pyrophyllite powder* (grounded finely) to elicit its effect on bed-bugs. For this purpose, adult bed-bugs were exposed continuously for 24 hours to clean cloth pieces and to the cloth pieces uniformally treated with 0·5 gm. of pyrophyllite powder. The mortality rates, recorded at the end of the exposure period of 24 hours in insects exposed to clean cloth pieces and to the cloth pieces dusted with powder, were 2·85 and 15·25 per cent respectively. The difference in the mortality rates was found to be not significant (Table I). In the comparative control tests, however, use of pyrophyllite powder should be made.

TABLE I.

Mortality rates in bed-bugs exposed for 24 hours to clean cloth pieces, and to cloth pieces treated with pyrophyllite powder (0·5 gm.)

Substratum	n	r	p
Cloth pieces, clean.	70	2	2·86
Cloth pieces treated with pyrophyllite powder.	59	9	15·25

n=number of bed-bugs exposed.
r=number of bed-bugs dead and/or moribund.
p=response rate (per cent).

(II) CRITERION OF RESPONSE IN TEST INSECTS.

In recording mortality in bed-bugs at the end of each test, the criterion of response was taken as dead and/or moribund. This criterion can only be considered valid if the moribund bed-bugs do not recover afterwards.

In preliminary tests carried out with 5·0 per cent DDT dust, nine bed-bugs were found moribund with 24 hours of continuous exposure. These insects were kept under observation on clean cloth pieces for a further period of 24 hours. All of them were found dead at the end of the observation period. In another series of tests with DDT, a total number of 170 bed-bugs were continuously exposed to varying concentrations of DDT for 24 hours, and 54 of them were found moribund (Table II). The moribund insects were kept separately under observation for a further period of 24 hours, on clean cloth pieces. All the insects were found dead

* Pyrophyllite powder is used for diluting the different insecticides supplied with the World Health Organization's body-lice kit (Wright and Brown, 1957).

except one of the batch exposed to the lowest concentration of DDT (0·04 per cent).

TABLE II.

Recovery of moribund bed-bugs after exposure to DDT for 24 hours.

Concentration of DDT (per cent.)	Number of bed-bugs exposed.	Number moribund at the end of exposure period.	Number of moribund bed-bugs recovering after 24 hours of holding.
5·0	43	21	Nil
1·0	46	13	Nil
0·2	41	15	Nil
0·04	40	5	1
Total	170	54	1
Control	40	Nil	Nil

This suggested that there is little possibility of the moribund insects to recover afterwards. Therefore, in recording mortality, the moribund insects, for all practical purposes, can be considered as dead.

(*III*) OPTIMUM NUMBER OF BED-BUGS TO BE EXPOSED TO EACH INSECTICIDAL CONCENTRATION.

To estimate the optimum number of bed-bugs to be exposed at a time to any insecticidal concentration, tests were carried out with gamma BHC and DDT by varying the number of insects during the exposure period in each test. In the tests with gamma BHC, 40, 20 and 10 bed-bugs were exposed for 15 minutes to cloth pieces treated with 0·1 per cent gamma BHC. After the exposure, the insects were kept for 24 hours in test tubes, each containing a piece of cloth. The number of insects during the holding period was 40 per tube in all the cases. The data obtained indicated that mortality rates observed in all the three sets of experiments did not differ significantly from each other (Table III). Similar results were

TABLE III.

Results of susceptibility of bed-bugs exposed to gamma BHC (0·1 per cent) for 15 minutes. Mortality recorded after 24 hours of holding.

Test series	n	r	Expected r	$Or-Er$	$(Or-Er)^2$	$\frac{(Or-Er)^2}{E}$
A 40 bugs exposed at a time.	40	40	39	1	1	0·026
B 20 bugs exposed at a time.	40	40	39	1	1	0·026
C 10 bugs exposed at a time.	40	37	39	−2	4	0·102
	120	117				$0·154 = \chi^2_{(2)}$

The value of χ^2, with two degrees of freedom, was found not significant. Therefore, the hypothesis, that the response of bed-bugs to 0·1 per cent gamma BHC was independent of the different degrees of crowdedness during exposure, was considered tenable.

obtained in the tests with DDT when 10 and 20 bed-bugs were exposed continuously for 24 hours after which mortality in them was recorded (Table IV). From the results it may be concluded that 10 to 20 bed-bugs may be exposed at a time to a treated cloth piece.

(*B*) FIELD EVALUATION OF THE TECHNIQUE.

To assess the utility of the World Health Organization's body-lice kit for estimating the insecticide susceptibility of bed-bugs, studies were conducted in Mettupalaiyam Town of Madras State and Gundlupet Town of Mysore State during June-July, 1960. Mettupalaiyam Town was only once sprayed with DDT in 1959. Gundlupet Town was regularly sprayed with DDT from 1954 to 1957 and with gamma BHC in 1958 and 1959 for malaria control/eradication purposes.

Tests were carried out on active adult bed-bugs* of both sexes collected from the two places against DDT, gamma BHC and pyrethrins, following the procedure already described (Sharma and Singh, 1960 *loc. cit*). The data were analysed by the probit analysis method (Finney, 1952) wherever applicable, and different parameters were estimated (Tables V, VI and VII).

RESULTS.

The results of experiments are summarised below under the appropriate headings.

(*I*) LINEARITY OF RESPONSE AND BATCH VARIABILITY.

In all, three, four and three tests were carried out against DDT, gamma BHC and pyrethrins, respectively, with bed-bugs collected from the two localities (Graphs 1, 2 and 3). Out of these, in one test with DDT and two tests with gamma BHC, critical range of mortality in insects was not obtained. In one test with DDT (48 hours' exposure) though critical range of mortality in insects was observed, yet the probit/log concentration relationship was found to be non-linear. This may be due to the selection of resistant strain of bed-bugs as discussed elsewhere. In each of the remaining six tests, critical range of mortality in insects was recorded and the probit/log concentration relationship was found to be linear (Graphs 1, 2 and 3). In all these cases, the low values of the chi-square, with the available degree of freedom (Table VIII), suggested that the probit/log concentration regression lines were good fit straight lines and the differences in mortality rates in the different replicate tests were well within the range of normal variations. This meant that with the technique employed, consistent results in different replicate tests were obtained in the field.

---

* The bed-bug populations of the two localities consisted of both the species, *Cimex lectularius* and *C. hemipterus*. The relative prevalence of *C. lectularius* was found to be 52·9 and 43·9 per cent in Gundlupet Town and Mettupalaiyam Town, respectively.

TABLE IV.

Susceptibility of bed-bugs to DDT. Mortality recorded after 24 hours of continuous exposure.

Concentration of DDT (Per cent.)	Series A: Ten bed-bugs per test during exposure.							Series B: Twenty bed-bugs per test during exposure.						
	n	r	p $C=5\cdot0$	Sxx	Sxy	Syy	X^2 (6)	n	r	p $C=5\cdot0$	Sxx	Sxy	Syy	X^2 (2)
5·0	20	20	100·0	106·6724	337·1833	1194·2784	2·95	20	20	100	64·4815	237·2613	991·1308	0·43
				85·3333	316·0200	1170·3364					50·8497	222·6712	975·0795	
				21·3391	21·1633	23·9420					13·6318	14·5901	16·0513	
1·0	20	16	78·9					20	18	89·5				
0·2	20	16	78·9					20	16	87·9				
0·04	20	7	31·5					20	10	47·4				
Control	20	1	..											

$\bar{x}(A) = 1\cdot5147$; $\bar{y}(A) = 5\cdot6096$ $\bar{x}(B) = 1\cdot2955$; $\bar{y}(B) = 5\cdot6730$.

$$\begin{array}{r} 21\cdot3391 \\ 13\cdot6318 \\ \hline \Sigma Sxx = 34\cdot9709 \end{array} ; \quad \begin{array}{r} 21\cdot1633 \\ 14\cdot5901 \\ \hline \Sigma Sxy = 35\cdot7534 \end{array} ; \quad \begin{array}{r} 23\cdot9420 \\ 16\cdot0513 \\ \hline \Sigma Syy = 39\cdot9933 \\ 36\cdot5534 \\ \hline X^2 = 3\cdot4399 \end{array} \quad \frac{(\Sigma Sxy)^2}{\Sigma Sxx}$$

$b = 1\cdot0224 \pm 0\cdot17$.

The two regression lines are the lines through two mean points, (x, y), with slope; b; thus

$$YA = 5\cdot6094 + 1\cdot0224\,(x - 1\cdot5147)$$
$$= 1\cdot0224x + 4\cdot0947.$$

Similarly $YB = 1\cdot0224x + 4\cdot3485$.

Analysis of variance.

	D.F.	Sum of squares.	Mean squares.
Parallelism ...	1	0·0513	0·0513
Residual heterogeneity ...	4	3·3886	0·8471
	5	3·4399	

The sum of squares for parallelism and residual heterogeneity were not significant; as such the hypothesis, that the two regression lines were parallel, was considered tenable.

Estimation of relative difference in the mean response rates.

$V(M) = 0\cdot057319$.

$m(A) = 0\cdot885$
$m(B) = 0\cdot637$
Difference $(MAB) = 0\cdot248$ $\therefore SE = 0\cdot239$.

The SE was almost equal to MAB; hence the difference between the values of the mean response rates, observed in tests with two different levels of crowdedness, was considered not significant.

TABLE V.

Results of susceptibility of bed-bugs to DDT using the World Health Organization's test-kit for body-lice. Mortality in insects was recorded at the end of 24 hours' or 48 hours' continuous exposure.

Locality	Period of exposure	Serial number of the test.	Concentration of DDT (Per cent.)									
			5·0		1·0		0·2		0·04		Control.	
			n	*r*	*n*	*r*	*n*	*r*	*n*	*r*	*n*	*r*
Mettupalaiyam Town (Madras State)	24 hours' continuous exposure.	1	10	9	10	7	10	3	10	2	10	2
		2	10	8	10	8	10	8	10	4	10	nil
		3	15	10	15	10	15	7	15	8	15	1
		4	10	7	10	6	10	3	10	5	10	3
		5	10	9	10	10	10	6	10	4	10	1
		6	15	10	15	12	12	7	15	6	15	2
	Total	6	70	53	70	53	70	34	70	29	70	9
	p		72·1		72·1		41·0		32·7		C=12·9	
Gundlupet Town (Mysore State)	24 hours' continuous exposure.	1	15	2	15	3	15	nil	15	3	15	1
		2	15	1	15	1	15	3	15	2	15	nil
		3	15	5	15	1	15	2	15	nil	15	1
		4	15	3	15	7	15	3	15	3	15	1
	Total	4	60	11	60	12	60	8	60	8	60	3
	p		14·0		15·8		8·7		8·7		C=5·0	
	48 hours' continuous exposure.	1	15	14	15	12	8	4	15	10	15	5
		2	15	13	15	5	15	5	15	9	15	3
		3	15	13	15	4	15	6	15	5	15	2
		4	15	9	15	11	15	9	15	7	15	2
	Total	4	60	49	60	32	53	24	60	31	60	12
	p		77·1		41·6		31·6		39·6		C=20·0	

n = number of bed-bugs. *r* = number of bed-bugs dead and/or moribund.
p = corrected mortality rate. *C* = control mortality rate.

(*II*) SUSCEPTIBILITY AND RESISTANCE OF BED-BUGS TO DDT, GAMMA BHC AND PYRETHRINS.

The MLC values of DDT and gamma BHC for the bed-bugs of Mettupalaiyam Town were 0·334 and 0·069 per cent, respectively. Similar values for the insects of Gundlupet Town, were more than 5·0 and more than 2·5 per cent, respectively (Table VIII). This suggested that the insects of Gundlupet Town, compared to those of Mettupalaiyam Town, were resistant to both DDT and gamma BHC. The regression co-efficient values and the probit/log concentration curves also confirmed this conclusion. In the case of Mettupalaiyam Town, the flat probit/log concentration line suggested that there too the bed-bug population contained a large proportion of individuals resistant to DDT and gamma BHC (Graphs 1 and 2).

TABLE VI.

Results of susceptibility of bed-bugs to gamma BHC, using the World Health Organization's test kit for body-lice. Mortality in insects was recorded after 24 hours of holding.

Locality.	Period of exposure.	Serial number of the test.	Concentration of gamma BHC (per cent.)									
			2·5		0·5		0·1		0·025		Conrol.	
			n	r	n	r	n	r	n	r	n	r
Mettupalaiyam Town (Madras State.)	½ hour	1	10	6	10	5	10	3	10	6	10	1
		2	10	10	9	8	10	5	10	4	10	2
		3	10	10	10	8	8	7	10	7	10	3
		4	10	10	10	5	10	5	10	4	10	1
		5	15	10	15	14	15	9	15	8	15	3
		6	10	8	10	5	10	9	10	4	10	1
	Total	6	65	54	64	45	63	38	65	33	65	11
	p		79·7		64·2		52·2		40·8		C=16·9	
	4 hours	1	10	10	10	10	10	8	10	6	10	2
		2	10	10	10	9	10	9	10	8	10	1
		3	10	8	10	10	10	8	10	6	10	2
	Total	3	30	28	30	29	30	25	30	20	30	5
	p		91·9		95·9		80·1		59·9		C=16·7	
Gundlupet Town (Mysore State)	½ hour	1	15	1	15	nil	15	1	15	nil	15	1
		2	15	4	15	nil	15	1	15	nil	15	2
		3	15	5	15	3	15	1	15	1	15	1
	Total	3	45	10	45	3	45	3	45	1	45	4
	p		17·1		C=6·1		(The value of the control mortality rate was estimated from the data of three lower dosages of the insecticide and control).					
	4 hours	1	15	9	15	7	15	2	15	5	15	2
		2	15	11	15	8	15	3	15	nil	15	3
		3	15	8	15	11	15	3	15	2	15	3
		4	15	11	15	5	15	3	15	1	15	1
		5	15	12	15	5	15	6	15	4	15	2
	Total	5	75	51	75	36	75	17	75	12	75	11
	p		62·5		39·0		9·4		1·5		C=14·7	

n=number of bed-bugs exposed. r=number of bed-bugs dead and/or moribund.
p=corrected mortality rate. C=control mortality rate.

In the tests with pyrethrins, the MLC was 0·011 per cent with one hour of exposure for the bed-bugs of Mettupalaiyam Town. Similar value for Gundlupet

TABLE VII.

Results of susceptibility of bed-bugs to pyrethrins using the World Health Organization's test kit for body-lice. Mortality in insects was recorded after 24 hours of holding.

Locality.	Period of exposure.	Serial number of the test.	Concentration of pyrethrins (per cent).									
			0·2		0·4		0·008		0·0016		Control	
			n	*r*	*n*	*r*	*n*	*r*	*n*	*r*	*n*	*r*
Mettupalaiyam Town (Madras State)	1 hour	1	10	10	10	8	10	6	10	2	10	1
		2	10	10	10	6	10	6	10	1	10	nil
		3	10	10	10	8	10	5	10	4	10	1
		4	10	10	10	6	10	4	10	3	10	2
	Total	4	40	40	40	28	40	21	40	10	40	4
	p		100·0		66·7		47·3		16·7		10·0	
Gundlupet Town (Mysore State)	½ hour	1	..	..	15	15	15	2	15	2	15	2
		2	..	..	15	14	15	2	15	1	15	1
		3	..	..	15	15	15	8	15	3	15	nil
		4	..	..	15	15	15	6	15	1	15	2
		5	..	..	15	14	15	7	15	1	15	2
	Total	5			75	73	75	25	75	8	75	7
	p				97·0		26·5		1·5		*C*=9·3	
	1 hour	1	15	15	15	13	15	8	15	2	15	nil
		2	15	15	15	14	15	14	15	nil	15	nil
		3	15	15	15	15	15	13	15	11	15	3
	Total	3	45	45	45	42	45	35	45	13	45	3
	p		100·0		92·8		76·2		23·8		*C*=6·7	

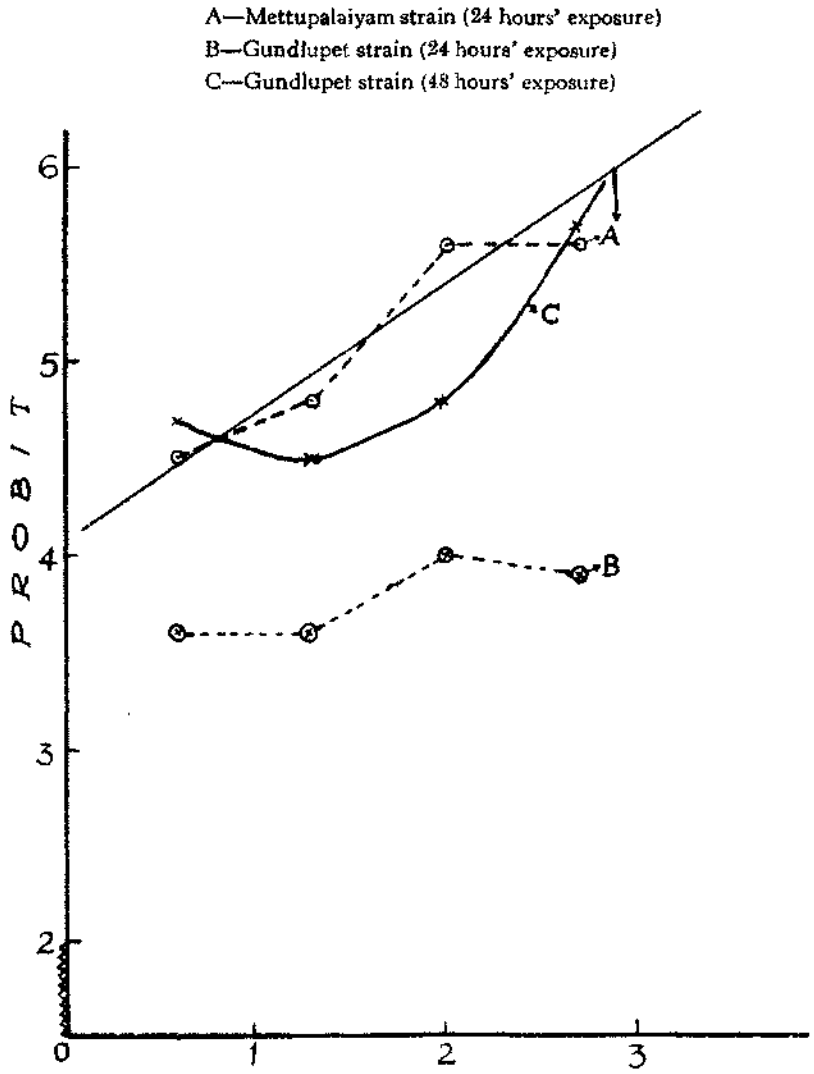
n=number of bed-bugs exposed. *r*=number of bed-bugs dead and/or moribund.
p=corrected mortality rate. *C*=control mortality rate.

Town was found to be 0·0038 per cent. The Mettupalaiyam Town strain of bed-bugs, therefore, for some reasons showed higher tolerance than the Gundlupet Town strain (Graph 3). From the available information, it was ascertained that pyrethrum was never used in that area. This aspect of the problem requires further studies to elucidate the exact nature of tolerance of bed-bugs to pyrethrins.

(*III*) COMPARATIVE TESTS WITH THE WORLD HEALTH ORGANIZATION'S PROTOTYPE TEST-KIT FOR BED-BUGS.

Susceptibility tests were also carried out against DDT and dieldrin with the World Health Organization's prototype test-kit for bed-bugs at Mettupalaiyam Town and Gundlupet Town. Mortality in the insects was recorded daily for five days. The results of the tests are shown in Table IX. It may be seen from the

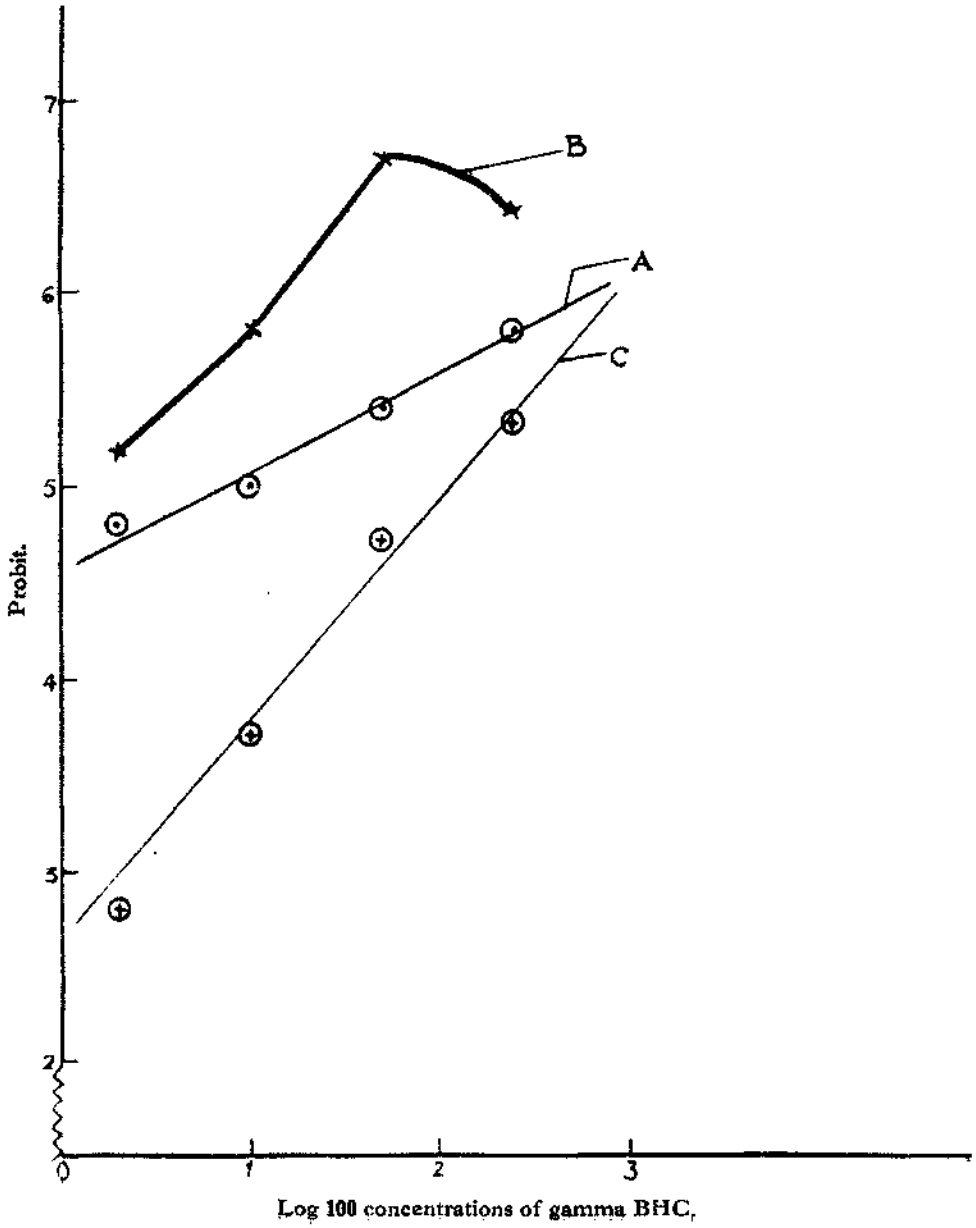
GRAPH 1.
Relationship of probit of mortality and log concentrations of DDT.



data presented that the mortality rates in insects in control tests were very high (42·5 to 80·0 per cent) on the last day of exposure. There was a progressive rise in the mortality rate from the first day to the fifth day of the tests. The results were found to be in conformity with the earlier observations (Sharma and Singh, 1960 *loc. cit.*). This technique, therefore, has serious limitations for field use.

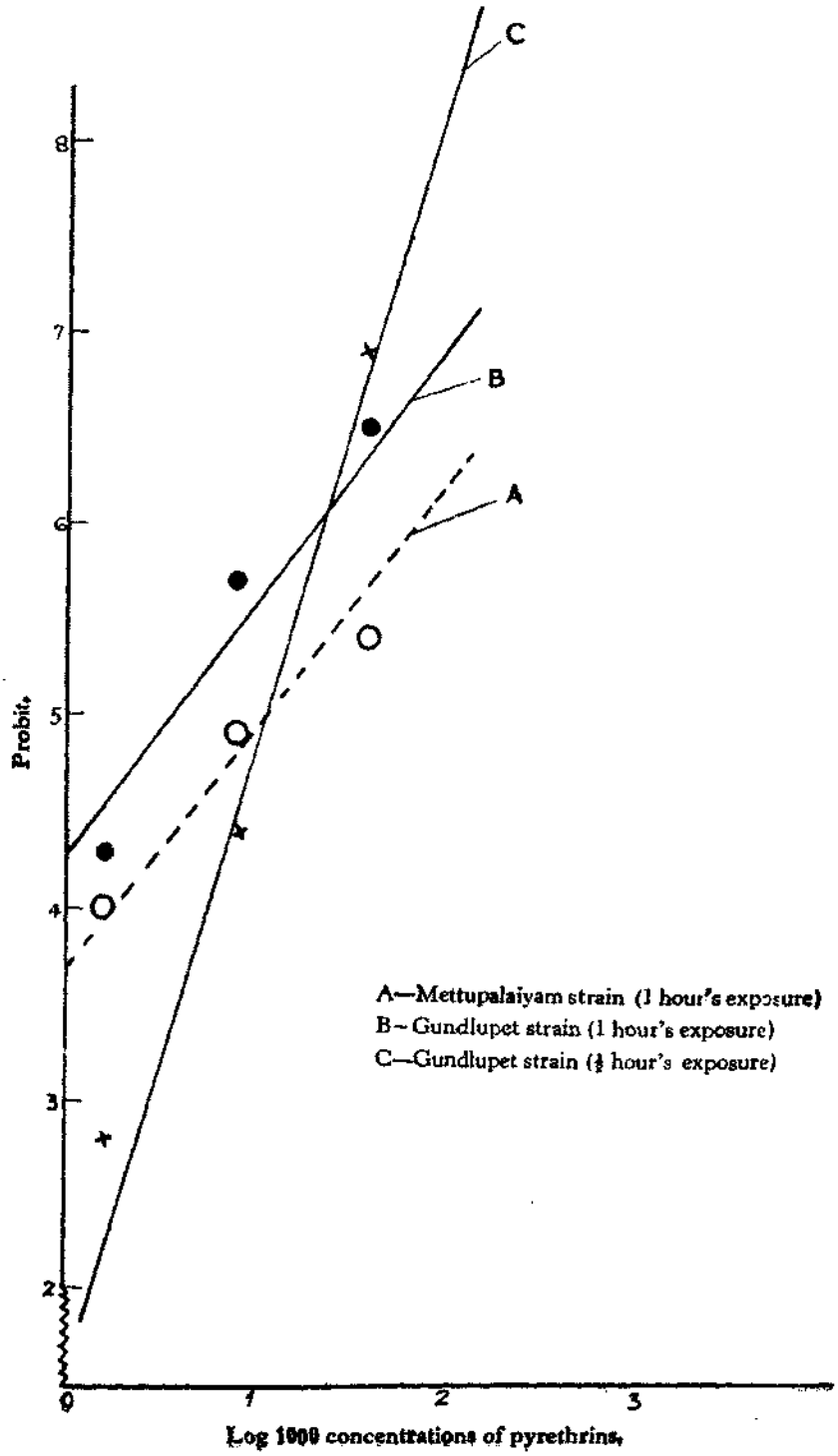
GRAPH 2.
Relationship of probit of mortality and log concentrations of gamma BHC.

A—Mettupalaiyam strain (½ hour's exposure)
B—Mettupalaiyam strain (4 hours' exposure)
C—Gundlupet strain (4 hours' exposure)



GRAPH 3.

Relationship of probit of mortality and log concentrations of pyrethrins.



TABLE

Summary of statistical analysis of the

Locality.	Insecticide.	Exposure period (hours.)	Holding period (hours.)	Equation of the probit/log concentration regression line.	Factors by which concentration was multiplied.	Goodness of fit of the probit/log concentration regression line.
Mettupalaiyam Town (Madras State)	DDT	24	Mortality was recorded at the end of exposure period.	$y=0{\cdot}5529x+4{\cdot}2427$	×100	χ^2 (22)+3·631
Gundlupet Town (Mysore State)	DDT	24	-do-	..	..	..
	DDT	48	-do-	..	×100	..
Mettupalaiyam Town (Madras State)	Gamma BHC	½	24	$y=0{\cdot}4871x+4{\cdot}5905$	×100	χ^2 (22)+3·330
Gundlupet Town (Mysore State)	Gamma BHC	½	24	..	..	..
Mettupalaiyam Town (Madras State)	Gamma BHC	4	24	..	..	χ^2 (22)+3·330
Gundlupet Town (Mysore (State)	Gamma BHC	4	24	$y=1{\cdot}096x+2{\cdot}7212$	×100	χ^2 (18)=0·742
Mettupalaiyam Town (Madras State)	Pyrethrins	1	24	$y=1{\cdot}247x+3{\cdot}6891$	×1090	χ^2 (14)=2·59
Gundlupet Town (Mysore State)	Pyrethrins	1	24	$y=1{\cdot}629x+4{\cdot}0614$	×1000	χ^2 (10)=1·51
do-	Pyrethrins	½	24	$y=3{\cdot}404x+1{\cdot}3444$	×1000	χ^2 (18)=0·10

VIII.

susceptibility test data of bed-bugs.

Regression coefficient value (*b*).	Variance of '*b*'	Variance of '*m*'	LC 50* with 95 per cent fiducial limit.	Remarks.
0·55	0·0141	0·033244	0·532—**0·234**—0·1030	
..	..	..	..	The data were not analysed for estimation of the mean and variance of the tolerance distribution of the bed-bug population to DDT, as the mortality rate in the highest concentration of DDT (5·0 per cent) was less than 50·00 per cent.
..	..	..	..	The data were not analysed for estimation of the mean and variance of the tolerance distribution of the bed-bug population to DDT as the provisional graph showed curvilinear relationship between the probit of mortality rate and log concentration of DDT.
0·49	0·0152	0·055420	0·197—**0·069**—0·024	
..	..	..	>2·50	The data were not analysed for estimation of the mean and variance of the tolerance distribution of the bed-bug population to gamma BHC as the corrected mortality rate in the highest concentration of gamma BHC (2·5 per cent) was less than 50 per cent (17·1 per cent).
..	..	..	<0·02	The data were not analysed for estimation of the mean and variance of the tolerance distribution of the bed-bug population to gamma BHC as the corrected mortality rate in the lowest concentration of gamma BHC (0·02 per cent) was more than 50 per cent.
1·10	0·0476	0·011953	1·96—**1·2**—0·73	
1·25	0·0309	0·010911	0·018—**0·011**—0·007	
1·63	0·0616	0·007921	0·0056—**0·0038**—0·0025	
3·40	0·1966	0·001760	0·0143—**0·0119**—0·0098	

*LC_{50} values are shown in bold type.

TABLE IX.

Results of susceptibility of bed-bugs to DDT and dieldrin using the World Health Organisation's provisional technique for bed-bugs. Mortality in insects was recorded daily for five days.

Insecticide and concentration.	Numbers of replicates.	METTUPALAIYAM TOWN (MADRAS STATE)														
		1 day.			2 days.			3 days.			4 days.			5 days.		
		n	r	p'	n	r	p'	n	r	p'	n	r	p'	n	r	p'
DDT 4·0 per cent.	2	20	5	25·0	20	11	55·0	20	16	80·0	20	17	65·0	20	18	90·0
DDT 2·0 per cent.	2	20	2	10·0	20	4	20·0	20	5	25·0	20	7	35·0	20	7	35·0
DDT 1·0 per cent.	2	20	1	5·0	20	2	10·0	20	6	30·0	20	12	60·0	20	15	75·0
DDT 0·5 per cent.	2	20	3	15·0	20	5	25·0	20	9	45·0	20	13	65·0	20	14	70·0
DDT 0·25 per cent.	2	20	1	5·0	20	4	20·0	20	5	25·0	20	9	45·0	20	9	45·0
Control	2	20	2	10·0	20	3	15·0	20	5	25·0	20	6	30·0	20	13	65·0
Dieldrin 4·0 per cent.	2	20	8	40·0	20	18	90·0	20	19	95·0	..	..	..	20	20	100·0
Dieldrin 1·6 per cent.	2	15	4	26·7	15	8	53·3	15	9	60·0	..	..	..	15	14	93·3
Dieldrin 0·8 per cent.	2	20	4	20·0	20	8	40·0	20	15	75·0	..	..	..	20	20	100·0
Dieldrin 0·4 per cent.	2	20	4	20·0	20	7	35·0	20	13	65·0	..	..	.	20	19	95·0
Dieldrin 0·2 per cent.	2	20	4	20·0	20	6	30·0	20	7	35·0	..	..	..	20	19	95·0
Dieldrin 0·1 per cent.	2	20	4	20·0	20	6	30·0	20	10	50·0	..	..		20	16	80·0
Dieldrin 0·05 per cent.	2	20	2	10·0	20	4	20·0	20	5	25·0	..	..	..	20	17	85·0
Control	2	20	nil	0·0	20	2	10·0	20	6	30·0	..	..	..	20	16	80·0

(*Contd.*)

n=number of bed-bugs exposed.

r=number of bed-bugs dead.

p'=observed mortality rate.

TABLE IX. (*Concld.*)

Insecticide and concentration.	Numbers of replicates.	GUNDLUPET TOWN (MYSORE STATE)														
		1 day.			2 days.			3 days.			4 days.			5 days.		
		n	r	p'	n	r	p'	n	r	p'	n	r	p'	n	r	p'
DDT 4·0 per cent.	2	40	11	27·5	40	18	45·0	40	19	47·5	40	21	52·5	40	25	62·5
DDT 2·0 per cent.	2	40	11	27·5	40	14	35·0	40	21	52·5	40	26	65·0	40	29	72·5
DDT 1·0 per cent.	2	40	7	17·5	40	10	25·0	40	13	32·5	40	14	35·0	40	18	45·0
DDT 0·5 per cent.	2	40	5	12·5	40	10	25·0	40	12	30·0	40	14	35·0	40	17	42·5
DDT 0·25 per cent.	2	40	10	25·0	40	14	35·0	40	16	40·0	40	19	47·5	40	24	60·0
Control	2	40	6	15·0	40	8	20·0	40	14	35·0	40	16	40·0	40	20	50·0
Dieldrin 4·0 per cent.	2	40	8	20·0	40	14	35·0	40	21	52·5	40	25	62·5	40	28	70·0
Dieldrin 1·6 per cent.	2	40	10	25·0	40	12	30·0	40	18	45·0	40	22	55·0	40	24	60·0
Dieldrin 0·8 per cent.	2	40	11	27·5	40	15	37·5	40	18	45·0	40	20	50·0	40	24	60·0
Dieldrin 0·4 per cent.	2	40	6	15·0	40	8	20·0	40	14	35·0	40	23	57·5	40	25	62·5
Dieldrin 0·2 per cent.	2	40	9	22·5	40	12	30·0	40	17	42·5	40	18	45·0	40	19	47·5
Dieldrin 0·1 per cent.	2	40	4	10·0	40	7	17·5	40	13	32·5	40	18	45·0	40	22	55·0
Dieldrin 0·05 per cent.	2	40	4	10·0	40	6	15·0	40	11	27·5	40	15	37·5	40	17	42·5
Control	2	40	4	10·0	40	6	15·0	40	8	20·0	40	11	27·5	40	17	42·5

n=number of bed-bugs exposed.

r=number of bed-bugs dead.

p'=observed mortality rate.

SUMMARY.

Insecticide susceptibility tests on bed-bugs in the laboratory and field were carried out with the World Health Organization's body-lice test-kit with a view to standardizing it and to assess its suitability for field use. The technique was found to give satisfactory performance in the field. With this technique, DDT and gamma BHC resistance in the bed-bugs of Gundlupet Town (Mysore State) was detected. The World Health Organization's prototype test-kit for bed-bugs was found unsuitable for field use due to high control mortality in the insects.

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STUDIES ON THE INCIDENCE AND TRANSMISSION OF FILARIASIS IN MONGHYR (BIHAR).

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INTRODUCTION.

UNDER the auspices of the National Filaria Control Programme (Raghavan, 1955), observations were made in Monghyr Town and surrounding villages under the Filaria Control Unit, Monghyr, between the period May, 1957 to May, 1958, to determine the extent of filariasis.

Monghyr, situated at a latitude of 25°23′ North and longitude 86°28′ East on the south bank of the River Ganges, is an ancient city of historic fame and is the headquarter of the district of the same name. It has a population of 75,000 (according to the 1951 Census) and an area of 6·14 sq. miles.

TOPOGRAPHY.

The district of Monghyr lies between 24°22′—25°49′ North latitude and 85°36′—86°51′ East longitude. The area of the district is 3,975 sq. miles. It has a population of 28,49,127 (1951 Census). River Ganges divides this district into two parts—the North and South Monghyr. The soil of the southern part is hard and rough and the area is mainly covered with forest and hills. The area lying on the western part of South Monghyr is cultivated and has soft soil.

SOCIO-ECONOMIC CONDITION.

Business, trade and cultivation are the main occupations. Socio-economically, majority of the population is poor.

WATER SUPPLY AND DRAINAGE.

Water from the River Ganges is filtered and supplied to the majority of the municipal wards. In Mohallas Bindwara, Mohdipur and Chuabagh area, no domestic tap water is available but the municipal street taps cover these areas. Majority of

the houses have their own wells for domestic use. No tap water supply exists outside the Municipal areas of Monghyr and Jamalpur.

The drainage system of Monghyr is inadequate and inefficiently maintained. The storm water drains are mostly kutcha. Night soil is disposed of by manual carriage to the trenching ground situated outside the urban areas. Fouling by defecation in the storm-water drains is a common feature. The excreta of the jail of about 1,000 persons is discharged into the River Ganges.

CLIMATIC CONDITIONS.

These are favourable for transmission of bancroftian filariasis during the greater part of the year.

PREVIOUS HISTORY OF FILARIASIS.

No filaria survey had been conducted previously in this district but filariasis and elephantiasis were prevalent in the past (O'Malley, 1909). Enquiry, made from persons above 60 years of age, reveals that it is on the increase since the last World War. They associate this disease with urbanisation. Some of the local people believe that this disease is caused by drinking impure water and associate elephantiasis cases with goitre.

PRESENT SURVEY.

A parasitological, clinical and entomological survey was undertaken in the urban areas. Only parasitological survey was carried out in the area outside the town. Blood smears were obtained between 8·30 p.m. and 11·30 p.m. from persons of both sexes and of all ages by random sampling.

RESULTS.

For the purposes of detailed study, an area covering a population of 30,875 persons was surveyed. It comprised Wards XIV, XVII, XX and a part of Ward I known as Belan Bazar, Hetran Bazar, Kasim Bazar, Chauabagh and Bundwara in the Monghyr Municipality.

In this area 4,589 persons, representing 14·8 per cent of the population, were examined. The cumulative infection rate was 11·9 per cent, i.e., 547 persons were positive for microfilariae in their blood. One year old children of both sexes were harbouring microfilariae in their blood. Three hundred seventy-seven (12·8 per cent) males and 170 (10·59 per cent) females had microfilariae—positive blood smears) (Table I, Graph 1). Thus infection was higher in males than in females.

A progressive rise in the infection rate was noted up to the age 20 years. Above this age, there was a decline (Table I, Graph 2). Up to the age 10 years, females showed higher infection rate than males, and above this age up to 30 years there was no significant difference in the infection rates in the two sexes. Again, above the age 30 years males had higher infection rate than females.

TABLE I.

Incidence of filariasis (Urban Area).

	Below 1 year :			1 year—5 years :			6 years—10 years :			11 years—20 years :			21 years—30 years :			31 years—40 years :			41 years—50 years :			51 years and above :			Total.		
	M.	F.	Both sexes.	M.	F.	Both sexes	M.	F.	Both sexes.	M.	F.	Both sexes.	M.	F.	Both sexes.	M.	F.	Both sexes.	M.	F.	Both sexes.	M.	F.	Both sexes.	M.	F.	Both sexes.
Number of persons examined	22	24	46	472	356	828	355	183	538	635	271	906	678	354	1032	409	245	654	215	145	360	131	94	225	2,917	1,672	4,589
Number of persons positive for microfilaria	0	0	0	23	28	51	30	17	47	161	55	261	80	39	119	43	19	62	26	10	36	14	2	16	377	170	547
Percentage of microfilaria infection	0	0	0	4·8	7·8	6·1	8·4	9·2	8·7	25·3	20·2	28·8	11·7	11·01	11·5	10·5	7·7	9·4	12·09	6·8	10·0	10·6	2·1	7·1	12·8	10·16	11·9
Number of persons positive for disease	0	0	0	5	4	9	20	4	24	127	39	166	207	75	282	121	56	177	64	24	88	37	17	54	581	219	800
Percentage for disease	0	0	0	1·06	1·1	1·08	5·6	2·1	4·4	20	14·3	18·3	30·5	21·1	27·3	29·5	22·8	27·06	29·7	16·5	24·4	28·2	18·08	2·4	19·9	13·03	17·11
Positive for both	0	0	0	1	0	1	2	1	3	18	5	23	29	9	38	25	5	30	16	7	23	12	6	18	103	33	136
Percentage for both	0	0	0	0·2	0	0·1	0·5	0·5	0·5	2·8	1·8	2·5	4·2	2·5	3·6	6·1	2·04	4·5	7·4	4·8	6·13	9·1	6·3	8·0	3·5	1·9	2·9

M = Male. F = Female.

TABLE II.

Incidence of filariasis (Rural Area).

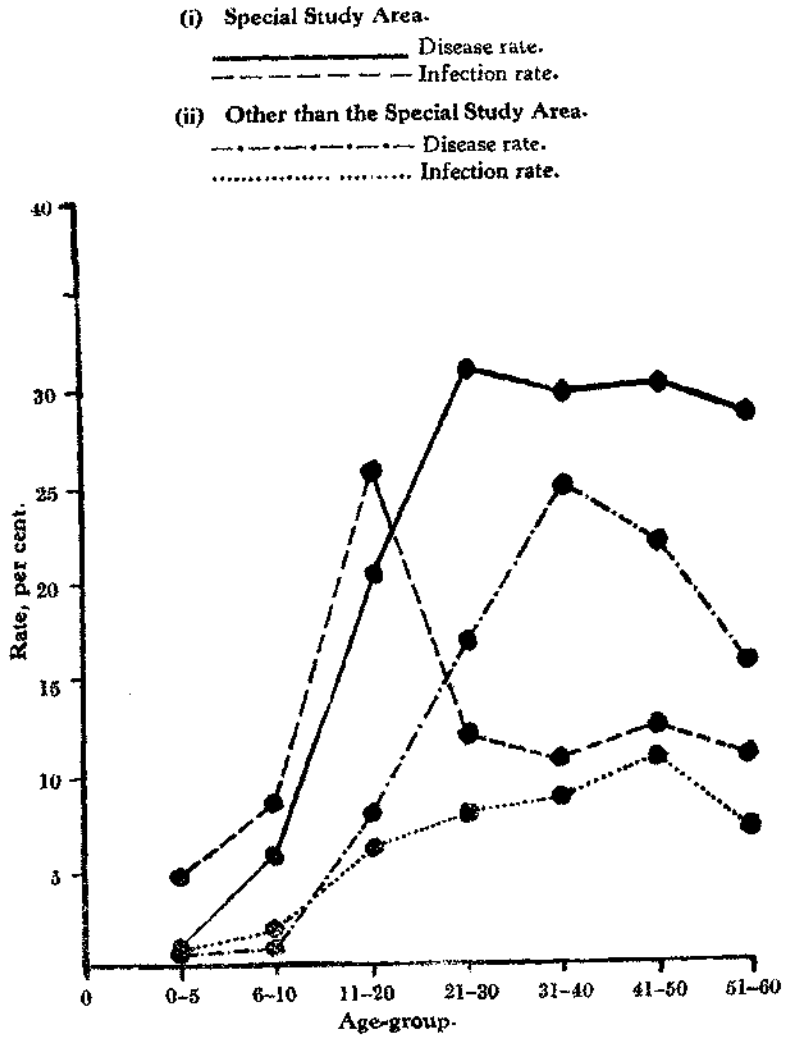
	Below one year :			1 year—5 years :			6 years—10 years :			11 years—20 years :			21 years—30 years :			31 years—40 years :			41 years—50 years :			51 years above :			Total :		
	M.	F.	Both sexes.	M.	F.	Both sexes.	M.	F.	Both sexes.	M.	F.	Both sexes.	M.	F.	Both sexes.	M.	F.	Both sexes.	M.	F.	Both sexes.	M.	F.	Both sexes.	M.	F.	Both sexes.
Number of persons examined	10	12	22	772	582	1,294	1,129	501	1,630	1,382	492	1,874	1,113	598	1,716	541	327	268	364	281	595	186	170	356	5,502	2,913	8,415
Number of persons positive for microfilaria	0	0	0	7	8	15	22	13	35	70	11	81	84	26	110	47	10	57	33	8	41	13	6	19	276	82	358
Percentage of microfilaria infection	0	0	0	0·9	1·2	1·08	1·9	2·6	2·1	5·05	2·2	4·3	7·5	4·3	6·4	8·5	3·05	6·5	10·4	3·4	6·8	3·9	3·5	5·3	5·1	2·7	4·3
Number of persons positive for disease	0	0	0	5	2	7	11	9	20	119	16	126	127	25	212	135	25	160	79	18	97	29	12	42	556	108	664
Percentage for disease	0	0	0	0·7	0·3	0·5	0·9	1·7	1·2	7·9	3·2	6·7	16·7	5·02	12·3	24·9	7·6	18·4	21·7	7·7	16·3	15·5	7·6	11·7	10·2	3·6	7·9
Positive for both	0	0	0	0	0	0	1	0	1	10	4	14	23	4	27	20	3	23	12	2	14	5	1	6	71	14	85
Percentage for both	0	0	0	0	0	0	0·08	0	0·06	0·7	0·8	0·7	2·05	0·8	1·5	3·6	0·9	2·7	3·2	0·8	2·3	2·6	0·5	1·6	1·3	0·4	1%

M = Male. F = Female.

GRAPH I.

Incidence of filarial disease and infection among males.

(Urban area)

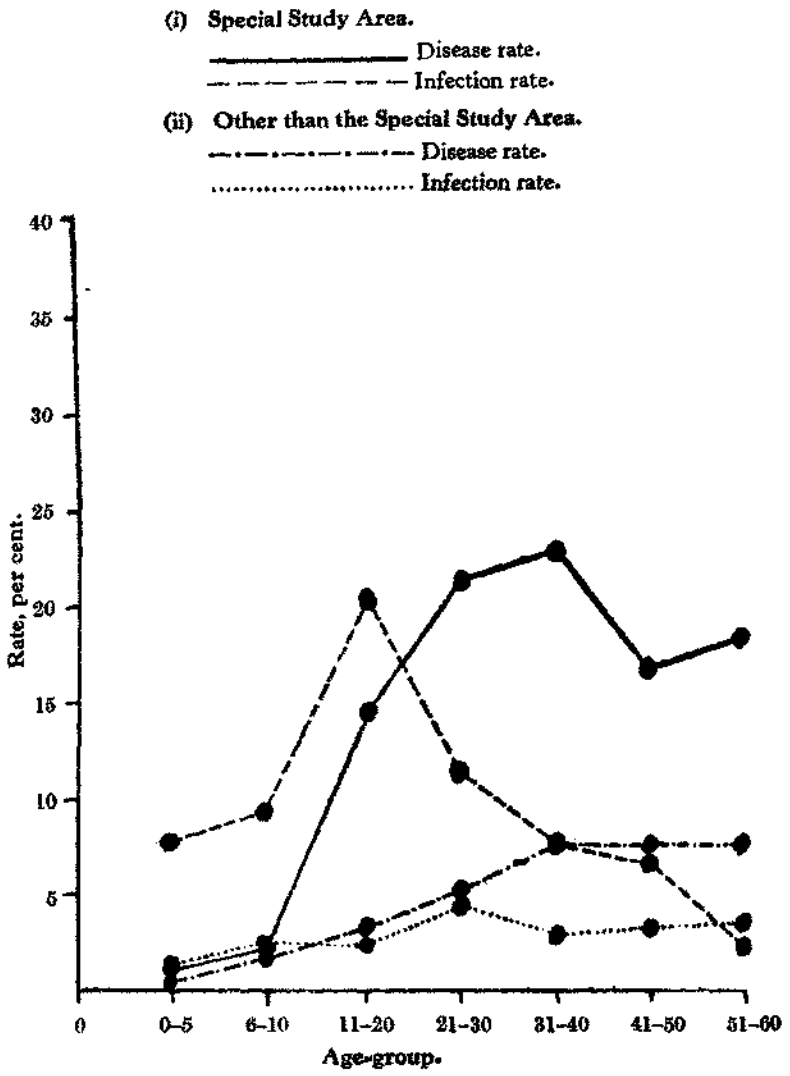


Amongst the persons examined, 581 males (19·9 per cent) and 219 females (13·03 per cent) had disease manifestations. The cumulative disease rate was 17·11 per cent. A progressive rise in disease rate was noted up to the age 40 years (Table I, Graph 3) and thereafter it was on the decline. The disease rate was higher in males in the all age-groups than in females. The youngest person to show disease was a boy of 2 years. Swelling of extremities (upper, lower, or both) (temporary or permanent), hydrocele, other genital manifestations including

GRAPH 2.

Incidence of filarial disease and infection among females.

(Urban area)

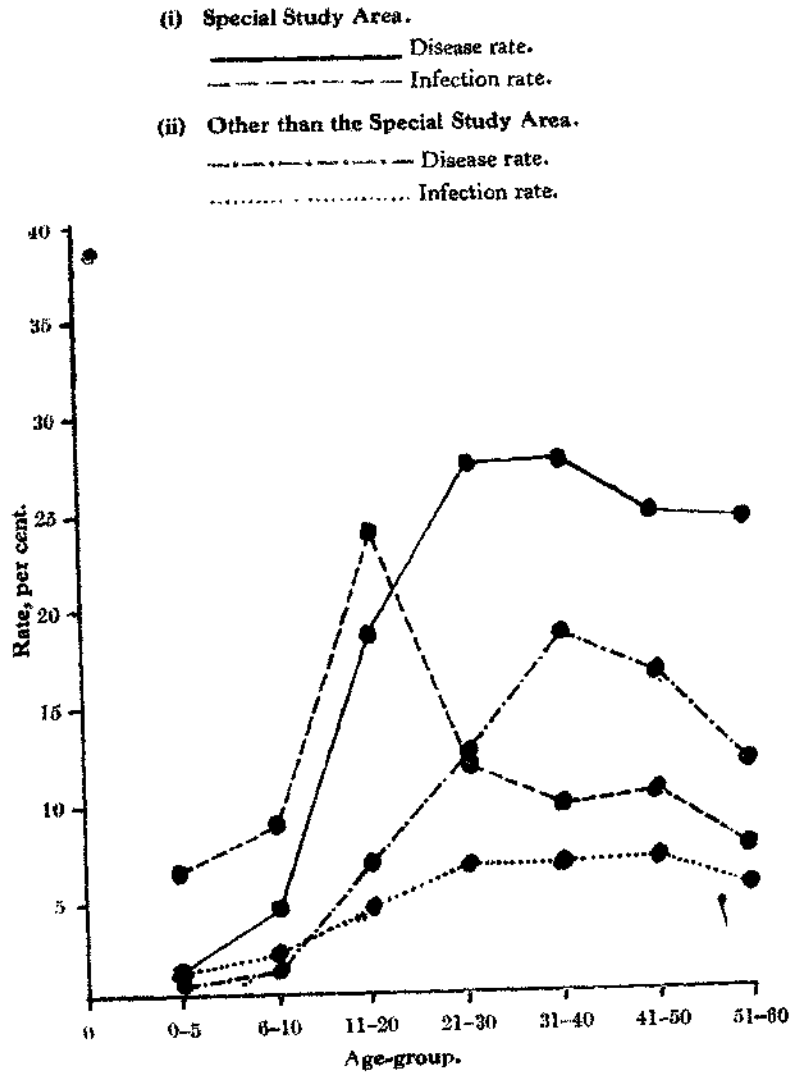


elephantoid scrotum, epididymo-orchitis, etc., and filarial fever were recorded as disease symptoms during the survey. One hundred-and-three (3·5 per cent) males and 33 (1·9 per cent) females were positive for both infection and disease. The youngest age and sex, exhibiting both disease and infection, was a 5 years old male (Table I).

GRAPH 3.

Incidence of filarial disease and infection among both sexes.

(Urban area)



The endemicity rate in the urban area was noted to be 26·6 per cent.

The average infestation of microfilariae per 20 c.mm. of blood for the community was recorded as 19·1. The average infestation was higher in males than in females in all age-groups. The maximum infestation was noted at the age 7 years and was considerable above the age 8 years (Table II).

RURAL AREA.

This area included Jamalpur and part of the Monghyr Municipality as well as villages lying in the jurisdiction of the Police Stations Monghyr, Kharagpur and Surajgarha. Out of 8,415 persons examined, 358 (4·3 per cent) were found positive for microfilariae, and of them 276 (5·4 per cent) were males and 82 (2·8 per cent) females. Thus infection was relatively commoner in males. The infection rate was observed to be low in the lower age-groups and rose up to the age 30 years, and afterwards it was more or less constant (Table II, Graph 3). A girl, aged one year, was positive for infection.

664 (7·9 per cent) persons were recorded to have disease manifestations. Of them, 556 (10·9 per cent) were males and 108 (3·6 per cent) females. The disease rate was higher in males. There was a progressive rise in the disease rate up to the age 40 years and thereafter remained almost level (Table II, Graph 3). Signs of early filarial disease were noted in a boy aged 2 years. Both the disease manifestation and infection were noted in 85 (0·99 per cent) persons. The filarial endemicity rate for the population examined was 11 per cent.

The average microfilarial infestation per 20 c.mm. of blood was higher in males than in females except for the age-groups 6, 7, 8 and 10 years. The average count of microfilaria per 20 c.mm. of positive blood in the community examined was 17·2.

ENTOMOLOGICAL SURVEY.

During the period of observation mentioned above, mosquitoes were collected in three types of shelters, viz., human dwellings, mixed dwellings and cattle sheds during April and May, 1958, only.

Culex fatigans (Weid.) was found to be the abundant domestic species. It was found to breed in stagnant drains and cesspools with high degree of organic pollution and also in the ditch around the fort of Monghyr.

The results of the dissection given below showed that only *Culex fatigans* carried the natural infection of *Wuchereria bancrofti*.

Number of *Culex fatigans* dissected	... 372
Number of *Culex fatigans* positive	... 129
Percentage of *Culex fatigans* infected	... 32·5

DISCUSSION.

The present study in the entire area shows that, of 8,419 males examined 653 (7·7 per cent) showed microfilariae in blood and 1137 (13·5 per cent) disease manifestations. The corresponding figures among 4,585 females were 252 (5·4 per cent) and 327 (7·1 per cent), respectively. As the protean manifestations of

disease were also taken into account, the actual rates noted may not be so accurate. Similar observations were made by Mackenzie (1925) and King *et al.* (1929).

The youngest persons to show microfilaria in blood were boys and girls aged 1 year whereas the youngest age at which the disease was noted was 2 years.

Filarial infection, as determined by blood examination, showed rising incidence up to the age 20 years, followed only by a minor drop. This is in agreement with the records made by Krishnaswami (1955).

The microfilarial infestation per 20 c.mm. of blood in positive persons ranged from 1 to 129. The average infestation rate was found to be 19·1 per 20 c.mm. of blood. Agewise analysis showed no relationship. However, the higher density among the adults (9·5 to 35·3 per 20 c.mm.) is a pointer to the magnitude of the reservoir of infection, specially in the urban areas of Monghyr.

A comparison of the infection rate and average infestation of the communities in the urban and rural areas bring out clearly that the spread of bancroftian filariasis is from the urban to the surrounding rural areas. This indicates that the control measures, mainly anti-larval, should be vigorously taken up in the urban areas to check the disease at its source. The feasibility of extending such anti-larval measure to rural areas should also be tried.

TABLE III.

Average infestation of microfilaria of Wuchereria bancrofti *in 20 c.mm. of blood.*

Age group.	Urban Area :		Rural Area :	
	Males	Females	Males	Females
Below 1 year ...	..	..	..	..
1 year ...	12·5	8·3	..	4·0
2 years ...	11·0	20·7	2	..
3 years ...	30·3	26·9	49·5	3
4 years ...	56·0	37·0	5·5	..
5 years ...	10·4	17·7	4	2
6 years ...	11·5	9·5	2	4
7 years ...	23·0	57·2	1	4·3
8 years ...	8·8	19·6	2·6	46·9
9 years ...	37·5	8·0	12	1
10 years ...	30·3	12·9	10·3	15·0
11—20 years ...	9·5	10·7	42·1	19·7
21—30 years ...	24·06	23·8	9·7	7·8
31—40 years ...	32·6	23·8	13·3	7·9
41—50 years ...	35·5	28·0	10·5	7·6
Above 51 years ...	20·3	34·5	14·9	10·8
Average of the total	19·1		17·2	

SUMMARY.

The filarial disease rate in the urban areas of Monghyr and Jamalpur was found to be 17·11 per cent and the microfilarial infection rate, 11·9 per cent. The corresponding figures for the rural areas were 7·9 per cent and 4·3 per cent.

The average microfilarial infestation per 20 c.mm. of positive blood in the town and rural areas was 19·1 and 17·2, respectively.

The filarial endemicity rate for the town and rural areas was 26·6 and 11 per cent., respectively.

The youngest persons to show the infection and the disease were 1 year and 2 years old children respectively, of both sexes.

W. bancrofti was the only species of filaria recorded and *Culex fatigans* (Weid.) was the only mosquito exhibiting the natural infection with microfilaria.

ACKNOWLEDGEMENTS.

The authors are greatly indebted to Dr. S.M. Hassan, Director of Health Services, Bihar, for his kind permission to publish this paper.

Thanks are also due to the staff of the Filaria Control Unit, Monghyr, for their active co-operation during the survey.

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A NOTE ON THE ABSENCE OF ACTION OF HETRAZAN* AND ALVO-SULPHONE (DDS)† ON THE MICROFILARIAE OF *DIROFILARIA REPENS* IN DOG AND THE DEVELOPING FORMS IN THE MOSQUITO.

BY

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[July 1, 1961.]

INTRODUCTION.

Of the two common filarial infections of the dog, namely *Dirofilaria immitis* and *Dirofilaria repens*, the former has been used to a greater extent than the latter to study the action of drugs. Little work appears to have been done on the action of hetrazan on *D. repens* infection of the dog except for McGaughey's (1952) work in Ceylon. Hawking *et al.* (1950) reported that hetrazan had no action against *D. repens* infection developing in the mosquitoes.

Results of studies on the action of hetrazan and DDS on microfilariae of *D. repens*‡ and their development in mosquitoes are reported here.

MATERIALS AND METHODS.

Thick blood smears (20 c.mm. each) were taken daily from the ear lobe of each of the two dogs (Serial numbers 16 and 30) used in these experiments. Two such smears were taken in the morning and evening from each of the animals. The mean of the number of microfilariae in the four smears in each case was taken to be the daily infestation.

Each dog was given hetrazan orally for five days. Drug was given at the rate of 5·6 mg./kg. body weight to Dog No. 16 and 3·8 mg./kg. to Dog No. 30. Tablets of the drug were placed in the centre of a cooked piece of mutton which the dog ingested immediately under observation of the author.

*Aedes aegypti*** mosquitoes were fed daily on dog No. 30, one day before drugging with hetrazan, during the five days of drugging and also on the day after the cessation of the treatment. The fully fed mosquitoes were held in the insectary under identical conditions. A few mosquitoes from all the batches were dissected daily and the progress of development of the parasite in the mosquito was

* 1-diethylcarbamyl-4-methylpiperazine hydrochloride.

† DDS or Alvo-sulphone is a preparation of 4 : 4′ diaminodiphenyl sulphone of the Imperial Chemical Industries.

‡ The filarial infection in the two dogs under experiment was identified as that of *D. repens* after examination of microfilariae in the blood as described by Dalip Singh *et al.* (1958) and also by their development in *A. stephensi* as reported by Webber and Hawking (1955).

** From the laboratory colony.

TABLE I.

Number of microfilariae (D. repens) *per 20 c.mm. blood in dogs before, during, and after the administration of Hetrazan andAlvo-sulphone (DDS).*

Number of days	c	c	c	c	1	2	3	4	5	6	7	8	9	10	11	12	13
Dog No. 30 No. of mf.	60	47	24	37	DEC 74	DEC 137	DEC 102	DEC 58	DEC 142	113	79	..	..	122	..	56	32
	..	..	120	110	DDS 60	DDS 95	DDS 67	DDS 74	DDS 25	62	60	65	41	55	109	83	85

Number of days	14	15	16	17	18	19	20	24	30	36	43	61	64	71	76	83	89	95
Dog No. 30 No. of mf.	93	67	..	131	..	..	..	86	45	91	119	130	132	124	119	133	181	215
	88	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..

Number of days	c	c	c	c	1	2	3	4	5	6	7	8	9	10	11	12	13
Dog No. 16 No. of mf.	..	..	31	48	DEC 45	DEC 47	DEC 29	DEC 44	DEC 19	76	17	36	70	50	28	53	56
	..	..	55	33	DDS 83	DDS 48	DDS 56	DDS 25	DDS 32	16	39	47	23	35	28	47	56

Number of days	14	15	16	17	18	19	20	21	22	23	24	25
Dog No. 16 No. of mf.	..	14	..	57	51	37	..	..	75	38	65	87
	41	32	30	59	37	..	51	..	..	..	..	..

c = Days of control observation before administration of drug.
DEC = Days on which Diethylcarbamazine (Hetrazan) was administered.
DDS = Days on which Alvo-sulphone was administered.
.. = Days on which blood smears were not taken.

observed till the larvae had reached the infective stage. Observation on the batch of mosquitoes that had fed on the day prior to the commencement of therapy, served as control.

One hundred and ninety-seven infective larvae of *D. repens* were recovered from the batch of mosquitoes fed on Dog No. 30 on the 5th day of drugging with Hetrazan. They were inoculated subcutaneously into the right groin of a normal pup (Serial number 47) weighing 2·2 kg. to observe their further development in this host.

About 4 and 8 months respectively after the completion of Hetrazan treatment, DDS was administered to Dogs Nos. 16 and 30. The dosage schedule for the two dogs was 5·6 and 6·3 mg./kg., respectively. The daily mean count of microfilariae in the blood films was determined in a manner similar to that in the case of Hetrazan treatment. Observations regarding the development of microfilariae in the mosquitoes were not carried out in this case.

The results recorded in Table I show that both Hetrazan and DDS had no effect on the microfilariae (*D. repens*) in the dogs under experiment.

TABLE II.

Time taken by microfilariae (D. repens) *to reach infective larval stage in* Aedes aegypti *mosquitoes fed before, during, and after the administration of Hetrazan to Dog No. 30.*

Time taken for appearance of infective larvae after blood meal	Before drug (control)	DAYS OF DRUG					
		1st	2nd	3rd	4th	5th	6th day, no drug.
Days	14	13	12	11	16	13	12

The results of dissection of mosquitoes showed that Hetrazan did not inhibit the growth of the parasite (*D. repens*) in *Aedes aegypti* as the microfilariae took more or less the same time to reach infective larval stage as in the control.

Pup No. 47 showed microfilariae of *D. repens* in its peripheral blood seven months after the inoculation of infective larvae taken out of mosquitoes fed on Dog No. 30 after 5 days of Hetrazan treatment.

DISCUSSION.

The dose of Hetrazan given in these experiments was rather low as it was intended to give a similar dose of Hetrazan to dogs with *D. repens* infection, as advocated by Raghavan (1955) for mass therapy with diethylcarbamazine against bancroftian filariasis in the National Filaria Control Programme in India.

Although Hetrazan was found to be effective against the microfilariae of *D. immitis* of dog (Hewitt *et al.*, 1947 and 1948; Foley, 1950), in the dosages administered it has been found to be ineffective on the *D. repens* infection in dogs during the present studies. These findings are in agreement with that of

McGaughey's (1952 *loc. cit.*) work in Ceylon, as his results in the treatment of filariasis of dogs with Hetrazan were also disappointing. The reason, perhaps, was that most, if not all, cases of dog filariasis encountered in Ceylon were of *D. repens.* Adult worms of *D. repens* were actually found in 2 out of the 16 dogs treated.

Canet and Jahan (1950) tried Hetrazan on three dogs with filarial infection. The microfilariae in these dogs were stated to resemble those of *D. immitis*, possibly those of *Acanthocheilonema dracunculoides*, but accurate identification of the parasite was not possible. The drug was stated to be ineffective on this infection.

It is evident that Hetrazan is effective specifically only against *D. immitis* and not *D. repens* in the dog. The lack of effect on *D. repens* is most likely due to the physiology of the parasites and not of the dog because it has action on the other filarial infection of dog, namely *D. immitis.*

It is also of interest to observe that Hetrazan or its metabolites, circulating in the blood on the days of therapy when *Aedes aegypti* mosquitoes were fed on the dog, showed no inhibitory effect on the developing forms in the mosquitoes. The infective larvae from mosquitoes fed on the dog on the fifth (last) day of Hetrazan therapy, when inoculated into the normal pup, underwent normal development. Microfilariae appeared in the peripheral blood of this animal seven months after the inoculation. Six to seven months is the normal period for this infection to develop in the dog (Webber and Hawking, 1955). Wilson (1961) stated that the action of Hetrazan on *B. malayi* (Semi-periodic) infection in cats is to reduce the number of circulating microfilariae, at the same time it does not effect their infectivity to mosquitoes and the larvae, which develop in the mosquitoes, are capable of infecting cats.

From the foregoing it can be concluded that Hetrazan in the dosages administered had no effect on the microfilariae (*D. repens*) with respect to (*a*) the number circulating in the peripheral blood of the dogs, (*b*) their development in the *Aedes aegypti* mosquitoes fed on the fifth day of treatment on Dog No. 30. There is also an indirect evidence to show that Hetrazan had no effect on the adult worms (*D. repens*) as the microfilaria count in both the dogs showed gradual rise after treatment (Table I).

DDS on the *D. repens* infection in dogs in the present studies was also found to be ineffective.

Sewell and Hawking (1950) studied *in vivo* action of some sulphones against microfilariae and adult worms of *L. carinii* and found them to be ineffective both against microfilariae and adult worms. DDS was tried on 150 patients and found to have some effect in reducing the number of filarial fever attacks and in some cases the interval between the attacks was also prolonged; the drug was however found to be ineffective against microfilariae of *B. malayi* (Malaria Institute of India, 1960).

SUMMARY.

1. *In vivo* action of Hetrazan and Alvo-sulphone (DDS) against the microfilariae of *D. repens* was studied in two dogs. Both the drugs were found to be ineffective.

2. It was also observed that Hetrazan had no inhibitory effect on the microfilariae of *D. repens* developing in the *Aedes aegypti* mosquitoes fed on the infected dog on the fifth day of drugging with Hetrazan.

3. Infective larvae taken out of the above mentioned mosquitoes, when inoculated into a normal pup, the pup showed microfilariae (*D. repens*) in its peripheral blood after seven months, the normal time for this infection to develop in dog.

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STUDIES ON *PLASMODIUM BERGHEI* VINCKE AND LIPS, 1948.

Part XXXII. Effect of the lowering and raising body temperature of the host (white rat)* on the course of *P. Berghei* infection.†

BY

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[June 26, 1961.]

THE body temperature of normal white rats is 37°C. with a cyclical variation of temperature of about 0·6°C. on either side, with the maximum at midnight and the minimum at 1 p.m. (Verain and Verain, 1955). In white rats infected with *P. berghei*, five-hour fluctuations with rises of 1·5° to 2°C. lasting for about one hour have been observed. This does not reveal any cycle of temperature that can be ascribed with certainty to the development of the parasite (Verain and Verain, 1955 *loc. cit.*). In the present investigation, attempts were made to determine the effect on the course of infection of *P. berghei* of hypothermia and temperature rise maintained in the host.

MATERIALS AND METHODS.

Ninety-six rats of both sexes, each weighing between 150 and 298 gm., were utilised in this investigation. No choice was exercised regarding age or sex of the animals used.

Hypothermia in rats.—The method was modified from that described by Vogel (1959). The infected white rats were starved over-night. Each animal received 5 mg./kg. largactil‡ subcutaneously. Within 10 minutes after injection the animals were immersed for two to three minutes in a bowl of ice-water, keeping only the head above water. Thereafter, the animals were transferred, one each to a glass jar fitted with air-tight lid. The glass jars were then placed in a cold chamber (refrigerator) with a constant temperature of 10°C. The jars were opened every

* White rats were obtained from the colony maintained at the Malaria Institute of India, Delhi.

† The strain was the same as Strain (II) of Ramakrishnan *et al.*, 1953. The inoculations were given intraperitoneally at one million parasites per animal as described by Krishnaswami *et al.* (1954).

‡ Largactil (May and Baker Ltd.) 2·5 p.c. W/v of chlorpromazine.

10 minutes for 10 seconds. Thus, under the combined influence of chlorpromazine and altered CO_2 concentration in a low ambient temperature, it was possible to produce hypothermia.

The treatment brought down the rectal temperature from the normal 37°C. to 15°—20°C. The animals were kept in the cold chamber for a period of three hours. Thereafter the animals were placed in the open at a room temperature of 30°—33·5°C., depending on the season. The animals revived in the course of about three hours and the rectal temperature returned to normal (37°C.) in the course of 8 to 10 hours. Eight, out of forty-one rats put under hypothermia, failed to revive and had to be discarded. Thus, in the experimental rats it was possible to produce hypothermia lasting for a period of 8 to 10 hours.

The effect of hypothermia was studied in three different groups of infected rats. In one group, the rats were put under hypothermia lasting 8 to 10 hours a day on the zero and first day of infection. In the second group, similar treatment was given on the third and fourth day of infection and in the third group, on the seventh and eighth day of infection.

Largactil only.—Largactil 5 mg./kg. subcutaneously was given to three different groups of infected rats. The first group received largactil on the zero and first day of infection, the second group received the same treatment on the third and fourth day of infection, while in the third group largactil was given on the seventh and eighth day of infection. The treated rats were kept at room temperature and rectal temperature recordings did not show any appreciable change.

Raised body temperature.—Injection of TAB* 0·05 ml. per animal subcutaneously produced a rise of rectal temperature by 1·0°C. to 2·2°C. seven to twelve hours following the injection. Such a rise of temperature lasted for a period of eight to thirty-six hours.

The effect of rise of body temperature was studied in three different groups of infected rats. In one group the rats received TAB injection on the zero day of infection, in the second group the animals received TAB injection on the third day of infection and in the last group TAB was given on the seventh day of infection. All the experimental animals treated this way, registered a rise in the rectal temperature.

Course of infection.—Stained thin blood smears were examined daily and the parasites were counted against 10,000 erythrocytes. A control group of infected rats, without any treatment, was examined to compare the normal course of infection.

RESULTS.

The control group had eleven rats. Parasite count was made on all these animals from first to eleventh day of infection. As within this same sub-group large variation in the count was observed, geometric mean, instead of the arithmetic

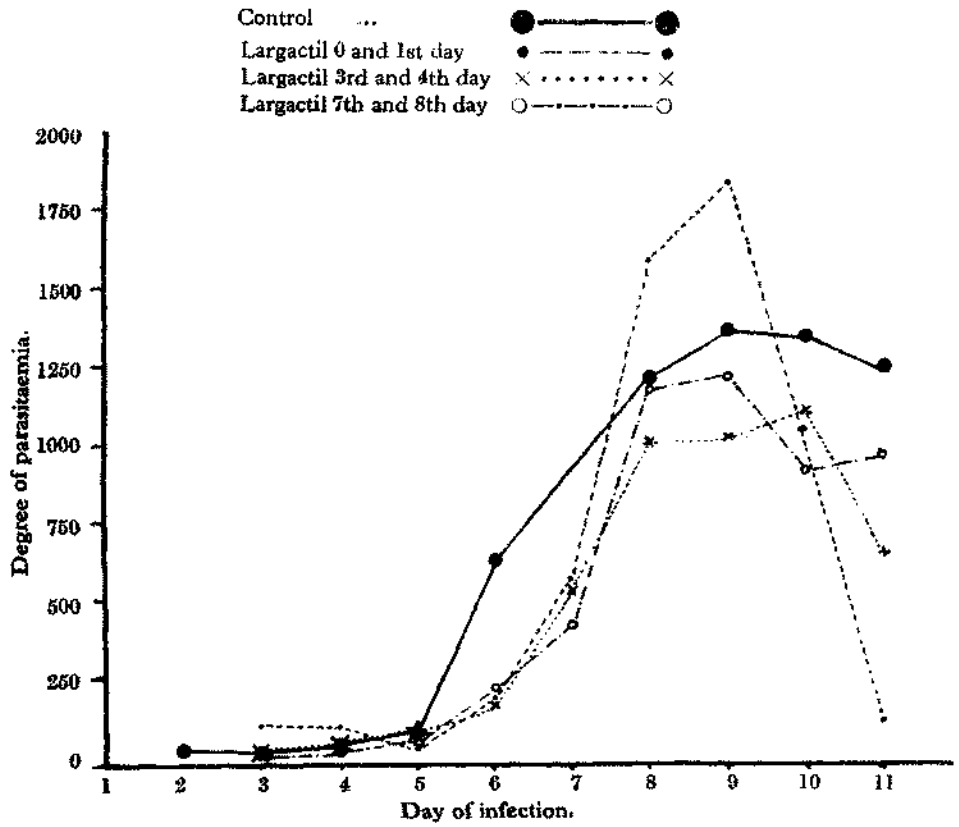
* (Typhoid paratyphoid A & B vaccine manufacture by Bengal Immunity Ltd.).

mean, was calculated. In all, there were three treatments, i.e., largactil only, largactil and hypothermia, and hyperthermia. The observations in these groups and in the control group are mentioned in Table I. For comparison of results, the geometrical mean parasite counts of the treated groups are represented graphically along with that of the control group in Graphs 1 to 3.

The average daily parasitaemia of the groups which received largactil only (Graph 1) shows that largactil treatment on the zero and first day of infection produced no change except a depression on the sixth and seventh day of infection. With largactil administration on the 3rd, 4th as well as 7th and 8th day of the infection, a slight depression of parasitaemia was observed during the periods following the drug administration.

GRAPH 1.

Degree of parasitaemia in the rats receiving largactil during the course of infection and in the control rats.



The depression of parasitaemia in the hypothermic rats on the zero and first day of infection was observed throughout the observation period and was more pronounced than with largactil alone (Graph 2). Hypothermia on the 3rd and 4th

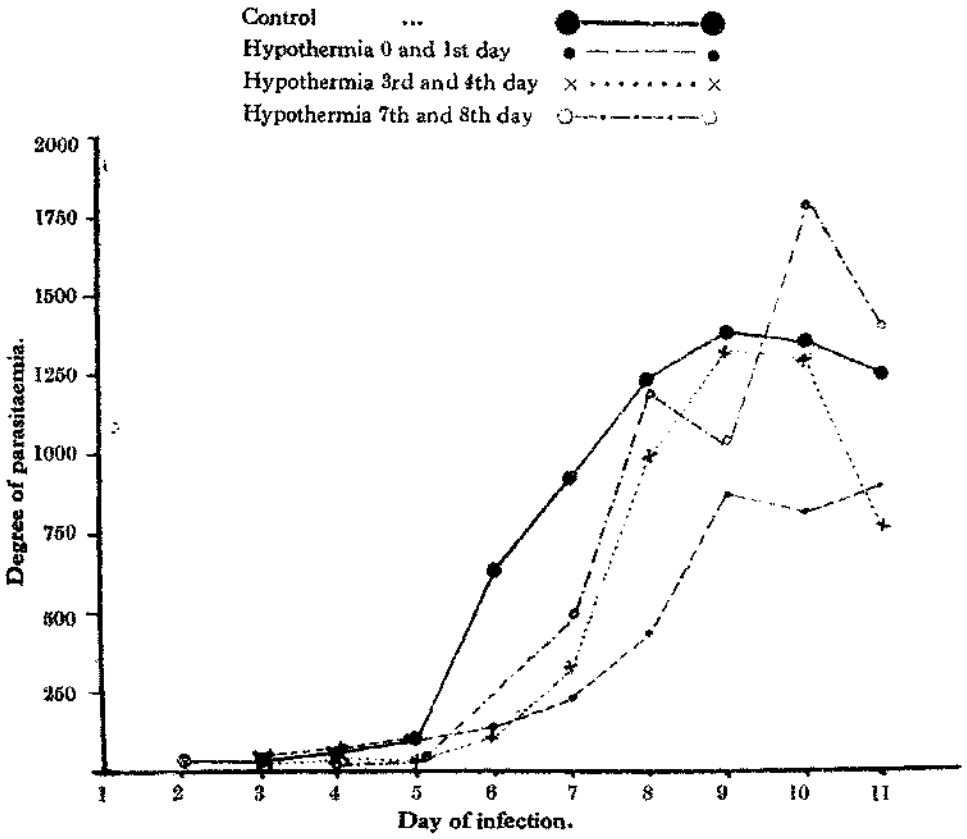
TABLE I.

Course of infection in the control and the experimental white rats. Geometric average of the daily count of parasites against 10,000 RBCs of the individuals represents the parasitaemia of the group.

Particulars of the treatment groups.	Number of animals used.	GEOMETRIC AVERAGE OF THE PARASITAEMIA (DAYS OF INFECTION) :										
		1	2	3	4	5	6	7	8	9	10	11
Control	11	6·7	28·7	20·2	50·6	89·3	630·9	928·9	1247·4	1377·2	1352·1	1256·0
Largactil only on the 0 and 1st day of infection	6		7·3	105·2	101·4	46·3	106·8	594·3	1607·0	1849·0	1052·0	113·8
Hypothermia on the 0 and 1st day of infection	8	2·3	2·3	15·9	52·2	93·5	123·0	225·9	445·6	864·9	796·1	895·3
Hyperthermia on the 0 and 1st day of infection	7		11·0	84·5	60·5	44·1	233·8	714·5	1161·4	1166·8	785·2	1224·6
Largactil only on the 3rd and 4th day of infection	6		4·4	15·1	62·6	81·3	184·5	548·3	1004·6	1044·7	1119·4	656·2
Hypothermia on the 3rd and 4th day of infection	8		6·5	28·9	49·2	46·5	118·6	328·8	984·0	1330·5	1312·2	767·4
Hyperthermia on the 3rd and 4th day of infection	8	1·9	6·9	27·2	48·5	41·0	162·2	372·4	843·3	909·9	1276·4	1042·3
Largactil only on the 7th and 8th day of infection	8	1·9	10·7	23·6	37·6	57·0	212·3	444·6	1177·6	1213·4	924·7	972·7
Hypothermia on the 7th and 8th day of infection	7		2·3	4·1	13·6	32·6	254·7	460·3	1199·5	1011·6	1774·2	1367·7
Hyperthermia on the 7th and 8th day of infection	7		3·3	19·9	30·9	99·3	81·1	381·1	389·1	445·6	690·2	701·5

GRAPH 2.

Degree of parasitaemia in the rats under hypothermia and in the control rats.



day of infection did not show any marked depression of parasitaemia compared to the group receiving largactil only on the corresponding days of infection. Similarly, hypothermia on the 7th and 8th day of infection produced depression comparable to the group which received largactil only on the corresponding days of infection.

Induction of hyperthermia depressed the parasitaemia but the depression was very much more marked with hyperthermia induced on the 7th and 8th day of infection (Graph 3).

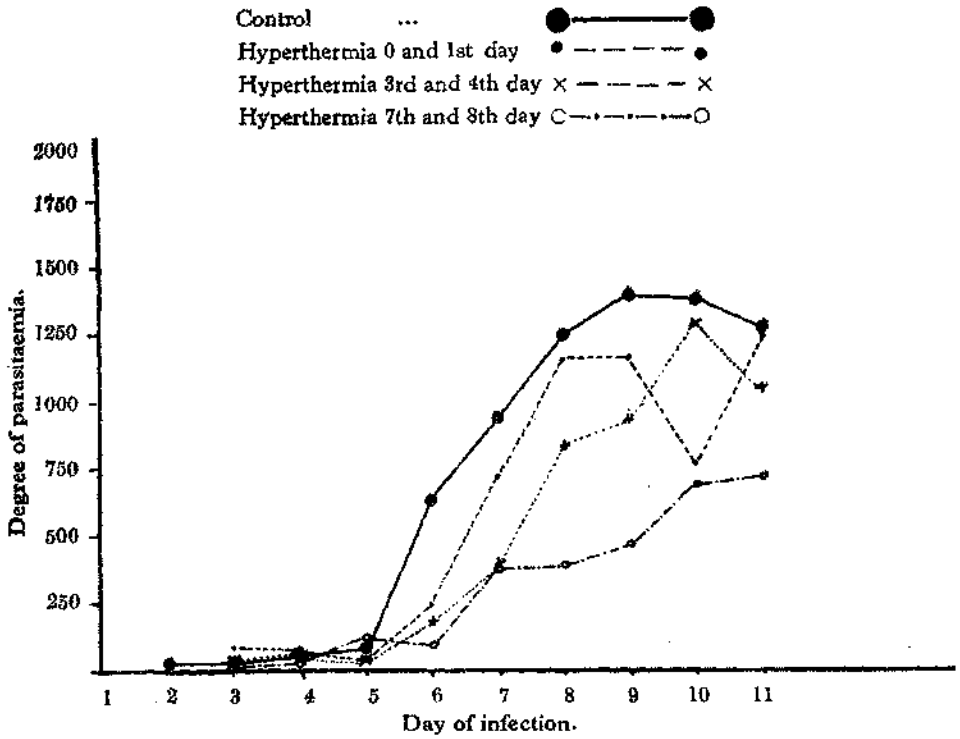
DISCUSSION.

Using a dosage of 40 mg./kg. of promethazine* on the zero and first day of *P. berghei* infection, a rise of parasitaemia has been claimed. This rise in parasitaemia is attributed to the inhibition of the development of the macrophages and their phagocytic activities by the antihistamine (Pautrizel and Martrenchar, 1955).

* Another phenothiazine compound similar to chlorpromazine (Largactil).

GRAPH 3.

Degree of parasitaemia in the rats under hyperthermia and in the control rats.



In the present investigation it has been revealed that a small dose of 5 mg./kg. of largactil, administered at different stages in the course of *P. berghei* infection, produces only a slight depression of parasitaemia as compared to the untreated control animals. Probably the dose was much too low to produce a depression of the phagocytic activity of the macrophages and their development.

Hypothermia on the zero and first day of infection, involving a total period of 16 to 20 hours, produces marked depression of parasitaemia, which is much more pronounced than with largactil alone and thus could be attributed to the hypothermia of the host.

Although preservation of malaria parasites under low temperature is a well known technique (Russell, West and Manwell, 1946), it is likely that some labile parasites are damaged under the condition of low temperature of the host. Such damaged parasitic substance within the host may provide the requisite antigenic stimulus to the early appearance of immunity. This may be the reason for the depression of parasitaemia noticed in rats placed under the hypothermic condition on the zero and first day of infection. It has been shown that birds, injected with

malaria parasites subjected to −3°C. for seventy-three to ninty-six hours, are protected against subsequent infection (Redmond, 1939).

Under hypothermia there is a reduction of the oxygen consumption by the host (Fairley, 1961). The low oxygen consumption by the host, however, is not likely to depress the parasitaemia. On the contrary, the prepatent period in *P. berghei* infection is shortened and the grade of parasitaemia is enhanced in hypoxic rats (Hughes and Tatum, 1956), the authors believe due to the functional inhibition of defensive mechanism of the reticuloendothelial system of the host.

A change in the body temperature, with the resulting comprehensive alteration of the physiological and biochemical state, is likely to alter the endocrine system profoundly (Bigelow and Sidlofsky, 1961). Thus it is possible that the endocrine imbalance of the host in the initial stages of infection, has an inhibitory influence on parasite multiplication. Determination of the course of infection in *P. cathemerium* in duck embryos, incubated at lowered temperature (30°C.), revealed that the infection developed more slowly and never reached the peak seen in the infected embryos incubated at 37°C. and the worker suggested that it could be due to the reduced ability of the parasite to penetrate new host cells (McGhee, 1959).

Hypothermia induced later in the course of infection, i.e., on the 3rd and 4th or 7th and 8th day of infection, does not produce any discernible decline in parasitaemia greater than that with largactil alone. This may probably be due to the established nature of the host parasite relationship when greater amplitudes of variations are accommodated.

Hyperthermia of the host, lasting 8 to 36 hours at different stages in the course of *P. berghei* infection, shows depression of parasitaemia which is very much marked when applied late in the course of infection. It is difficult to state whether this inhibition is primarily due to the rise of body temperature or due to histamine liberation which is likely to stimulate the development of the macrophages and their phagocytic activities, resulting in the greater destruction of parasitized red cells.

SUMMARY.

1. The effect of largactil, hypothermia and raised body temperature of the host (white rat), on the course of *P. berghei* infection, was investigated.

2. Hypothermia on the zero and first day of infection depressed the parasitaemia more than that produced by largactil alone.

3. Hyperthermia, produced by the TAB injection, depressed the parasitaemia wherever applied in the course of infection. This depression of parasitaemia was marked when treatment was applied late in the course of infection.

ACKNOWLEDGEMENT.

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STUDIES ON INSECTICIDE-RESISTANT ANOPHELINES.

5. Lipoid content of female *Anopheles atroparvus*.

BY

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[November 9, 1961.]

In a previous note (Neri, Ascher and Mosna, 1958 ; Part 3 of this series), a preliminary report on determinations of lipoid content in two resistant and two normal strains of *Anopheles atroparvus* was presented. In the present study, fat assays were extended to all our DDT-resistant strains and a dieldrin-selected strain of *A. atroparvus*. Since the normal parent strain (Sens.—Roma) of *A. atroparvus*, from which all the resistant strains had been developed, had yielded inconsistent results in the preliminary work, further repetitions were done with this strain. There was furthermore included in this study a normal and a DDT-resistant strain of *A. stephensi*.

A short survey of the literature regarding a possible correlation between lipoid content and resistance in mosquitos has been given in Part 3 of this series.

MATERIAL AND METHODS.

The susceptible, DDT-resistant and dieldrin-selected strains of *A. atroparvus* have already been described at length previously (Mosna, Rivosecchi and Ascher, 1958 ; Mosna, Palmieri, Ascher, Rivosecchi and Neri, 1959). Selection pressure on these strains has been applied steadily since the end of 1955 and fat determinations were carried out on the 32nd selected generation of RAFM and RL (Neri, Ascher and Mosna, 1958) and on the 38th to 42nd generation of the other strains (this report). To improve the larval breeding it was necessary as from the 32nd generation to introduce some modifications in the larval food. There was a change of the type of fine wheat bran, and a switch-over from commercial "piscidin"

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powder to whole dried and ground Daphniae. The ratio of wheat bran to Daphniae powder was also changed and is now 2 : 1. Thus the results obtained previously (Neri, Ascher and Mosna, 1958) are not directly comparable with the present ones, apart from considering the difference in number of generations selected in the resistant strains.

1. Strains of *A. atroparvus* (*cf* Parts 1 and 2).

SUSCEPTIBLE REFERENCE STRAINS.

I. Sens.—Hamburg.

II. Sens.—Roma.

DDT-RESISTANT STRAINS.

III. RAFM—adult female and male selection.

IV. RL—larval selection.

V. RLAF—larval and adult female selection.

DIELDRIN-SELECTED STRAIN.

VI. R/dieldrin—larval selection with dieldrin.

2. Strains of *A. stephensi*.

VII. Sens.—*A. stephensi*—originating from a colony maintained at the Ross Institute, London.

VIII. DDT-resistant—*A. stephensi*—derived from Strain VII by selection, in each generation since the end of 1955, of the adult females by exposure to DDT by the Busvine and Nash method.

Determination of lipoid content was done on samples consisting of 2,000 females. Soxhlet extractions from samples of each strain were carried out as described previously by :

(*a*) acetone extraction, and

(*b*) ether extraction followed by re-extraction of the residue with acetone.

Full details of the extraction procedures were quoted previously (Neri, Ascher and Mosna, 1958).

RESULTS.

The results of the preliminary study (Neri, Ascher and Mosna, 1958) are summarized briefly in Table I. According to this Table the strain Sens.—Hamburg has the lowest fat content, followed by Sens.—Roma, with a discrepancy, however, between the two results. The DDT-resistant strains RL and especially RAFM have a higher lipoid content.

Tables II and III show the results of the present assays on the other resistant strains of *A. atroparvus* and several replicates of Sens.—Roma. Again, the DDT-resistant strain RLAF has a much higher lipoid content than Sens.—Roma. The strain R/dieldrin (larval selection only, of about three times resistance to dieldrin

in both adult and larval stages ; adult resistance to DDT about twice) has practically the same lipoid content as the normal strain.

Also a DDT-resistant strain of *A. stephensi* tends to have a higher percentage of lipoids than its normal parent strain. It was noted that in general the lipoid level of *A. stephensi* was higher than that of *A. atroparvus* raised under the same conditions.

TABLE I.

Fat determinations, A. atroparvus (*32nd generation*).

(After Neri, Ascher and Mosna, 1958—Part 3).

Number.	Strain.	Mode of extraction.	Percentage of fat extracted, calculated on fresh weight.			Percentage of *total* fat extracted, calculated on fresh weight.
			a	b	b′	
			Susceptible strains			
I	Sens.-Hamburg	a	4·50			*4·50*
	Sens.-Hamburg	b+b′		3·39	0·91	*4·30*
II	Sens.-Roma	a	6·016			*6·016*
	Sens.-Roma	b+b′		3·094	0·974	*4·986*
			Resistant strains			
III	RAFM	a	6·752			*6·752*
	RAFM	b+b′		6·026	0·760	*6·786*
IV	RL	a	6·30			*6·30*
	RL	b+b′		4·85	1·31	*6·16*

DISCUSSION.

It appears from this and the previous study on this subject that the DDT-resistant strains of *A. atroparvus* developed in this laboratory tend to have a higher lipoid content than the normal parent strain or a normal reference strain (Sens.—Hamb.). This does not hold true for a strain selected in the larval stage with dieldrin for the same number of generations, which had about the same low lipoid content as the normal parent strain, which might be due to the low resistance level of the strain R/dieldrin. But it is also quite possible that DDT-resistance is correlated with other biological factors than resistance to dieldrin or lindane. Some evidence in such a direction was given by McKenzie and Hoskins (1954) working with the house-fly. This might explain the reason of absence of higher fat content in a dieldrin-selected strain found by us, and also in a strain of *A. gambiae* resistant to dieldrin and lindane reported by Bradbury, Campbell and O'Carrol (1958).

SUMMARY.

Continuing the previous work, further fat determinations were carried out on DDT-resistant and dieldrin-selected strains of *A. atroparvus* and a DDT-resistant strain of *A. stephensi*, which were compared with normal reference strains. The DDT-resistant strains developed in our laboratory tend to have a higher fat content

TABLE II.

Fat determinations, A. atroparvus (40th to 42nd generation).

Number.	Strain.	Mode of extraction.	Fresh weight of 2000♀♀, grams.	Weight of fat extracted, grams.			Percentage of fat extracted, calculated on fresh weight.			Percentage of *total* fat extracted, calculated on fresh weight.	Busvine and Nash LC_{50}(†), done in same generation as fat determination.		
											DDT		Dieldrin
				a	b	b′	a	b	b′		1 hour's contact.	24 hours' contact.	1 hour's contact.
II	Sens.-Roma	a	5·2933	0·2146	..	..	4·054	..	..	*4·054*			
	Sens.-Roma	a	5·0396	0·1698	..	..	3·373	..	..	*3·373*	3·3 (900)		0·18 (1200)
	Sens.-Roma	b+b′	4·5839	..	0·1414	0·0122	..	3·085	0·266	*3·351*			
V	RLAF	a	5·7742	0·3248	..	..	5·625	..	..	*5·625*			
	RLAF	a	5·2762	0·3150	..	..	5·970	..	..	*5·970*		>4·0 (2500)	
	RLAF	b+b′	6·2418	..	0·3584	0·0055	..	5·742	0·088	*5·830*			
VI	R/dieldrin	a	3·0997(*)	0·1380	..	..	4·452	..	..	*4·452*			
	R/dieldrin	a	3·3354(*)	0·1675	..	..	5·022	..	..	*5·022*			0·42 (900)
	R/dieldrin	b+b′	4·5739	..	0·1741	0·0260	..	3·806	0·568	*4·374*			

TABLE III.

Fat determinations, A. stephensi (38th to 39th generation)

VII	Sens.— *A. stephensi*	a	4·2807	0·2474	..	..	5·779	..	..	*5·779*			
	Sens.— *A. stephensi*	b+b′	4·2813	..	0·2814	0·0145	..	6·573	0·339	*6·912*	1·9 (1500)		
VIII	DDT—res. *A. stephensi*	a	4·1790	0·3261	..	..	7·803	..	..	*7·803*			
	DDT—res. *A. stephensi*	b+b′	4·4118	..	0·3657	0·0050	..	8·289	0·113	*8·402*		3·9 (1800)	

(*) Raised at high larval density.
(†) The figure in parentheses is the number of females employed in determining the LC50.

than the normal parent strains. This did not hold true for a dieldrin-selected srain of *A. atroparvus*.

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FILARIASIS IN KERALA STATE.

Part VII. Survey of Ponani (Palghat District).

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[November 15, 1961.]

INTRODUCTION.

PONANI, a minor natural port, is a Panchayat town about ninety miles north of Cochin It is the headquarters of Ponani Taluk in Palghat District, Kerala State.

The Panchayat, comprising an area of about two sq. miles, is bounded in the west by the Arabian Sea and in the north by the river Bharatha Puzha which falls into the sea at this place. The other two slides are in contiguity of the surrounding villages of Pallapuram in the south and Esuvathuruth in the east. The Canoli Canal, flowing parallel to the sea through the width of the Panchayat, is subject to tidal action and connects the Panchayat with other coastal towns in Kerala State.

The Panchayat is divided into 13 wards. The first nine wards are located between Canoli Canal in the east and the sea on the west, in an area comprising less than one sq. mile. The other four wards lie on the eastern side of the canal (Map 1). Ward 13 includes some of the adjacent rural areas lying on the east and south east, recently added to the Panchayat.

In Ponani there are a Taluk Headquarters Hospital, a Leprosy Clinic, an isolation shed, one high school and seven elementary school, several Madarasas (where Holy Koran is taught), a Mounathial Islam association (for religious conversions), and about 20 mosques with about 39 attached open burial grounds.

TOPOGRAPHY, SOIL AND HYDROGRAPHIC FEATURES.

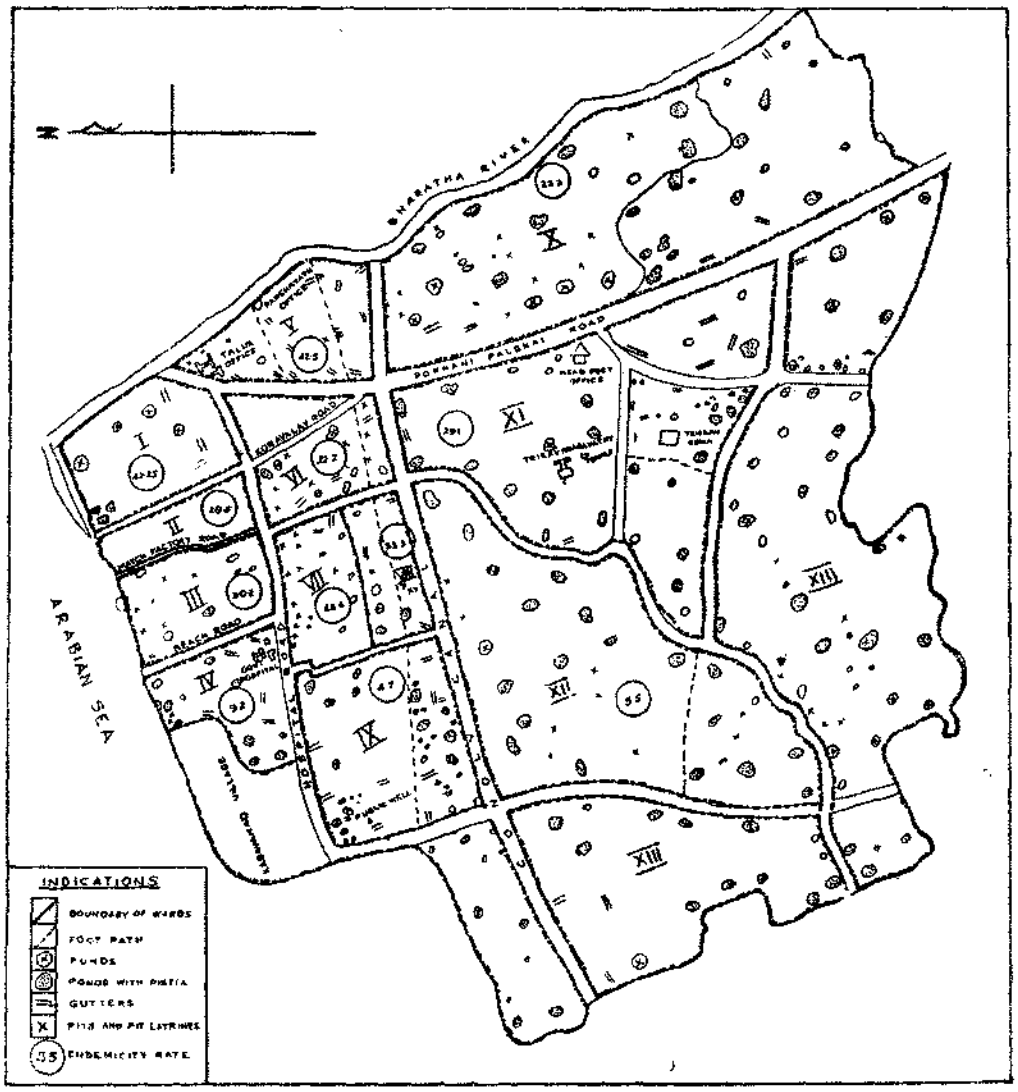
The town is almost at the sea level. The sub-soil water-level ranges between 5 and 12 feet. The soil is sandy except in the eastern limits of the Panchayat where it is of laterite type. There are eight stone pitched tanks attached to the mosques. They are covered with floating green algae. In addition there are many Kucha ponds, some of which are overgrown with Pistia and other aquatic plants. In some of the disused ponds, cocoanut husks are allowed to rot for coir making.

POPULATION, OCCUPATION, HABITS AND HOUSING CONDITION.

The population of the Panchayat (1951 census) is 23,610. About eighty per cent of them (Muslims) live in wards 1 to 9. The rest four wards (10 to 13) are

MAP 1.

Plan of the Ponani Panchayat (Palghat District).



inhabited predominantly by Hindus. The main occupations of the people are fishing, cocoanut cultivation and coir making. People are mostly static, except for some who go as sailors on cargo boats during fair weather periods. There is no migration of population from other areas to Ponani.

Muslim women observe strict *purdah*. They move about only at night for social visits when they carry with them umbrellas to cover their face from the men folk. It is customary for people to keep awake until as late as 1 or 2 a.m. and get up between 8 and 9 a.m. Early marriages are very common and polygamy is the rule among the Muslims. The literacy rate is low.

The houses in Wards 1 to 8 (except Ward 4) are crowded and constructed without any planning or civic sense. Cocoanut trees are grown in close proximity to the houses. In the rest of the wards, the houses are scattered over extensive cocoanut groves and other trees. In the Panchayat area, 48 per cent of the houses are made of mud walls, 46 per cent with brick walls and 6 per cent with wooden walls. The houses are either with thatched (with cocoanut leaves) or tiled roofs and these two types existed in almost equal proportion. Almost all the houses in the thickly crowded localities are dark, stuffy, heavily curtained, and ill-ventilated with a musty smell inside. In the wards located along the sea beach, majority of the houses are small huts with thatched roofs and walls.

WATER SUPPLY AND SANITATION.

There is no protected water supply to the Panchayat. Water from wells (which exist in the back of almost every house) and from ponds is being used by the people.

Sanitary conditions in Wards 1 to 9 are very poor. People invariably resort to open air defaecation or use pit latrines in the little backyard of the houses. There are few trench latrines for public use with poor disposal arrangements.

Drainage is poor. The sandy soil facilitates absorption. The sullage and ablution water from the houses are lead to a soakage pit behind each house which forms a source of prolific mosquito breeding. There is on open-built drain, about a mile long, in the bazar area which is ill-maintained.

SOCIO-ECONOMIC CONDITION.

The annual income of the Panchayat is about Rs. 21,000 of which nearly half the amount is spent on conservancy and general sanitation. Filariasis is the most menacing Public Health Problem and has been hampering the social and economic progress of the people. There is no provision for anti-mosquito measures.

METEOROLOGICAL DATA.

The average annual rainfall for the years 1951 to 1956 (derived from both the south-west and north-east monsoons) was 102-8 inch. Temperature during the same period ranged between 70° and 90° *F*.

HOSPITAL STATISTICS.

The average total attendance in a year in the out-patient department of the local hospital for the period 1948-1956 was 48,000. Out of these, nearly 2,000 on an average (4·2 per cent of the total treated) had filarial complaint. The monthly average attendance for the disease did not show any seasonal fluctuation.

FILARIA SURVEYS.

PREVIOUS SURVEYS.

An entomological unit of the Madras State carried out a rapid survey of the Panchayat in January, 1953. (Regional Malaria Organisation, Coimbatore; Survey Report, 1953). They reported that amongst 460 persons examined there was an infection rate of 13·7 per cent, a disease rate of 19·7 per cent and endemicity rate of 33·4 per cent. They had included lymphangitis and fever among disease conditions. The only infection noted was *W. bancrofti.*

Culex fatigans (per man-hour density 63·6) was incriminated as the vector wtih an infection rate of 22·7 and infectivity rate of 6·8 per cent. *Mansonioides annulifera,* though recorded during the survey, was not found infected.

PRESENT SURVEY.

METHODS AND MATERIALS.

The investigations reported here refer to a rapid filaria survey carried out in February, 1957, of Wards 1 to 12 in the Panchayat to estimate the prevalence and degree of the disease in the area.

(*a*) *Human filariasis survey.*—This was conducted between 8.30 p. m. and 12 midnight by a house-to-house visit in a representative area in each ward. All members of those houses from where cooperation could be obtained were examined. 20 c.mm. blood smears from a pricked finger were collected from all the people examined. On the following day, these slides were stained and examined. The species of microfilariae and their numbers in each positive slide were noted. Along with the collection of blood smears, clinical history and any disease manifestations of each individual were also recorded. In addition to the above, hourly blood smears for a period of 24 hours were collected from four microfilaria carriers to determine the periodicity status of the microfilaria in the area.

(*b*) *Animal filariasis survey.*—Blood samples were obtained from domestic birds and animals for study of possible filaria infection in them.

(*c*) *Entomological survey.*—Adult mosquitoes were collected from living quarters from all the wards, between 7 and 10 a. m., using sucking tubes and the density per man-hour catch was estimated. The mosquitoes were identified, dissected and examined for filarial infection. Larval survey of the breeding places in the wards was also carried out.

The blood meals from *C. fatigans* (the vector) were sent to the Malaria Institute of India, Delhi, for precipitin studies for determining food preferences of the vector.

RESULTS OF THE SURVEY.

The population at the time of survey was estimated to be 27,000. Altogether 1,555 people (about 5·5 per cent of the population) in the whole Panchayat (excepting Ward 13) were surveyed. The filarial infection, disease and endemicity rates of the different wards of the Panchayat are shown in Table I. The overall infection rate was 14·4 per cent., with the maximum incidence of 22·2 per cent in Ward 7 and minimum of 1·6 per cent in Ward 9. Microfilarial density per positive smear was on an average 21·4 for the whole Panchayat. The maximum density was in Ward 10 and the same was 34 on an average. The count in the individuals ranged from 1 to 182. The endemicity rate for the whole Panchayat was 27·9 per cent. Ward 7 was found to be the maximum infected area, with an endemicity rate of 44·4 per cent.

TABLE I.

Filarial infection, disease and endemicity rates in different wards of the Ponani Panchayat.

Ward Number.	Population (Approximate).	Number examined.	infection rate (per cent).	Average infestation.	Disease rate*.	Percentage with both infection and disease.	Endemicity rate (per cent).
1	2,500	71	21·12	4·5	25·35	4·22	42·25
2	1,570	137	16·8	14·7	12·4	0·7	28·5
3	1,500	185	21·08	26·2	11·4	2·2	30·2
4	2,000	163	6·1	8·9	3·06	..	9·2
5	1,750	181	19·3	26·0	25·4	3·2	42·5
6	2,500	177	15·8	26·7	18·6	2·2	32·2
7	2,470	90	22·2	5·5	26·7	4·4	44·4
8	2,070	174	10·9	15·2	24·1	1·7	33·3
9	2,400	64	1·6	7·0	3·1	..	4·7
10	2,000	117	16·2	36·0	5·9	..	22·2
11	3,240	141	9·3	22·8	12·8	..	29·1
12	3,000	55	3·6	34·0	1·8	..	5·5
Total	27,000	1,555	14·4	21·4	15·0	1·4	28·0

*Swellings only.

The species involved was *W. bancrofti* excepting in two individuals, one in Ward 3 and another in Ward 10, in whom *B. malayi* infection was present.

The particulars for the different age-groups in the two sexes are noted in Table II. Infection was noted even at the early age of three. Infection rate gradually increased with the age up to the age-group 21-30 and remained more or less stationary thereafter. Disease rate increased in both sexes with advance in age; starting from 1·3 per cent in the children between 1 and 5 years to 25·6 per cent in persons above 50 years. A 7·2 fold increase in the incidence of elephantiasis was

TABLE II.

Filarial infection and filarial disease (elephantiasis, hydrocoele and other genital affections) in relation to age in the two sexes.

Age-group (Years).	Males:					Females:					Both Sexes:				
	Number examined.	Infection.		Disease rate Per cent.	Endemicity rate. (per cent.)	Number examined.	Infection.		Disease rate Percent.	Endemicity rate. (per cent.)	Number examined.	Infection.		Disease rate Per cent.	Endemicity rate (per cent).
		Percentage.	Average infestation per person infected.				Percentage.	Average infestation per person infected.				Percentage.	Average infestation per person infected.		
1—5	40	5	6·5	..	5·0	40	12·5	8·6	2·5	15·0	80	8·8	8	1·3	10·0
6—10	83	8·4	22·1	2·4	10·8	80	20·0	22·1	2·5	22·5	163	14·1	22·1	2·5*	16·0
11—20	134	14·2	15·3	8·2	19·4	214	12·1	18·1	9·3	21·0	348	12·9	16·9	8·9**	20·4
21—30	124	19·4	27·9	15·3	32·3	249	16·5	22·9	17·7	32·9	373	17·4	24·8	16·9†	32·7
31—40	82	15·9	26·8	21·9	36·6	162	9·9	13·1	17·3	25·9	244	11·9	19·1	18·9††	29·5
41—50	59	13·6	12·9	25·4	35·6	132	14·4	14·8	25·8	37·9	191	14·1	13·5	25·6‡	37·2
Above 50	75	17·3	35·9	24·0	41·3	81	18·5	29·1	27·2	43·2	156	17·9	30·8	25·6‡‡	42·3
Total	597	14·4	24·0	14·1	26·6	958	14·4	19·8	15·8	29·0	1555	14·4	21·4	15·0§	28·1

Microfilarial density per individual surveyed:—

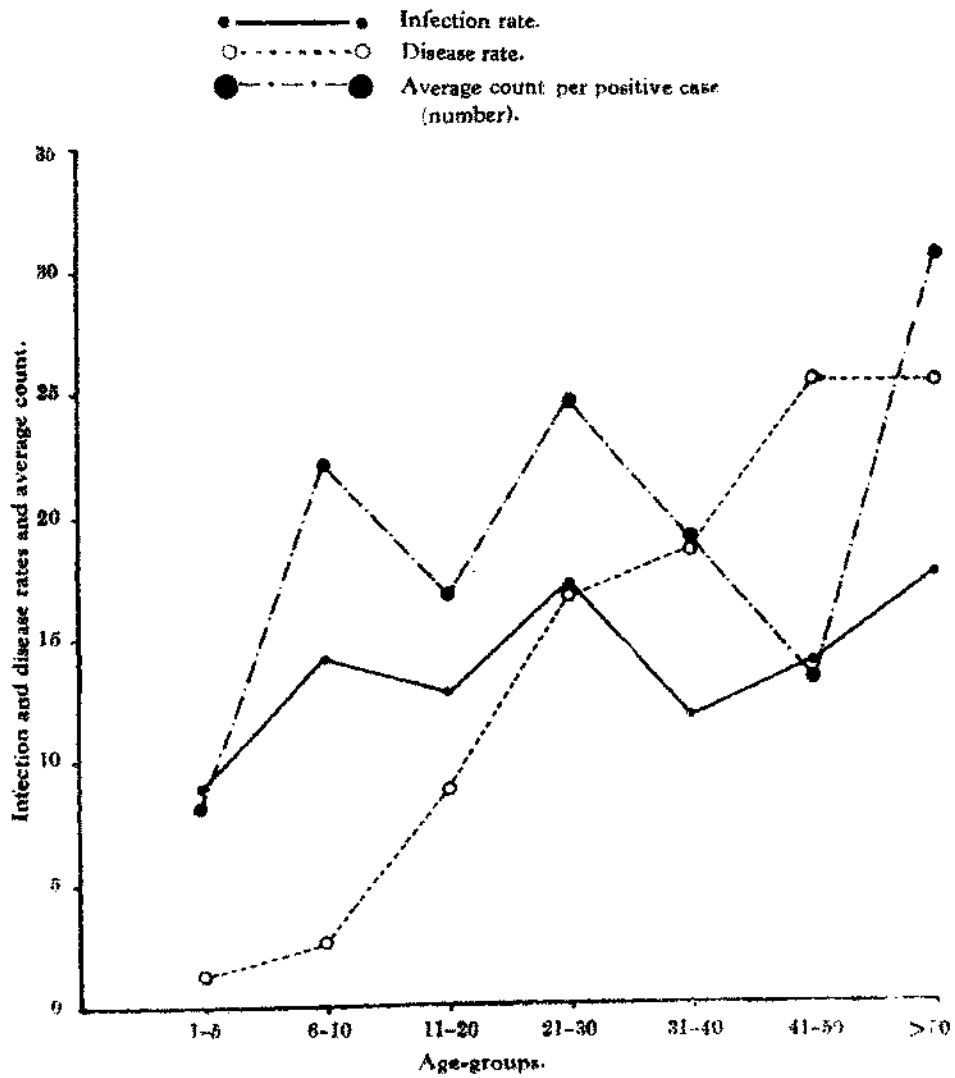
Males	*Females*	*Both Sexes*
3·5	2·8	3·1

*Two elephantiasis and two hydrocoele/genital affections.
**Thirty elephantiasis and one hydrocoele/genital affections.
†Fifty-two elephantiasis and eleven hydrocoele/genital affections.
††Thirty-nine elephantiasis and seven hydrocoele/genital affections.
‡Forty-five elephantiasis and four hydrocoele/genital affections.
‡‡Thirty-four elephantiasis and six hydrocoele/genital affections.
§Elephantiasis rate alone 13·1 per cent.

noted as the age-group advanced from 6-10 years to 11-20 years and this rate of increase was maximum recorded between any two successive groups. Though 6 to 10 years old children, persons in the age-group 21-30 years and older people (50 years and above), had comparatively higher microfilaria load among the positives, the average density in general had an overall correlation with the infection rates in the different age-groups (*vide* Graph 1).

GRAPH 1.

Filaria infection and disease and microfilaria load per positive case in the different age-groups.



The frequency distribution of the different degrees of microfilarial density among the carriers in the two sexes is furnished in Table III. In 38·8 per cent of the positive cases, the count was only 1 to 5. Broadly speaking, the microfilarial density groups and the percentages of the infected persons falling under those different groups were found to be correlated in an inverse proportion. The first three microfilarial density groups (1-20 counts) alone accounted for 74·5 per cent of the total persons harbouring microfilariae and only 3·6 per cent of the infected persons were having the highest count of 101 to 200. Both males and females behaved similarly in the frequency distribution pattern of the infestation levels.

TABLE III.

Frequency distribution of microfilarial density among the positives.

Microfilaria count.	MALES :		FEMALES :		TOTAL :	
	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.
1—5	31	36·0	56	40·6	87	38·8
6—10	17	19·8	26	18·6	43	19·2
11—20	14	16·3	23	16·7	37	16·5
21—30	7	8·1	8	5·8	15	6·7
31—50	7	8·1	16	11·6	23	10·3
51—100	5	5·8	6	4·3	11	4·9
101—200	5	5·8	3	2·2	8	3·6
Total	86	100·00	138	100·0	224	100·0

Microfilarial counts in the 20 c.mm. blood samples, collected hourly from four microfilaria carriers, are presented in Table IV. From this Table, the nocturnal periodicity is evident in all.

The youngest age at which disease was recorded was 5 years. The relative incidence of the disease manifestations, and the extent of concomitant infection associated with each, are detailed in Table V. 3·7 per cent of the males surveyed (8·34 per cent of the total with disease manifestations) had hydrocele. 13·6 per cent of those who had hydrocele and 9·3 per cent of those who had limb swellings, harboured microfilariae as well. The incidence of microfilaria infection in persons with filarial disease was only about two thirds of the incidence in persons without disease. (9·8 per cent in the former as against 15·2 per cent in the latter).

Information in regard to the number of houses surveyed, grouped in relation to the actual members in each house recorded and the incidence of either infection or disease or both among the respective groups, is furnished in Table VI. Filarial affection was recorded in 41·3 per cent of the houses in the entire area and 35·4 per cent of the people living in those houses had either disease or infection.

TABLE IV.

Microfilaria count (per 20 c.mm. of blood) from 4 microfilaria carriers at Ponani.

Time.	Count in carrier :			
	I	II	III	IV
6 P.M.	0	1	0	12
7 P.M.	0	0	4	78
8 P.M.	2	5	7	92
9 P.M.	6	11	6	130
10 P.M.	8	26	4	288
11 P.M.	11	55	14	330
12 P.M.	7	55	14	355
1 A.M.	6	55	8	122
2 A.M.	3	122	5	165
3 A.M.	15	101	15	307
4 A.M.	13	130	12	138
5 A.M.	10	155	12	97
6 A.M.	6	151	13	113
7 A.M.	2	67	7	32
8 A.M.	2	88	2	24
9 A.M.	5	88	1	8
10 A.M.	2	14	0	13
11 A.M.	1	5	0	1
12 A.M.	0	8	1	6
1 P.M.	0	4	0	0
2 P.M.	0	1	0	0
3 P.M.	0	0	0	0
4 P.M.	0	0	0	4
5 P.M.	0	0	0	7

TABLE V.

Incidence of individual disease manifestations among persons with clinical history and the extent of concomitant infection among them in Ponani.

Disease manifestation.	Incidence of filaria disease :		Cases showing microfilaria :		
	Number.	Percentage.	Number.	Percentage.	Average infestation.
Lower extremity ...	190	72·5	19	10·0	16·3
Upper extremity ...	7	2·7	..	..	..
Both extremities ...	6	2·3	..	..	.
Hydrocele ...	22	8·34	3	13·6	60·3
Genital (Female) ...	2	0·8	..	..	..
Chyluria ...	1	0·4	1	10·0	4·0
Hydrocele plus genital plus other swellings ...	6	2·2	..	..	..
Filarial fever ...	28	10·7	5	17·9	7·6
Total	262	100·00	28	10·7	22·05

TABLE VI.

Number of families surveyed and found having filarial infection or disease in Ponani Panchayat.

Members in each family	Number of houses surveyed in :		Number of families with infection or disease in :		Percentage of the families surveyed showing infection or disease :		Percentage of the persons in the affected families with infection or disease.	
	All wards	Wards 5 and 6	All wards	Wards 5 and 6	All wards	Wards 5 and 6	All wards	Wards 5 and 6
1	27	5	4	3	14·8	60·0	100	100
2	26	6	6	4	23·1	66·7	58·3	37·5
3	26	6	10	4	38·4	66·7	46·6	33·3
4	26	5	10	4	38·4	80·0	32·5	43·75
5	29	12	12	12	41·4	100·0	48·3	29·2
6	13	5	9	4	69·2	80·0	25·9	29·2
7	6	3	3	3	50·0	100·0	38·1	38·1
8	14	3	6	3	42·9	100·0	16·6	20·8
9	8	4	4	4	50·0	100·0	47·2	47·2
10 and more	21	3	17	3	81·0	100·0	31·6	46·7
Total	196	52	81	44	41·3	84·6	35·3	41·5

ENTOMOLOGICAL SURVEY.

The following species of mosquitoes were caught from houses in Wards 1 to 12 of the Panchayat :—

C. fatigans.
C. sitiens.
C. vishnui.
Armegeres obturbans.
Ficalbia sp.
Mansonioides annulifera.
Mansonioides uniformis.
Mansonioides indiana.
A. subpictus.
A. vagus.

Mansonioides species was obtained only from Wards 9 to 12. Per man-hour density of mosquitoes during the survey was as under :—

All mosquitoes.	...	35
C. fatigans	...	22
Mansonioides sp.	...	3

Mosquitoes caught were dissected and examined. The results of examination are depicted in Table VII. Only in *C. fatigans, M. uniformis* and *Armegeres obturbans* infection was noted. In the last named species, only unsheathed microfilariae were noted in the abdomen. Development in thorax was evident in the other two

species, out of which only in *C. fatigans* infective larval forms were present. The infectivity rate was 4.5 per cent in this species. It would be noted that infective mosquitoes were caught only from Wards 5 to 8 and 10. The results of dissection of the mosquitoes by wards are furnished in Table VIII.

Breeding places.

Intense breeding of *C. fatigans* occurred in the following types of water collections within the Panchayat.

1. *Soakage pits.*—Each house has one or two soakage pits within the courtyard or in close vicinity, to which the domestic sullage water is drained. Similar pits are found outside mosques also.

2. Built-up cess pools, uncovered or partially covered, exist in the backyard of houses. These are never emptied or cleaned. The excess water keeps overflowing and gets absorbed in the sandy soil.

3. Cemented tanks, close to urinals within the houses, are used for storing water for ablution purposes in connection with routine daily prayers.

4. There are more than 300 trench type of latrines within the Panchayat. Most of them are improperly built, with no outlet for the wash water to sink in the sub-soil.

5. Pit latrines in houses.

6. Ill-kept kucha and pucca open drains.

7. Artificial containers, such as arecanut soaking cisterns.

8. Disused wells.

9. Ponds used for soaking cocoanut husk.

Breeding of *Mansonioides sp.* occurred in ponds overgrown with pistia and many of those were seen in Wards 9 to 13.

Precipitin test carried out at the Malaria Institute of India, Delhi, with the blood meal collected from mosquitoes indicated that the anthropophilic index of the vector species (*C. fatigans*) was 86 per cent.

ANIMAL SURVEY.

A blood survey of the following animals within the Panchayat yielded negative results.

Goats	...	13
Fowls	...	70
Cats	...	7
Dog	...	1

TABLE VII.

Mosquito dissection results.

Serial Number.	Species dissected.	Number dissected.	Number with filarial infection, including microfilariae in abdomen.		Number with filarial infection, excluding microfilariae in abdomen.		Mosquitoes positive for infective larvae.	
			Number.	Percentage.	Number.	Percentage.	Number.	Percentage.
1	*C. fatigans* ...	201	33	16·4	25	12·4	9	4·5
2	*M. annulifera* ...	26	14	53·8	7	26·9	..	..
3	*M. uniformis* ...	1	..	..	..	..	..	..
4	*M. indiana* ...	6	..	..	..	..	..	..
5	*Armegeres spp.* ...	1	1	100	..	..	..	..

TABLE VIII.

Results of dissection of vector mosquitoes in different wards of Ponani Panchayat.

Ward numbers.	1	2	3	4	5	6	7	8	9	10	11	12
Infection rate *C. fatigans*	8·3	13·8	14·3	0	15·8	8·2	23·1	17·6	14·3	7·1	0	0
Infection rate *Mansonioides*	..	0·0	..	0·0	0·0	0·0	0·0	0·0	33·3	31·7	0·0	0·0
Infectivity rate *C. fatigans*	..	..	..	..	5·4	3·3	7·7	9·7	..	7·1	..	..
Infectivity rate *Mansonioides*	..	..	..	..	..	..	..	..	..	..	..	..

DISCUSSION.

Ponani Panchayat is highly endemic for *W. bancrofti*. The endemicity rates recorded during the present rapid survey are considered to be very much less than the actual figures as many of the Muslim ladies do not come out for examination in the presence of strangers. Eliciting genital swellings in males is also similarly difficult. With these limitations, the survey revealed that about 45 per cent of the population, surveyed in some of the wards, are having either chronic disease manifestation or infection or both.

Previous preliminary filaria survey of 460 persons in some of the highly infected wards of the Panchayat had revealed an infection rate of 13·7 per cent, disease rate of 19·7 per cent and endemicity rate of 33·4 per cent. Though the results of the previous and the present surveys may not be comparable due to difference in the sampling and other survey techniques, it is gathered that the overall incidence of the disease in the area has remained almost steady.

During the present study, two persons of whom one was a girl of 6 years, were found to be infected with *B. malayi*. Further, developmental forms of the parasite could be obtained in *M. annulifera* caught from Wards 9 and 10. Thus, transmission of *B. malayi* infection in the wards, bordering the periphery of the Panchayat, is indicated. During the previous survey, only *W. bancrofti* infection was detected.

The higher endemicity was observed in Wards 1, 5 and 7 having the highest density of population. The only wards with somewhat low incidence of the disease were Wards 4, 9 and 12 which are bordering the southern limits of the Panchayat, with much less population pressure. To study the effect of crowding of population, the wards are grouped in three categories; Group 1 (Wards 1, 5, 6, 7 and 8) being the most densely populated, Group 2 (Wards 2, 3, 10 and 11), the less crowded and Group 3 (Wards 4, 9 and 12) the least crowded. The incidence of filariasis in these three different groups is compared with the crowding status and the differences among them are found to be highly significant, indicating thereby that the density of population at risk in an area is one of the important factors which goes to decide the degree of endemicity of bancroftian filariasis.

Transmission potential (Krishnaswami *et al.*, 1959) is positively correlated with the microfilarial infestation up to a count of about 100 per 20 c.mm^3. of blood. Levels above this are commonly associated with low transmission potential. The distribution of microfilarial infestation among the positives in the community at Ponani was found to be less than one hundred per 20 c.mm. among 96·4 per cent of the carriers, indicating thereby that almost all infected persons are good potential infectors to vector mosquitoes. This aspect, along with the finding that most of the microfilaria carriers are without any clinical manifestation (filarial disease), indicates the favourable conditions prevailing within the Panchayat for the intense transmission of the disease.

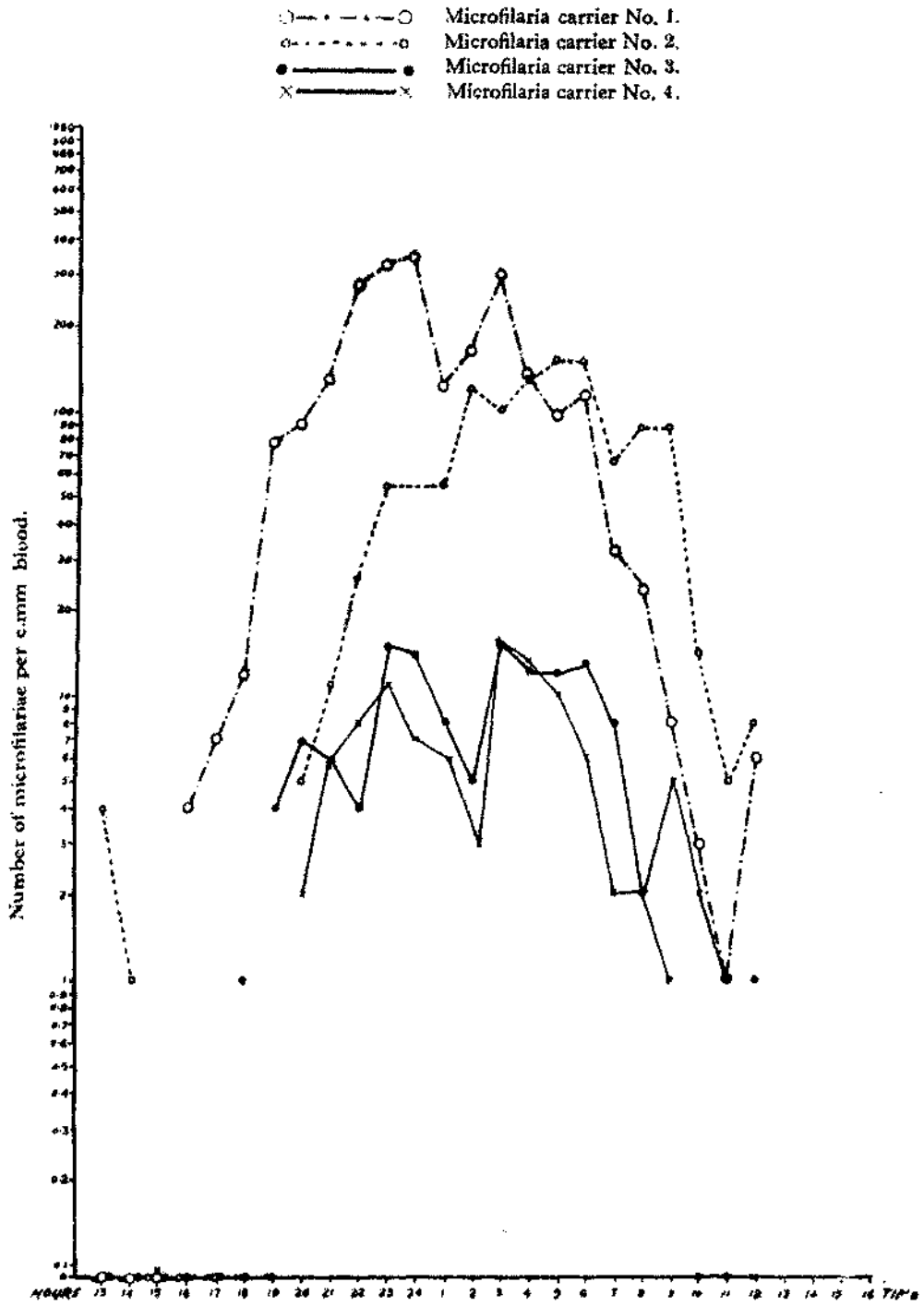
Among the various causes for the high incidence of filariasis in Ponani, special mention must be made of (1) the very poor insanitary conditions of the locality, (2) very poor economic condition of the people, (3) man's creation of innumerable breeding places within and outside human habitations, (4) the existence of a large number of open burial grounds within the heart of the densely populated wards which act as a source of organic pollution of soil in the general area, (5) the customs of the people of heavily curtaining of houses in observance of the *purdah* system, thus, converting the houses sufficiently dark to afford attractive resting places for adult *C. fatigans*, (6) the late hours up to which the people keep awake in the night, thereby affording opportunities for repeated infection by the bites of infective mosquitoes even among persons who use mosquito nets and (7) absence of any sort of mosquito control operations.

As could be seen from Graph 2, there are invariably two peaks in microfilarial count; the first peak occurring at about 12 midnight (11 to 12 night) and the second at about 3 A. M. (3 to 5 A. M.). The rates of increase and decrease are almost the same in all the cases studied excepting for the variation in between the two peaks, as observed by the parallel lines in the logarithmic graph. The maximum microfilaria count recorded during the 24 hour observation period was 355 (very high), 155 (high), 15 (low) and 15 respectively in the Carriers 1 to 4. In all the cases peripheral blood was free from microfilariae for varying periods during the day-time hourly examination. They were completely absent during three consecutive examinations (1 to 3 P. M.) in the very high count carrier, four times (3 to 5 P. M. and at 7 P. M.) in the high count person and eight times (between 10 A. M. and 7 P. M.) in the remaining two low count carriers. From these findings it is felt that, in endemic areas (1) even though microfilariae exhibit strict nocturnal periodicity, detection of the parasite in very low numbers at least, during day-time in a few odd cases is not anything strange, (2) such cases that show the parasite during day-time, can be considered to be harbouring comparatively a high load of embryos in the peripheral blood at night-time and only due to the spilling-over effect in such high microfilaria states, day-time detection of the embryo is made possible, (3) the frequency of this occurrence during day-time is more in very high microfilaria carriers and less in low count persons and (4) the time when there is the least possibility of detecting microfilariae even in very high count persons, is in the afternoon at about 3 P. M.

Among the persons having filarial disease as well as infection, those with hydrocele had the highest load of microfilaria; the average infestation among them being 60.3 as against the average of 22.05 for the entire group. But it is admitted that the number of hydrocele cases, showing microfilariae, was only three in the present series. Further, information such as the duration and degree of genital swelling, whether unilateral or bilateral etc., is not available. However, studies of this nature during filariasis surveys are indicated to throw more light on this important epidemiological aspect.

GRAPH 2.

Two peaks in microfilarial count. (Logarithmic scale).



Of the persons surveyed, 13·1 per cent had elephantiasis. Of the families surveyed in the entire Panchayat, 41·3 per cent had either filarial infection or disease. Actually 84.6 per cent of the total families surveyed in Wards 5 and 8 together were affected with the disease. This shows the magnitude of the problem.

SUMMARY.

A filariasis survey of Ponani, a coastal township in Kerala State, was carried out in the second half of 1957, during which 1,555 persons were examined. The infection, disease and endemicity rates were 14·4, 15·0 and 28 per cent respectively. The average infestation was 21·4 per about 20 c.mm. of blood. The species of filarial infection prevalent was *W. bancrofti*. But two cases of *B. malayi* were also observed, which were presumably of local origin. Periodicity of microfilariae (*W. bancrofti*) was strictly nocturnal.

C. fatigans was incriminated as the main vector, with an infection rate of 16·4 per cent and infectivity rate of 4·5 per cent. Average number of infective larvae per positive mosquito was 1·2. Developmental forms up to pre-infective stages were found in *Mansonioides sp.*, indicating thereby the possibility of *B. malayi* transmission to a minor degree in some of the semiurban wards.

Some of the reasons for the high incidence of bancroftian filariasis in the place are discussed.

ACKNOWLEDGEMENT.

The active cooperation and help rendered by Dr. T. V. Sundaresan, District Medical Officer of Health, Palghat (now Assistant Director, Malaria and Filariasis, Kerala State), Mr. Thangal, the President and Mr. Sankaremenon, Executive Officer, Ponani Panchayat, are gratefully acknowledged.

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A STUDY OF *ANOPHELES BARBIROSTRIS* VAN DER WULP IN THE COLLECTION OF THE MALARIA INSTITUTE OF INDIA WITH FIRST RECORD OF *A. BARBUMBROSUS* STRICKLAND AND CHOWDHURY AND *A. VANUS* WALKER IN INDIA.

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WHILE going through the adult *Anopheles* collections at the Malaria Institute of India for morphological abnormalities occurring in the Indian *Anopheles* (Wattal *et al.*, 1960) it was noticed that *A. barbirostris* in the collections represented more than one species. Therefore, it was decided to study the group further and not to include this species in the work published then (Wattal *et al.*, 1960 *loc. cit.*).

As a result of an examination of 983 adult specimens previously identified as *A. barbirostris*, including 9 female specimens identified previously as *A. barbirostris* var. *ahomi*, it has been found that the *A. barbirostris* as previously recorded in India is composed of *A. barbirostris* (*senso stricto*), *A. barbumbrosus*, and *A. vanus*. Besides these, *A. barbirostris* var. *ahomi* adults in the collections resemble *A. barbumbrosus* whereas the larva as described by Chowdhury (1929) differs from the larva of *barbumbrosus*. Therefore, it appears to be a distinct species (Reid, 1961) related to *barbumbrosus*. The larval slides of *A. barbirostris* in the Institute's collection also show that both *A. barbirostris* and *A. barbumbrosus* are represented in the Indian fauna. However, no larval slides of *A. vanus* could be discerned amongst the slides available in the collection.

Christophers (1933), who had access to the collections, described *A. barbirostris* Van der Wulp, 1884, with *vanus* Walker, 1860, and *martini* Laveran, 1902, as probable synonyms. However, recent work of Bonne-Wepster and Swellengrebel (1953) and that of Stone *et al.* (1959) treat *A. vanus* Walker as a distinct species. Christophers (1933 *loc. cit.*) mentioned that among the species closely resembling *A. barbirostris*, neither *A. barbumbrosus* nor *A. bancrofti* was recorded from the Indian area. The impression that *A. barbirostris* was the sole representative of the group in the Indian fauna has since been generally accepted as is evidenced by the published keys and the distribution records of Indian *Anopheles* (Puri, 1931, 1949, 1955*a*, 1955*b* ; Wattal, 1961 ; and Wattal and Kalra, 1961).

Christophers (1933 *loc. cit.*) distinguished the *barbirostris* sub-group and its closely related species on the basis of the following key : –

1. Femora and tibiae distinctly speckled with white scales; centre of mesepimeron without a patch of white scales ; white scales present on venter of abdomen 2

— Femora and tibiae not so marked; centre of mesepimeron with a patch of white scales ; white scales on venter present or absent ... 3

2. Fringe spots at 4·1, 4·2, 5·1 and 5·2 ; leaflets of phallosome small, the shorter ones broadly conical (Australia) ..... *bancrofti.**

— Not so ; a fringe spot may be present at 5·2, leaflets of phallosome simple, without serrations. Larva : oc with 60 or more branches ; ic with some fine lateral branches in apical half ; lateral hair of abd. seg. VI long. (Philippines, Celebes ; recorded from Ceylon†) .....*pseudobarbirostris*‡

3. Commonly without a dark interruption at apical spot (4); oc of larva with 50 or more branches 4

— Commonly with a dark interruption of apical spot ; venter of abdomen without pale scales ; leaflets of phallosome simple, without serrations ; oc with 11-22 br. only.

(Celebes, Dutch East Indies) .. ...*barbumbrosus* (*pallidus*).

4. Venter of abdomen with white scales ; leaflets of phallosome with marked serrations ; ic simple, or one or rarely both may be bifurcate; lateral hair of seg. VI in larva short (Oriental Region) ..... *barbirostris.*

— Venter of abdomen without white scales ; ic with fine lateral branches in apical portion (Assam) ..... *barbirostris* var *ahomi.*

Among the Indian specimens examined in the collection, none belonged to the '*bancrofti* group'. The dark interruption of apical pale spot, as mentioned by Christophers (1933 *loc. cit.*) for distinguishing *barbirostris* from *barbumbrosus*, does not appear to be a valid character for identification because both *barbirostris* and *barbumbrosus* may show it. Therefore, for the present study the following key was adopted for differentiating *barbirostris* from *barbumbrosus* and *vanus*.

1. White scattered scales present on venter of abdomen ; wings with narrow fringe spot at vein 3; leaflets of phallosome with serrations; oc with more than 30 br. 2

— Venter of abdomen without white scales ; wings with fringe spot at vein 3 to 4·1, leaflets of phallosome longer with serrations on one side only ; oc with 11-22 hr. *barbumbrosus***.

* Also recorded from New Guinea and Bismarck Archipelago (Stone *et al.*, 1969).

† Reid (1961) has examined the specimens from Ceylon and is of the view that they are related to *barbumbrosus* and are not *pseudobarbirostris* (*bancrofti* group).

‡ Also recorded from New Guinea and Ceram (Bonne-Wepster and Swellengrebel, 1953).

** The outer sutural hair in *barbumbrosus* has 7-8 fine branches, whereas it has 2-3 branches in *barbirostris*.

2. Leaflets of phallosome large, the larger ones serrated on both edges; oc of larva with 50 or more branches . .. *barbirostris**

— Leaflets of phallosome longer, with serrations on one edge only, oc with less than 50 branches..... *vanus**.

The distribution of *barbirostris*, *barbumbrosus*, *vanus* and *ahomi* specimens in the collections, as determined by the above mentioned key, is indicated in Table I and Maps 1 and 2. The characters of these species, as observed in the present study, are discussed in the following paragraphs.

A. barbirostris Van der Wulp, 1881.

This species has been recorded from all of the Indian States. Out of the 983 adult specimens examined, 896 were found to be those of *barbirostris*. In these specimens the apical pale area on the wing was found to be continuous in some specimens (Plate I, Fig. *a*) and with an interruption in others (Plate I, Fig. *b*). However, in either case the white fringe spots were confined to Vein 3 and 5·2 only. The white scales on the venter of the abdomen were found to show differences. In a majority of the specimens there were broad white scales present on the venter. In most of the specimens the broad scales were scattered uniformly on the sternites (Plate I, Fig. *c*). In some cases the broad scales were confined to anterior three segments only while the remaining segments had broad or narrow scales in the centre of the sternites (Plate I, Fig. *d*). There were 126 specimens which had narrow white scales on the venter confined to the centre of the sternites only (Plate I, Fig. *e*).

Bonne-Wepster and Swellengrebel (1953) have used the white tarsal band 3-4 of the hind legs to distinguish *barbirostris* from its closely related species *vanus*. This character does not seem to be constant and the three types of banding, encountered during the present study in *barbirostris*, are indicated in Plate I, Fig. *f* to *h*.

The phallosome was observed to have 4-5 pairs of stout leaflets (Plate II, Fig. *a*). The larger ones are distinctly serrated on both sides and are about one half the length of the phallosome (Plate II, Fig. *b*). The ninth tergite did not present a uniform appearance in the different specimens examined. Whereas, in a few specimens, the ninth tergite had swollen base and short, stout tapering processes (Plate II, Fig. *c*), in a majority of the specimens this tergite looked more like *vanus* in that the bases were less swollen, the lateral processes were slightly longer, non-tapering and blunt, with a slight constriction in the middle (Plate II, Fig. *d*).

A. vanus Walker, 1860.

Only 3 male specimens in the collections could be identified as *vanus*. It seems that females of this species are indistinguishable from *barbirostris*.

* Bone-Wepster and Swellengrebel (1953) used tarsal joint on 3-4 on the hind legs and the ninth tergite as additional characters to distinguish *barbirostris* from *vanus*. However, in the present study it was found that these characters are not constant and could not form the basis differentiating the species.

TABLE I.

Specieswise distribution of specimens labelled as Anopheles barbirostris *in the collection of the Malaria Institute of India.*

Serial number.	Country/State.	Locality.	SPECIES : *Barbirostris.* Narrow* scales	*Barbirostris.* Broad* scales.	*Vanus*	*Barbumbrosus.*	*ahomi*
	India						
1.	Andaman and Nicobar Islands	Andaman Island	41	..	..	..	..
2.	Andhra Pradesh	Ramkote	..	1	..	..	..
		Tirumalai Hills	..	..	..	1	..
3.	Assam	Jeypore	..	3	..	..	..
		Marianbari	..	..	1	29	..
		Nalabari	15	..	..	..	..
		Sibsagar	..	1	..	..	..
		Lakhimpur	..	..	..	..	9
		Other areas	7	11	..	..	..
4.	Bihar	Madhubani	4	16	..	..	..
		Pandaul	1	..	..	..	..
		Purnea	..	48	..	..	..
		Singhbhum	..	3 6	..	13	..
5.	Kerala	Kozhikode (Calicut)	..	8	..	..	..
		Travancore	..	1	..	..	..
6.	Madhya Pradesh	Jabalpur	..	..	..	2	..
		Other areas	..	1	..	..	..
7.	Madras	Madras	..	02	..	2	..
8.	Maharashtra	Bombay	..	126	..	..	..
		Nagpur	..	5	..	1	..
		Sholapur	..	1	..	..	..
9.	Manipur	Imphal	..	4	..	..	..
10.	Mysore	Bangalore	..	12	..	1	..
		Belgaum	..	4	..	15	..
		Bellary	..	12	..	..	..
		Coorg	..	14	..	..	..
		Hubli	..	30	..	..	..
		Kampti	..	1	..	..	..
		North Kanara	..	4	..	..	..
		Yellapur	30	..	..	..	..
		Other areas	..	1	1	..	..
11.	Panjab	Unknown	...	23	..	..	..
12.	Tripura	Agartala	18	2	..	..	..
13.	Uttar Pradesh	Terai	..	9	..	..	..
14.	West Bengal	Birnagar	..	2	..	..	..
		Burdwan	4	..	..	..	..
		Calcutta	..	2	..	..	..
		Sukna	..	..	..	3	..
		Other areas	..	49	..	..	..

*Refers to the scattered white scales on the venter.

(Continued)

Table I (Concld.)

Serial Number.	Country/State.	Locality.	Species: *Barbirostris.* Narrow* scales.	*Barbirostris.* Broad* scales.	*Vanus.*	*Barbumbrosus.*	*Ahomi.*
15	India	Unknown	6	9	1	..	..
16	Burma	Laboe	..	2	..	..	..
		Maymo	..	..	..	4	..
		Namsang	..	1	..		..
		Other areas	..	24	..	..	..
17	Indo China	Unknown	..	1	..	..	..
18	Indonesia	Sumatra	.	2	..	..	..
19	Malaya	Unknown	..	7	..	..	..
20	Pakistan	Dacca	..	..	..	1	..
		Parbatipur	..	8	..	..	..
		Tarpassa	..	3	..	..	..
		Other areas in East Pakistan	..	8	..	..	..
21	Philippines	..	..	..	..	3	..
22	Thailand	..	..	6	..	..	..
	Total	..	126	770	3	75	9

*Refers to the scattered white scales on the venter.

Bonne-Wepster and Swellengrebel (1953) distinguished *vanus* from *barbirostris* on the basis of the hind tarsal band 3-4 narrowly white (Plate I, Fig. *h*), leaflets of phallosome being longer and more numerous, with serrations only on one side and the oc in larva with somewhat less than 50 branches. As already mentioned, the hind tarsal band 3-4 in *barbirostris* shows *vanus*-like variation. Similarly the hind tarsal band 3-4 in *vanus* may be broad (Plate I, Fig. *g*). Therefore, the shape of the phallosome (Plate II, Fig. *e, f*) seems to be the only valid character on which the adults of these species can be differentiated. The ninth tergite (Plate II, Fig. *g*) closely resembles *barbirostris* (Plate II, Fig. *d*).

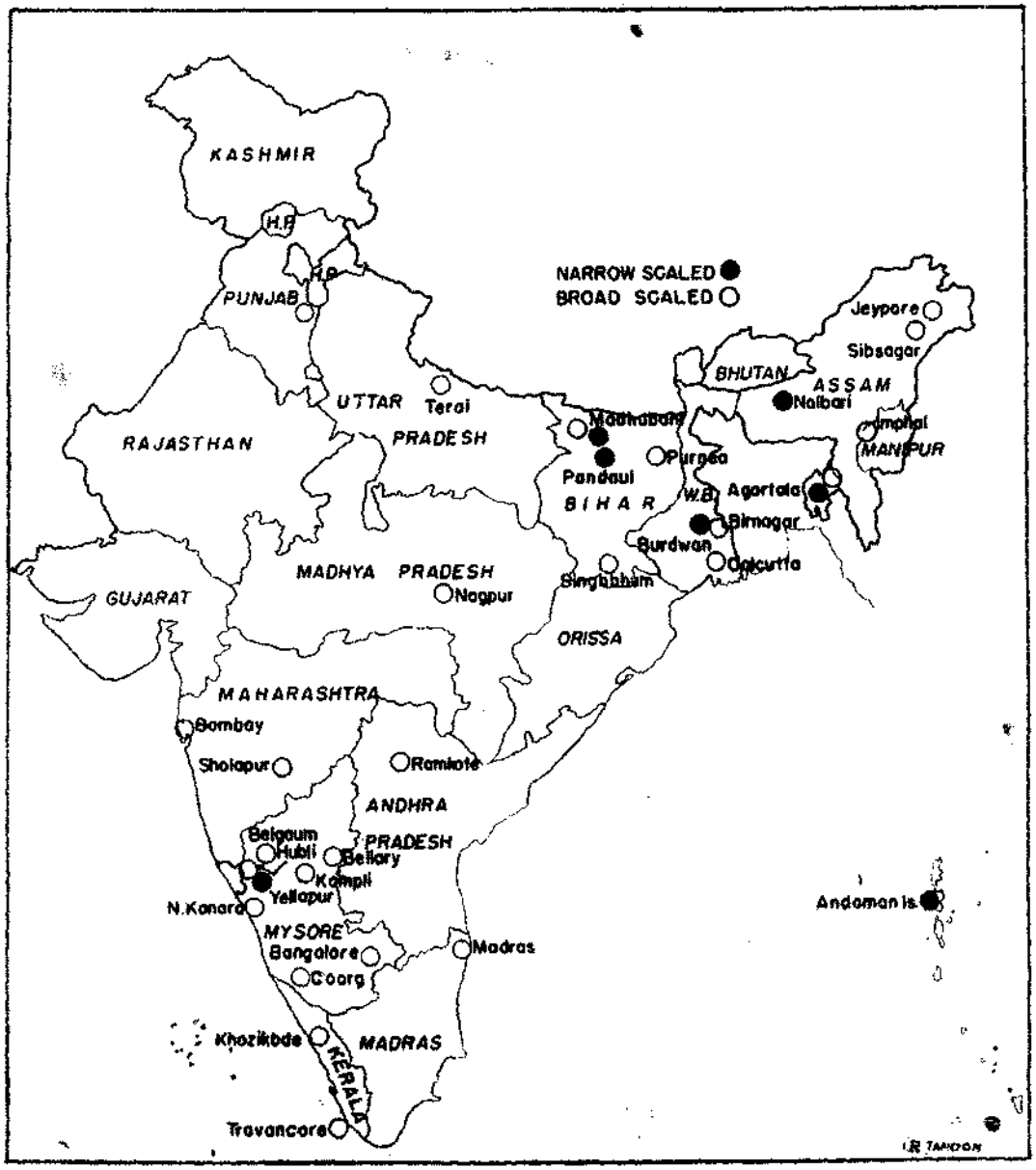
The specimens of *vanus* have been collected from Marianbari (Assam) and Mysore. It is likely that the species is distributed over a wider area along with *barbirostris*.

A. barbumbrosus Strickland and Chowdhury, 1927.

Seventy-five adult specimens in the Malaria Institute of India collections, previously labelled as *barbirostris*, were found to be those of *barbumbrosus*. Sixty-seven of these specimens are from India and 1 from Dacca in East Pakistan, 4 from Burma and 3 from the Philippines (Table I). The wing in a majority of the specimens showed a dark interruption in the apical white area (Plate III, Fig. *a*) and in some specimens the wing was without this interruption (Plate III, Fig. *b*).

MAP 1.

Distribution of A. barbirostris *(broad and narrow scaled specimens) in India as determined from the adult collection of the Malaria Institute of India.*



MAP 2.

Distribution of A. barbumbrosus, A. vanus and A. ahomi in India as determined from the adult collection of the Malaria Institute of India.

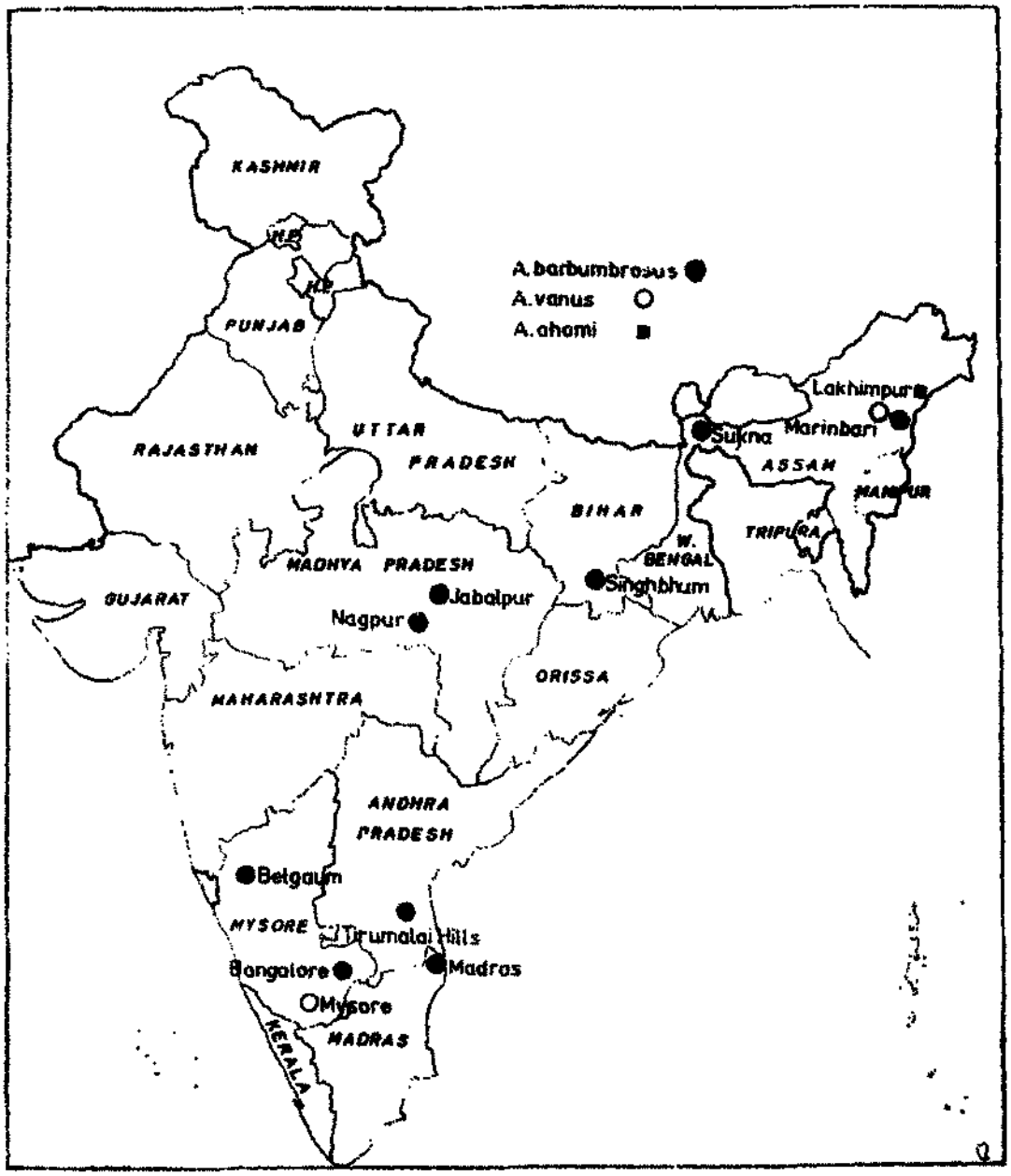
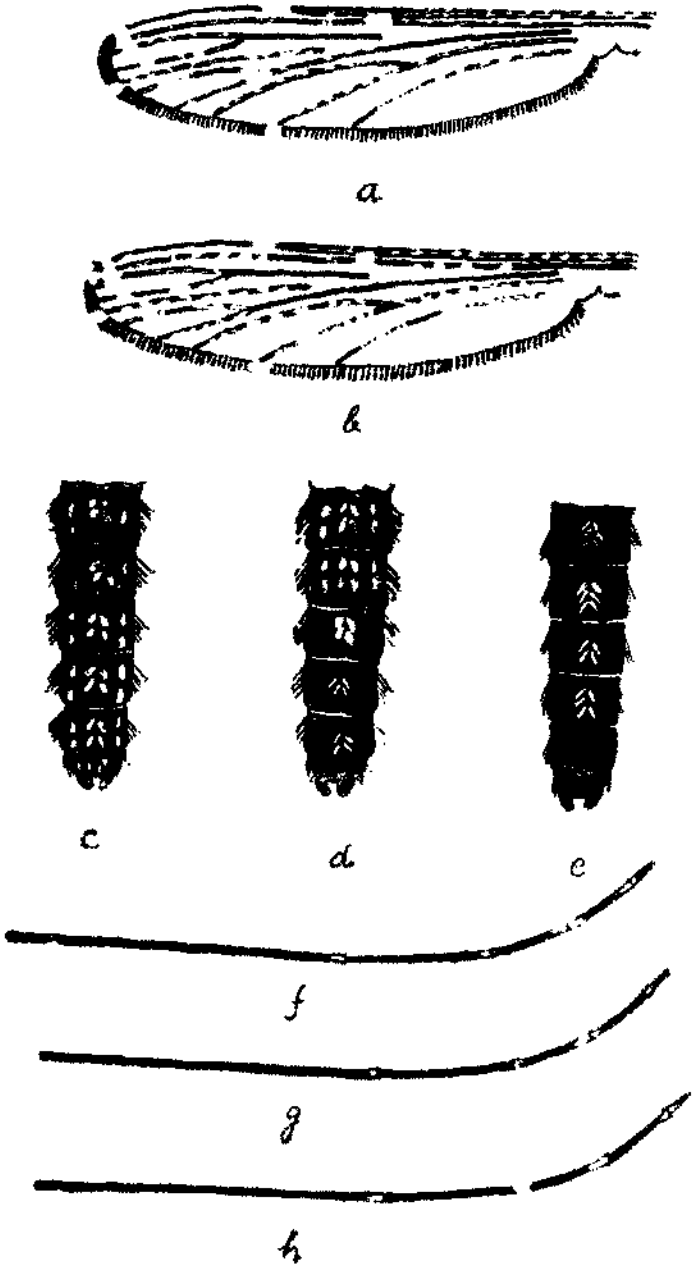


PLATE I.

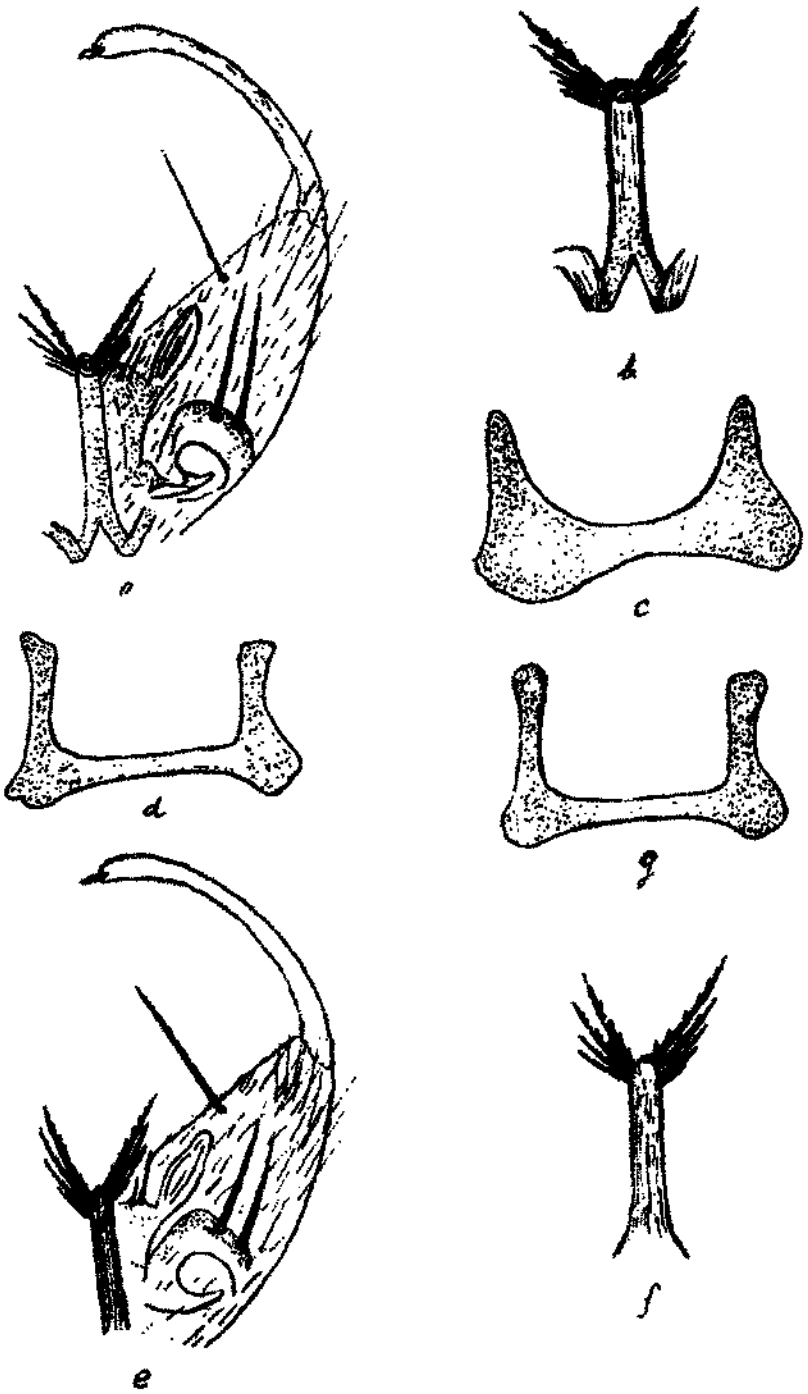
A. barbumbrosus.



a, b. Wing c, d, e. Abdominal segments f, g, h. Hind tarsus

PLATE II.

A. [illegible] (Figs. [illegible]) A. ramus (Figs. [illegible])



Figs. [illegible] A. [illegible]

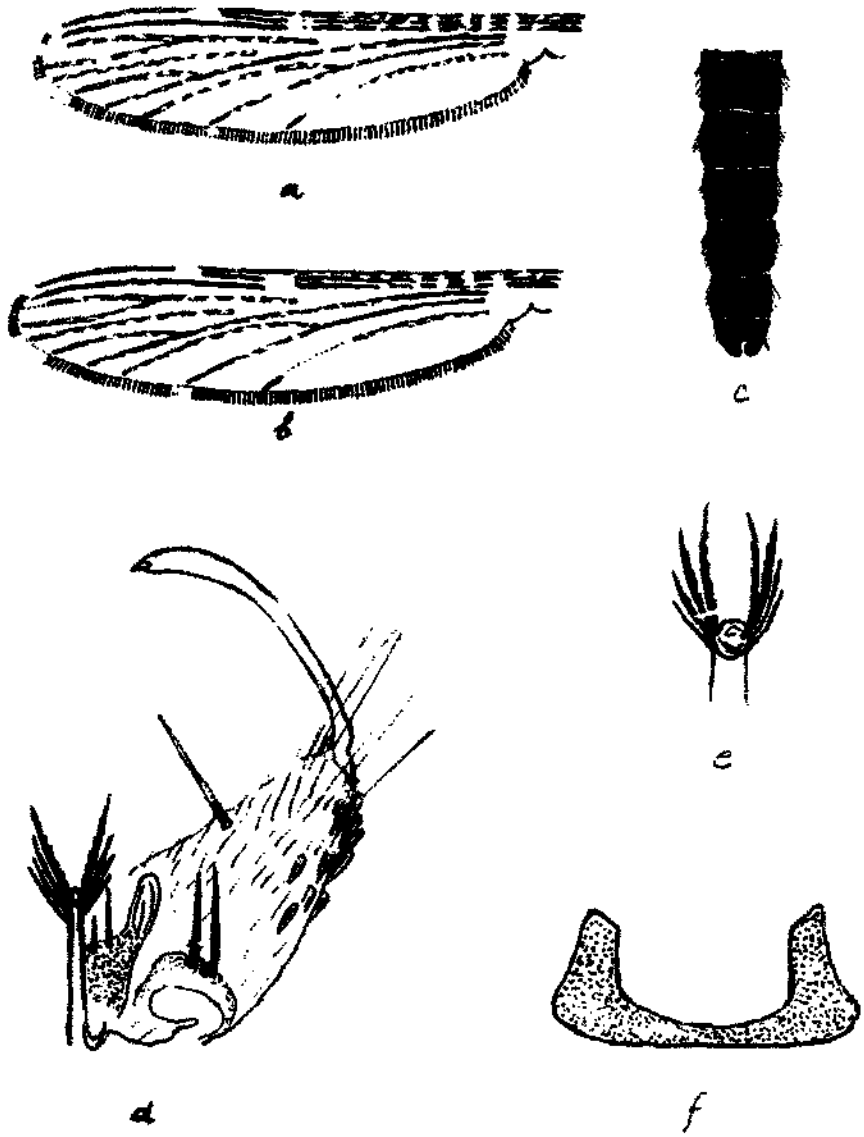
(a) Male terminalia (b) Phallosome showing leaflets; (c) Ninth tergite.

Fig. (e, f, g) A. ramus.

(e) Male terminalia; (f) Phallosome showing leaflets; (g) Ninth tergite.

PLATE III

A. barbumbrosus.



(a & b) Wing ; (c) Abdominal sternites ; (d) Male terminalia.
(e) Phallosome showing leaflets ; (f) Ninth tergite.

However, the most critical character in the wing, distinguishing it from '*barbirostris* group' is the white fringe spot extending from an area below V. 2·2 to V. 4·1. The venter of the abdomen is without white scales (Plate III, Fig. *c*). The phallosome was observed to have 4 to 5 pairs of long leaflets with distinct serrations on one edge only (Plate III, Fig. *c, d*). The larger leaflets were found to be longer than one half the length of the phallosome. The ninth tergite had slightly swollen bases with short stout processes (Plate III, Fig. *f*).

***A. barbirostris* var. *ahomi* Chowdhury, 1929.**

Christophers (*1933 loc. cit.*) provisionally considered the specimens described by Chowdhury as *barbirostris* var. *ahomi* under *barbirostris*. As already mentioned, there are only 9 female specimens labelled as var. *ahomi* in the collections. The wing of these specimens resembles that of *barbumbrosus* (Plate III, Fig. *a*). These specimens also resemble *barbumbrosus* in not having any white scales on the venter of the abdomen (Plate III, Fig. *c*). Since no male specimens were available the genitalia could not be studied. However, the larvae in var. *ahomi* are known to have ic with fine lateral branches on the apical half (Strickland and Chowdhury, 1931) whereas in *barbumbrosus* the ic are without any branches (Strickland and Chowdhury, 1927). Also, the oc in *barbumbrosus* have few branches (11-22) whereas in var. *ahomi* the branching of oc resembles that of *barbirostris*. Therefore, it seems that *barbirostris* var. *ahomi* is a distinct species nearer *barbumbrosus* than *barbirostris*.

Subject to a study of the male genitalia it may be well to consider *ahomi* as a distinct species.

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A NOTE ON THE RELATIVE TOXICITY OF ORGANO-PHOSPHORUS INSECTICIDES AGAINST HOUSEFLIES.

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INTRODUCTION.

MANY reports are available now about the quick selection of insecticide-resistant strains of houseflies to chlorinated hydrocarbons following use of these insecticides for their control. In the year 1957, houseflies were reported to be resistant to DDT almost all over the world except India and Ceylon (Brown, 1958). Recently in India also, houseflies have been found to be resistant to DDT and dieldrin†. Organophosphorus compounds have been used successfully where the flies were resistant to chlorinated hydrocarbon insecticides. Extensive trials, using residual treatment of organophosphorus compounds for the control of resistant houseflies, have been reported but the results of these trials were found to vary considerably, depending on geographic location, the insecticidal formulation used, method of its application and criteria used in the evaluation of results (Schoof and Kilpatrick, 1958).

Organophosphorus compounds have not been so far evaluated for the control of houseflies in India. Studies were, therefore, undertaken to evaluate some of the organophosphorus compounds against them. Diazinon, malathion, trithion and a new organophosphorus compound, baytex, were evaluated in the studies reported. Baytex was reported to have long residual effectiveness and low mammalian toxicity (Jung, 1959) and was found to be quite a promising insecticide for the control of *C. fatigans* (Cerf and Lebrun, 1959; Ramakrishnan *et al.*, 1960).

(*i*) *Relative toxicity of organophosphorus compounds to adult houseflies.*—Topical application method was used for assessing the toxicity of these compounds to adult houseflies (*Musca domestica nebula*). Five to six days old laboratory-bred‡ houseflies, maintained on milk, were used for these studies. Various concentrations of alcoholic solutions of the insecticides (technical) were applied on the thorax of anaesthetised flies at a dosage of 1 microlitre per fly by means of a micrometre syringe. The flies were then held for 24 hours when mortality in them was recorded (Table I). The temperature during the application and holding period

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† Annual Report of the Malaria Institute of India for the year 1959.

‡ The technique of rearing the houseflies described by Wattal *et al.* (1959) was followed.

ranged from 24 to 27°C. The results were analysed by the probit analysis method (Finney, 1952). The LD50 in μg./fly with fiducial limits of different insecticides for the female and male flies are depicted in Table II. The LD50 values of baytex and diazinon for female flies were 0·035 and 0·046 μg. per fly respectively. These figures did not differ significantly from each other. The LD50 values of these two insecticides were found to be almost the same for the male flies also; these compounds were equally toxic to both sexes of the adult housefly. The LD50 values of malathion for the male and female houseflies were 0·094 and 0·19 μg. per fly respectively. Though malathion was found to be slightly more toxic than diazinon against *C. fatigans* (Ramakrishnan *et al.*, 1960), it was less toxic than diazinon against adult houseflies. The toxicity of trithion was found to be of the same order as that of malathion. The order of toxicity of these compounds against the adult houseflies, therefore, was baytex = diazinon > malathion = trithion.

(*ii*) *Relative toxicity of organophosphorus compounds against housefly larvae.*—The relative toxicity of the four compounds to housefly larvae was assessed by exposing the larvae on filter papers impregnated with the respective insecticides. Filter papers (Whatman No. 1, diameter 11 cm.) were impregnated with 1 c.c. of varying concentrations of solutions of technical insecticides in ethylene dichloride. Petridishes (10 cm. diameter) were lined with the treated filter papers. Third-instar larvae (25 in each case) were exposed to the impregnated papers for one hour after which they were removed and kept in the holding glass jars under observation. Two to four replicates were performed at each concentration of the insecticides.

The toxic effects of the poisons in the case of larvae were manifest quite late; even after 24 hours of exposure, signs of life were observed in them. In the case of adults, on the other hand, the end point of reaction was reached within a few hours after the application of insecticide. Therefore, it was not considered advisable to record the mortality in the larvae after 24 hours or 48 hours of exposure. The larvae which failed to pupate, were considered dead. The observations with various concentrations of insecticides are presented in Table III. The results were analysed by the probit analysis method and the MLC values with fiducial limits are also depicted in Table III. Baytex with MLC of 0·0057 per cent was the most toxic and trithion with MLC > 0·05 per cent, was least toxic. Diazinon which was found to be as toxic as baytex against adult houseflies, was about one third as toxic against the larvae. The order of toxicity of the insecticides against the larvae was baytex > diazinon > malathion > trithion.

(*iii*) *Residual persistence of organophosphorus insecticides on mud surfaces.*—Mud panels were prepared from the clay used in the construction of rural houses. The panels, when completely dry, were sprayed with suspensions in water prepared from water dispersible powders* of baytex, diazinon, malathion and trithion at the doses of 200, 100 and 50 mg. of each of the insecticides per sq. ft. of surface.

* The water dispersible powders used were baytex 40 per cent, diazinon 50 per cent, malathion 25 per cent and trithion 50 per cent.

TABLE I.

24 hours' percentage mortality in houseflies with different dosages of organophosphorus insecticides.

BAYTEX :			DIAZINON :			MALATHION :			TRITHION :		
(μg/fly)	P (males)	P (females)	(μg/fly)	P (males)	P (females)	(μg/fly)	P (males)	P (females)	(μg/fly)	P (males)	P (females)
0·015	26·3 (50)	9·3 (75)	0·015	26·0 (75)	5·5 (75)	0·06	23·3 (50)	8·2 (50)	0·03	11·1 (150)	..
0·03	67·1 (75)	37·0 (100)	0·03	69·5 (75)	19·5 (75)	0·125	72·9 (50)	26·6 (50)	0·06	37·0 (150)	..
0·06	97·2 (75)	89·3 (75)	0·06	95·8 (75)	62·5 (75)	0·25	93·02 (50)	67·4 (50)	0·125	83·7 (150)	21·3 (100)
0·125	100·0 (75)	100·0 (75)	0·125	100·0 (75)	87·5 (75)	0·5	97·7 (50)	87·7 (50)	0·25	98·6 (150)	86·2 (100)
..	..	..	..	..	..	..	..	..	0·5	..	97·9 (100)
..	C=2·7	C=0	..	C=4·0	C=4·0	..	C=4·0	C=2·0	..	C=10·0	C=6·0

P=Corrected percentage mortality.
C=Mortality in control (per cent).
Note :—Figures in parenthesis indicate the number of flies tested at each dosage.

TABLE II.

Median lethal dosages, with fiducial limits, of different insecticides for the male and female houseflies.

Insecticide.	Sex.	MLD (μg/fly).	Fiducial limits of LD50 for 95 per cent probability.
Baytex	Male	0·024	0·021—0·027
	Female	0·035	0·032—0·038
Diazinon	Male	0·023	0·021—0·026
	Female	0·046	0·037—0·053
Malathion	Male	0·094	0·079—0·11
	Female	0·19	0·17 —0·22
Trithion	Male	0·073	0·066—0·079
	Female	0·18	0·17 —0·19

TABLE III.

Percentage mortality in hosuefly larvae exposed to different concentrations of organophosphorus insecticides.

Insecticide	Concentration (per cent)						M.L.C.	Fiducial limits of M.L.C.
	0·0015	0·003	0·006	0·0125	0·025	0·05		
Baytex	22·0 (100)	32·0 (100)	50·0 (100)	86·0 (100)	96·0 (100)	..	0·0057	0·0044–0·0065
Diazinon	..	..	8·0 (50)	34·0 (100)	70·0 (100)	..	0·017	0·015–0·019
Malathion	..	..	..	8·0 (100)	24·0 (100)	52·0 (100)	0·048	0·041–0·057
Trithion	..	..	..	0·0 (50)	12·5 (50)	38·0 (50)	>0·05	

Figures in parenthesis indicate the number of housefly larvae tested.

TABLE IV.

24 hours' percentage mortality in houseflies (♀♀) exposed to the insecticide-treated mud-panels, for one hour.

Insecticide	Dosage (mg/sq.ft.)	Days after treatment									
		1	10	20	30	40	50	60	70	80	90
Baytex	200	100·0	100·0	98·0	96·0	97·0	95·0	87·7	10·0	4·0	..
	100	100·0	100·0	97·0	66·0	76·0	10·0	..	..	..	..
	50	100·0	100·0	40·0	9·0	..	..	..	..	..	..
Diazinon	200	100·0	100·0	93·0	91·0	77·0	10·0	..	..	..	..
	100	100·0	100·0	95·0	18·0	7·0	..	..	..	..	..
	50	100·0	100·0	10·0	5·0	..	..	..	..	..	..
Malathion	200	100·0	100·0	79·0	83·0	65·0	5·0	..	..	..	..
	100	100·0	100·0	79·0	25·0	..	..	..	..	..	..
	50	100·0	100·0	19·0	5·0	..	..	..	..	..	..
Trithion	200	100·0	78·0	33·0	4·0	..	..	..	..	..	..
	100	100·0	17·0	9·0	0·0	..	..	..	..	..	..
	50	96·0	9·0	..	..	..	..	..	..	..	..

Houseflies (♀♀) were exposed to the treated panels for one hour in accordance with the technique described by Pal (1951). Twenty insects were used in each case, and five replicate tests were carried out for each insecticidal dosage. Mortality in insects was recorded after 24 hours of holding. The tests were repeated on the same panels after intervals of 10 days. From the results (Table IV) it may be seen that baytex was the most persistent of all the insecticides tested; the dosage of 200 mg. per sq. ft. produced more than 50 per cent mortality in insects for 60 days. The initial effect of all the three dosages, 50, 100 and 200 mg. was found to be similar, but the residual persistence was found to increase with the increase in dosage. Diazinon, which by topical application was intrinsically found to be as toxic as baytex, was found to be less persistent and more than 50 per cent mortality in insects was observed only up to 40 days after treatment. Malathion, though intrinsically less toxic than diazinon, showed residual persistence almost equal to it. On the other hand trithion, which was found to be as toxic as malathion by topical application, was much less persistent in its toxicity on the mud panels.

(*iv*) *Field evaluation of baytex and trithion as residual sprays for the control of housefiies.*—The fly density was found to be very high in the villages which were sprayed for assessing the effectiveness of these compounds against *C. fatigans* (Ramakrishnan *et al.*, 1960 *loc. cit.*). So the effect of these compounds on the fly population was also simultaneously recorded. The investigations were carried out in three villages of Meerut District, Uttar Pradesh, during March-June 1960. These villages were completely isolated from each other and consisted mainly of houses with mud-walls and roofs made of thatch or wooden planks. About 20 per cent of the structures in each of the villages were pucca. Particulars in regard to the number of houses, total population and the previous history of insecticidal spraying of exprimental villages, are depicted in Table V. Two villages were sprayed, one with baytex and the other with trithion while the third was kept unsprayed for purposes of comparison. The villages were sprayed at a dosage

TABLE V.

Particulars of experimental villages.

Name of the village.	Number of houses.	Population.	Previous spraying history.	Insecticidal application.
Garhi	50	330	Sprayed with DDT since 1958. Three rounds of DDT spraying have been carried out. Last sprayed in Oct. 1959.	Baytex—40 W (40 per cent water dispersible powder)
Milkh	30	280	Sprayed with DDT since 1958. Three rounds of DDT spraying have been carried out. Last sprayed in Oct. 1959.	Trithion 50 per cent water dispersible powder.
Guldahar	100	750	Sprayed with DDT since 1958. Three rounds of DDT spraying have been carried out. Last sprayed in Oct. 1959.	Comparison village (unsprayed).

of 100 mg. of each insecticide per sq. ft. of surface. The efficacy of the residual treatments was determined by making weekly grill counts of the houseflies at each village. Five counts were made at each of the four general locations preliminarily determined to be attractive to houseflies. The fly index for a village was taken as the average of four maximum counts, one from each location. The observations (Table VI) showed that there was a sharp decline in the fly density in the village sprayed with baytex. It remained at a very low level for about 40 days, after which an increase was noticed. In the village sprayed with trithion no significant effect on the fly population was observed.

TABLE VI.

Fly index in the villages sprayed with Baytex and Trithion.

Days after treatment	Village Garhi sprayed with Baytex 100 mg./sq.ft	Village Milkh sprayed with Trithion 100 mg./sq.ft.	Control village Guldahar (Unsprayed)
−7	250	180	120
0	300	250	150
Sprayed			
1	5	126	115
8	3	220	180
15	7	230	185
25	10	..	220
32	9	..	85
40	25	..	75
48	60	..	80

SUMMARY AND CONCLUSIONS.

Studies were carried out to determine the relative toxicity of baytex, diazinon, malathion and trithion against houseflies. Baytex and diazinon were found to be equal in their intrinsic toxicity when tested by the topical application method and were found to be more toxic than the other two compounds tested.

Baytex was found to be the most persistent insecticide on mud surfaces. Field tests with baytex and trithion indicated that baytex at a dosage of 100 mg. per sq. ft. kept the density of houseflies low for a period of about 40 days. Trithion at the same dosage did not yield any significant reduction in the fly density.

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ANOPHELES STEPHENSI AS A CARRIER IN RURAL BENGAL.

BY

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AND

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[June 2, 1961.]

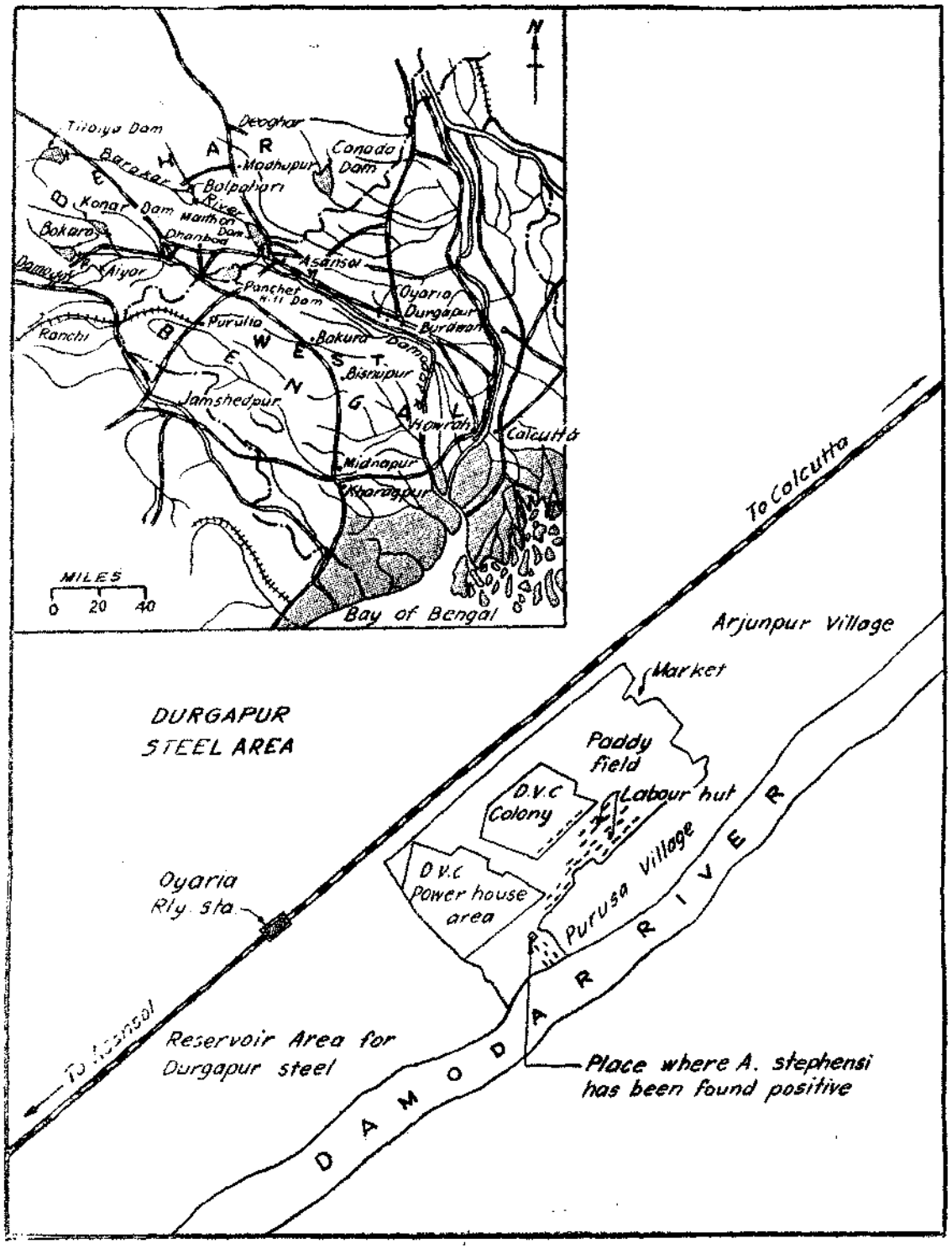
IN July, 1959, a report was received of malaria cases coming for treatment at the DVC Dispensary at Oyaria, District Burdwan. As the number of cases was unusually high, it was decided to start a thorough investigation of the cause of this outbreak. This was possible on August 17, 1959, and was continued to September 30, 1959. A rapid malaria survey was undertaken at the Oyaria Camp (site of the Durgapur Thermal Power Station, DVC) and of the surrounding two villages, Pursa and Arjunpur. As the malaria cases mainly came from the labour huts outside the DVC colony, these were also included in the survey. It may be mentioned here that this area had been included in the National Malaria Control Programme since 1953.

LOCATION.

The DVC camp site at Oyaria comprises about 2 square miles under the Police Station Faridpur in the District of Burdwan, West Bengal. It lies at a latitude of 23°5′ North and longitude of 87°5′ East and altitude of 240 feet above the mean sea level. The place is situated on the left bank of the Damodar River which is a few furlongs away to the south of the colony. There are 3 villages, *viz.*, Pursa, Arjunpur and Angadpur in between the Damodar River and the southern limit of the Oyaria colony. At the north, lies the Eastern Railway track and on the other side of the railway track is the site of the Durgapur Steel Plant. On the east, it is bounded by a market called Maya Bazar and on the west it is bounded by the site of the proposed reservoir for the steel plant and a kutcha road leading to Oyaria Railway Station (E. Rly.) The camp is situated about 2 miles to the south of the 118th mile post of the Grand Trunk Road from Calcutta (Map 1). The area has got facilities for natural drainage and is comparatively free from large marshes and extensive low pockets during the rainy season.

The construction of the camp started towards the end of the year 1957. There were temporary buildings, huts, asbestos and tin barracks inside the camp and the population was a mixed one coming from different States of India. Besides the DVC quarters, there were labour huts situated between the camp and Village

MAP I.
Oyaria Camp.



Pursa and a few inside the DVC colony. The huts were constructed by the labourers on their own with straw and bamboo. They are very small and dark. The labourers used to cook meals within these huts and thus these were not suitable for shelters of mosquitoes during the day time when cooking started. The population of the DVC camp was about 2,500.

CLIMATE.

March to June is dry period. This is followed by the monsoon from July to September. The maximum temperature rises up to 114°F. and the minimum temperature is about 48°F. Cold months are December, January and early part of February. The maximum precipitation is during the month of July or August. In 1959 the maximum temperature in May was 106·7° F. and relative humidity during the months of June to October remained high. The rainfall during the year was higher, in comparison with the records of the preceding four years, with a maximum precipitation of 18·4 inch in September, 1959.

Records of previous surveys.—The villages Pursa and Arjunpur were surveyed once by the DVC in 1951 in connection with the survey conducted for the Durgapur Barrage Pond area and also in 1957 when this site was selected for the Thermal Power Station. Spleen and parasite rates for these two years may be seen from Table I.

TABLE I.

Spleen and parasite rates in 1951 and 1957.

Name of the area	Spleen rate : (Per cent.)		Parasite rate : (Per cent.)	
	1951	1957	1951	1957
Pursa village ...	84·6	2·6	15·6	0·6
Arjunpur village ...	86·2	3·0	30·4	1·2

Both P. *vivax* and P. *falciparum* were present during these surveys. The predominant parasite was P. *vivax*. The main vector species of anophelene found during the survey in 1957 was *A. culicifacies*. No. *A. philippinensis* was found. Of the 8 other anophelene species collected during this time, the most predominant was A. *hyrcanus*.

RESULTS OF THE SURVEY UNDERTAKEN IN THE YEAR 1959.

Malaria cases were mainly confined among the occupants of the labour huts situated just outside the camp and near the Village Pursa. Two villages, with 290 holdings and a population of 1200, were surveyed along with the residents of the camp of the DVC. Children between the age of 2 and 10 years were examined for spleen and blood films. 30 children from Arjunpur, 48 children from Pursa and 50 children from the DVC colony were examined for this purpose. The spleen and parasite rates are depicted in Table II.

TABLE II.

Spleen and parasite rates in the year 1959.

Name of village.	Spleen rate.	Parasite rate.
Pursa ...	8·3	6·2
Arjunpur ...	3·3	3·3
Oyaria, DVC. ...	4·0	6·0

HOSPITAL CASES.

During the period of survey, 194 blood films from the malaria cases attending at the DVC dispensary at Oyaria were examined, out of which 119 were found positive with *P. vivax* infection.

The attendance of malaria cases at the Oyaria Hospital from July to October, 1959, is indicated in Table III.

TABLE III.

Attendance of malaria cases at the Oyaria Hospital.

Month (1959)	Old cases.	New cases.
July	Nil	215
August	7	60
September	200	173
October	30	20

ENTOMOLOGICAL DATA.

Adult mosquitoes.—4,739 specimens of anopheline mosquitoes were collected from human dwellings and cattle-sheds during the survey. Out of these 1,613 were dissected. Sporozoites were detected from salivary glands of one *A. stephensi*. The incidence of anopheline species collected and dissected is shown in Table IV.

TABLE IV.

Anopheline species collected and dissected.

Name of species.	Number collected	Per man-hour density.	Number dissected.	Number found positive.
A. stephensi	196	1·1	193	1*
A. culicifacies	63	0·35	63	Nil
A. pallidus	3	0·001	2	Nil
A. annularis	47	0·27	44	Nil
A. vagus	647	3·76	134	Nil
A. subpictus	3,747	12·9	1,142	Nil
A. hyrcanus	36	1·2	35	Nil

*Infection was found in the salivary glands only. The sporozite rate was 0·52 per cent.

Larvae.—2,043 anopheline larvae were collected during the survey. The nature of the breeding places of the vector species is shown in Table V.

TABLE V.

Nature of breeding places of the vector species.

Name of species.	Number collected.	Nature of breeding places.	Name of the area.
A. stephensi	65	Empty containers, ditches and neglected pits.	DVC camps and labour huts.
A. culicifacies	3	The Damodar River bed and channels.	DVC camps and labour huts.

DISCUSSION.

On a perusal of the literature it is found that there is no record that *A. stephensi* is a vector of malaria in the rural area of West Bengal. Siddons (1946) incriminated *A. stephensi* as a vector in Calcutta. He dissected 678 guts and 1,052 glands, out of which 4 guts and 6 salivary glands were found infected. In the deltaic West Bengal, *A. philippinensis* has been a recognised vector species.

It was expected that *A. culicifacies* would be the vector in Oyaria Camp as this species was found breeding in large numbers in the pool of the Damodar River bed. The density of *A. culicifacies* during the survey period was rather low. The per man-hour catch of *A. stephensi* was more than that of *A. culicifacies*. It is, therefore, significant that *A. stephensi* has been incriminated as a vector in rural Bengal. This finding has surely created a new problem in the malaria eradication programme when it is a common knowledge that the chief vector of the area, i.e., *A. philippinensis* is gradually vanishing from the alluvial deltaic Bengal.

In this connection it may be noted that a similar area, i.e., colony of the Durgapur Barrage about 4 miles to the east, was urbanised in the year 1955. *A. stephensi* could not be collected there during the course of urbanisation or later even now.

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AN ENZOOTIC FOCUS OF SIMIAN MALARIA IN *MACACA RADIATA RADIATA* GEOFFROY OF NILGIRIS, MADRAS STATE, INDIA*.

BY

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[December 11, 1961.]

In view of the recently revived interest in simian malaria and its transmissibility to man (Eyles *et al.*, 1960), a malaria survey of monkeys† was carried out in the foot-hills of the Nilgiris. The location for the survey was chosen because the only existing records of natural simian malaria (in brown monkeys) in India were from the Nilgiris (Donovan, 1920; Mulligan and Swaminath, 1940). In 1942, a few monkeys purchased from a dealer in Gudalur showed natural infection with *P. inui* (Mohan, 1961). Besides, the examination of blood smears from nine monkeys captured in February, 1961, by the Pasteur Institute, Coonoor‡, for their virological work, showed six positive to malaria. A preliminary report has already been made by the authors (Ramakrishnan and Mohan 1961) of their finding natural infection in 13 out of 19 monkeys captured in the Kallar area in February, 1961. This paper reports the results of a detailed survey of the incidence of simian malaria in the Nilgiri Hills, establishing the presence of an enzootic focus.

The identity of the infections (*P. inui and P. cynomolgi*), mentioned in the preliminary report, was tentative. Infected monkey-blood from the Nilgiris has since been inoculated into clean *M. mulatta* monkeys at Delhi. Detailed studies have confirmed the presence of natural infections of *P. inui* (Halberstadter and Prowazek, 1907) and *P. cynomolgi* (Mayer, 1907) in *Macaca radiata radiata* Geoffroy** in the Nilgiris area. Description of the morphology of the parasites, their asexual erythrocytic periodicity and immunological status with reference to other strains of *P. cynomolgi* will form the subject of another paper.

While trapping, it was noted that the younger monkeys in the group were driven away by the group leader. Usually it was the group leader—a full-grown

* The work was mainly financed from the regular budget of the Malaria Institute of India and partly from grants of the Indian Council of Medical Research and the World Health Organization.

† The permission accorded by Shri C. A. R. Bhadran, I. F. S., Chief Conservator of Forests, Madras State, to capture a limited number of monkeys in the Nilgiris, is gratefully acknowledged.

‡ The authors are indebted to the Director, Pasteur Institute of South India, Coonoor, for his permission to examine the monkeys at the Pasteur Institute.

** The authors are indebted to the Director of Zoological Survey of India, Calcutta, for the identification of the monkeys as *Macaca radiata radiata*.

adult or the "second in command"—that was captured. Shooting monkeys in the Nilgiris is prohibited.

Between February and December, 1961, 43·4 per cent of the monkeys were found positive to infection out of a total of 53 captured in different localities in the Nilgiri mountains (*vide* Map 1). Table I depicts the captures altitude-wise, and the presence or absence of infection in them. Unfortunately, the weight of all the monkeys was not recorded and, therefore, it was not possible to classify the infection rate according to weights which could be approximately correlated to the age of the monkeys. Four of the bigger monkeys did not show parasites in the blood till after splenectomy. This indicated that the infections were chronic in them.

TABLE I.

Number of Macaca radiata radiata *captured at different altitudes in the Nilgiris and found infected with* P. cynomolgi *and* P. inui (*vide Map I*).

Region.	Altitude range above mean sea-level, in meters, at which monkeys were trapped.	NUMBER OF MONKEYS :		NUMBER SHOWING :		
		Trapped and examined.	Found infected.	*P. inui.*	*P. cynomolgi.*	Mixed. infection.
Kallar-Coonoor Road	457—1235	19	13*	9	..	4*
Coonoor Town	1707	21	3	3	..	..
Mettupalayam-Kotagiri Road	792—924	9	5†	3	..	2†
Gudalur Town	1066	4	2‡	1	..	1
Total	..	53	23	16	..	7

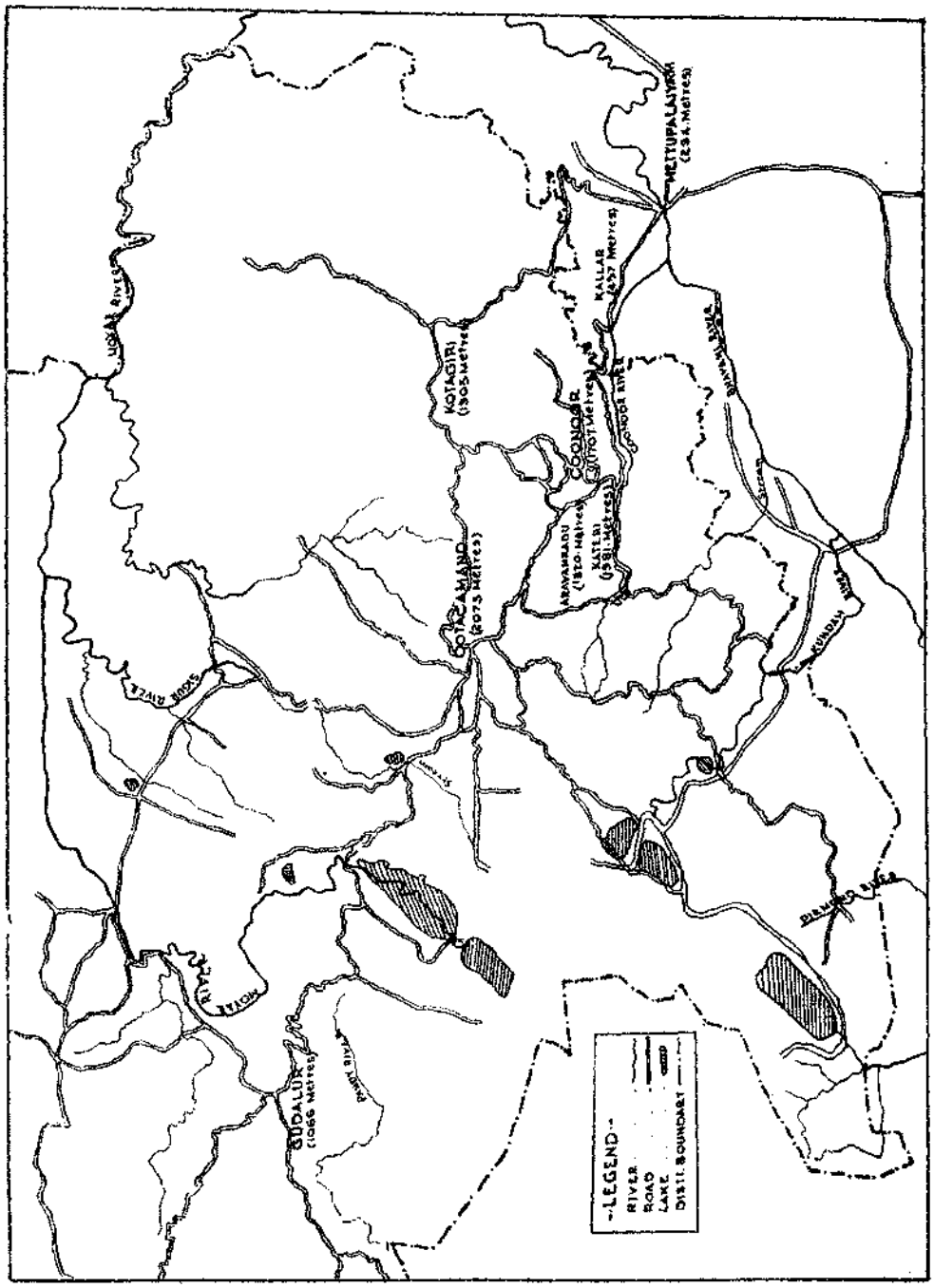
*Three monkeys were splenectomised. In one, the latent infection became mixed patent infection. In the second, the scanty mixed infection was confirmed. The third, which was negative prior to splenectomy, showed mixed infection after splenectomy.

†Two of the monkeys showed patent parasitaemia only after splenectomy; one showed *P. inui* and the other mixed infection.

‡Two monkeys were splenectomised; one showed a patent *P. inui* infection and the other a mixed infection.

In general the infection in the positive slides was scanty except in those from splenectomised monkeys. Positive monkeys were found at all altitudes of capture between 457 and 1,707 meters. To fix an exact altitude beyond which transmission would cease may not be easy, in view of the well-known migratory habits of monkeys.

The predominant patent infection was seen to be *P. inui*. Sixteen (70 per cent) out of the twenty-three positive monkeys showed this infection. Pure *P. cynomolgi* infection was not encountered in the course of the survey. The remaining seven showed mixed infection. In view of the fact that of the seven monkeys splenectomised five showed mixed infection, the true prevalence of *P. cynomolgi* infection may have been higher than actually seen.



MAP I.
Nilgiri Hills.

In the course of search for the natural vector for the simian malaria, night catches of mosquitoes were made around two huts (only two huts existed) in an areca-nut grove in Kallar. It was in this area that many of the positive monkeys (Table I) were captured. A total of 878 female mosquitoes were caught between March and December, 1961, and the results of dissection of their salivary glands are shown in Table II.

TABLE II.

Number and species and results of dissection of the 878 female mosquitoes caught around the two huts at Kallar in the year 1961.

Species of the female mosquito.	Number caught and dissected.	
A. culicifacies ...	1	Negative
A. fluviatilis ...	4	
A. hyrcanus ...	6	
A. jeyporiensis ...	1	
A. maculatus ...	21	
A. pallidus ...	1	
A. splendidus ...	6	
A. subpictus ...	38	
A. tessellatus ...	317	
A. vagus ...	17	
A. varuna ...	4	
Aedes spp. ...	36	
Mucidus spp. ...	1	
Culex gelidus ...	2	
Culex vishnui ...	208	3 specimens found infected with sporozoites, presumably of avian origin.
Culex spp. other than *gelidus* and *vishnui*).	215	Negative

Most of the mosquitoes were caught from the vegetation around the huts. None was found at any part of the night in a cage with a monkey at ground-level or another with a rabbit about 20 feet above the ground. With the exception of 3 specimens of *C. vishnui* which showed sporozoites in the salivary glands, all the others were negative. The sporozoites in the salivary glands of *C. vishnui* were confirmed by staining. The source of the infection, however, could not be identified for want of clean monkeys. The finding merits further investigation in view of Mulligan's (1935) record that in the course of experimental feeding of mosquitoes on monkeys with *P. cynomolgi* gametocytes, the occurrence of oocysts in the stomach of *C. vishnui* was encountered on one occasion. This mosquito is not a listed vector of any of the avian malaria parasites (Boyd, 1949).

Female *A. fluviatilis* and *A. stephensi* mosquitoes, obtained from the colonies maintained at the Coonoor Branch of the Institute, were experimentally fed in different lots on monkeys which showed good infections predominantly of *P. cynomolgi* or a mixed infection of both *P. inui* and *P. cynomolgi*. The mosquitoes were dissected ten days later. Batches of the fed mosquitoes were dissected separately for oocysts and sporozoites. The presence of oocysts in many was confirmed. The results in Table III, however, show only the proportion of mosquitoes

that showed sporozoites. Their presence was confirmed by staining. In most of the infected mosquitoes the sporozoite infection of the glands was scanty in comparison to the degree of oocyst infection in the mid-gut. Whether this was due to the partial suitability of the vector or the time interval between feeding and dissection, can only be determined by further work.

TABLE III.

Results of dissection of the laboratory-bred mosquitoes fed on monkeys showing infection of P. cynomolgi *and/or* P. inui.

Number of monkeys used for feeding.	Spleen : Intact/ removed.	Source of infection.	Parasite species.	A. STEPHENSI :		A. FLUVIATILIS :	
				Number dissected.	Number showing sporozoites.	Number dissected.	Number showing sporozoites.
1	Intact	Infective blood.	Mixed infection*.	22	6	43	15
1	Removed	Sporozoites.	Mixed infection*.	60	13	6	6
1	Removed	Infective blood.	P. *cynomolgi*.	16	11	91	12
1	Intact	Infective blood.	P. *cynomolgi*.	9	3	48	11
			Totals and percentage	107	32 (31 per cent)	188	40 (24 per cent)

*Mixed infection : *P. cynomolgi* and *P. inui*.

Fifteen specimens of *A. stephensi*, which had an infective blood meal 13 days previously, were fed on a clean *radiata* monkey. That the monkey was clean, was proved by the fact that its blood was negative to infection after splenectomy. Two of the mosquitoes were dissected immediately after engorgement and sporozoites were seen in their salivary glands. That the sporozoites were of both *P. cynomolgi* and *P. inui* was shown by the monkey developing a patent erythrocytic infection of both species, 11 days after the inoculation.

DISCUSSION.

The above mentioned survey has revealed for the first time that a considerable amount of natural simian malaria, at least in one focus, is prevalent in India. It has also recorded for the first time, the presence of *P. cynomolgi* as a natural infection in India. The extent of simian malaria in the country, its natural vector(s), susceptibility of man to the simian infections and the related information, are being investigated.

Natural infection in the brown monkeys has seldom been recorded from India previously. The general impression was that natural simian malaria was extremely rare in India. This was mostly due to the fact that not a single natural infection has been encountered in the thousands of *Macaca mulatta* monkeys that have been utilized for research at the Malaria Institute of India since about 1930, when Sinton and Mulligan commenced research on simian malaria in India. Mulligan and

Swaminath (1940 *loc. cit.*), while recording a natural infection of *P. inui* in one monkey, stated that they had not encountered any natural malaria infection in the many hundreds of *sinicus* and *rhesus* monkeys examined by them previously.

The first record of a natural simian infection in a brown monkey from India was made by Donovan (1920 *loc. cit.*). He reported negative results of the examination of blood smears from 76 *Silenus sinicus* (synonyms : *Macaca sinicus*; *Macaca radiata radiata*) monkeys shot in some of the valleys of the Nilgiri Hills—the same locality as of the present survey. As an addendum to the paper, however, he recorded the finding of a *Plasmodium* in a blood-slide from a *sinicus* monkey which was sent to him subsequently and that the parasites resembled *P. cynomolgi*. Sinton and Mulligan (1933) critically reviewed the identity of simian malaria parasites and from the meagre data in Donovan's publication, placed the parasite he recorded in the *P. inui* group with which *P. cynomolgi* was at that time confused.

The second record of a natural infection by Mulligan and Swaminath (1940 *loc. cit.*) was in a young *S. sinicus* captured from Kallar, also in the Nilgiris. They identified the parasite as *P. inui*.

The present survey showed that in the valleys of the Nilgiris, simian malaria is enzootic. Out of 53 monkeys captured (Table I), a total of 23 were found to be infected, but in four of them the infection was patent only after splenectomy. The parasite rate, therefore, was 19/53 or 35·4 per cent. The high parasite rate, the scanty parasitaemia in general, and the chronicity of infection revealed by the post-splenectomy patency in all the 4 animals which had negative pre-splenectomy blood smears, showed the enzootic nature of the infection in the valleys of Nilgiris.

Mulligan and Swaminath (1940 *loc. cit.*) drew attention to the fact that Kallar, the place of origin of the strain of *P. inui* recorded by them, was hyperendemic to human malaria and that the prevalent human infection was *P. malariae*. The human parasite rate was 51·2 per cent., and 76·7 per cent of the positive blood smears showed the presence of infection with the human quartan parasite. The chief or probably the sole vector of human malaria in Kallar area was *A. fluviatilis*.

The human malaria situation has completely changed since 1953 in the Kallar area, as elsewhere in India, on account of the National Malaria Control/Eradication Programme. For the last several years there has been no evidence of human malaria transmission in that area. As stated earlier, between March and December 1961, night catches of mosquitoes in Kallar resulted only in the capture of four specimens of *A. fluviatilis* (Table II). All of them were caught in the vegetation around the two huts. The point of interest, however, was that *A. fluviatilis* was found to be susceptible to the simian infection (Table III).

The search for the natural vector of the simian malaria has not yielded results so far. The continuation of work during the year was not possible due to the monsoon in the area and is to be taken up from January, 1962, onwards.

The previous impression that natural simian infection is rare in India, probably arose due to the fact that *Macaca mulatta* monkeys utilised for research

at the Malaria Institute of India, Delhi, were chiefly captured in "North India" from the Delhi and Lucknow areas. This is confirmed by Schmidt *et al.* (1961) who reported that since 1945 to July, 1960, they had used 5,600 *M. mulatta* monkeys from India for their research and that not one was found to be naturally infected. In their finding that out of 228 *M. mulatta* monkeys imported from East Pakistan ("probably Dacca") between October and December 1960, 22 were found to be naturally infected with *Plasmodium*, the identification of the infection has been tentatively made by them to be *P. inui* and *P. cynomolgi*, the former being more prevalent than the latter.

The discovery by Shortt *et al.* (1961) of a *Plasmodium* infection in *Macaca radiata* monkeys, captured around the campus of Osmania University of Hyderabad, reveals an additional focus of simian malaria. They have tentatively designated the infection to be due to *Plasmodium osmaniae*, a new species.

In view of the above and the results of the present survey, an intensive search in Indian monkeys for natural malaria infection is indicated. Mulligan and Swaminath (1940 *loc. cit*). drew attention of the possibility of natural simian malaria foci only in some foot-hill jungles.

From the point of view of *Macaca mulatta* as the animal of choice in experimental malariology, greater caution has to be exercised in future to detect natural infections, particularly with regard to monkeys obtained from certain localities.

SUMMARY.

An enzootic focus of simian malaria in *Macaca radiata radiata* Geoffroy in the Nilgiri Hills, Madras State, India, is described. The two natural infections found were *P. cynomolgi* and *P. inui*. The former is recorded for the first time in India and the latter confirms earlier records of many years ago.

ACKNOWLEDGEMENT.

Due credit is acknowledged for the help in the work in the field and laboratory rendered by Sarvashri Cornelius and Puran Singh, Laboratory Technicians and the two animal attendants, Sarvashri H. M. Giriappa and K. Dorai Raju. Special mention is made of Shri M. A. Aruldoss, the Van Driver, who enthusiastically participated in the capture of the monkeys.

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STUDIES IN THE EXPERIMENTAL TRANSMISSION OF *MF. BANCROFTI* AND *MF. MALAYI* BY BED-BUGS: NEGATIVE RESULTS.

BY

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[December 11, 1961.]

MANY workers at different times have examined the bed-bug in order to determine if it played any role as a vector of infections in man. The results have been consistently negative and have been summarised by Belding (1958) that it may act as a mechanical carrier but it is not a proved vector of human disease. Burton (1960), however, while working in highly filarial coastal area of Kerala State (India) collected 60 bed-bugs* from beds on which proved microfilaria carriers (both *B. malayi* and *W. bancrofti*) had slept. He reported that 11 (18.3 per cent) of the bed-bugs showed the developing forms (sausage or older forms) of filarial larvae. He even recorded the development up to infective larvae in a few of the bed-bugs which had fed on microfilaria carriers of *B. malayi* and *W. bancrofti*. If Burton's findings were correct, their epidemiological implications would be very great. Therefore, a planned investigation on the vectorial role of the bed-bug in human filariasis was urgently indicated. The results of such studies are recorded here.

The studies were carried out at Ernakulam—the same area where Burton made his observations and at Barabanki in Uttar Pradesh. Both the areas are endemic to filariasis. In addition, investigations were made with one carrier of *W. bancrofti* in Delhi, a non-endemic area. The bed-bugs from the clean colony maintained at the Malaria Institute of India, Delhi, were used for the experimental feeding on the microfilaria carriers.

**Cimex lectularius* and *Cimex hemipterus*.

The results are presented in Table I.

TABLE I.

Results of dissections of bed-bugs fed on the W. bancrofti *and* B. malayi *carriers.*

Date and place of feeding.	Type of carrier on whom fed.	Interval (days) between feeding and dissection.	Number dissected.	Number of bed-bugs showing :	
				Microfilariæ.*	Developing stages.
Delhi					
Oct. 29, 1960	*W. bancrofti*	1 to 16	18	Nil	Nil
Dec. 17, 1960	*W. bancrofti*	1 to 16	23	Nil	Nil
Barabanki					
Jan. 30, 1961	*W. bancrofti*	4 to 28	60	8	Nil
Ernakulam					
Feb. 6, 1961	*W. bancrofti*	8 to 29	51	12	Nil
Feb. 8, 1961	*B. malayi*	1 to 36	79	3	Nil
Feb. 19, 1961	*B. malayi*	1 to 23	85	Nil	Nil
Feb. 19, 1961	*B. malayi*	9 to 24	71	4	Nil
Feb. 22, 1961	*W. bancrofti*	6 to 20	44	15	Nil
Feb. 22, 1961	*W. bancrofti*	24 to 25	115	Nil	Nil
Feb. 22, 1961	*W. bancrofti*	24 to 25	264	29	Nil
Mar. 2, 1961	*W. bancrofti*	2 to 8	39	Nil	Nil
Mar. 2, 1961	*W. bancrofti*	2 to 23	75	Nil	Nil
Mar. 2, 1961	*B. malayi*	2 to 12	96	1	Nil
Mar. 2, 1961	*B. malayi*	2 to 12	1,527	57	Nil

*Microfilariae were found alive inside the bed-bugs for 7 to 12 days, and only in one bed-bug up to the 20th day of feeding.

In addition, bed-bugs, collected from different areas from beds on which microfilaria carriers had slept were dissected on the day of collection. The results are set out in Table II.

TABLE II.

Results of dissection of bed-bugs collected from beds on which microfilaria carriers had slept.

Place.	Species of infection.	Number of bed-bugs. dissected.	Number of bed-bugs showing :	
			Microfilariæ*	Developing stages.
Jaunpur† (Uttar Pradesh) ...	*Mf. bancrofti*	555	4	Nil
Kamareddy‡ (Andhra)	*Mf. bancrofti*	594	6	Nil
Thuravoor (Kerala)	*Mf. malayi*	415	3	Nil

*Microfilariae were found alive inside the bed-bugs for 7 to 12 days, and only in one bed-bug up to the 20th day of feeding.

†Reported by Mathur (1961).

‡Reported by Hassan (1961).

From the above Table II, it would be noted that only a small number of bed-bugs, collected from the beds on which microfilaria carriers had slept, contained microfilariae. In no bed-bug, however, developmental stages were noted.

It would appear that Burton's (1960) observations caused workers in other countries also to investigate the vectorial role of the bed-bug in human filariasis. Negative results have been recorded by Nelson (1961) working with *W. bancrofti* in East Africa, and Wharton (1961) working with *B. malayi* in Malaya.

Observations made by Burton (1960 *loc. cit.*) have not been confirmed by the work carried out by the Malaria Institute of India and workers elsewhere. The innocence of the bed-bug as a vector of human filariasis has been re-established.

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STUDIES ON THE BLOOD-PARASITES OF THE WHITE-THROATED MUNIA, *UROLONCHA MALABARICA* LINNAEUS.

Plasmodium (relictum) praecox Grassi and Feletti, 1890.

BY

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INTRODUCTION.

During the course of studies on the cryptic (occult) trypanosomes in birds (Grewal *et al.*, 1957*a*: 1957*b*) the author selected a tiny little bird, the white-throated munia, *Uroloncha malabarica* (vernacular: *Chiruka* or *Charchara* or *Piddi*), weighing 12-15 grammes, for the convenience of giving comparatively little quantity of the infective (material meta-trypanosomes from the infective mosquitoes, *Culex fatigans*) to see the flagellates in its blood without much of difficulty. This bird is found practically all over India (up to about 6,000 ft. elevation in the Himalayas) and in the drier parts of Ceylon. It occurs in West Pakistan, but apparently not in East Pakistan, Assam, Burma or in the districts in Bengal situated north and east of the Bay of Bengal. On the west, it is found in Baluchistan. Routine blood examination of these birds, caught in nature, revealed three different parasites patent in the blood. They were :- (1) *Plasmodium* sp. (2) *Trypanosoma* spp. (3) *Haemoproteus* sp.

These parasites are of great importance, and this is the first record regarding the occurrence of these forms in this bird in India.

A total of 69 birds was examined for patent infections in the blood. Of these, one showed only malaria parasite, none showed *Haemoproteus* alone, and twenty four showed trypanosomes alone. One bird had mixed infections of malaria parasite and trypanosomes; one had *Haemoproteus* and trypanosomes, while two had mixed infections of all the three types of parasites. The results are summarised in Table I.

Table I shows that the parasite, *P. (relictum) praecox*, dealt with in this paper, does not infect many birds in nature.

In this paper, it is proposed to deal with the morphology of *Plasmodium (relictum) praecox*. A few observations on its development in the mosquito, *Culex fatigans*, and the cross-infection of the parasite of white-throated munia, *Uroloncha malabarica*, and the house-sparrow, *Passer domesticus*, with each other's strain of parasites, are also presented.

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TABLE I.

Patent natural infection of the white-throated munia, Uroloncha malabarica, *with* Plasmodium, Hæmoproteus *and* Trypanosoma.

Name of the infection/infections, number of infected bird/birds and their experimental numbers.

	SINGLE INFECTION :			MIXED INFECTION :					
Number of birds examined.	P.	H.	T.	P and H.	P and T.	H and T.	P, H and T.	Total number of infected birds.	Total number of uninfected birds.
69	1 (31)	..	24 (1, 8, 9, 15, 16, 17, 18, 20, 23, 24, 25, 33, 34, 35, 39, 43, 44, 51, 56, 57, 61, 66 and 68.)	..	1 (22)	1 (59)	2 (28, 32)	29	40

Abbreviations : P. = *Plasmodium (relictum) præcox.*
H. = *Hæmoproteus.*
T. = *Trypanosoma sp.*

Note :— *Figures in brackets indicate the serial number of bird.*

MATERIAL AND METHODS.

All the birds used for this work were wild-caught ones purchased from the local bird market.

Mosquitoes, *Culex fatigans,* were obtained from the laboratories of the Malaria Institute of India where they are maintained on raisins or glucose for regular use.

In all cases, the material for examination consisted of thin blood films and impression smears of organs. The smears were dried in air, fixed in methyl alcohol for 2-3 seconds, and stained with Giemsa's or J. S. B. (1956) stains. Blood was taken from the pectoral vein with the help of a very fine needle. Mosquito dissections were examined as fresh preparations.

EXPERIMENTAL DATA.

Morphology of the stained parasites.—The youngest form is tiny rounded body consisting chiefly of chromatinic substance which is in the form of a tiny dot or strand taking red stain. Soon a vacuole is formed. The cytoplasmic content increases and the parasite assumes the shape of a stout ring, resembling the ring stage of human *Plasmodium.* Growing parasite undergoes various changes in shape.

Pigment appears in all excepting the ring stages. It is in the form of dark brown granules, either diffusely scattered or appears in discrete granules in the asexual forms. In some instances, the nucleus of the host cell is displaced to one side which is brought about due to the growth of the parasite and in a few cases it is expelled out of the red cell. Division forms, in which the chromatin divides into 2, 4 or 8 deeply staining granules, are quite common.

The mature schizonts are round and vary considerably in size. The highest number of merozoites in one schizont is up to 18.

Round or slightly oval gametocytes are numerous. Elongate gametocytes, with their tendency to surround the nucleus of the host cell, as described by Wolfson (1939) in the case of *P. (relictum) praecox* in duck, *Anas buschas domestica*, are not seen. The pigment in the gametocytes is practically always discrete and has a refractile appearance.

The cytoplasm of the female—or the macro-gametocyte—takes a deep blue stain and the nucleus is more compact and stains deep red. The male—or the micro-gametocyte—takes a faint pinkish stain. Its nucleus is diffuse and occupies much of the body of the parasite. The cytoplasm is, often, so faintly stained that it appears almost transparent.

Multiple infection of the red blood corpuscles is not very common. It was observed only twice throughout this investigation, which is, most probably, due to the light infection. Parasitaemia has never been high (42 parasites per 200 oil immersion, 1/12 in. objective and x6 ocular, fields, with a maximum of 9 parasites per 10,000 red blood corpuscles). With a continuous search for a long time in smears taken from a bird showing satisfactory infection, every stage of growth of the parasite was found. In blood preparations, made after ten days of the appearance of parasite in the erythrocytes, number of gametocytes has always been higher than the asexual stages. In birds experimentally infected by the inoculation of sporozoites, the incubation period is about ten days, after which for a week or ten days there is an increase in the number of parasites and after that the infection begins to decline. Scanty infection persists for a long period and eventually it appears to die out altogether within another 2—3 months. The presence of the tissue parasites cannot be overlooked because generally exo-erythrocytic forms remain in the body of the infected birds for a long period without showing any blood parasitaemia. Even the xenodiagnosis, performed on such birds, gave negative results.

After comparative study of the detailed morphology of the strain of *P. (relictum) praecox* obtained from the white-throated munia (*U. malabarica*) and the one obtained from house-sparrow (*P. domesticus*) the author believes that there is parallelism in their morphology, proving the two strains to be belonging to the same species.

Biological experiments.—Cross-infections, by inoculating infected blood, from the naturally infected house-sparrow, *P. domesticus*, were given to 'clean'

white-throated munia, *U. malabarica*. Also, sporozoites collected from the salivary glands of mosquitoes, fed on infected birds (*U. malabarica*), were inoculated intraperitoneally into birds of the same species (munia to houses-parrow and vice-versa). The results of these experiments are summarised in Tables II, III, IV and V.

TABLE II

Results of blood-induced infection from house-sparrow, Passer domesticus, *to the white-throated munia,* Uroloncha malabarica.

Number of the bird.	Number of blood examination prior to inoculation.	Result.	Date of inoculation.	Source of inoculum.	Date of Examination after inoculation.	Result.
27	2	Negative	Feb. 24, 1958	Sparrow No. 5 M.	Feb. 26 to March 17, 1958.	Negative
37	7	Negative	April 15, 1958	Sparrow No. 1 FTC.	April 21, 22, 23, 1958.	Positive
38	6	Negative	—Do—	—Do—	April 21, 22, 23, 29, 1958.	Positive

Table II shows that the strain of *P. (relictum) praecox* from sparrow, *P. domesticus*, by the intraperitoneal blood-induced infection, is infective to the white-throated munia, *U. malabarica*.

The observations recorded in Table III show that *P. (relictum) praecox* from house-sparrow, *P. domesticus*, by the inoculation of sporozoites, is infective to the white-throated munia, *U. malabarica*.

The observations show that the house-sparrow (*P. domesticus*) is susceptible to the infection of (*P. relictum*) *praecox*, from the white-throated munia (*U. malabarica*).

DISCUSSION.

Plasmodium (relictum) praecox is one of the bird malaria parasites, which has been extensively studied. Grassi and Feletti (1890) described *Haemoproteus praecox* from man and birds, believing that it was the same species in both cases, but soon (1891) changed the name of the parasite in birds to *H. relicta*. Celli and San Felice (1891) described three types of parasites observed in the blood cells of birds. This was probably mixed infection with *Plasmodium* and *Haemoproteus*. Labbè (1894) settled the problem by putting the birds' malaria parasite in the new genus *Proteosoma*. This nomenclature was accepted by many malariologists, although the specific name *relictum* was given in preference to Labbés' proposed name *grassii*. Marchiafava and Celli (1889) included the avian parasites, as well as those in man, under the genus *Plasmodium*.

TABLE III.

Infection of white-throated-munia, Uroloncha malabarica, *by the inoculation of sporozoites from the mosquitoes,* Culex fatigans, *fed on naturally, infected house-sparrow,* Passer domesticus.

Serial number of the bird.	*Number of blood examinations prior to inoculation.	Result.	Date of inoculation.	Source of inoculum.	Quantity given.	Date of examination after inoculation.	Result.
10	3	Negative	December 28, 1957.	Sparrow 12 F.	4 infected salivary glands.	January 9, 1958.	Positive
29	3	Negative	March 13, 1958.	Sparrow 4 FTC.	4 infected salivary glands.	March 21, 22, 26, 1958.	Positive
30	3	Negative	March 13, 1958.	Sparrow 4 FTC.	5 infected salivary glands.	March 21, 22, 26, 1958 and April 14, 25, 1958.	Positive
†34	6	Negative	April 8, 1958.	Sparrow Number 5	2 infected salivary glands.	April 10 to April 29, 1958.	Negative
†35	6	Negative	April 8, 1958.	Sparrow Number 5	2 infected salivary glands.	April 10 to April 29, 1958.	Positive
†36	6	Negative	April 8, 1958.	Sparrow Number 5	2 infected salivary glands.	April 10 to April 29, 1958.	Negative

*Thin blood smears were examined and twenty minutes were devoted for each observation.
†Tissues (liver, lungs, kidney, spleen and brain) of birds Numbers 34 and 36 were examined, but no E.E. forms were observed.

Coatney (1937) found a strain of *P.* (*relictum*) *praecox* in the mourning dove and a pigeon. Das Gupta and Siddons (1941) described a *Plasmodium* from the Malay chestnut-bellied munia (*Munia atricapilla atricapilla*) and called it *P.* (*relictum*) *praecox* var. *munia*. Redmond (1939) found that the virulence of different strains of *P. relictum* was correlated with the immune reactions of the same strain, and the most virulent strains produced a great degree of protection against superinfection with other strains. Jaswant Singh *et al.* (1952) recorded *P. relictum* in weaver bird, *Ploceus philipinus*.

The incidence of infection in any series of birds examined also depends to a great extent upon the type of avian host. Plasmodium spp. are known to occur

TABLE IV.

Infection of the house-sparrow (P. domesticus) *by the inoculation of sporozoites from the mosquitoes,* C. fatigans, *fed on the naturally infected white-throated munia* (U. malabarica).

Sparrow Number.	Number of blood examinations prior to inoculation.	Result.	Date of inoculation.	Source of inoculum.	Quantity given	Date of examination after inoculation.	Result.
2	Repeated examinations for a period of 1½ month	Negative	April 9, 1958.	*C. fatigans* fed on the white-throated munia, Number 32 on March 19, 1958	4 infected salivary glands.	April 22, 29, 1958.	Positive*
4	Repeated examinations for a period of 1½ month	Negative	April 9, 1958.	*C. fatigans* fed on the white-throated munia, Number 32 on March 19, 1958	4 infected salivary glands.	April 17, 1958.	Positive
6	Repeated examination for a period of 1½ month	Nagative	April 9, 1958.	*C. fatigans* fed on the white-throated munia, Number 32 on March 19, 1958	3 infected salivary glands.	April 22, 1958.	Positive

*The bird died on April 29, 1958, due to some unknown cause.

TABLE V.

Infection of white-throated munia (U. malabarica) *by the inoculation of sporozoites from the mosquitoes* (C. fatigans) *fed on naturally infected birds of the same species. (Control Experiment).*

Number of the bird.	Numer of blood examination.	Result.	Date of xeno-diagnosis.	Result.	Date of inoculation.	Source of inoculum.	Quantity given.	Date of examination after inoculation.	Result.
26	2	Negative	Feb. 10, 1958.	Negative	Feb. 17, 1958.	*C. fatigans* fed on munia Number 22, on Jan. 7, 1958.	8 infected salivary glands.	Feb. 24, 26, 27, 1958.	Positive
33	3	Negative	..	Negative	March 28, 1958.	*C. fatigans* fed on munia Number 32, on March 19, 1958.	4 infected salivery glands.	April 22, 25, 29, 1958.	Positive
39	3	Negative	Feb. 16, 1958.	Negative	April 25, 1958.	*C. fatigans* fed on munia Number 32, on March 19, 1958.	5 infected salivary glands.	April 29 to June 13, 1958.	Negative
40	3	Negative	Feb. 16, 1958.	Negative	April 25, 1958.	*C. fatigans* fed on munia Number 32, on March 19, 1958.	5 infected salivary glands.	April 29 to June 13, 1958.	Negative

Sporozoites obtained from the mosquitoes fed on infected white-throated munia (*U. malabarica*) were infective to some and non-infective to other birds of the same species.

more frequently in the passerine birds than in any other group. Sparrows have generally been found to be heavily infected in most regions where examinations have been made. Pigeons, doves, geese, ducks, turkeys and chickens, however, seem to be rarely infected in nature, although other malaria parasites have been found in all of them.

Little agreement exists among avian malariologists as to how many valid species actually have been found to date. Sergent and Cataney (1932), Manwell (1935 : 1936 : 1938), Giovannola (1934 : 1939) and Brumpt (1938) have enumerated the distinguishing characteristics of avian plasmodia.

Various specific names were added to the list of malaria parasite from birds. *P. majoris* (Laveran, 1902), *P. vaughani* (Novy and MacNeal, 1904), *P. passeris* (Johnston and Cleland, 1909), *P. wasielewskii* (Brumpt, 1910), *P. biziurae* (Gibruth, Sweet and Marullaz, 1914) were some of the plasmodia described from birds under these names. All these species, according to Wenyon (1926), do not differ much from *P. (relictum) praecox.*

Manwell (1938) classified the avian plasmodium spp. on the basis of the following morphological characters in the vertebrate host:—

1. Forms of the gametocytes,
2. Character of the pigment of gametocytes,
3. Form of the schizonts,
4. Number of merozoites, and
5. Alterations in the host cell.

The following points can also be taken into consideration:—

1. Host-parasite relationship,
2. The length of the asexual cycle,
3. Pathogenicity,
4. Reaction to anti-malaria drugs,
5. Cross-immunity reactions,
6. Climatic conditions controlling development in the mosquitoes,
7. Geographical distribution, and
8. Types of cells parasitized.

Huff (1937) gave the name *P. (relictum) praecox* var. *matutinum* to a malaria parasite from an American robin which produces 10 to 22 merozoites. Hewitt (1940) listed various strains of *P. (relictum) praecox* occurring in sparrow, robin, quail, finch, pigeon and wood-thrust. Das Gupta and Siddons (1941) believed that malaria parasite of the Malay chestnut-bellied munia (*Munia atricapilla atricapilla*) represented a separate strain or variety of bird plasmodium and called it *P. (relictum) praecox* var. *muniae.*

Manwell (1933), Coatney (1938*a*), Wolfson (1939), Jaswant Singh *et al.* (1951) and Grewal and Chowdhary (unpublished work) have shown the possibility of

infecting birds like common-fowl, canary, duck, domestic pigeon, weaver-bird and nightingale with *P. (relictum) praecox*. They found certain biological differences in this parasite. Jaswant Singh *et al.* (1951) were unable to infect red-munia and pigeons with this parasite, although Coatney (1938) felt that pigeons were better birds for experimental studies on *P. (relictum) praecox* as they were highly susceptible to the infection with this parasite.

Huff *et al.* (1959) reported that Strain I P derived from a Nebraska pigeon produced sporozoites in mosquitoes, but attempts to infect pigeons from these succeeded only 8 times in 72 trials. The same strain of malaria parasite behaved differently in the two species of mosquitoes, *Culex pipiens* and *C. tarsalis*. Strain I B derived from an Iowa pigeon gave rise to low infection in pigeons infected with sporozoites. It also behaved like the previous strain in the two species of mosquitoes (50 per cent of *C. tarsalis* became infected, while only 6 per cent of *C. pipiens* developed low infection, proving the former as a better host than the latter.)

Since *P. (relictum) praecox* is one of the species usually used for experimental work, and since it appears to occur more commonly than other species in nature, it is quite possible that a number of strains have been described. Some of these strains have been defined and maintained in canaries, while others need a little more careful study before a distinct differentiation is made. It does not seem necessary that a new strain designation be made when a known strain is transferred from one type of experimental host to another closely related one, and shows little change in its essential characteristics.

In nature, the two types of birds are found more or less in the same environments. It appears very likely that the insect vector seems to pass on the same strain to the birds of the different species, and the two species of birds become naturally infected with *P. (relictum) praecox*. The slight variations in the two strains might be due to factors related to the host. Such variations in the blood picture, following infection with the same strain of *P. berghei* in different hosts (rats, mice and squirrels), have been recorded (Ramakrishnan and Satya Prakash, 1950).

After studying the morphological characters of the parasite under discussion, the present author has no doubt in calling it *P. (relictum) praecox*. Although pre-erythrocytic schizogony has not been studied, the erythrocytic cycle of the two strains of malaria parasite from the white-throated munia and sparrow is found to be more or less identical. Although the biological characteristics of this parasite were studied, no attempts were made to go into the details of its behaviour in the two hosts, *U. malabarica* and *P. domesticus*, and to find out the differences if any in the two strains.

This report adds another host, the white-throated munia, *Uroloncha malabarica*, to the already existing and growing list of vertebrate hosts of *P. (relictum) praecox*.

SUMMARY.

Natural blood infection of the white-throated munia, *Uroloncha malabarica*, with *Plasmodium (relictum) praecox* is described.

The morphological and biological characters of this bird's malaria parasite are discussed. Results of experiments performed on birds, the white-throated munia (*U. malabarica*) and the house-sparrow (*P. domesticus*), by inter transfer of infections, both blood-induced and sporozoite-induced, are reported.

Previous history of *P.* (*relictum*) *praecox* has been reviewed.

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ANNOUNCEMENT

INDIAN COUNCIL OF MEDICAL RESEARCH.

(Colonel Amir Chand Trust Fund Prizes for Medical Research)

LIEUT.-COLONEL AMIR CHAND, lately Principal of Lady Hardinge Medical College, New Delhi, donated Rs. 50,000 to the Indian Council of Medical Research for creating a Prize Fund. From the interest earned by this sum, prizes are awarded for the best published research work in medical sciences. The Governing Body of the Council has constituted a Trust called the "COLONEL AMIR CHAND TRUST" for the administration and management of the Fund.

The prizes are awarded annually on an all-India basis for the best published research work in any subject in the field of medical sciences including clinical research. The term 'Clinical Research' covers research into the mechanism and causation of disease and its prevention and cure, and includes work on patients in hospitals, field studies in epidemiology and social medicine and observations in general practice.

It has been decided to award four prizes in 1962, each of Rs. 300 to Indian nationals of not more than 40 years of age on 1st January, 1962, for the best research papers in medical sciences published by them during 1961 in Indian or Foreign Journals as a result of work done in any Institution in India. These prizes will be known as 'SHAKUNTALA AMIR CHAND PRIZES'.

Those eligible for the prizes are MEDICAL OR NON-MEDICAL GRADUATES.

Selections for the award of the prizes will be made by a Selection Board.

In the case of a joint authorship of a publication, the prize shall be divided between the authors in such proportion as the Selection Board may decide. The role of the person who applies for the prize should be clearly indicated so as to make it easy to determine whether the major part of the work has been done by that person.

The award of the prizes will be announced at the meetings of the Council's Advisory Committees in November/December, 1962.

CANDIDATES for award of prizes are requried to submit TEN REPRINTS of their papers published during 1961. These should be sent to the DIRECTOR, INDIAN COUNCIL OF MEDICAL RESEARCH, P.O. BOX 494, NEW DELHI, so as to reach him NOT LATER THAN 1st SEPTEMBER, 1962. The PAPERS should be accompanied by a short biographical sketch and two copies of passport-size photographs of the candidate/candidates concerned.

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STUDIES ON THE OVICIDAL ACTION OF ORGANOPHOSPHORUS COMPOUNDS AGAINST *CULEX FATIGANS*.

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INTRODUCTION.

THE assessment of susceptibility to insecticides of different stages in the life cycle of an insect is important for purposes of implementation of practical control measures against it. So far as the mosquitoes are concerned, such measures are adopted either against the larvae or the adults. The toxicity of the insecticides, therefore, is generally determined against these stages only. The egg of the mosquito is considered as the most resistant stage and very little information is available on its susceptibility to insecticides. Ramakrishnan *et al.* (1960) reported the relative toxicity of various organophosphorus insecticides against the larvae, pupae and adults of *C. fatigans*. It was, therefore, considered of interest to study the comparative susceptibility of *C. fatigans* eggs also to organophosphorus compounds, *viz.*, parathion, baytex, diazinon, malathion, phosdrin and trithion.

In the early embryonic stages the nervous system of an insect is not differentiated and the cholinesterase (Ch E) occurs in eggs only in the later stages. Since the organophosphorus compounds are known to act by the inhibition of cholinesterase (Ch E), workers engaged in the study of their mode of action are interested to determine the stage of embryonic development at which the compounds exert the toxic effect. Using parathion, Schwartz (1950) and TEPP, Lord and Potter (1951) reported death in early embryo of *Lepinotursa decemlineata* and *Diataraxia oleracea* respectively. On the basis of these findings, Lord and Potter (1954) suggested that organophosphorus compounds might kill by some mechanisms other than the inhibition of Ch E. Whereas Speyer (1950), Smith (1955) and Mahrotra and Smallman (1957) observed continued embryonic development to later stages in the eggs treated with organophosphorus insecticides. During the course of these studies, therefore, the stage of embryonic development at which the poison exhibited the toxic effect was also recorded to understand the mode of action of these compounds. Factors which could affect the insecticidal toxicity, such as the effect of age of the egg, temperature during and after treatment, site of penetration of the

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poison through the egg shell, etc., were also studied. The results of these investigations are presented in this communication.

RELATIVE TOXICITY OF ORGANOPHOSPHORUS COMPOUNDS AGAINST *C. FATIGANS* EGGS.

Egg rafts, obtained from the *C. fatigans* colony* 30 to 60 minutes after laying, were floated in various concentrations of suspension/solution of each insecticide in water prepared by mixing 250 c.c. of water and 1 c.c. of alcoholic solution of the insecticide. These were kept exposed to the insecticide till the hatching of the control eggs kept in clean water was complete†. The eggs which had been treated with the poison were counted under the microscope. Those which failed to hatch were considered dead and the ones which hatched, even though eventually the larvae died, were considered unaffected‡.

The percentage of unhatched eggs at various concentrations of each insecticide, is shown in Table I. The results analysed by the probit analysis method

TABLE I.

Percentage of unhatched C. fatigans *eggs (obtained 30 to 60 minutes after laying) when exposed to various insecticide concentrations.*

Insecticide.	Concentration (p.p.m.)	Number of eggs.	Percentage unhatched.	Range of percentage unhatched eggs per raft.
Parathion	0·03	739 (5)	2·9	0–7·1
	0·06	812 (5)	49·0	25·0 – 85·8
	0·125	1153 (5)	98·0	95·2 –100·0
	0·25	807 (5)	100·0	..
Baytex	0·5	830 (5)	1·4	0 – 3·5
	1·0	845 (5)	99·8	99·0 –100·0
	2·0	609 (5)	100·0	..
Diazinon	1·0	579 (5)	0·34	0 – 0·75
	4·0	738 (5)	32·8	33·5 – 87·2
	10·0	840 (5)	86·9	58·0 –100·0
	40·0	752 (5)	100·0	..
Malathion	1·0	473 (4)	0	0
	4·0	513 (4)	0·6	0 – 2·3
	10·0	627 (5)	9·7	0 – 55·3
	20·0	530 (5)	71·2	30·2 – 85·5
	40·0	795 (5)	99·5	98·9 –100·0
Phosdrin	2·5	491 (4)	27·3	2·4 – 86·2
	5·0	549 (5)	78·3	9·1 –100·0
	10·0	587 (5)	99·3	94·0 –100·0
	20·0	619 (5)	100·0	..
Trithion	1·0	632 (5)	0·8	0 – 5·3
	4·0	682 (5)	8·2	0·71– 18·9
	10·0	599 (5)	10·2	3·3 – 25·0
	40·0	681 (4)	2·6	0 – 7·2

Figure in parenthesis indicate the number of rafts tested.

* Maintained at the Malaria Institute of India, Delhi.

† The experiments were carried out at water temperature of 22 to 24°C. The incubation period at this temperature was found to vary from 48 to 52 hours.

‡ Eggs found unfertilized were discarded from the results.

(Finney, 1952) are depicted in Table II. Parathion with Lc50 of 0·063 parts per million (p.p.m) was found to be the most toxic of all the organophosphorus compounds tested. With trithion, only 2.6 per cent eggs did not hatch at a dosage as high as 40 p.p.m. It was the least toxic compound against the eggs. The Lc50 values of baytex, malathion, diazinon and phosdrin were found to be 0·68, 5·1, 15·9 and 3·4 p.p.m. respectively (Table II). The order of toxicity of various compounds, therefore, was parathion > baytex > phosdrin > diazinon > malathion > trithion. The dosages of these compounds for producing the ovicidal effect, however, were found to be much higher than those required to cause mortality in larvae (Ramakrishnan *et al.* 1960); thus showing that the eggs were much less susceptible to the toxic action of the insecticides.

TABLE II.

Equation of the fitted pr-lc regression line, median lethal concentration (Lc50) with fiducial limits and regression co-efficient values regarding C. fatigans *eggs (obtained 30 to 60 minutes after laying) exposed to different insecticides.*

Insecticide.	Equation of the fitted pr-lc line.	Lc50 (ppm.)	Fiducial limits of Lc50 for 95 per cent probability.	Regression coefficient.	Factor by which dosage was multiplied.
Parathion	y = −0·2346 + 6·56x	0·063	0·061 − 0·064	6·56 ± 0·21	100
Baytex	y = −8·9994 + 16·87x	0·68	0·65 − 0·70	16·87 ± 0·91	10
Diazinon	y = −0·6557 + 3·31x	5·1	4·9 − 5·4	3·31 ± 0·03	1
Malathion	y = −2·0661 + 5·88x	15·9	15·4 − 16·4	5·88 ± 0·11	1
Phosdrin	y = 2·4634 + 4·77x	3·40	3·26 − 3·54	4·77 ± 0·20	1
Trithion	..	>40·0	..	..	..

STAGE OF EMBRYONIC DEVELOPMENT AT WHICH THE POISONS EXERT THE TOXIC EFFECT.

The eggs of *C. fatigans* are transparent and, therefore, the stage of embryonic development so far as the complete development is concerned could be ascertained by visual inspection through the microscope. Examination of the treated eggs showed that irrespective of the insecticidal concentration used, there was complete embryonic development.

Potter *et al.* (1957) while studying the effect of TEPP on the eggs of *Pieris brassicae* found that at high insecticidal concentrations it was possible to obtain complete kill in the early stages of embryonic development. As the dosage was reduced the proportion of eggs continuing development increased. Lord and Potter (1951 *loc. cit.*) also obtained similar results with the eggs of *Diataraxia oleracea* and *Anagasta kubniella*. It was considered probable by Smith and Wagenknecht (1959) that there might be two modes of action of organophosphorus compounds, the most sensitive being the Ch E inhibition and the other, when superimposed at a higher concentration, would arrest embryonic development before occurrence of Ch E. Potter (1958) reported the difference as high as 100 between the dosage which stopped development and which prevented hatching for paraoxon.

Effect of increase in concentration of the poisons on the development of mosquito egg was, therefore, studied. The embryonic development was found to continue in the eggs continuously exposed just after laying to concentration of 240 p.p.m. of parathion (i.e. about 4,000 times the Lc50 value). The development progressed also in the eggs which were floated in very high concentration (240 p.p.m.) of baytex. Similarly in the case of phosdrin at a concentration as high as 4,000 p.p.m. (800 times the Lc50 value) the embryonic development did not stop.

This was further substantiated by treating the eggs with pure parathion and phosdrin. Eggs after 12 to 13 hours of laying were floated for one hour in pure parathion and phosdrin and were then washed with water and floated in clean water. Out of the 5 rafts treated with parathion, one raft submerged in water on floatation. The rafts, after the incubation period of 54 hours, were examined. There was complete embryonic development in the eggs which were floating on water, whereas in the raft which submerged under water, about 25 per cent of the eggs were completely developed and the rest were partially developed. But almost the same effect was observed in the untreated eggs which were kept similarly submerged. In the eggs treated with phosdrin for one hour, all the eggs submerged in water and the development was partial in most of them. The eggs which were treated with pure phosdrin for two minutes, then washed with water and floated, did not submerge. In these, complete embryonic development was found to occur. The results obtained indicated that the compounds tested did not prevent embryonic development even at very high concentrations. It is possible that the shell of the mosquito egg is impermeable to these insecticides and the quantity of any chemical which might have penetrated was too small to prevent development.

EFFECT OF AGE OF EGGS ON THEIR SUSCEPTIBILITY TO INSECTICIDES.

Studies were carried out with *C. fatigans* eggs to see the effect of age, if any, on their susceptibility to insecticides, parathion, baytex, diazinon and phosdrin. Eggs 30 to 60 minutes and 30 to 31 hours after laying were exposed to the suspensions/solutions of insecticides in water for 4 hours, after which these were washed and kept for hatching in clean water. The total number of eggs and the number which did not hatch were counted after complete hatching took place in the untreated eggs. The results are presented in Table III. The results analysed by the probit analysis method are depicted in Table IV.

From Table IV it may be seen that the Lc50 values of parathion for 30 to 60 minutes and 30 to 31 hours old eggs were found to be 0·79 p.p.m. and 0·37 p.p.m. respectively. This showed that the older eggs were slightly more susceptible to the insecticides. In the case of baytex no significant difference in susceptibility between the eggs of two ages was observed. However, in the case of diazinon and phosdrin, the older eggs were found to be about 8 and 20 times more susceptible respectively than the younger eggs.

TABLE III.

Effect of age of C. fatigans *eggs on their susceptibility to insecticides.*

Insecticide.	Concentration of insecticide in ppm.	AGE : 30-60 MINUTES OLD		AGE : 30-60 HOURS OLD	
		Number of eggs.	Percentage unhatched	Number of eggs.	Percentage unhatched.
Parathion	0·25	872 (5)	0·0	1976 (16)	4·1
	0·5	802 (5)	8·7	1100 (8)	91·0
	1·0	1011 (5)	74·4	2527 (16)	99·84
	2·0	1015 (5)	99·6	2318 (16)	100·0
Baytex	0·5	324 (4)	0·0	946 (8)	0·0
	1·0	693 (5)	0·7	729 (8)	0·3
	2·0	960 (5)	72·7	966 (8)	90·8
	4·0	721 (5)	91·2	1001 (8)	99·2
Phosdrin	5·0	..	..	1011 (8)	0·0
	10·0	..	..	1287 (8)	9·7
	20·0	..	..	1126 (8)	69·2
	40·0	540 (5)	0·0	815 (8)	91·4
	80·0	..	..	910 (8)	100·0
	160·0	736 (5)	12·7	..	..
	640·0	527 (5)	62·4	..	..
	4000·0	855 (4)	100·0	..	..
Diazinon	40·0	..	..	623 (5)	4·2
	80·0	..	..	491 (4)	34·6
	160·0	632 (5)	5·5	756 (5)	50·4
	320·0	947 (5)	10·0	680 (5)	78·9
	640·0	938 (5)	23·5	..	..
	1280·0	940 (5)	50·0	..	..

Figure in parenthesis indicate the number of rafts tested.

TALBE IV.

Equation of the fitted pr-lc line, median lethal concentration (Lc50) and regression co-efficient values regarding the insecticide susceptibility of C. fatigans *eggs of different ages.*

Age of eggs. (in hours)	Insecticide.	Equation of the fitted lc-pr line.	Lc50 (ppm.) (Fiducial limits for 95 per cent probability.)	Regression coefficient.	Factor by which dosage was multiplied.
½—1	Parathion	Y = −0·9976 + 6·65x	0·79 (0·78 − 0·81)	6·65 ± 0·21	10
30—31	Parathion	Y = −0·0270 + 9·21x	0·37 (0·35 − 0·39)	9·21 ± 0·71	10
½—1	Baytex	Y = −0·0600 + 4·20x	1·60 (1·43 − 1·83)	4·20 ± 1·7	10
30—31	Baytex	Y = −5·0510 + 8·57x	1·49 (1·30 − 1·70)	8·57 ± 1·4	10
½—1	Phosdrin	Y = −1·3200 + 2·36x	476·4 (436·5 − 518·8)	2·36 ± 0·13	1
30—31	Phosdrin	Y = −0·8515 + 4·72x	17·42 (16·94 − 17·61)	4·72 ± 0·21	1
½—1	Diazinon	Y = −0·9636 + 1·91x	1351·0 (1108·0 − 1645·0)	1·91 ± 0·9	1
30—31	Diazinon	Y = −0·3331 + 2·46x	147·2 (140·7 − 154·0)	2·46 ± 0·11	1

The increased susceptibility of the older eggs to diazinon and phosdrin could not be attributed to any change in the inherent susceptibility of the embryonic material as the susceptibility of eggs of both the age-groups was almost the same to parathion and baytex. The pick up (or penetration) of the poison by the eggs of different ages, due to the same reason, could also be not very great. This was further confirmed by bioassay. Eggs 12 to 13 hours old and 36 to 37 hours old were exposed for 4 hours to 4,000 p.p.m. of phosdrin. These were washed with water and grounded thoroughly. The grounded material was then mixed in 10 c.c. of water. Fifteen first-stage larvae were introduced and the mortality in them was noted after the observation period of 24 hours. From the results (Table V) obtained it was observed that the mortality produced in the larvae by the insecticide obtained from the younger and older eggs was almost the same.

The data thus obtained clearly proved that the eggs were susceptible to the insecticides at all stages of development. As the toxic effect of the poison was manifested at the very late stage of embryonic development, there was longer time lag for the younger eggs to excrete or detoxify the poison. The difference observed in the case of two groups of poisons, i.e., parathion and baytex on the one hand and diazinon and phosdrin on the other might, therefore, be on account of the latter group of insecticides being liable to quicker detoxification and excretion. It is of interest to note that the poisons of the latter group are more soluble in water and unstable to alkali.

EFFECT OF TEMPERATURE ON THE TOXICITY OF PARATHION TO *C. FATIGANS* EGGS.

The toxicity of the insecticide to the insect eggs was found to be affected by the temperature during treatment and incubation period and the magnitude of the effect mainly depended upon the nature of the species against which the poison was applied and the type of the poison. Studies were, therefore, carried out with parathion to elicit the relationship, if any, between temperature and its ovicidal action against *C. fatigans*. The effect of continuous high temperature was studied by exposing egg-rafts (after 30 to 60 minutes of laying) continuously at 30 to 32°C. in suspension/solution of parathion in water. Effect of temperature during exposure on the toxicity of insecticide was also studied by exposing the eggs (12 to 13 hours after laying) for 4 hours at 30 to 32°C. and 18 to 20°C. The eggs after exposure were washed and kept for hatching at both the temperatures to study the effect of temperature, if any, during the reaction period. The eggs were examined after the incubation period and the results are presented in Tables VI and VII.

From Table VII it may be seen that the Lc50 of parathion for the eggs which after 30 to 60 minutes of laying were under continuous exposure at 32 to 34°C., was 0·0076 p.p.m. The Lc50 of parathion for the eggs which were similarly treated at 22 to 24°C. was, however, found to be 0·063 p.p.m. (Table II). Parathion was, therefore, found to be more toxic to the eggs exposed at high temperature*.

* The incubation period was 22 to 24 hours at 32 to 34°C. as against 46 to 52 hours at 22 to 24°C.

TABLE V.

Percentage mortality in the C. fatigans *first-instar larvae exposed to insecticide (phosdrin). Material obtained from grounded eggs of different age.*

Age of eggs.	Total number of eggs grounded after 4 hours exposure to phosdrin 4000 pp.m.	Percentage mortality in larvae after 24 hours exposure to insecticide (phosdrin) material obtained from grounded eggs.
12—13 hours old	200	0·0
	1000	86·0
36—37 hours old	200	0·0
	1000	93·0

TABLE VI.

Effect of temperature on the toxicity of parathion to C. fatigans *eggs.*

Eggs (after 30 to 60 mts. of laying) under continuous exposure in parathion at 30-32°C.			Eggs (after 12-13 hrs. of laying) exposed for 4 hrs. to parathion at 30-32° C. washed with water and then kept at 30 to 32° C.			Eggs (afrer 12-13 hrs. of laying) exposed for 4 hrs. to parathion at 30-32° C°. washed with water and then kept at 18 to 20° C.			Eggs (after 12-13 hrs. of laying) exposed for 4 hrs. to parathion at 18-20° C. and washed with water and then kept at 30 to 32°C.			Eggs (after 12-13 hrs. of laying) exposed for 4 hrs. to parathion at 18-20° C. washed with water and then kept at 18 to 20° C.		
Concentration	n.	p.	Concentration	n.	p.	Concentration	n.	p.	Concentration	n.	p.	Concentration	n.	p.
0·0037	761 (4)	15·9	0·125	671 (5)	0·0	0·125	548 (5)	0·0	0·25	1207 (10)	0·0	0·25	2531 (20)	0·15
0·0075	679 (4)	45·5	0·25	718 (6)	27·3	0·25	658 (6)	16·4	0·5	2281 (16)	20·1	0·5	2439 (19)	7·3
0·015	912 (5)	91·0	0·5	1029 (6)	98·7	0·5	719 (6)	97·2	1·0	2267 (16)	88·6	1·0	2347 (20)	48·6
0·03	459 (4)	100·0	1·0	765 (6)	100·0	1·0	841 (6)	100·0	2.0	1739 (10)	100·0	2·0	2438 (15)	99·8
0.06	824 (5)	100·0												

N. B. Concentration = dosage of insecticide in ppm.
n = number of eggs.
p = percentage unhatched.
Figuers in parenthesis indicate the number of rafts tested.

The Lc50 (0·35 p.p.m.) of parathion with the eggs exposed at higher temperature (30 to 32°C.) for 4 hours and kept thereafter at low temperature was found to be significantly less than the Lc50 (0·95 p.p.m.) obtained with eggs exposed and kept at low temperature, thereby showing a higher rate of penetration at high temperature. The Lc50 value, obtained with the eggs which were exposed and kept at high temperature, was found to be 0·29 p.p.m. which was only slightly less than the Lc50 value of 0·35 p.p.m. with the eggs exposed at high temperature and kept at low temperature. This difference is barely statistically significant.

The toxicity of parathion was also found to be more in the case of eggs which were exposed at low temperature and kept at high temperature (Lc50, 0·67 p.p.m.) than the eggs which were exposed and kept at low temperature (Lc 50, 0·95 p.p.m.).

TABLE VII.

Equations of the fitted lc-pr line, median lethal concentrations (Lc 50) and regression co-efficient values of parathion regarding C. fatigans *eggs exposed at different temperatures.*

Type of treatment.	Equation of the fitted lc-pr line.	Lc 50 (ppm.) (with fiducial limits for 95 per cent probability).	Regression coefficient.	Factor by which dosage was multiplied.
Continuous exposure of eggs (30 minutes after laying) to parathion at high temperature.	Y=1·5656+3·90x	0·0075 (0·0069—0·0083)	3·90±0·15	1,000
Eggs (12-13 hours after laying) exposed to parathion for 4 hours at high temperature and then kept at high temperature	Y=0·4883+9·75x	0·2902 (0·2883—0·2920)	9·75±0·37	10
Eggs (12-13 hours after laying) exposed to Parathion for 4 hours at high temperature and then kept at low temperature	Y=0.1810+9·75x	0·3478 (0·3449—0·3508)	9·75±0·37	10
Eggs (12-13 hours after laying) exposed to parathion at low temperature and then kept at high temperature.	Y=—0·7087+6·94x	0·6667 (0·6573—0·6759)	6·94±0·13	10
Eggs (12-13 hours after laying) exposed to parathion at low temperature and then kept at low temperature.	Y=—0·6095+5·73x	0·95 (0·87— 1·04)	5·73±0·15	10

Pradhan and Ranga Rao (1957) also found more effect at the higher post treatment temperature with parathion. The effect of post-treatment temperature in the

present studies was not found to be very pronounced probably due to the fact that the reaction period at high temperature was less than at low temperature. The incubation period at high temperature was less than that at low temperature.

The effect of pre-treatment (preconditioning) of eggs at high temperature was also studied. Eggs (after 30 to 60 minutes of laying) were kept at 30 to 32°C. for 4 hours, and then exposed to the insecticide at low temperature (18 to 20°C.) for 4 hours after which these were washed and kept at low temperature. There was no significant difference between the Lc50 values obtained with the eggs which were preconditioned at high temperature and those kept continuously at low temperature.

The results obtained indicated that the pretreatment temperature did not produce any effect on the insecticidal toxicity of parathion whereas the temperature during exposure considerably affected its toxicity to *C. fatigans* eggs. The interpretation of the effect of post-treatment temperature, however, is difficult because of the variation in the reaction period at different temperatures but the data collected indicated that the post-treatment higher temperature resulted in slightly higher kill.

SITE OF PENETRATION OF POISON THROUGH THE EGG.

Beament (1948) has shown that a range of materials with widely different chemical properties enter the eggs of *Rhodinus prolixus* only through the micropyles and not through the general shell surface. The micropylar area appears to be an area where the entry to the embryonic material is comparatively easy. In all the above experiments, only the micropylar area was in contact with the insecticides. It was, therefore, of interest to find if the insecticides could penetrate to the mosquito embryo through the rest of the egg shell.

If the poison was penetrating through the whole shell surface, it was likely to be more toxic against the eggs which were kept under submergence than those which were kept floating in the insecticide treated water because in the former case greater surface area of the egg was exposed to the insecticide. Experiments were, therefore, carried out in which 12 to 13 hours old egg rafts were floated and submerged for 30 minutes in various concentrations of parathion and baytex in water. The eggs after the exposure were washed with water and floated in clean water till the hatching in control eggs was complete. The Lc50 values of parathion for the submerged and floated eggs were 1·79 p.p.m. and 3·23 p.p.m. respectively (Tables VIII and IX). The results obtained with baytex were also similar. The data collected, therefore, indicated that the insecticide not only penetrated through the micropyle but also through the general surface of the chorion.

Salkeld and Potter (1953) suggested that the liquids might again entrance to the embryo through the micropyle more easily than through the general surface of the chorion. Experiments were carried out, in which egg rafts (12 to 13 hours after laying) were placed for 4 hours, with micropylar area upward and micropylar area downward on a cotton pad soaked with various concentrations of parathion.

TABLE VIII.

Toxicity of baytex and parathion to C. fatigans eggs in relation to surface exposed.

Eggs floated for 30 minutes in parathion.			Eggs submerged for 30 minutes in parathion.			Eggs floated for 30 minutes in baytex.			Eggs submerged for 30 minutes in baytex.			Eggs kept with micropyle downward on cotton soaked with parathion.			Eggs kept with micropyle upward on cotton soaked with parathion.		
Con.	n	p	Con.	n	p	Con.	n	p	Con.	n	p	Con.	n	p	Con.	n	p
1·0	630 (4)	0·0	1·0	479 (4)	8·4	2·0	787 (5)	0·0	2·0	696 (5)	23·4	0·25	558 (4)	0	0·25	558 (4)	0·2
2·0	556 (4)	11·9	2·0	340 (4)	65·0	4·0	766 (5)	39·4	4·0	836 (5)	77·0	1·0	818 (5)	1·8	1·0	526 (4)	0·2
4·0	595 (4)	69·7	4·0	527 (4)	95·4	10·0	713 (5)	98·3	10·0	877 (5)	99·7	4·0	666 (5)	87·3	4·0	966 (5)	88·9
												20·0	825 (5)	100·0	20·0	1,030 (5)	100·0

N.B. Con. = Concentration in p.p.m.
n = Number of eggs.
p = Percentage unhatched.
Figures in parenthesis indicate the number of rafts tested.

TABLE IX.

Equation of the fitted lc-pr line, median lethal concentration (Lc50) and regression coefficient values regarding toxicity of parathion and baytex to C. fatigans *eggs in relation to the surface area exposed.*

Type of treatment.	Equation of the fitted lc. pr line.	Lc50 (p.p.m.) (with fiducial limits for 95 per cent probability).	Regression Coefficient.	Factor by which dosage was multiplied.
Eggs floated in parathion for 30 minutes.	Y = −3·5566 + 5·67x	3·23 (3·11 − 3·34)	5·67 ± 0·31	10
Eggs submerged in parathion for 30 minutes.	Y = −1·4621 + 5·16x	1·79 (1·72 − 1·86)	5·16 ± 0·21	10
Eggs floated in baytex for 30 minutes.	Y = 0·8872 + 6·3x	4·49 (4·37 − 4·62)	6·3 ± 0·2	1
Eggs submerged in baytex for 30 minutes.	Y = 2·7734 + 4·95x	2·82 (2·73 − 2·91)	4·95 ± 0·2	1
Eggs kept with micropyle downward on cotton soaked with parathion for 4 hours.		2·3		
Eggs kept with micropyle upward on cotton soaked with Parathion for 4 hour.		2·3		

The eggs after 4 hours of exposure were washed with water and floated in clean water. These were examined after the incubation period. From the results (Tables VIII and IX) it may be seen that the Lc50 values of parathion obtained in both the cases were not significantly different from each other. This suggested that the penetration of the insecticide through the micropylar area was similar to that through the rest of the egg surface.

MECHANISM OF OVICIDAL ACTION OF ORGANOPHOSPHORUS COMPOUNDS.

The absence of any evidence of inhibition of embryonic development even at very high insecticidal concentrations suggested the following possible explanations on the mode of action :

(*i*) The poison by contact with egg shell brought about changes in its outer layer which made it unbreakable or;

(*ii*) The poison after contact penetrated into the egg and was stored somewhere and was available to the embryo only at the time of hatching or;

(*iii*) Although the poison was available to the embryo the vulnerable system which it attacked was not present before the completion of embryonic development, or the system it attacked did not play any vital role until the point of hatching.

It was considered likely that certain changes in the egg chorion were brought about on contact with the compounds due to which it was not breakable by the egg breaker and that the primary larva, though not affected in any way, was not able to come out of the shell and died. When the egg chorion was dissected at the completion of embryonic development, the primary larvae from the treated eggs, however, were not in a normal condition. They were in paralytic state. The larvae dissected from the untreated eggs were normal in all respects. These observations suggested that it was not due to any effect on the egg shell that resulted in the ovicidal action of the compounds.

Beament (1946) and Salkeld and Potter (1953 *loc. cit.*) observed many membranes in between the embryo and exochorion in eggs of some insects studied by them. Christopher (1946) in studying the egg structure of *C. fatigans* noted three layers, *viz.*, the exochorion, endochorion and vitteline membrane. It was presumed that poison on penetration was stored somewhere in between these membranes, thus not reaching the embryo. On the completion of embryonic development, probably due to some change in the permeability of the membranes, the embryo came in contact with the poison and was so affected that it could not break the egg shell, the poisons thus acting as larvicides and not as ovicides. It was, therefore, expected that the relative toxicity of the various compounds against the eggs would be similar to that against the primary larvae during a short period of contact. To confirm the validity of this assumption, toxicity of these compounds was determined against first-instar larvae (6 to 8 hours old). Fifty larvae were exposed to the suspensions/solutions of insecticides in water obtained by mixing 1 c.c. of alcoholic solution of

insecticide in 250 c.c. of water. Four replicates were made at each concentration. The mortality in the larvae was observed at regular intervals. From the results (Table X) it may be seen that the order of toxicity of the compounds was parathion > malathion = diazinon = phosdrin = baytex > trithion when the kill was recorded after 30 minutes interval. Considering the larval kill obtained after an interval of 4 hours the relative toxicity of various compounds against first-instar larvae was found to be parathion > phosdrin > malathion > diazinon = baytex > trithion. The order of toxicity was not similar to that observed against the eggs (Tables I and II). The order to toxicity against the eggs was parathion > baytex > phosdrin > diazinon > malathion > trithion. However, against the first-instar larvae, parathion was found to be the most toxic and trithion the least, similar to what was observed against the eggs. But so far as the other poisons were concerned, the relation did not hold good. It appears likely that in the ovicidal effect, certain other factors, different from those of the larvicidal action, were playing part in the toxicity of the poisons against the eggs.

TABLE X.

Percentage mortality in the first-instar C. fatigans *larvae at varying time intervals when exposed to different insecticidal concentrations.*

Concentration. (p.p.m.)	¼ hour.	½ hour.	1 hour.	1½ hours.	2 hours.	3 hours.	4 hours.
Parathion							
0·015	0	0	0	0	0	76	100
0·03	..	..	..	1	34	100	100
0·06	..	0	0	81	99	100	..
0·25	0·0	10·0	100·0				..
1·0	4·0	100·0	..				
4·0	86·0	100·0					
Baytex							
0·25					0·0	4·0	13·0
1·0	0	0·0	4·0	..	36·0	84·0	98·0
4·0	0	94·0	100·0	..			
Diazinon							
0·25	..	..	..		3·0	..	17·5
1·0	0·0	0·0	0·0		14·0	..	97·0
4·0	4·5	63·5	100·0		..	..	..
Malathion							
0·06						0·0	6·0
0·25			0·0		6·0	56·0	94·0
1·0	0·0	0·0	3·0		66·0	97·0	100·0
4·0	61·0	97·0	100·0				
Phosdrin							
0·25					0·0	76·0	100
1·0	1·0	0·0	7·0		100·0	100·0	
4·0	0·0	96·0	100·0		..	..	
Trithion							
0·25							0·0
1·0	0·0	0·0	0·0		0·0	1·0	20·0
4·0	0·0	6·0	72·0		100·0	..	..

The data obtained gave some indication against the validity of the hypothesis of the storage of the poisons in between the membranes away from embryo but it was, however, not conclusive. It was possible that the penetration or the pick up of the different poisons was different in the eggs and larvae and hence the difference in the comparative toxicity. Experiments were, therefore, carried out to elucidate it further by studying the effect of temperature on the toxicity of parathion to *C. fatigans* eggs at the different stages of development. It was considered that if the assumption that the poison was available only at the completion of the embryonic development was correct then the eggs treated with poison kept very near to the point of hatching at higher temperature should show higher or lower mortality, depending upon the positive or negative temperature coefficient of the poison against the larvae. This effect of temperature is not expected to be observed in the case of eggs kept at high temperature long before the hatching.

The higher temperature was found to cause an increased toxicity of parathion to *C. fatigans* eggs but it may not be strictly applicable to the larvae. Thevasagayam (1957) found low mortality in *C. fatigans* larvae when exposed to parathion at high temperature. Pradhan and Ranga Rao (1957 *loc. cit.*) observed increased toxicity at post-treatment high temperature. It was, therefore, considered necessary to study the effect of temperature on the toxicity of parathion to the first-instar larvae of *C. fatigans*.

Experiments were carried out by following the method similar to one described above at two temperatures 30—32°C. and 16—18°C. The tests were carried out when the larvae were continuously exposed to the various insecticide concentrations at the two temperatures. The mortality was assessed at intervals of 24 hours, 48 hours and 72 hours. In another series of tests larvae were exposed for 1 hour at 20°C. and then kept under observation at 30—32°C. and 16—18°C. The results are presented in Tables XI and XII. From the results it may be seen that Lc50 of parathion for the larvae continuously exposed at higher temperature (0·0005 p.p.m.) was significantly less than the value (0·0011 p.p.m.) obtained at the temperature 16 to 18°C. Low Lc50 value (0·022 p.p.m.) was observed for the larvae which after exposure at low temperature were kept at higher temperature than the Lc50 (0·03 p.p.m.) for larvae which were kept at the temperature of exposure. The speed of action of parathion was found to be more at higher temperature than that at lower temperature (Table XIII).

It was considered, therefore, that if the assumption of the availability of the poison at the completion of the embryonic development was correct, then the eggs kept near the point of hatching at higher temperature should show higher mortality whereas the eggs which are kept at higher temperature long before the completion of embryonic development should not show any such effect. Eggs rafts after 12 to 13 hours of laying were, therefore, exposed at 18 to 20°C. for 4 hours in the various concentrations of suspension/solutions of parathion in water. The eggs after exposure were washed with water and divided into two batches. One batch was

TABLE XI.

Effect of temperature on the toxicity of parathion to the first-instar C. fatigans *larvae.*

Type of treatment.	Concentration. (p.p.m).	Percentage mortality after 24 hours.		Percentage mortality after 48 hours.		Percentage mortality after 72 hour	
		HT	LT	HT	LT	HT	LT
Continuous exposure	0·00037	11·5 (200)	0·0 (200)	60·0 (200)	14·0 (200)	63·0 (200)	15·0 (200)
	0·00075	99·5 (200)	4·0 (200)	100·0 (200)	97·5 (200)	..	98·0 (200)
	0·0015	100·0 (200)	99·0 (200)	..	100·0 (200)	..	..
	0·003	100·0 (200)	100·0 (200)	..	..	..	..
	0·02	36·0 (100)	10·0 (100)	..	..	..	..
Exposed at low temperature for 1 hour and kept at high temperature and low temperature for reaction	0·03	90·0 (100)	30·0 (100)	..	..	..	..
	0·04	100·0 (100)	91·0 (100)	..	..	..	..
	0·06	100·0 (100)	100·0 (100)	..	..	..	..

TABLE XII.

Equations of the fitted lc-pr lines, median lethal concentrations (Lc50) and regression coefficient values of parathion regarding C. fatigans *larvae exposed and kept at different temperatures.*

Type of treatment.	Equation of the fitted lc-pr line.	Lc50 (p p.m) (fiducial limits for 95 per cent probability).	Regression coefficient.	Factor by which dosage was multiplied.
Continuous exposure at high temperature	Y=−4·1433+13·24x	0·00049 (0·00047−0·00052)	13·24±0·80	10,000
Continuous exposure at low temperature	Y=−8·6334+13·24x	0·0011 (0·0010 −0·0011)	13·24±0·80	10,000
Exposed for 1 hour at low temperature and then kept at high temperature	Y=1·9802+9·21x	0·022 (0·021 −0·023)	9·21±1·3	1,000
Exposed for 1 hour at low temperature and then kept at low temperature	Y=0·6069+9·21x	0·030 (0·027−0·033	9·21±1·3	1,000

kept at the higher temperature of 33 to 34°C. for 4 hours near the completion of embryonic development, i.e., after 50 hours of laying, whereas the other batch was kept after about 30 hours of laying at 33 to 34°C. for 4 hours and then kept again at a temperature of 18 to 20°C. These were examined when hatching in the untreated control eggs was complete. The results obtained are presented in Table XIV. The Lc50 (0·67 p.p.m.) of the insecticide obtained for the eggs kept at high temperature

TABLE XIII.

Speed of action of parathion at higher and lower temperature to the first-instar C. fatigans *larvae.*

Type of treatment.	Concentration (p.p.m.)		Percentage mortality after the observation period:											
			30 mts.	45 mts.	60 mts.	75 mts.	90 mts.	105 mts.	120 mts.	150 mts.	180 mts.	210 mts.	240 mts.	300 mts.
Continuous exposure	0·06	HT	15	92	100	..								
		LT	0	0	0	3	81	98	99	100	..			
	0·03	HT	0	4	65	95	100	..		..				
		LT	0	0	0	0	1	17	34	96	100	..		
	0·015	HT	0	0	0	0	20	29	53	94	..	..	100	..
		LT	0	0	0	0	0	0	0	15	76	..	100	..
	0·0075	HT	0	0	0	0	0	0	0	3	..	..	73	..
		LT	0	0	0	0	0	0	0	0	..	..	0	63
Exposed at low temperature for 1 hour and kept at high temperature and low temperature	0·06	HT							60	100	..	..	..	..
		LT							15	56	..	98	..	100
	0·04	HT							25	82	..	92	..	96
		LT							0	1	..	9	—	40

HT = high temperature. LT = low temperature.

TABLE XIV.

Effect at post-treatment high temperature on the toxicity of parathion to C. fatigans *eggs.*

Concentration (p.p.m.)	Exposed at LT for 4 hours (after 12 hours of laying) and then kept for 4 hours at HT after 30 hours of incubation period.		Exposed at LT for 4 hours (after 12 hours of laying) and then kept for 4 hours at HT after 50 hours of incubation period.	
	Number of eggs.	Percentage unhatched.	Number of eggs.	Percentage unhatched.
0·25	729 (5)	0·0	603 (5)	0·0
0·5	552 (5)	6·0	570 (5)	18·1
1·0	830 (5)	88·3	890 (5)	90·2
2·0	900 (5)	99·8	550 (5)	100·0
Lc50	0·73		0·67	
(With fiducial limits for 95 per cent probability)	(0·67–0·80)		(0·65–0·69)	

LT = low temperature. HT = high temperature.

N.B. Figures in parenthesis indicate the number of rafts tested.

near the completion of embryonic development was found to be significantly less than the value (0·95 p.p.m., Table VII) obtained for the eggs kept continuously at low temperature. The Lc50 obtained with eggs kept for four hours at high temperature after 30 hours of incubation period was also less (0·73 p.p.m.).

The results obtained, therefore, indicated that the poison was available to the embryo but the toxic effect was manifested only at the completion of embryonic development. Studies are in progress to confirm it. Mahrotra (1960) found the inhibition of ChE in the *Musca* eggs treated with parathion as soon as the enzyme appeared* and quite a few hours before hatching; thereby showing that the poison was available to the embryo all the time and acted only when the vulnerable system appeared.

DISCUSSION AND CONCLUSIONS.

Organophosphorus insecticides exert the toxic action by the inhibition of Ch E. Presumably, when Ch E is inhibited, acetylcholine (Ach) accumulates, thus subjecting the organism to excessive level of Ach which induces toxicity. Doubts were first expressed about the applicability of this hypothesis in insects. The pertinent evidence in favour of this hypothesis has been reviewed by Smallman (1956). But lot of information has accumulated during recent years which suggests about the possibility of inhibition of other enzymes also. The evidence in favour of this has also come from the work on ovicidal action of organophosphorus compounds.

Eggs were found to be susceptible at all stages of development but in the early embryonic stage the nervous system is not differentiated and the Ch E is not present. The embryonic development, however, was found to continue to the point of hatching even in the eggs treated at an early stage. On this basis, Smith and Wagenknecht (1956 *loc. cit.*) suggested that the poison after penetration was stored in the embryo till the development of vulnerable system, i.e., Ch E which it attacked. But Potter *et al.* (1957 *loc. cit.*) observed the stoppage of development even before differentiation of nervous system in the egg of *Pieris brassicae* treated with TEPP. They further found the inhibition of certain other enzymes apart from Ch E by the organophosphorus compounds. It was, however, observed by them too that high proportion of the eggs continued to develop when treated with low dosages of TEPP. These workers did not think it probable that the poison was stored in the embryo as such, till the development of functional nervous system. Smith and Wagenknecht (1959 *loc. cit.*) suggested that the most sensitive system attacked by organophosphorus compounds was Ch E. If the massive dosage of the poison was applied, it might interfere with other system. Potter (1958 *loc. cit.*)

* The appearance of Ch E in the insect eggs was found to coincide with the development of functional nervous system and generally after half of the incubation period (Smith and Wagenknecht, 1959). In *Culex molestus*, the development of nervous system was found to occur after about half of the incubation period(Christopher, 1960),

found difference between the dosage which stopped development and that which prevented the hatching of the egg.

Present studies carried out on the ovicidal action of various organophosphorus compounds against *C. fatigans* revealed that the embryonic development continued to the point of hatching, irrespective of the insecticidal concentration used. The primary larvae in the treated eggs showed symptoms of life even 3 to 4 hours after hatching in the untreated eggs was complete. In the eggs treated at the concentration of about 4,000 times the Lc50 value of parathion, the development continued to the point of hatching. With other organophosphorus compounds also the embryonic development did not stop. Even in the eggs treated with pure parathion and phosdrin, the embryonic development continued. If it is assumed that systems other than Ch E of the nervous system are involved in the toxic action by the organophosphorus compounds, the observations made in the case of *C. fatigans* eggs could be expected if either (*i*) certain changes took place in the egg shell by contact with the poison, making it unbreakable or (*ii*) the poison was stored somewhere and it was available to the embryo only very near to the point of hatching. Experiments carried out with the eggs suggested that none of these assumptions was correct. The present investigations revealed that the poison penetrated the egg shell after contact and it was all the time available to the embryo but toxicity of the organophosphorus compounds, however, was manifested only at the time of hatching.

Lack of any effect on embryonic development in the early stages even with the massive dosages of insecticides, suggested that either the physiological system of *C. fatigans* eggs was not similar to the system of *Pieris brassicae* or the quantity of the poison which penetrated was not sufficient to interfere with other systems. The latter possibility cannot be completely ruled out even though the poisons were tested at very high concentrations as the quantities which penetrated might not be proportional to the dosages applied (Hewlett, 1958). Most of the data collected, however, suggested that it was very near to the point of hatching that the toxic action of the compounds was manifested and probably the Ch E of the nervous system was involved. The delayed lethal action is likely due to the inhibition of Ch E below the level critical for the larvae to hatch as suggested by Mahrotra (1960). However, the larvae obtained by dissecting the egg was in a paralytic state, thereby indicating that apart from hatching the larvae was so affected that it was not able to live. Further detailed physiological studies on the mosquito eggs and eggs of other insects with organophosphorus compounds are needed to elucidate the mechanism of the action precisely.

SUMMARY.

Various organophosphorus compounds, *viz.*, parathion, baytex, malathion, diazinon, phosdrin and trithion were tested for their ovicidal action against *C. fatigans*. Parathion was found to be the most effective of all the organophosphorus compounds tested. Complete embryonic development was found to occur

in the eggs treated even at the early stages of development. Even very high concentrations of the poisons did not inhibit embryonic development. The results suggested that the organophosphorus compounds, after contact, penetrated into the egg and were available all the time to the embryo. The toxic effect of these compounds, however, was manifested only at the point of hatching, thereby supporting the hypothesis that the Ch E of the nervous system was involved.

Effect of age of the egg on its susceptibility to insecticides was studied. The older eggs were found to be more susceptible to insecticides, phosdrin and diazinon, than the younger eggs. In the case of baytex no significant difference in susceptibility with age was observed.

The penetration of the poison was found to occur not only through the micropylar region but also through the rest of the surface of the egg shell. Further the penetration was found to be more at higher temperature. The dosages of these compounds for producing the ovicidal effect, however, were found to be much higher than those required to cause mortality in the larvae.

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SUSCEPTIBILITY OF SOME INSECT VECTORS OF DISEASES TO SYNTHETIC CONTACT INSECTICIDES.

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INTRODUCTION.

SINCE the discovery of DDT and BHC and their world wide application in the control of insect pests of agricultural, veterinary and medical importance, a number of newer synthetic contact insecticides have been marketed. These include aldrin, dieldrin, endrin, chlordane, toxaphene, Lethane 384, thanite, allethrin, hexaethyl tetraphosphate (HETP), tetraethyl pyrophosphate (TEPP), diazinon, malathion and Pestox 3. Available information on the susceptibility of different species of insects to the newer insecticides has been reviewed by Negherbon (1959). It is, however, observed that the susceptibilities are not strictly comparable since different species of insects have been employed as test subjects and different techniques of bioassay of insecticides have been used by different workers. It appeared of interest, therefore, to investigate the comparative efficiency of these materials by different techniques against the housefly, *Musca nebulo* Linn. and the mosquitoes, *Culex fatigans* Wied. and *Aedes aegypti* Linn. This paper describes the work carried out on the susceptibility of these vectors of diseases to the modern synthetic contact insecticides in relation to DDT, lindane and pyrethrins.

MATERIALS AND METHODS.

Insecticides.—The insecticides used in these investigations were: aldrin, dieldrin, endrin, chlordane, toxaphene, Lethane 384, thanite, allethrin, hexaethyl tetraphosphate (HETP), tetraethyl pyrophosphate (TEPP), Pestox 3, diazinon, malathion, DDT, lindane and pyrethrins. These insecticides were obtained from the trade in India or from manufacturers in the U.K. or U.S.A.

Test insects.—The following species of insects, drawn from laboratory cultures, were used as test insects :

(*i*) The housefly, *Musca nebulo* Linn.—Adult female houseflies, four to

five days old, were used. The method of rearing the insect was the one described by Basden (1949) for *M. domestica.*

(ii) The mosquito, *Culex fatigans* Wied.—Adult female mosquitoes, forty to sixty hours old, were used. The insect was reared by the method described by Newman *et al.* (1949).

(iii) The mosquito, *Aedes aegypti* Linn.—Adult female mosquitoes, forty to sixty hours old, were used. These were reared in the same manner as *C. fatigans.*

Experimental procedure.—The susceptibility of *M. nebulo, C. fatigans* and Aedes *aegypti* to various synthetic contact insecticides was assessed by two different procedures as described below :

(i) *Topical application.*—The insecticide dissolved in acetone was applied on the dorsal thoracic region of the insect by means of 'Agla' microsyringe*. Mortality was recorded after twenty four-hours. There were forty insects in each assay. The temperature and relative humidity during the experiments were 26 to 32°C. and 40 to 90 per cent., respectively.

(ii) *Residual films.*—'Whatman' filter papers (No. 1, 11 cm.) were laid flat on pin points and one ml. of the insecticidal solution in acetone was delivered on each filter paper with a hypodermic syringe. The filter papers were then allowed to dry for three hours. The assessment was carried out by confining 25 insects for one hour under a clean funnel (7·5 cm. diameter) placed over the treated filter paper. Observations on the mortality were recorded twenty-four hours thereafter. There were two replicates in each assay. The temperature and relative humidity during the experiments were 29 to 32°C. and 50 to 86 per cent., respectively.

RESULTS.

The results obtained in the various experiments were subjected to probit analysis (Finney, 1952) and are summarized in Tables I—VI. The LD_{50} and LD_{90} of the insecticides have been estimated from probit/log concentration curves. The relative susceptibility of the vectors to the insecticides has been determined based on LD_{50}.

DISCUSSION.

It will be noted from the data presented in Tables I—VI that the order of susceptibility of the vectors to various insecticides varies with the species of the insect and the technique.

M. nebulo.—When the insecticides were applied topically, this insect was most susceptible to lindane followed by dieldrin, endrin, HETP, diazinon, aldrin, pyrethrins and TEPP, whereas on residual film the insect was most susceptible to endrin followed by dieldrin, aldrin and lindane.

* Manufactured by Messrs. Burroughs Wellcome & Co., London.

TABLE I.

Susceptibility of M. nebulo *to insecticides applied topically on the insect.*

Insecticides.	Regression equation.	LD_{50} μg./insect.	LD_{90} μg./insect.	Relative susceptibility* compared to :		
				DDT.	Lindane.	Pyrethrins.
Lindane ...	Y=4·2360x+13·7075	0·009	0·018	1102·2	1·0000	6·796
Dieldrin ...	Y=5·6836x+15·9887	0·012	0·020	829·0	0·7521	5·111
Endrin ...	Y=2·8510x+10·4386	0·012	0·035	782·2	0·7097	4·823
HETP ...	Y=6·0319x+16·0179	0·015	0·024	650·9	0·5906	4·013
Diazinon ...	Y=6·3697x+16·5979	0·015	0·024	642·3	0·5828	3·960
Aldrin ...	Y=4·3074x+12·5690	0·018	0·035	554·2	0·5029	3·417
Pyrethrins ...	Y=2·1068x+ 7·5766	0·060	0·243	162·2	0·1472	1·000
Chlordane ...	Y=6·1231x+12·0348	0·071	0·115	136·6	0·1239	0·842
TEPP ...	Y=4·1621x+ 9·4093	0·087	0·177	111·2	0·1009	0·686
Malathion ...	Y=2·7432x+ 7·6521	0·108	0·317	89·9	0·0816	0·554
Allethrin ...	Y=2·2931x+ 6·8540	0·155	0·563	62·4	0·0566	0·385
Toxaphene ...	Y=4·6554x+ 8·0390	0·222	0·419	43·6	0·0396	0·269
Thanite ...	Y=4·3794x+ 2·2651	4·212	8·262	2·3	0·0021	0·014
Lethane 384 ...	Y=4·1458x+ 2·1614	4·839	9·858	2·0	0·0018	0·012
DDT (tech). ...	Y=2·7743x+ 2·2625	9·699	28·090	1·0	0·0009	0·006
Pestox 3 ...	Y=8·6555x−11·0000	70·550	99·220	0·1	0·0001	0·001.

*Based on LD_{50}. Y=Probit kill. x=log concentration.

TABLE II.

Susceptibility of C. fatigans *to insecticides applied topically on the insect.*

Insecticides.	Regression equation :	LD_{50} μg/insect.	LD_{90} μg./insect.	Relative susceptibility* compared to :		
				DDT.	Lindane.	Pyrethrins.
Malathion ...	Y=6·0799x+20·7591	0·003	0·004	81·42	6·577	8·962
TEPP ...	Y=4·0943x+15·1932	0·003	0·007	66·16	5·344	7·281
Diazinon ...	Y=2·9436x+11·5160	0·006	0·017	34·70	2·803	3·820
Dieldrin ...	Y=5·1540x+15·1204	0·001	0·019	19·42	1·569	2·138
Endrin ...	Y=4·1501x+13·0881	0·011	0·023	18·73	1·513	2·062
Lindane ...	Y=3·2444x+10·7316	0·017	0·043	12·38	1·000	1·363
HETP ...	Y=8·9340x+20·5023	0·018	0·026	11·51	0·929	1·266
Pyrethrins ...	Y=2·3871x+ 8·8977	0·023	0·080	9·09	0·734	1·000
Allethrin ...	Y=2·8903x+ 8·9395	0·043	0·120	4·88	0·394	0·537
Aldrin ...	Y=3·8847x+10·1880	0·046	0·099	4·58	0·370	0·504
Chlordane ...	Y=3·7548x+ 9·0270	0·085	0·186	2·50	0·202	0·275
Toxaphene ...	Y=5·5198x+ 9·9960	0·125	0·212	1·70	0·137	0·187
DDT (tech) ...	Y=4·2060x+ 7·8357	0·212	0·427	1·00	0·081	0·110
Pestox 3 ...	Y=2·5627x+ 4·7107	1·206	2·766	0·18	0·014	0·019
Thanite ...	Y=4·5146x+ 4·1946	1·508	2·900	0·14	0·011	0·016
Lethane 384 ...	Y=4·9136x+ 3·3313	2·186	3·990	0·10	0·008	0·011

Y=Probit kill ; x=log concentration *Based on LD_{50}

TABLE III.

Susceptibility of Aedes aegypti *to insecticides applied topically on the insect.*

Insecticides.	Regression equation.	LD_{50} μg./insect.	LD_{90} μg./insect.	Relative susceptibility* compared to :		
				DDT.	Lindane.	Pyrethrins.
TEPP ...	Y=3·5972x+15·4218	0·001	0·003	32·69	1·692	10·077
Malathion ...	Y=3·5967x+15·3045	0·001	0·003	30·36	1·571	9·357
Endrin ...	Y=2·5467x+12·1297	0·002	0·005	26·56	1·375	8·188
Lindane ...	Y=4·2426x+16·2887	0·002	0·004	19·32	1·000	5·955
Dieldrin ...	Y=1·4692x+ 8·8766	0·002	0·017	18·48	0·957	5·696
HETP ...	Y=7·8644x+20·1956	0·012	0·017	3·63	0·188	1·120
Diazinon ...	Y=8·0401x+20·4933	0·012	0·017	3·60	0·186	1·110
Allethrin ...	Y=3·2411x+11·0994	0·013	0·033	3·24	0·168	1·000
Pyrethrins ...	Y=2·0244x+ 8·8146	0·013	0·056	3·24	0·168	1·000
Aldrin ...	Y=2·3266x+ 9·3331	0·014	0·049	3·10	0·161	0·956
DDT (tech) ...	Y=2·0894x+ 7·8667	0·043	0·174	1·00	0·052	0·308
Chlordane ...	Y=2·3358x+ 7·6476	0·074	0·260	0·58	0·030	0·178
Toxaphene ...	Y=3·7580x+ 8·4160	0·123	0·270	0·34	0·018	0·106
Thanite ...	Y=3·6851x+ 7·4145	0·221	0·493	0·19	0·010	0·059
Pestox 3 ...	Y=2·7967x+ 6·6604	0·255	0·732	0·17	0·009	0·051
Lethane 384 ...	Y=2·3057x+ 5·2831	0·754	2·711	0·06	0·003	0·017

Y=Probit kill ; x=log concentration. *Based on LD_{50}

TABLE IV.

Susceptibility of M. Nebulo† *to residual films of insecticides.*

Insecticides.	Regression equation.	LD_{50} μg./sq.cm.	LD_{90} μg./sq. cm.	Relative susceptibility* compared to :	
				Lindane.	Pyrethrins
Endrin ...	Y=4·7796x+6·8503	0·41	0·76	1·3163	1228·1
Dieldrin ...	Y=4·5755x+6·3203	0·52	0·98	1·0492	978·9
Aldrin ...	Y=3·4293x+5·9223	0·54	1·27	1·0026	935·5
Lindane ...	Y=4·1749x+6·1179	0·54	1·09	1·0000	933·0
Chlordane ...	Y=5·6356x+3·4480	1·89	3·18	0·2864	267·2
Diazinon ...	Y=6·1617x+2·3087	2·73	4·41	0·1974	184·2
TEPP ...	Y=5·4983x−1·6056	15·90	27·20	0·0339	31·7
Lethane 384 ...	Y=3·5284x−1·2517	59·13	136·50	0·0091	8·5
Malathion ...	Y=5·2622x−4·9449	77·61	136·00	0·0070	6·5
HETP ...	Y=5·1126x−4·6963	78·80	140·30	0·0069	6·4
Allethrin ...	Y=1·3865x+2·3398	82·91	696·60	0·0065	6·1
Pyrethrins ...	Y=2·5982x−2·0208	503·70	1568·20	0·0011	1·0
Thanite ...	Y=2·3626x−3·2310	3047·20	10624·30	0·0002	0·2

Y=Probit kill ; x=log concentration. *Based on LD_{50}

†*Susceptibility of M. nebulo* to DDT in terms of LD_{50} or LD_{90} could not be investigated by this technique since even high concentration of the insecticide (20 per cent solution in acetone) failed to effect appreciable mortality of the insects. A deposit of 2104 μg./sq.cm. of DDT, which could be obtained on filter papers, produced only 34 per cent kill of the insects.

TABLE V.

Susceptibility of C. fatigans† *to residual films of insecticides.*

Insecticides.	Regression equation.	LD_{50} μg./sq.cm.	LD_{90} μg./sq.cm.	Relative susceptibility* compared to:	
				Lindane.	Pyrethrins.
Lindane ...	Y=2·4442x+10·0835	0·01	0·03	1·0000	3254·70
Chlordane ...	Y=0·6679x+ 5·5274	0·16	13·47	0·0489	166·44
Aldrin ...	Y=1·1701x+ 5·8927	0·17	2·15	0·0481	156·51
Dieldrin ...	Y=2·1045x+ 5·9938	0·34	1·37	0·0246	80·14
Endrin ...	Y=1·0430x+ 5·4746	0·35	5·94	0·0237	77·01
Diazinon ...	Y=3·1128x+ 4·9448	1·04	2·69	0·0080	25·94
Allethrin ...	Y=3·5346x+ 4·4827	1·40	3·23	0·0059	19·28
Malathion ...	Y=6·6353x+ 2·4376	2·43	3·80	0·0034	11·10
TEPP ...	Y=1·3531x+ 4·3415	3·07	27·15	0·0027	8·81
Toxaphene ...	Y=1·1547x+ 3·7485	12·13	156·20	0·0007	2·23
Thanite ...	Y=4·2713x− 0·8294	23·07	46·11	0·0004	1·17
Pyrethrins ...	Y=1·9674x+ 2·1835	27·01	121·10	0·0003	1·00
Lethane 384 ...	Y=3·1252x+ 0·0097	39·52	101·60	0·0002	0·08
HETP ...	Y=2·6975x− 0·9004	154·00	459·70	0·00005	0·18
Pestox 3 ...	Y=2·4507x− 2·9230	1711·00	5700·30	0·00001	0·02

Y=Probit kill. x=log concentration. *Based on LD_{50}

†Susceptibility of *C. fatigans* to DDT in terms of LD_{50} or LD_{90} could not be investigated by this technique since even high concentration of the insecticide (20 per cent solution in acetone) failed to effect appreciable mortality of the insects. A deposit of 2104 μg./sq.cm. of DDT, which could be obtained on filter papers, produced only 25 per cent kill of the insects.

TABLE VI.

Susceptibility of Aedes aegypti *to residual films of insecticides.*

Insecticides.	Regression equation.	LD_{50} μg./sq.cm.	LD_{90} μg./sq. cm.	Relative susceptibility* compared to:		
				DDT.	Lindane.	Pyrethrins.
Lindane ...	Y= 1·7948x+ 7·8108	0·03	0·14	689·25	1·0000	272·05
Dieldrin ...	Y= 2·7845x+ 8·5405	0·05	0·16	349·78	0·5074	138·06
Endrin ...	Y= 1·5142x+ 6·8157	0·06	0·44	296·06	0·4295	116·86
Chlordane ...	Y= 1·1311x+ 5·9324	0·15	2·04	124·88	0·1812	49·29
Aldrin ...	Y= 1·5865x+ 6·2879	0·15	0·99	121·40	0·1761	47·92
Allethrin ...	Y= 2·1900x+ 5·1248	0·88	3·37	21·35	0·0309	8·43
Malathion ...	Y= 4·2837x+ 5·1775	0·91	1·81	20·59	0·0299	8·13
Diazinon ...	Y= 1·6867x+ 4·6550	1·60	9·21	11·69	0·0170	4·61
Toxaphene ...	Y= 1·1967x+ 4·3995	3·18	37·39	5·90	0·0086	2·33
TEPP ...	Y= 8·4828x− 2·2556	7·17	10·15	2·61	0·0038	1·13
Pyrethrins ...	Y= 3·9097x+ 1·6039	7·39	15·72	2·53	0·0037	1·00
DDT (tech) ...	Y= 2·1885x+ 2·2155	18·72	72·09	1·00	0·0015	0·40
Thanite ...	Y= 5·2648x− 2·7833	30·09	52·70	0·62	0·0009	0·25
Lethane 384 ...	Y= 2·9773x− 0·1149	52·24	140·70	0·36	0·0005	0·14
HETP ...	Y= 2·5738x+ 0·0480	83·95	264·20	0·22	0·0003	0·09
Pestox 3 ...	Y=12·4308x+32·2010	983·10	1246·50	0·02	0·00003	0·01

Y=Probit kill. x=log concentration. *Based on LD_{50}

C. fatigans.—When the insecticides were applied topically, this insect was most susceptible to malathion followed by TEPP, diazinon, dieldrin, endrin, lindane, HETP, pyrethrins, allethrin, aldrin and chlordane, whereas on residual film the insect was most susceptible to lindane.

Aedes aegypti.—When the insecticides were applied topically, this insect was most susceptible to TEPP followed by malathion, endrin, lindane, dieldrin, HETP, diazinon, allethrin, pyrethrins, aldrin, DDT, and chlordane, whereas on residual film the insect was most susceptible to lindane followed by dieldrin and endrin.

No specific information is available in the literature on a suitable method of grading the susceptibility of vectors to insecticides when assessed by different methods against different species of insects. In view of this, a system has been developed which consists in giving marks based on the value of LD_{99}* obtained in the different techniques. The least toxic insecticide in any one method against any one species is given one mark, the next best two marks and so on. With this marking system, grading of the insecticides, based on the susceptibility of the vectors, has been possible. The gradings are shown in Table VII. It will be seen that in the overall assessment, lindane was the most toxic to the three species of insects investigated, followed by dieldrin, endrin, diazinon, malathion, aldrin and TEPP. The remaining insecticides were much less toxic, the least effective being DDT and Pestox 3.

SUMMARY.

The susceptibility of the housefly, *Musca nebulo* Linn. and the mosquitoes, *Culex fatigans* Wied. and *Aedes aegypti* Linn. to certain synthetic contact insecticides, *viz.*, aldrin, dieldrin, endrin, chlordane, toxaphene, Lethane 384, thanite, allethrin, hexaethyl tetraphosphate (HETP), tetraethyl pyrophosphate (TEPP), Pestox 3, diazinon and malathion was investigated in relation to DDT, lindane and pyrethrins. The LD_{50} and LD_{90} were estimated from probit/log concentration curves. It was found that lindane was the most effective insecticide when topically applied on the insects or as residual film on filter paper and was followed by dieldrin, endrin, diazinon, malathion, aldrin and TEPP. The remaining insecticides were much less effective, the least effective being DDT and Pestox 3.

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* Although LD_{90} is statistically less precise than the value of LD_{50} (Busvine, 1957) the former was employed for the grading because of its importance in the field use.

TABLE VII.

Grading of the susceptibility of vectors to insecticides.

Insecticides.	Topical application:			Residual film:			Total number of marks.*	Average.†
	M. nebulo.	*C. fatigans.*	*Aedes aegypti.*	*M. nebulo.*	*C. fatigans.*	*Aedes aegypti.*		
Lindane	16	10	14	12	16	16	84	14·0
Dieldrin	15	13	10	13	15	15	81	13·5
Endrin	11	12	13	14	10	14	74	12·3
Diazinon	14	14	11	9	13	9	70	11·7
Malathion	7	16	15	7	11	12	68	11·3
Aldrin	12	8	8	11	14	13	66	11·0
TEPP	9	15	16	8	8	8	64	10·7
Chlordane	10	6	5	10	9	11	51	8·5
Allethrin	5	7	9	4	12	10	47	7·8
HETP	13	11	12	5	3	2	46	7·7
Pyrethrins	8	9	7	3	5	7	39	6·5
Toxaphene	6	5	4	1‡	4	6	26	4·3
Thanite	4	2	3	2	7	5	23	3·8
Lethane 384	3	1	1	6	6	3	20	3·3
DDT (tech.)	2	4	6	1‡	1‡	4	18	3·0
Pestox 3	1	3	2	1‡	2	1	10	1·7

*Marks obtained on the basis of LD_{50} of insecticides.

†Total number of marks scored divided by the number of techniques employed.

‡Susceptibility of *M. nebulo* and *C. fatigans* to these insecticides in terms of LD_{50} or LD_{90} could not be investigated by the 'residual film' method since even high concentrations of the insecticides failed to effect appreciable mortality of the insects (footnotes to Tables IV and V refer). Hence these insecticides have been given one mark for purposes of grading.

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SUSCEPTIBILITY STATUS OF RAT-FLEAS (*X. CHEOPIS*) TO D.D.T., B.H.C. AND DIELDRIN IN PUNJAB, BIHAR AND UTTAR PRADESH*.

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EXTENSIVE use of residual insecticides, particularly in the field of malaria eradication, has resulted in the emergence of resistance to the insecticides in some insects of public health importance. Patel *et al.* (1960), Mohan (1960) and Sharma and Joshi (1961*a*) have reported resistance in rat-fleas in certain parts of Maharashtra, Mysore and Delhi, where DDT has been sprayed for several years under the National Malaria Control/Eradication Programmes. These findings necessitated the determination of the susceptibility status of rat-fleas to chlorinated hydrocarbon insecticides in different parts of the country. The tests were carried out on rat-fleas in the towns of Ambala (Punjab), Bettiah and Chapra (Bihar) and Saharanpur (Uttar Pradesh). The results obtained are presented in this paper.

MATERIAL AND METHODS.

The fleas for tests were collected from rats, trapped alive by the municipal health staff of the above mentioned towns which had been under DDT spraying. The rats were killed by strangulation before placing them in a clean white enamelled basin and the fleas were carefully collected by means of an aspirator, and transferred into a clean jar. They were held under observation for one to two hours before the tests, and the injured or dead specimens were discarded. The prevalent species of rat-fleas encountered in these places was *X.cheopis*†. About ten fleas were used in each test.

The World Health Organization provisional technique [*World Health Organization Tech. Rep. Series* (1960), No. 191] for determining the insecticide susceptibility of rat-fleas, was employed. DDT and dieldrin impregnated papers, supplied by the World Health Organization, were used for exposures. Gamma BHC papers were prepared in the laboratory, following the Busvine and Nash (1954) method. From these treated papers, strips of the size 5 cm. × 2 cm. were

* These studies were carried out under the auspices of the Indian Council of Medical Research enquiry "Assessment of susceptibility of insects of public health importance". The work was partially financed by a grant from the Indian Council of Medical Research.

† In Ambala (Punjab), a single specimen of *X. astia* (♀) was also found.

cut. At the end of the exposure period of one hour, the fleas were removed and kept in clean tubes for 24 hours, after which the mortality was recorded. In Bihar and Uttar Pradesh, fleas were exposed continuously for 24 hours to DDT to get the critical range of mortality*. The data obtained† were analysed using the probit analysis method (Finney, 1952) and wherever possible, median lethal concentration and regression coefficient values were visually estimated from the graphs.

RESULTS.

The history of the insecticide treatment under the National Malaria Eradication Programme in the areas investigated is recorded in Table I. The results of the susceptibility tests for DDT, gamma BHC and dieldrin are tabulated in Tables II, III and IV for Punjab, Bihar and Uttar Pradesh, respectively.

TABLE I.

History of insecticidal treatments and the flea index of different areas where flea susceptibility tests were carried out.

Place where the tests were carried out.	Date of testing.	Flea index.	History of spraying in terms of rounds of spray per year.
Ambala (Punjab)	April 11 to April 20, 1961.	6·5	Two rounds of DDT @ 100 mg./sq. ft. from 1955 to 1960.
Bettiah (Bihar)	Aug. 13 to Aug. 21, 1961.	4·5	One round of DDT @ 100 mg./sq. ft. from 1954 to 1956.
			Two rounds of DDT @ 100 mg./sq. ft. from 1957 to 1961.
			[Part of the city was sprayed with BHC in 1957 (one round)].
Chapra (Bihar)	Aug. 23 to Aug. 28, 1961.	0·8	One round of DDT @ 100 mg./sq. ft. from 1959 to 1961.
Saharanpur (Uttar Pradesh)	Sep. 8 to Sep. 15, 1961.	7·03	Two rounds of DDT @ 100 mg./sq. ft. from 1958 to 1961.

The median lethal concentrations for DDT of the flea population from Ambala (Punjab), Bettiah (Bihar) and Saharanpur (Uttar Pradesh) were estimated to be more than 4 per cent with one hour exposure period (Tables II, III and IV).

In Bettiah (Bihar) and Saharanpur (Uttar Pradesh), (Tables III and IV), the fleas had to be exposed to the DDT-impregnated papers for 24 hours to obtain a critical range of mortality as one-hour exposure period proved inadequate. The LC_{50} values were 1·38 and 1·6 per cent., respectively for Bettiah and Saharanpur, with 24 hours' exposure. These results indicated high resistance among the fleas tested for DDT resistance.

* The mortalities in fleas were recorded after the completion of the 24 hours exposure without having any further holding period.

† The mortalities were corrected with control mortalities using Abbots' formula.

Susceptibility tests were also carried out at Chapra Town (Bihar) which had received only three rounds of DDT spray (Table I). In this town, sufficient number of fleas could not be obtained as the flea index was very low. Consequently, the few fleas obtained were exposed to 2·0 per cent and 4·0 per cent DDT for 1 hour (Table III). Hundred per cent mortality was obtained in both the concentrations, indicating thereby that the fleas of Chapra Town were still susceptible to DDT.

TABLE II.

Results of susceptibility tests of the rat-flea, X-cheopis, *from Punjab.*

LOCALITY—AMBALA.

Insecticide.	Concentration, per cent.	Number of fleas exposed:			Number of fleas dead.			Corrected mortality.	LC_{50}, per cent.	Regression coefficient.
		Total.	Male.	Female.	Tota l.	Male.	Female.			
DDT (one hour's exposure)	0·5	32	17	15	6	6	0	11·9	>4	..
	1·0	35	19	16	9	7	2	19·5		
	2·0	37	18	19	13	10	3	29·7		
	4·0	55	18	37	18	14	4	27·1		
	Control	39	10	29	3 (C=7·7)	2	1			
Gamma BHC (one hour's exposure)	0·0025	30	16	14	10	6	4	25·9	0·011	1·2
	0·01	26	12	14	14	8	6	48·7		
	0·04	30	12	18	24	10	14	77·8		
	Control	20	10	10	2 (C=10)	2	0			
Dieldrin (one hour's exposure)	0·05	31	12	19	14	8	6	31·5	0·125	1·5
	0·1	31	16	15	17	13	4	43·5		
	0·2	33	12	21	26	11	15	73·5		
	0·4	28	18	10	28	18	10	100		
	Control	20	11	9	4 (C=20)	3	1			

C = per cent mortality in control.

The fleas from the three investigation areas were also tested for their susceptibility to gamma BHC and Dieldrin. Except for the use of a single round of BHC spray in Bettiah, these insecticides had not been used in the other areas under any insect control programme.

The LC_{50} values for *X. cheopis* against BHC were 0·011, 0·027 and 0·02 per cent; while the LC_{50} values of Dieldrin were 0·125, 0·07 and 0·05 per cent., respectively, from Ambala, Bettiah and Saharanpur (Tables II, III and IV).

Sharma and Joshi (1961*b*) have estimated the LC_{50} of a susceptible strain of *X. cheopis* to be 0·4 per cent, 0·01 per cent and 0·035 per cent., respectively for DDT, gamma BHC and Dieldrin, with one hour's exposure. From the above

mentioned findings it is seen that the fleas from the investigation areas appear to be susceptible to BHC and Dieldrin.*

TABLE III.

Result of susceptibility test of the rat-flea, X-cheopis, *from Bihar.*

Insecticide.	Concentration, per cent.	Number of fleas exposed: Total.	Male.	Female.	Number of fleas dead: Total.	Male.	Female.	Corrected mortality.	LC_{50}, per cent.	Regression coefficient.
				LOCALITY—BETTIAH.						
DDT (one hour's exposure)	0·5	10	4	6	0	0	0	..	>4	..
	1·0	10	4	6	0	0	0	..		
	2·0	17	6	11	1	0	1	5·9		
	4·0	31	15	16	6	6	0	19·4		
	Control	22	6	16 (C=0)	0	0	0			
DDT (24 hours' exposure)	0·5	30	16	14	5	3	2	16·6	1·38	2·5
	1·0	30	12	18	10	5	5	33·3		
	2·0	30	16	14	19	13	6	63·3		
	4·0	30	14	16	26	14	12	86·7		
	Control	30	11	19 (C=0)	0	..	..	..		
Gamma B.H.C. (one hour's exposure)	0·005	28	11	17	3	2	1	7·6	0·027	1·6
	0·01	35	17	18	10	7	3	26·2		
	0·02	39	16	23	18	7	11	44·5		
	0·04	38	16	22	21	10	11	53·8		
	Control	30	15	15 (C=3·3)	1	1	0	..		
Dieldrin (one hours' exposure)	0·05	20	12	8	4	4	0	20	0·07	3·3
	0·1	35	19	16	26	16	10	74·3		
	0·2	30	15	15	27	15	12	96		
	0·4	20	6	14	20	6	14	100		
	0·8	10	6	4	10	6	4	100		
	Control	30	10	20 (C=0)	0	..	..	..		
				LOCALITY—CHAPRA						
DDT (one hour's exposure)	2·0	19	7	12	19	7	12	100	<2·0	
	4·0	11	6	5	11	6	5	100		
	Control	9	2	7 (C=0)	0	..	..	..		

C = per cent mortality in control.

FLEA INDEX.

Flea index was determined in all the areas investigated. It was seen that in Ambala, Bettiah and Saharanpur, where the fleas were highly resistant, the flea index was 6·5, 4·5 and 7·03, respectively. But in Chapra, where the fleas were

* Patel *et al.* (1960) reported LC_{50} values of 0·035 per cent and 0·13 per cent for gamma BHC and Dieldrin for the susceptible strain from Maharashtra.

susceptible, the flea index was 0·8. Roy and Brown (1954) have opined that the *X. cheopis* index of 1·0 per rat is enough to start an epidemic of plague.

TABLE IV.

Result of susceptibility test of the rat-flea, X. cheopis, from Uttar Pradesh.

Insecticide.	Concentration, per cent.	Number of fleas exposed: Total.	Male.	Female.	Number of fleas dead. Total.	Male.	Female.	Corrected mortality, per cent.	LC_{50}, per cent.	Regression coefficient.
				LOCALITY—SAHARANPUR.						
DDT (one hour's exposure)	1·0	20	13	7	3	3	0	15·0	>4·0	..
	2·0	18	10	8	5	4	1	27·8		
	4·0	18	9	9	5	3	2	27·8		
	Control	30	13	17	0 (C=0)	..	..	..		
DDT (24 hours' exposure)	0·5	50	28	22	8	7	1	9·2	1·6	2·5
	1·0	50	24	26	18	13	5	30·8		
	2·0	50	25	25	30	20	10	56·3		
	4·0	58	30	28	50	29	21	85·1		
	Control	80	30	50	6 (C=7·5)	3	3	..		
Gamma BHC (one hour's exposure)	0·005	50	26	24	10	3	7	18·4	0·02	2·1
	0·01	47	24	23	22	18	4	45·7		
	0·02	49	24	25	18	12	6	35·4		
	0·04	39	22	17	25	18	7	63·4		
	0·08	20	12	8	17	11	6	84·7		
	0·1	20	7	13	20	7	13	100·0		
	Control	50	8	42	1 (C=2)					
Dieldrin (one hour's exposure)	0·025	27	8	19	5	3	2	9·4	0·05	3·3
	0·05	40	20	20	25	19	6	58·3		
	0·1	50	27	23	39	21	18	75·5		
	0·2	47	24	23	47	24	23	100·0		
	0·4	30	16	14	30	16	14	100·0		
	Control	60	34	26	6 (C=10)	5	1	..		

C = per cent mortality in control

CONCLUSIONS.

From the above mentioned observations, it is quite apparent that wherever the insecticides have been used for some years, the fleas, without any exception to the area, have developed resistance. The situation regarding flea resistance to DDT and the high flea indices are serious and warrant strict vigilance.

SUMMARY.

Flea susceptibility tests were conducted in certain towns of Punjab, Bihar and Uttar Pradesh against DDT, BHC and Dieldrin. The fleas were found to be highly resistant to DDT wherever the insecticide had been used for some time. Incidentally flea index was found to be very high in all the places where the fleas had become resistant to DDT.

ACKNOWLEDGEMENT.

Thanks are due to the Public Health Directorates of the Punjab, Bihar and Uttar Pradesh States. The help rendered by Dr. Gurdial Singh, District Medical Officer of Health, Ambala (Punjab), Dr. S.C. Shukla, District Medical Officer of Health, Champaran (Incharge Bettiah Municipality) and Dr. G.S. Nigam, Medical Officer of Health, Municipal Board, Saharanpur, is gratefully acknowledged.

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A TOXIC REACTION IN MALE RAT-FLEAS (*XENOPSYLLA CHEOPIS* AND *XENOPSYLLA ASTIA*) EXPOSED TO DDT, GAMMA BHC AND DIELDRIN—PROTRUSION OF THE INTROMITTENT ORGAN*.

BY

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[January 8, 1962.]

EXCITATION, discoordinated movements, paralysis and convulsions are the signs observed in insects prior to death due to poisoning by chlorinated hydrocarbon insecticides. Protrusion of the intromittent organ, though occasionally observed with the male *X. cheopis* (Patton and Evans, 1929), has not so far been reported as of frequent occurrence in rat-fleas poisoned by DDT, gamma BHC and Dieldrin. During the routine identification of rat-fleas subjected to susceptibility tests with these insecticides, it was observed that in many of the dead male fleas the two chitinous rods of the intromittent organ, from inside the aedoeagus, were found thrust out of the body between the clasper and the ninth sternum (Plate I).

The susceptibility tests were carried out in accordance with the World Health Organization provisional test technique for rat-fleas [*World Health Organization Technical Report Series (1960) No. 191*]. The pre-impregnated DDT, dieldrin and control papers, supplied by the World Health Organization, were used for this purpose. In the case of tests with gamma BHC, filter papers were impregnated locally following the procedure of Busvine and Nash (1954). The mortality rates in the test insects were recorded at the end of a holding period of 24 hours after exposure to the impregnated papers. The dead and live fleas (the latter killed by chloroform vapour) were identified sex and species-wise.

Susceptibility tests in the field were carried out in four different areas. Particulars in regard to the susceptibility status of the fleas, and the history of the insecticide spraying, are stated in Table I. Confirmatory tests in laboratory were carried out with the Delhi strain of fleas (susceptible) (Sharma and Joshi, 1961) colonized at the Malaria Institute of India, Delhi. The results of the field and laboratory observations are presented and discussed in this paper.

* The studies on which this paper is based were partly financed by the Indian Council of Medical Research. The authors are indebted to Mr. T. Thimmiah for technical assistance.

PLATE I.

Photo-micrograph of male flea (*X. cheopis*) showing the intromittent organ thrust out from inside the aedoeagus.

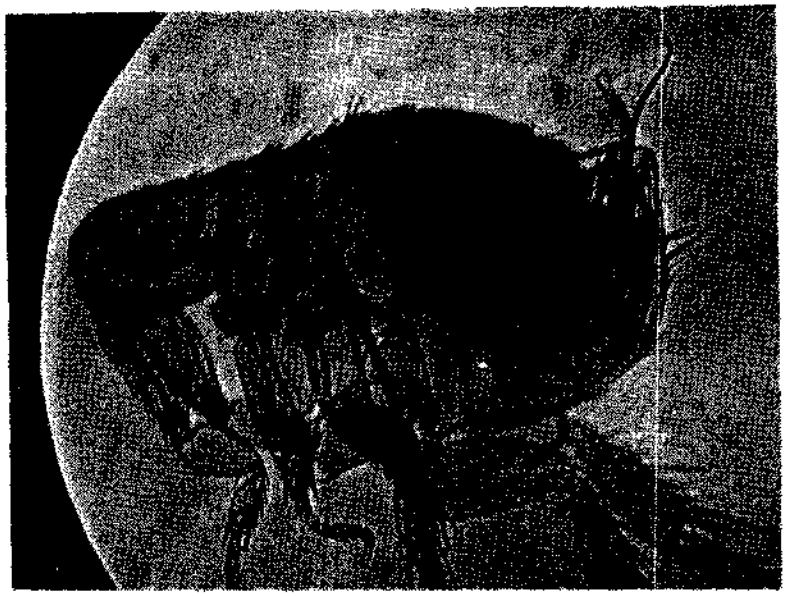

TABLE I.

Details regarding the investigation areas, the spraying history of insecticide and susceptibility status of rat-fleas.

Name of the investigation area.	History of spraying with chlorinated hydrocarbon insecticides.	Susceptibility status of rat-fleas.
Mettupalaiyam Town (Coimbatore District, Madras State)	Sprayed with DDT since 1959.	More or less normal susceptibility.
Coonoor Circle (Nilgiri District, Madras State)	Insufflation of rat burrows and dusting of all rat harbourages with 10 per cent DDT dust since 1948. In the case of infected villages, houses were also sprayed with 2·5 per cent DDT suspension in addition to routine anti-plague measures.	High resistance to DDT. Also tolerance to dieldrin and gamma BHC although, according to the information given by the District Health Officer, neither dieldrin nor gamma BHC has been used.
Gundlupet Town (Mysore State)	Sprayed with DDT since 1954 in addition to gamma BHC in 1958 and 1959 for the control of plague.	Resistance to DDT and tolerance to dieldrin and gamma BHC.
Masti and Challaganahalli villages (Kolar District, Mysore State).	Sprayed with DDT from 1954 to 1960 except for the years 1953 and 1957 when gamma BHC was sprayed.	Resistance to DDT with some evidence of tolerance to gamma BHC.

(*A*) FIELD OBSERVATIONS.

The results of the susceptibility tests, carried out in the field, with *X. cheopis* and *X. astia* against DDT, gamma BHC and dieldrin are depicted in Tables II, III and IV, respectively. Both *X. cheopis* and *X. astia* from Mettupalaiyam were more susceptible to DDT and dieldrin than fleas of the other areas. The higher mortality rates of the male *X. cheopis* of Mettupalaiyam were found to be related to higher protrusion rates*. The probit/log concentration curve, with respect to protrusion rate due to DDT and dieldrin in male *X. cheopis* of Mettupalaiyam, was found to be linear (Graphs 1 and 2). Similar linear relation between probits of protrusion rate and dosages of gamma BHC was also observed for *X. cheopis* of Coonoor (Graph 3, Fig. *a*, *b*) and Gundlupet. However, in each case higher mean and/or variance of responses were observed when the protrusion rate was the criterion and not the mortality rate. The reason for this may be that the former phenomenon was a reversible one than the latter and/or the populations were more homogeneous with respect to the latter than the former.

In the case of male *X. astia*, protrusion of the intromittent organ was also observed only in dead fleas. The protrusion rate was the lowest with DDT and highest with dieldrin and intermediate with gamma BHC within the range of dosages tried. *X. astia* of Mettupalaiyam was found to exhibit a higher protrusion rate than those of Coonoor and Gundlupet at similar concentrations of dieldrin. Similarly, higher rates of protrusion due to gamma BHC were observed with *X. astia* of Gundlupet than that of Coonoor. The higher protrusion rates were found to correlate with the higher susceptibility status of the species. The pattern of relationship between the protrusion rate and the dosages of dieldrin and gamma BHC in *X. astia* was found to be more or less similar as observed with *X. cheopis* but in the former case the effect was less pronounced than in the latter.

(*B*) LABORATORY STUDIES.

Tests were carried out in the laboratory to study the effect of the different insecticides, inert chemicals and temperature on the rate of protrusion of the chitinous rods of intromittent organs in the male fleas of the colony strain of *X. cheopis*. The fleas were subjected to varying treatments such as exposure to chlorinated hydrocarbon insecticides, anaesthetics and high and low temperatures.

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* Occasionally a few cases of protrusion of the intromittent organ were observed in fleas found dead in the control series. It was ascertained that the protrusion was not due to the action of reagents used for mounting the fleas. Pattan and Evans (1929) recorded occasional occurrence of protrusion of the intromittent organ in *X. cheopis*. This, however, was not reported due to exposure to any insecticide.

Values, where the number of specimens used were less than five, were omitted from the computation.

TABLE II.

Protrusion of the two chitinous rods of the intromittent organ in X. cheopis *and* A. astia *exposed to different concentrations of DDT.*

Place.	Species.	0·25 PER CENT × 1 HOUR :			0·5 PER CENT × 1 HOUR :			1·0 PER CENT × 1 HOUR :		
		Number examined.	Per cent mortality.	Percentage of specimens with protrusion*.	Number examined.	Per cent mortality.	Percentage of specimens with protrusion*.	Number examined.	Per cent mortality	Percentage of specimens with protrusion*.
Mettupalaiyam	X. *cheopis*	23	34·8	26·1	19	58	26·3	6	100	33·3
Coonoor	X. *cheopis*	..	..	..	..	..	..	1	0	0
Gundlupet	X. *cheopis*	..	..	..	..	..	..	..	..	..
Masti and Challaganahalli villages	X. *cheopis*	..	..	..	..	..	..	29	24·1	10·3
Mettupalaiyam	X. *astia*	17	35·3	0	13	53·8	7·7	21	81	0
Coonoor	X. *astia*	..	..	..	..	..	..	29	0	0
Gundlupet	X. *astia*	..	..	..	..	..	..	..	..	..
Masti and Challaganahalli villages	X. *astia*	..	..	..	..	..	..	12	75	0

(*Contd.*)

TABLE II (*Concld.*)

Place.	Species.	2·0 PER CENT × 1 HOUR			4·0 PER CENT × 1 HOUR :			4·0 PER CENT × 24 HOURS :		
		Number examined.	Per cent mortality.	Percentage of specimens with protrusion*.	Number examined.	Per cent mortality.	Percentage of specimens with protrusion*.	Number examined.	Per cent mortality.	Percentage of specimens with protrusion*.
Mettupalaiyam	X. *cheopis*	5	100	60	4	100	0	9	100	44·4
Coonoor	X. *cheopis*	4	0	0	2	50	0	28	74·4	14·3
Gundlupet	X. *cheopis*	10	60	50	8	62·5	25	12	100	25
Masti and Challaganahalli villages	X. *cheopis*	33	30·3	3	29	51·3	25·6	2	100	100
Mettupalaiyam	X. *astia*	11	100	0	4	100	0	2	100	0
Coonoor	X. *astia*	68	11·8	0	39	28·3	0	74	54·1	0
Gundlupet	X. *astia*	9	0	0	12	33·3	0	24	100	4·2
Masti and Challaganahalli villages	X. *astia*	22	45·5	4·5	15	40	0	..	..	..

1. *Protrusion = Protrusion of the two chitinous rods of the intromittent organ.
2. Percentages are calculated from the total number of fleas exposed.

TABLE III.

Protrusion of two chitinous rods of the intromittent organ in X. cheopis *and* X-astia *exposed to different concentrations of dieldrin.*

Exposure period one hour.

Place.	Species.	0·025 PER CENT :			0·05 PER CENT :			0·1 PER CENT :		
		Number examined.	Per cent mortality.	Percentage of specimens with protrusion*.	Number examined.	Per cent mortality.	Percentage of specimens with protrusion*.	Number examined.	Per cent mortality.	Percentage of specimens with protrusion*.
Mettupalaiyam	*X. cheopis*	12	33·3	16·7	37	56·8	43·2	46	89·1	65·2
Coonoor	*X. cheopis*	..	..	..	..	..	..	4	100	100
Gundlupet	*X. cheopis*	..	..	..	2	0	0	..	..	..
Mettupalaiyam	*X. astia*	6	50	0	16	56·3	25	22	90·9	27·3
Coonoor	*X. astia*	..	..	..	..	..	..	18	38·9	11·1
Gundlupet	*X. astia*	..	.	..	2	0	0	12	41·7	8·3

(*Contd.*)

TABLE III (*Concld.*)

Place.	Species.	0·2 PER CENT :			0·4 PER CENT :			0·8 PER CENT :		
		Number examined.	Per cent mortality.	Percentage of specimens with protrusion*.	Number examined.	Per cent mortality.	Percentage of specimens with protrusion*.	Number examined.	Per cent. mortality.	Percentage of specimens with protrusion*.
Mettupalaiyam	*X. cheopis*	84	100	84·5	4	100	100	..	..	..
Coonoor	*X. cheopis*	10	70	70	10	100	30	..	..	..
Gundlupet	*X. cheopis*	30	70	43·3	19	100	73·7	8	100	0
Mettupalaiyam	*X. astia*	22	100	50	1	100	0	..	..	..
Coonoor	*X. astia*	33	72·7	27·3	20	80	40	..	..	..
Gundlupet	*X. astia*	9	100	0	1	100	0	11	100	0

1. *Protrusion = Protrusion of the two chitinous rods of the intromittent organ.
2. Percentages are calculated from the total number of fleas exposed.

TABLE IV.

Protrusion of the two chitinous rods of the intromittent organ in X. cheopis *and* X. astia *exposed to different concentrations of gamma BHC (lindane).*

Exposure period one hour.

Place.	Species.	0·025 PER CENT :			0·05 PER CENT ;			0·1 PER CENT :		
		Number examined.	Per cent mortality.	Percentage of specimens with protrusion*.	Number examined.	Per cent mortality.	Percentage of specimens with protrusion*.	Number examined.	Per cent mortality.	Percentage of specimens with protrusion*.
Coonoor	*X. cheopis*	10	20	0	23	56·5	8·7	13	84·6	15·4
	X. astia	22	18·2	0	13	30·8	7·7	22	90·9	9·1
		0·0625 per cent :			0·125 per cent :			0·25 per cent :		
Gundlupet	*X. cheopis*	3	33·3	0	14	78·6	72·9	13	84·6	61·5
	X. astia	27	85·2	0	27	85·2	11·1	16	100	18·8

(*Contd.*)

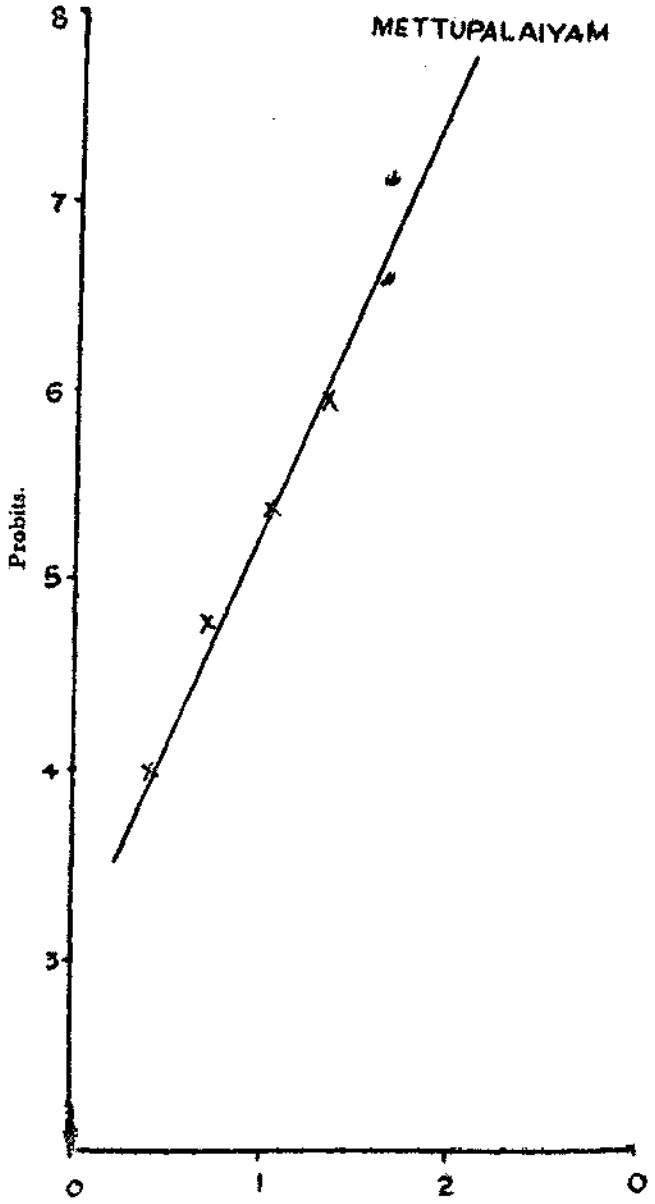
TABLE IV (*Concld.*)

Place.	Species.	0·2 PER CENT :			0·4 PER CENT :			0·8 PER CENT :		
		Number examined.	Per cent mortality.	Percentage of specimens with protrusion*.	Number examined.	Per cent mortality.	Percentage of specimens with protrusion*.	Number examined.	Per cent mortality.	Percentage of specimens with protrusion*.
Coonoor	*X. cheopis*	26	100	56·7	15	100	86·7	..	..	..
	X. astia	8	100	12·5	24	100	8·3	4	100	0
		0·5 per cent :			1·0 per cent :					
Gundlupet	*X. cheopis*	36	100	83·3	24	100	54·2	..	..	..
	X. astia	6	100	66·7	..	..	..	..	..	..

1. *Protrusion = Protrusion of the chitinous rods of the intromittent organ.
2. Percentages are calculated from the total number of fleas exposed.

GRAPH I.

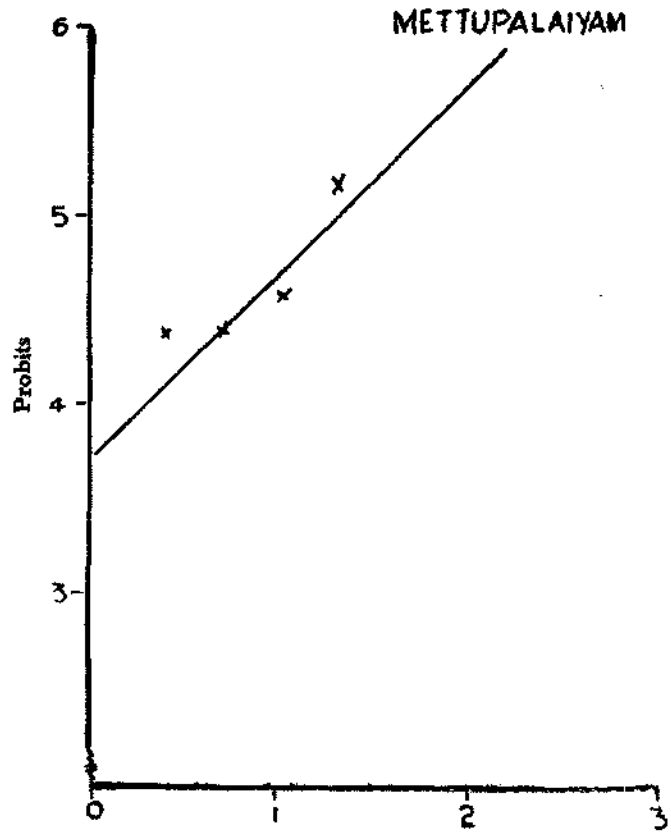
Relation between Log. Concentrations of dieldrin and probits of the protrusion rates of X. cheopis.



Log. 100 concentration of dieldrin.

GRAPH 2.

Relation between Log. Conentration of D.D.T. and probits of the protrusion rates of X. cheopis.



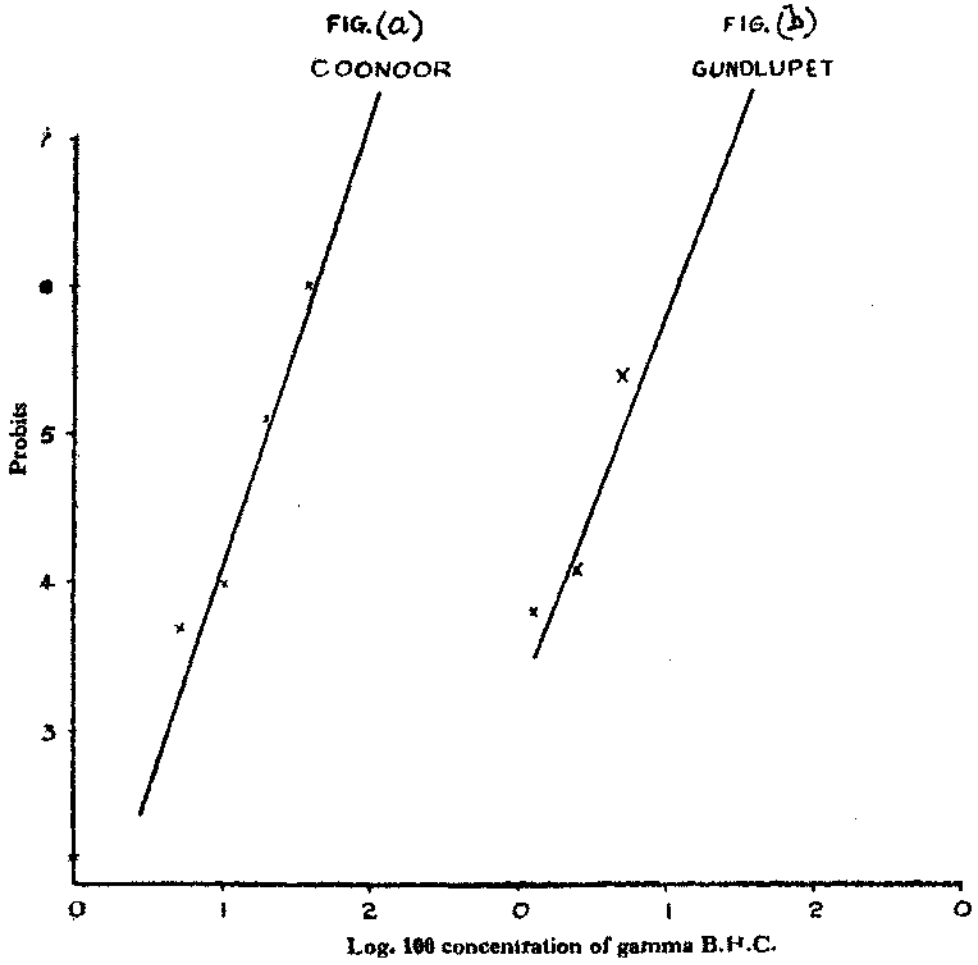
Log. 10 concentration of D.D.T.

In the series of tests with insecticides (DDT and Dieldrin) the adult fleas were exposed to DDT (4·0 per cent and 2 per cent) and Dieldrin (0·4 per cent and 0·2 per cent) for one hour and then kept under observation for 24 hours. The dead and live fleas from tubes, with papers containing different concentrations of insecticides, were separately processed* and mounted on slides. In the series of tests with anaesthetic reagents (acetone, benzene, ethyline dichloride, chloroform) the fleas were exposed to the vapours of these reagents and the affected fleas were kept for 24 hours, after which they were similarly processed for mounting. Fleas were also exposed to high temperature (45°C) and low temperature (3°C) (for 2 hours) and kept for 24 hours before treating them with reagents for mounting.

*The fleas were cleared in pottasium hydroxide (10 per cent solution) for 24 hours, dehydrated with alcohols and mounted in "Eucrose" (Bhatia's medium).

GRAPH 3.

Relation between Log. Concentration of B.H.C. and probits of the protrusion rates of X. cheopis *of* different localities.



The fleas were examined for the protrusion of the two chitinous rods of the intromittent organs in males. Details of the various treatments, number of fleas exposed, mortality and the protrusion rates are summarised in Table V.

The data revealed that none of the live male fleas (which were not exposed to insecticides) showed protrusion of the intromittent organ. However, varying rates of protrusion were observed in the dead male fleas which were exposed to DDT and Dieldrin only. Higher protrusion rates were found to be associated with the higher dosages of DDT and Dieldrin. No protrusion of the intromittent organ was observed in the fleas subjected to the other treatments. These observations are in conformity with the field observations discussed earlier.

TABLE V.

Protrusion of two chitinous rods of the intromittent organ in X. cheopis (*Laboratory colony*) *exposed to different treatments.*

Treatment exposed to :	Total Number of fleas exposed.	Number ♂♂	Alive ♂♂	Dead ♂♂	Mortality rate, per cent.	Number with protrusion.	Protrusion rate per cent.
DDT : 2·0×1	31	21	10	11	52·3	3	14·2
DDT : 4·0×1	32	28	2	26	92·8	5	17·8
Dieldrin : 0·2×1	32	18	12	6	33·3	1	5·6
Dieldrin :0·4×1	31	19	2	17	89·5	5	26·3
W.H.O. Control Paper	31	19	18	1	5·2	Nil	0·0
Acetone	29	10	Nil	10	100·0	Nil	0·0
Benzene	43	10	3	7	70·0	Nil	0·0
Carbon dioxide	29	8	6	2	25·0	Nil	0·0
Ethyline dichloride	30	17	Nil	17	100·0	Nil	0·0
Chloroform	30	20	Nil	20	100·0	Nil	0·0
Low temperature (3°C)	29	18	18	Nil	0·0	Nil	0·0
High temperature (45°C)	29	11	Nil	11	100·0	Nil	0·0
Clean Filterpaper	30	21	18	3	14·2	Nil	0·0
Test tube clean	15	15	15	Nil	0·0	Nil	0·0

DISCUSSION.

The above mentioned observations showed firstly that the protrusion of the intromittent organ was seen only in the dead fleas. Secondly, the protrusion was significant only when the mortality was caused by the insecticides like DDT, Dieldrin and not by other agents used in these studies. High incidence of the protrusion, due to poisoning by chlorinated hydrocarbon insecticides, could be the result of stimulation of the nervous system by these toxicants.

SUMMARY AND CONCLUSIONS.

The protrusion of the two chitinous rods of the intromittent organ from within the aedoeagus in *X. cheopis* and *X. astia* has been found to be a sign of acute toxicity to DDT, Dieldrin and gamma BHC.

The incidence of this toxic manifestation was found to be higher in the susceptible population of the fleas than the resistant ones.

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THE GIEMSA-COLOPHONIUM METHOD FOR STAINING PROTOZOA IN TISSUE SECTIONS.

BY

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AND

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[March 13, 1962.]

INTRODUCTION.

Two methods are in use today for the staining of sections by Romanowski stains ; that of Hewitt (1939) and that of Shortt and Cooper (1948). Many workers, using the Giemsa-colophonium method of Shortt and Cooper (1948 *loc. cit.*), have diverged in practice far from the original description in numerous details. In our experience, the Giemsa-colophonium method is the method of choice and in our hands the only staining method to give good and evenly brilliant staining. We feel that our present technique may be worth setting down for the guidance of interested workers.

THE METHOD.

1. The tissue piece is fixed in Carnoy's fluid (60 ml. ethyl alcohol, 30 ml. chloroform, 10 ml. glacial acetic acid) for 3 to 6 hours depending on the size and nature of the tissue piece. Thin pieces of tissue about ¼ to ½ cm. in thickness are preferable using about 3 hours fixation. The fixative should be replaced by fresh fixative about half way through the 3 hours. It is important that the alcohol and glacial acetic acid in the Carnoy's fluid should be completely free of water otherwise subsequent staining may be considerably affected.

2. The tissue is removed to 90 per cent alcohol (not 70 per cent alcohol) for two changes of an hour each. The tissue may remain in a third change of 90 per cent alcohol for up to 24 hours. If a longer period of storage is required, the tissue should be put into 70 per cent alcohol after all acetic acid has been removed by further washings in 90 per cent alcohol.

3. The tissue is dehydrated in 95 per cent alcohol and two changes of 100 per cent alcohol of one hour each (we usually use 100 per cent alcohol kept over anhydrous copper sulphate for the last change). Certain tissues or certain climates may require 3 changes of 100 per cent alcohol for complete dehydration. The clearing process will show if dehydration has been incomplete.

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† The London School of Hygiene and Tropical Medicine, London.

4. The tissue is cleared in clove oil overnight or xylene 4 to 6 hours. An opaque central portion will indicate incomplete dehydration.

5. If clove oil is used, then xylene for one hour removes the oil. The tissue is placed in 50 per cent wax in xylene, then in graded waxes and embedded.

6. Tissue sections 4 to 6 μ thick on slides are brought to water by treatment with xylene and alcohol. The sections are washed in water and placed in the Giemsa stain (10 ml. concentrated Giemsa stain, 10 ml. methyl alcohol, 10 ml. acetone, 100 ml. distilled water buffered by phosphate to pH 7·2). The sections are stained for one hour. We recommend "Revector" Giemsa or the National Analine Co. Giemsa.

7. The sections are washed individually with tap water and drained of all but one or two drops of water which are left covering the sections. Onto the level slide about ½ ml. of colophonium resin in acetone (15 per cent resin) is added to the water and the slide rocked and agitated to ensure mixture and even flow of the mixture over the sections. The mixture is left in agitated contact with the sections for 3 to 10 seconds. The mixture is poured off the slide and the sections quickly washed with colophonium in acetone 2 or 3 times until no green colour remains in the washings.

8. The slide is rapidly washed in 70 per cent xylene 30 per cent acetone.

9. The slide is repeatedly washed in xylene to remove all traces of acetone, then drained of xylene without allowing the sections to dry.

10. The section is mounted in green Euparal or a *neutral* plastic mountant.

(*N.B.*—The tissue and tissue sections must never be allowed to dry at any stage.)

If many specimens are to be prepared, the use of a "histokinete" or some other automatic device saves labour for Steps 1 to 5 above. The later steps require individual attention.

The critical part of this procedure is the differentiation of the Giemsa stain by the colophonium in acetone in Step 7. This consists of leaching out the blue colour, bringing out the underlying red colour and achieving a balance of blue cytoplasm and red nuclei in the protozoan examined. The water left on the slide assists the differentiation and the speed of differentiation is proportional to the amount of water used up to a limit of about 50 per cent water. Colophonium in acetone in the absence of water differentiates the Giemsa stain only weakly and slowly. The amount of water to be left on the slide and mixed with the colophonium in acetone is determined by experience and the desire for speed of differentiation; speed being balanced against convenience, evenness of colour removal and accuracy in determining a desirable colour contrast.

It is often best to allow the colophonium in acetone and water mixture to act for a few seconds and then to increase the amount of the former until flooding results in pure colophonium in acetone being in contact with the sections and acting

as a dehydrating step. Too prolonged contact with the mixture quickly removes essential stain and thus a gradual exclusion of water is an advantage.

Tissue with much nuclear material, such as spleen, requires longer and more radical differentiation than tissue with few nuclei such as brain. Larger parasites, such as exo-erythrocytic schizonts of malaria parasites, require more delicacy of touch and precision than smaller organisms such as flagellates or piroplasms to obtain the best effect.

Differentiation at Step 7 can be achieved by using dilute acetic acid (3 drops of glacial acetic acid in 100 ml. water) in place of colophonium. The slide is agitated in the dilute acetic acid for about 5 to 20 seconds, washed in water, well drained and swiftly dehydrated with colophonium in acetone or acetone. The use of acetic acid differentiation sometimes increases the brilliance of the nuclear staining but tends to be patchy in differentiation and brings about a pinkish cytoplasmic staining. The acetic acid differentiation is used with some success when fixatives, other than Carnoy, are used and colophonium resin gives very poor results.

Very brilliant results may be obtained by staining in half-strength Giemsa (i.e. 5 ml. of concentrated Giemsa stain in the formula in Step 6 above) overnight and following this by acetic acid differentiation and further differentiation with colophonium in acetone. When the acetic acid is washed off, very little water is left on the slide and differentiation by colophonium in acetone is made for 15 to 30 seconds, adding more colophonium in acetone until it is pure for dehydration.

For this staining method, fixation in Carnoy's fluid is essential for consistently good results although Lainson (1959) has used Bouin's fixative with success, especially on tissue and blood smears.

The technique is applicable for the demonstration of all protozoan parasites in tissue though in the case of the amoebae, the intestinal flagellates and the coccidia the haematoxylin stains give a better nuclear picture, probably also the combined Romanowski haematoxylin methods of Maximow, etc., as used by Huff and Coulston (1944), are superior in some respects for the cryptozoites of avian malaria parasites.

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TREATMENT OF HUMAN MALARIA BY DIAMINO-DIPHENYL-SULPHONE (DDS), SINGLY AND IN COMBINATION WITH PYRIMETHAMINE. A PRELIMINARY STUDY OF THEIR EFFECTS IN *P. VIVAX* AND *P. FALCIPARUM* INFECTIONS IN RAJASTHAN, INDIA.

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DDS has been shown to precipitate quick selection of resistance in *P. cynomolgi*. It has been further demonstrated that the DDS resistant parasites, while equally resistant to sulphadiazine, did not show any cross resistance to pyrimethamine (Ramakrishnan, Basu *et al.*, 1961). This finding was of considerable importance in favour of the combined use of these two drugs. Laboratory studies were commenced to investigate actual potentiation, if any, of DDS and pyrimethamine.

In the meantime, advantage was taken of a focal outbreak of malaria amongst the inhabitants of Jodhpur and of its environs (Rajasthan State) to investigate the role of DDS, singly and with pyrimethamine in the treatment of human malaria. The investigation was based in the Sursagar Government Dispensary, situated in the outskirts of Jodhpur City. The results of the preliminary trial carried out in October, 1961, are set out in this paper. The outbreak in this locality was due to an operational failure in DDT spraying under the National Malaria Eradication Programme.

PATIENTS, DOSAGE REGIMES AND PROCEDURE.

All fever cases reporting to the dispensary were examined microscopically. Only the cases found positive for malaria-parasites were treated according to one or the other of the regimes detailed below. All cases received a single dose treatment. The treatment was completely ambulatory and in no case was hospitalisation necessary.

The drug was administered in one single dose by mouth under strict supervision. Sulphone* tablets containing 100 mg. each and pyrimethamine† tablets

* Avlosulfon (Dapsone) Tablets B.P. 0·1 g. Imperial Chemical Industries (India) Private Ltd., Calcutta.

† Daraprim brand pyrimethamine B.P.C. Tablets 25 mg. Burroughs Wellcome & Co. (India) Private Ltd., Bombay.

containing 25 mg. each were used in this investigation. Proportionate fractional dosages (Table I) of the tablets were carefully ensured by the authors before actual administration.

TABLE I.

Single dose regimes for adults and proportionate dosage for different age-groups (Dose in mg.). (Doses for the age-groups not available for treatment in the different regimes, are omitted).

Age-group.	REGIME							
	I	II	III		IV		V	
	DDS only.	DDu only.	DDS and Pyrimethamine.		DDS and Pyrimethamine.		DDS and Pyrimethamine.	
A (Adults)	250	200	100	12·5	50	12·5	25	12·5
B (9 — 14 years)	175	150	..	..	33·0	8·3	16·6	8·3
C (5 — 8 years)	..	100	..	..	25·0	6·0	12·5	6·0
D (1 — 4 years)	75	50	..	..	12·5	3·0	6·2	3·0
E (Infants)	..	..	..	..	6·2	1·6	..	..

Five drug regimes were used. The smaller dosages of DDS were deliberately chosen for the purpose of eliciting the potentiation, if any, between this drug and pyrimethamine. The details of the regimes and the age-groupwise proportionate doses are shown in Table I. Only the dosages for the age-groups that were available under each regime, are stated in the Table.

The cases were classified age-wise into five groups—*A* to *E* (Table II). All cases had fever when they were first seen. Parasitaemia was investigated by the use of both thick and thin blood smears. The degree of parasitaemia was ascertained by counting the number of parasitized cells per 10,000 erythrocytes. Asexual parasite density index was calculated according to the method described by Bruce-Chwatt (1951 : 1958) initially and daily on the subsequent days of the observation period. The total erythrocyte count was, however, not determined and was assumed to be 5 million per c.mm. in each case.

No case choice was made regarding the adoption of a particular regime. The cases were treated in serial order as they became available, the first one with Regime I, the second with Regime II and so on till the sixth case was treated with Regime I and the same serial order was followed.

Due to the past endemicity of malaria in the area, it appeared that the different age-groups varied in the degree of acquired immunity to the infection. The infants in Group *E* had little or no immunity while the adults in Group *A* had substantial immunity ; the other age-groups had intermediate degree of immunity. This difference in the immunity status was reflected in the initial parasite density indices (Table II).

TABLE II.

Details of age, infection, parasite density index and pyrexia in respect of the five groups of subjects before treatment.

		AGE-GROUP :				
		A (15 years and above) (Adults)	*B* (9-14 years).	*C* (5-8 years).	D (1-4 years).	E (Infants).
Number	Male	21	10	6	5	1
	Female	8	2	2	3	1
	Total	29	12	8	8	2
Age in years	Mean	27·9	12·1	5·8	2·7	0·7
	Range	15—65	10—14	5—8	1½—4	5/12—1
Parasite species and number of cases		*P.v.* 14 *P.f.* 12 *P.v.*+*Pf.* 3 (Mixed)	*P.v.* 7 *P.f.* 5	*P.v.* 5 *P.f.* 2 *P.v.*+*P.f.* (Mixed) 1	*P.v.* 6 *P.v.*+*P.f.* (Mixed) 2	*P.v.* 2
Parasite density index		5·8	7·2	6·2	7·1	8·5
Pyrexia before treatment		Present in all the cases.				..

P.v. = *Plasmodium vivax.* *P.f.* = *Plasmodium falciparum.*

Each treated case was observed for five days (120 hours), following treatment, by daily clinical and parasitological examination which involved visits to the patients' homes or places of work. The cases which were cleared of asexual parasites by the regimes under investigation were transferred to the NMEP* surveillance organisation for radical treatment with primaquine after the expiry of 5-day observation. Cases, which were not cleared of asexual parasites by the fifth day of the trial dose or sometimes earlier, received the standard single dose treatment with chloroquine and were also transferred to surveillance organisation.

RESULTS.

The results have been analysed regime-wise with respect to the following criteria :

(*a*) Parasite density index (PDI), its initial value and the amplitude of its decline,

(*b*) Initial fever and its termination in hours,

(*c*) Sensitivity of the two species of parasites (*P. falciparum* and *P. vivax*),

(*d*) Complete clearance of asexual parasites in hours, and

(*e*) Effect on gametocytes in the peripheral blood.

The determination of the relapse pattern after treatment by the different regimes did not arise as radical treatment with primaquine of every positive case was obligatory under the National Malaria Eradication Programme.

* National Malaria Eradication Programme.

REGIME I.

Tables III, IV and V show the results of treatment by Regime I (250 mg. DDS—single adult dose). There were seven cases of *P. vivax*, four cases of *P. falciparum* and one of mixed infection of both the species.

(A) *P. VIVAX* INFECTION.

Pyrexia was cleared in all the seven cases within 72 hours. Asexual parasites were cleared within 72 hours of drug administration in five out of seven cases (Table III). The two cases in which the asexual parasites were not cleared, belonged to the age-group D (1 — 4 years). The parasitaemia in one of the cases ($1\frac{1}{2}$ years old) showed progressive decrease though the asexual parasites persisted even up to 96 hours of drug administration; in the other (4 years old) the parasitaemia dropped appreciably up to 48 hours, followed by a gradual increase which persisted up to 96 hours. Both these cases received a further single dose treatment with chloroquine. The asexual parasite density index decreased from 5·4 on D day to 1·8 on D+4 day (Table IV). Gametocytes were not cleared in two out of the four cases in which these were detected within 48—72 hours of drug administration (Table V).

(B) *P. FALCIPARUM* INFECTION.

Pyrexia was cleared in all the four cases within 72 hours. Asexual parasites were cleared in all the cases within 72 hours of drug administration (Table III). The parasite density index dropped from 5·0 on D day to 0 on D+3 day (Table IV). Gametocytes were not detected in any of the four cases during the period of observation (Table V).

(C) MIXED INFECTION.

Only one case (3 years old) of mixed infection (*P. vivax* and *P. falciparum*) was available under this regime. The case became afebrile within 24 hours. Asexual parasites of *P. falciparum* were cleared within 24 hours of drug administration while those of *P. vivax* persisted up to 120 hours (Table III). The asexual parasite density index declined from 7 to 5 on D+1 day but again (*P. vivax*) increased to 6 on D+4 day (Table IV). Gametocytes of both the species persisted in the circulation (Table V).

Regime I was effective in controlling the fever in all the cases of *P. vivax* infection but proved ineffective in securing complete disappearance of asexual parasites in three children with low immunity to malaria. Against *P. falciparum*, the regime was effective in clearing the parasites as well as fever within 72 hours. The case of mixed infection unmistakably demonstrated that *P. falciparum* was more sensitive to DDS than *P. vivax*.

REGIME II.

Tables VI, VII and VIII show the results of treatment by Regime II (200 mg. DDS—single adult dose) : There were seven cases of *P. vivax*, three of *P. falciparum* and two of mixed infections of both the species.

TABLE III.

Effect of a single dose of 250 mg. DDS on the clearance of asexual parasites (Regime I).

Age-group.	*P. vivax* infection clearance within hours.						*P. falciparum* infection clearance within hours.						*P. vivax* and *P. falciparum* (mixed) infection clearance within hours:	
	Number of cases.	24	48	72	96	Not cleared.	Number of cases.	24	48	72	96	Not cleared.	Number of cases.	
A (Adults)	3	..	2	1	..	..	3	..	2	1	..	..	..	
B (9 — 14 years)	1	..	..	1	..	..	1	1	..	..	..	..	..	
D (1 — 4 years)	3	..	1	..	..	2	..	..	..	..	..	..	1	One case aged 3 years. *P. falciparum* asexual parasite cleared within 24 hours. *P. vivax* asexual forms not cleared within 120 hours.
Total	7	..	3	2	..	2	4	1	2	1	..	..	1	

TABLE IV.

Asexual parasite density index on the day of treatment and on the following five days (Regime I).

	P. vivax.	*P. falciparum.*	*P. vivax* and *P. falciparum* (mixed).
On D day	5·4	5·0	7·0
On D + 1 day	4·2	2·2	5·0
On D + 2 day	1·7	1·2	5·0
On D + 3 day	1·5	0	5·0
On D + 4 day	1·8	0	6·0
On D + 5 day	Not observed	0	Not observed

TABLE V.

Effect on gametocytes (Regime I).

Type of infection.	Number of cases.	Gametocytes absent initially.	Appearance within hours of drug administration :						Clearance within hours of drug administration :			Total not cleared.
			24	48	72	96	120	Total.	72	96	120	
P. vivax	7	7	..	2	2	..	..	4	1	1	..	2
P. falciparum	4	4	..	..	..	..	..	Nil	..	..	..	..
Mixed (*P. vivax* and *P. falciparum*)	1	1	..	0/1	1/1	..	..	1/1	..	..	..	1/1

TABLE VI.

Effect of a single dose of 200 mg. DDS on the clearance of asexual parasites (Regime II).

Age-group.	*P. vivax* infection clearance within hours :							*P. falciparum* infection clearance within hours :				*P. vivax* and *P. falciparum* (mixed) infection clearance within hours :	
	Number of cases.	24	48	72	96	120	Not cleared.	Number of cases.	24	48	Not cleared.	Number of cases.	
A (Adults)	3	2	.	..	..	..	1	1	1	..	Nil	1	*P. vivax* asexual form not cleared within 120 hours. *P. falciparum* asexual form cleared within 72 hours.
B (9 — 14 years)	1	..	..	1	..	..	Nil	..	..	..	..	..	
C (5 — 8 years)	2	1		..	1	..	Nil	2	1	..	Nil	1	*P. vivax* asexual form cleared within 72 hours. *P. falciparum* asexual form cleared within 48 hours.
D (1 — 4 years)	1	..	..	..	..	..	1	..	..	1	..	..	
Total	7	3	..	1	1	..	2	3	2	1	Nil	2	

TABLE VII.

Asexual parasite density index on the day of treatment and on the following five days (Regime II).

	P. vivax.	*P. falciparum.*	*P. vivax* and *P. falciparum* (mixed).
On D day	7·3	4·0	6·0
On D + 1 day	3·5	1·0	4·0
On D + 2 day	1·5	0	3·0
On D + 3 day	1·4	0	2·5
On D + 4 day	1·0	0	1·5
On D + 5 day	1·0	0	2·5

TABLE VIII.

Effect on gametocytes (Regime II).

Type of infection.	Number of cases.	Gametocytes absent initially.	Appearance within hours of drug administration:						Clearance within hours of drug administration:					Total not cleared.
			24	48	72	96	120	Total.	24	48	72	96	120	
P. vivax	7	6	2	..	..	..	..	3	..	..	1	..	..	2
P. falciparum	3	3	2	..	..	..	..	2	..	..	..	..	..	2
P. vivax and *P. falciparum* (Mixed)	2	2	..	2 (0/1 and 1/0)	..	..	..	2	..	..	..	..	..	1/1

(A) *P. VIVAX* INFECTION.

All the seven cases became afebrile within 72 hours. Asexual parasites were cleared within 96 hours in 5 cases while in the other two cases (one adult, and one child aged 2 years) it continued to be patent up to 120 hours of drug administration (Table VI). The asexual parasite density index declined from 7·3 on D-day to 1·0 on D + 5 day (Table VII). Gametocytes were detected in three cases only and these continued to be observed up to 5 days after drug administration in two, while in one, the gametocytes disappeared within 72 hours (Table VIII).

(B) *P. FALCIPARUM* INFECTION.

The fever as well as the asexual parasites disappeared in all the three cases within 48 hours of drug administration (Table VI). The parasite density index dropped to zero from the initial value of 4·0 within 48 hours (Table VII). The gametocytes were not cleared within the period of observation in the two cases in whom such forms appeared (Table VIII).

(C) MIXED INFECTION.

Both the cases were rendered afebrile within 72 hours. In one, the asexual forms of both the species were cleared within 48—72 hours. In the other case (adult, aged 28 years) the asexual stages of *P. falciparum* were cleared within 72 hours but the corresponding stages of *vivax* persisted up to 120 hours (Table VI). The asexual parasite density index declined from the initial 5·0 to 1·5 within 96 hours of treatment but thereafter (*P. vivax*) registered a rise to 2·5 (Table VII). Gametocytes of both the species were not cleared (Table VIII).

Regime II was ineffective in two out of seven cases of *P. vivax* infection although one of the subjects (28 years) treated was semi-immune. The time taken for clearance of asexual forms in the other five cases was longer than under Regime I. Against *P. falciparum* infection the regime was found quite effective both in respect of fever and asexual parasites. In one case of mixed infection, the regime failed to clear the asexual forms of *P. vivax*. Like Regime I, this regime also proved to be inadequate for the treatment of *vivax* malaria as the asexual parasites persisted in three out of nine cases.

REGIME III.

Tables IX, X and XI show the results of treatment by Regime III (single adult dose of 100 mg. DDS combined with 12·5 mg. of Pyrimethamine) : There were six cases of *P. vivax* and six cases of *P. falciparum* infection.

(A) *P. VIVAX* INFECTION.

All the six patients were free from pyrexia within 48 hours. Asexual parasites were cleared in all the cases within 72 hours (Table IX). Asexual parasite density index recorded a drop from 6·8 to zero within 72 hours (Table X). Five cases had shown gametocytes either initially or following the treatment. The gametocytes were cleared in four within 72 hours but in one they persisted up to at least 120 hours (Table XI).

TABLE IX.

Effect of a single dose of 100 mg. DDS combined with 12·5 mg. of pyrimethamine on the clearance of asexual parasites (Regime III).

Age group.	*P. vivax* infection clearance within hours:							*P. falciparum* infection clearance within hours :						
	Number of cases.	24	48	72	96	120	Not cleared.	Number of cases.	24	48	72	96	120	Not cleared.
Adults	6	2	3	1	..	..	Nil	6	4	2	..	..	..	Nil
Total	6	2	3	1	..	..	Nil	6	4	2	..	..	..	Nil

TABLE X.

Asexual parasite density index on the day of treatment and on the five following days. (Regime III).

	P. vivax.	*P. falciparum.*
On D day	6·8	3·0
On D + 1 day	4·1	1·6
On D + 2 day	0·5	0
On D + 3 day	0	0
On D + 4 day	0	0
On D + 5 day	0	0

(B) *P. FALCIPARUM* INFECTION.

Fever as well as asexual parasites were cleared within 48 hours in all the six cases (Table IX). The asexual parasite density index was reduced to zero within 48 hours from the initial value of 3·0 (Table X). Crescents observed initially in three cases failed to clear up within 120 hours of the treatment (Table XI).

Under this combined regime, all the twelve cases appeared to be semi-immunes. Clearance of asexual parasites as well as fever in both types of infection was noted within 48—72 hours. The clearance of *P. vivax* gametocytes appeared to be better than that observed under Regimes I and II. The gametocytes of *P. falciparum*, however, persisted during the period of observation.

REGIME IV.

Tables XII to XIV show the results of treatment by Regime IV (single adult dose 50 mg. DDS combined with 12·5 mg. of pyrimethamine) : There were ten cases of *P. vivax*, five of *P. falciparum* and two of mixed infection.

(A) *P. VIVAX* INFECTION.

The fever disappeared in all the ten cases within 48 hours. Asexual parasites were cleared in all of them within 72 hours. The asexual parasite density index declined from the initial 7·4 to zero within 72 hours (Tables XII and XIII). Gametocytes were detected in nine out of ten cases and only in two they persisted at least up to 120 hours of drug administration (Table XIV).

TABLE XI.

Effect on gametocytes (Regime III).

Type of infection.	Number of cases.	Gametocytes absent initially.	Appearance within hours:						Clearance within hours:					Total not cleared.
			24	48	72	96	120	Total.	24	48	72	96	120	
P. vivax	6	3	2	..	..	..	..	5	1	2	1	..	..	1
P. falciparum	6	3	..	..	..	..	..	3	..	..	..	..	..	3

TABLE XII.

Effect of a single dose of 50 mg. DDS, combined with 12·5 mg. of pyrimethamine, on the clearance of asexual parasites (Regime IV).

Age-group.	*P. vivax* infection clearance within hours:					*P. falciparum* infection clearance within hours:					*P. vivax* and *P. falciparum* (mixed) infection clearance within hours:	
	Number of cases.	24	48	72	Not cleared.	Number of cases.	24	48	72	Not cleared	Number of cases.	
A (Adults)	1	1	..	..	Nil	3	1	1	1	Nil	1	*P. vivax* asexual forms cleared within 72 hours. *P. falciparum* asexual forms cleared within 72 hours.
B (9 — 14 years)	4	1	1	2	Nil	2	..	2	..	Nil	..	
C (5 — 8 years)	2	2	..	..	Nil	..	..	..	..	..	..	
D (1 — 4 years)	1	..	1	..	Nil	..	..	..	..	..	1	*P. vivax* asexual forms cleared within 72 hours. *P. falciparum* asexual forms cleared within 48 hours.
E (Infants)	2	..	2	..	Nil	..	..	..	..	..	..	
Total	10	4	4	2	Nil	5	1	3	1	Nil	2	

TABLE XIII.

Asexual parasite density index on the day of treatment and on the five following days. (Regime IV).

	P. vivax.	*P. falciparum.*	*P. vivax* and *P. falciparum* (mixed).
On D day	7·4	7·6	9·0
On D + 1 day	3·9	5·0	9·0
On D + 2 day	1·3	1·0	8·0
On D + 3 day	0	0	0
On D + 4 day	0	0	0
On D + 5 day	0	0	0

TABLE XIV.

Effect on gametocytes (Regime IV).

Type of infection.	Number of cases.	Gametocytes absent initially.	APPEARANCE WITHIN HOURS OF DRUG ADMINISTRATION :						CLEARANCE WITHIN HOURS OF DRUG ADMINISTRATION :					Total not cleared.
			24	48	72	96	120	Total	24	48	72	96	120	
P. vivax	10	2	1	..	..	..	..	9	3	2	1	..	1	2
P. falciparum	5	4	1	2	..	..	..	4	1	..	1	..	..	2
P. vivax and *P. falciparum* (mixed)	2	2	1/1 1/0	.. 1/1	..	..	..	2	..	..	..	..	.. 0/1	2

(B) *P. FALCIPARUM* INFECTION.

All the five cases were rendered free from fever as well as asexual parasites within 72 hours of drug administration. The asexual parasite density index was reduced to zero from the initial 7·6 within 72 hours (Tables XII and XIII). Crescents were observed in four cases and they continued to be present in two up to at least 120 hours of the drug administration (Table XIV).

(C) MIXED INFECTION.

Two cases which showed mixed infection were cleared within 48 to 72 hours of fever as well as asexual parasites of both species. The asexual parasite density index was reduced to zero from the initial 9·0 within 72 hours of the treatment (Tables XII and XIII). Gametocytes continued to be present up to 120 hours of drug administration in both the cases ; this involved both species in one case and crescents only in the other (Table XIV).

The cases treated under this regime represented all age-groups apparently with a wide range of immunity. The infants with negligible immunity on the one hand and the semi-immune adults on the other formed the extreme of the range. All the seventeen cases were rendered afebrile within 72 hours irrespective of the type of infection. Similarly clearance of the asexual parasites of both species was secured in all the seventeen cases within 72 hours of the drug administration.

The gametocytes of *P. vivax* were cleared in eight out of eleven cases which showed these forms. Crescents appeared to remain unaffected.

REGIME V.

Tables XV, XVI and XVII show the results of treatment by Regime V (single adult dose of 25 mg. DDS combined with 12·5 mg. of pyrimethamine) : There were four cases of *P. vivax*, one of *P. falciparum* and one of mixed infection.

(A) *P. VIVAX* INFECTION.

The pyrexia disappeared in all the four cases within 72 hours. Two cases were cleared of asexual parasites only on the D+5 day, whereas the other two continued to show the asexual parasites throughout the period of observation (Table XV). The asexual parasite density index declined from 6·7 on D-day to 2·0 on D+4 day but again rose to 2·5 on D+5 day (Table XVI). Gametocytes observed in four cases continued to be present during the period of observation (Table XVII).

(B) *P. FALCIPARUM* INFECTION.

Only one case, aged 10 years, was treated under this regime. The fever remained high and the asexual parasites persisted up to 72 hours of the drug administration, necessitating treatment with chloroquine. The asexual parasite density index during the period of observation declined from 9·0 on D-day to 0·8 on D+3 day (Tables XV and XVI). Gametocytes were not detected in this case.

TABLE XV.

Effect of a single dose of 25 mg. DDS, combined with 12·5 mg. of pyrimethamine, on the clearance of asexual parasites (Regime V).

Age-group.	*P. vivax* infection clearance within hours :							*P. falciparum* infection clearance within hours :					*P. vivax* and *P. falciparum* (mixed) infection clearance within hours :	
	Number of cases.	24	48	72	96	120	Not cleared.	Number of cases.	24	48	72	Not cleared.	Number of cases.	
A (Adults)	1	..	..	..	..	1	..	..	..	..	..	..	1	*P. vivax* asexual forms cleared within 96 hours. *P. falciparum* asexual forms cleared within 72 hours.
B (9 — 14 years)	1	..	..	..	..	..	1	1*	..	..	..	1	..	
C (5 — 8 years)	1	..	..	..	..	..	1	..	..	..	..	..	..	
D (1 — 4 years)	1	...	..	..	..	1		..	..	..	..	..	..	
Total	4	..	..	..	..	2	2	1	..	..	..	1	1	

*The case was observed up to 72 hours, the fever did not come down and the parasite count was also high. So he was given chloroquine treatment at the end of this period.

TABLE XVI.

Asexual parasite density index on the day of treatment and on the following five days (Regime V).

	P. vivax.	*P. falciparum.*	*P. vivax* and *P. falciparum* (mixed).
On D day	6·7	9·0	7·0
On D + 1 day	6·0	8·0	5·0
On D + 2 day	4·7	8·0	5·0
On D + 3 day	5·0	0·8	5·0
On D + 4 day	2·0	Not observed	0
On D + 5 day	2·5	Not observed	0

TABLE XVII.

Effect on gametocytes (Regime V).

Type of infection.	Number of cases.	Gametocytes absent initially.	Appearance within hours of drug administration :						Clearance within hours of drug administration :					Total not cleared.
			24	48	72	96	120	Total.	24	48	72	96	120	
P. vivax	4	3	..	1	1	..	1	4	..	..	..	..	..	4
P. falciparum	1	1	..	..	..	..	..	Nil	..	..	..	..	..	..
P. vivax and *P. falciparum* (mixed)	1	1	1/0	..	..	..	..	1	..	..	..	..	..	1

(*C*) MIXED INFECTION.

One case of mixed infection treated under this regime was rendered afebrile within 48 hours and showed clearance of asexual parasites (both species) within 72—96 hours. The asexual parasite density index was reduced from the D-day value of 7·0 to zero within 96 hours. The *vivax* gametocytes were not cleared within the period of observation (Tables XV to XVII).

A total of six cases were treated under Regime V. In the clearance of asexual parasites this regime proved ineffective in three cases. Such therapeutic failures involved both *P. vivax* and *P. falciparum* infections. In one case the fever persisted in spite of the treatment and recourse had to be taken to an alternate drug. The time taken for the clearance of asexual forms of *P. vivax* was long (120 hours) and, therefore, the regime was extremely slow in action even in cases where it was effective. The gametocytes of *P. vivax* remained apparently unaffected by this regime.

TOXICITY OF DDS SINGLY AND IN COMBINATION WITH PYRIMETHAMINE.

Only one case of vomiting was noted in a male, aged 20 years, treated under Regime II (DDS 200 mg.—single adult dose). The vomiting occurred 24 hours after drug administration and required no further palliative treatment. No skin rash was encountered. No toxic symptoms were noted either subjective or objective, unequivocally attributable to any of these regimes of treatment.

DDS-PYRIMETHAMINE COMBINATION.

A single dose of 250 or 200 mg. of DDS was invariably effective in all the *P. falciparum* infections but proved ineffective in clearing the asexual parasites in six out of seventeen *P. vivax* infections (Tables III and VI). Irrespective of the types of infection, however, the above dosage of DDS failed to clear the asexual parasites in six out of twenty-four cases.

Pyrimethamine 25 mg., given on two consecutive days, was shown to be ineffective to clear asexual parasites in two out of thirty-one *falciparum* malaria cases (Jaswant Singh *et al.*, 1952). Srivastava *et al.* (1953) showed that a single dose of 25 mg. pyrimethamine failed to clear parasites in one out of thirteen cases treated. Thus, the single dose of 25 mg. of pyrimethamine was not completely effective.

In the present series, 12·5 mg. of pyrimethamine combined with three levels of DDS dosages namely, 100, 50 and 25 mg., were tried. The failure to clear asexual parasites in persons having *vivax* and *falciparum* infections (compiled from Tables IX, XII and XV) was as shown in Table XVIII.

TABLE XVIII.

Failure of the combination of 12·5 mg. of pyrimethamine with DDS to clear asexual parasites.

Pyrimethamine 12·5 mg.	DDS :		
	100 mg.	50 mg.	25 mg.
P. vivax	0/6	0/12	2/5
P. falciparum	0/6	0/7	0/2
Both infections taken together	0/12	0/17	2/6

(Numerator = failures ; Denominator = number of cases treated).

In view of the above, a combination of 12·5 mg. pyrimethamine with either 100 or 50 mg. DDS was completely effective in 29 cases of *vivax* and *falciparum* malaria. In other words, a much smaller dose of each in combination was required for effective removal of asexual parasites than that needed with either given separately. This effect was further substantiated by the decline of parasite density indices recorded on 5 consecutive days as given below :

TABLE XIX.

Effect of the combination of 12·5 mg. of pyrimethamine with DDS in declining of the parasite density indices.

Regime.	Dosages and drugs (equivalent to adult dosage).	D day.	D + 1 day.	D + 2 day.	D + 3 day.	D + 4 day.	D + 5 day.
I	250 mg. DDS	5·4	4·2	1·7	1·5	1·8	..
II	200 mg. DDS	7·3	3·5	1·5	1·4	1·0	1·0
III	100 mg. DDS plus 12·5 mg. pyrimethamine	6·8	4·1	0·5	0·0	0·0	0·0
IV	50 mg. DDS plus 12·5 mg. pyrimethamine	7·4	3·9	1·3	0·0	0·0	0·0
V	25 mg. DDS plus 12·5 mg. pyrimethamine	6·7	6·0	4·7	5·0	2·0	2·5

DISCUSSION.

The comparatively smaller single doses (250 and 200 mg.) of DDS were deliberately chosen to determine the smallest effective dose. The Regime I (250 mg. DDS per adult) was found effective against *P. falciparum* in clearing the asexual parasites as well as fever in all cases within 72 hours. A single dose of 200 mg. of DDS in children of Northern Nigeria, with a mean age of 8·8 years, was shown to clear asexual *falciparum* parasites within 96 hours (Archibald and Ross, 1960). In the present series age for age, the dosage used was almost half of that used in

Northern Nigeria. It was noteworthy, however, that in a child aged 3 years with mixed infection, the asexual parasites of *P. falciparum* were cleared within 24 hours. But the same regime was found inadequate in clearing the asexual parasites in three out of eight *vivax* cases (mixed infection included). The comparatively virulent strain of *P. falciparum* appeared to be more sensitive to DDS than the benign strain of *P. vivax*. Similar higher sensitivity of the fatally virulent (Nuri strain) *P. knowlesi* to DDS was noted where the minimum effective dose (MED) was found to be as low as 0·25 mg./kg. compared to the MED against *P. cynomolgi* which was as high as 0·5 mg./kg. (Ramakrishnan, Basu *et al.*, 1961 *loc. cit.*). The over-all failure of the regime was seen in three cases out of the twelve treated and, therefore, 250 mg. DDS as a single dose was not considered adequate for routine therapy.

The Regime II (200 mg. DDS per adult) also proved effective in controlling both fever and parasitaemia due to *falciparum* ; all the five cases (mixed infection included) showing such infection were free from fever as well as asexual parasites within 48—72 hours. But, the regime failed to secure asexual parasite clearance in three out of nine *vivax* cases (mixed infection included). Thus, the over-all failure of the regime, irrespective of the types of infection, was seen in three out of twelve cases treated and, therefore, like the previous regime 200 mg. DDS as a single dose was not found adequate for routine therapy.

The Regime III (100 mg. DDS combined in 12·5 mg. pyrimethamine per adult) proved effective in complete clearance of asexual parasites of both species as well as termination of fever. The speed of action in respect of termination of fever was 48 hours in both types of infection. The speed of clearance of asexual stages of *falciparum* and *vivax* was 48 and 72 hours respectively. The clearance of *vivax* gametocytes also appeared to be quicker than with DDS alone due probably to the action of pyrimethamine on pregametocytes. No toxic symptoms were noted in the persons under this regime.

DDS and pyrimethamine (100 and 12·5 mg. respectively) also proved superior to Regime IV (50 and 12·5 mg. respectively) in that the PDI (parasite density index) showed greater amplitudes of decline in the former than in the latter. Although the patients under Regime III were apparently semi-immunes, that should not detract its value as even a lower combination (Regime IV, *vide infra*) had achieved good results in non-immunes. Regime III was found adequate as a single dose therapy and further trials of this regime could be profitably undertaken in other areas.

Judging by the speed of action, Regime III was found comparable to single dose therapy with 4-aminoquinoline group of drugs at a dosage level equivalent to 600 mg. of the chloroquine base (Srivastava 1953 ; Jaswant Singh, Ray and Misra, 1953). The well-known tardy action of pyrimethamine alone (Srivastava, 1953 ; Chaudhuri, 1953) was markedly improved upon by this combination. Such a

regime would be four times less expensive than the single dose therapy with chloroquine*.

A lower combination of DDS and pyrimethamine (Regime IV, 50 and 12·5 mg. respectively) also proved adequate and quick in both types of infection irrespective of the immunity factor. All the cases were rendered afebrile within 72 hours. The amplitude of decline in the PDI was slightly smaller than that in Regime III. A still lower combination of the two drugs (Regime V) was found unsuitable for adoption as the failures were high.

That DDS enhanced the activity of pyrimethamine, was a corroboration of the theoretical expectation. Ramakrishnan, Basu *et al.* (1961 *loc. cit.*) had shown that the DDS resistant strain of *P. cynomolgi* was equally cross-resistant to sulphadiazine while retaining its sensitivity to pyrimethamine, indicating that the former two acted at the identical point on the same metabolic pathway. The action of pyrimethamine had been shown to be greatly potentiated by sulphadiazine (Rollo, 1955 ; Hurly, 1959). Similar good result of DDS and pyrimethamine combination were obtained in human malaria in the present trials.

Individually, DDS and pyrimethamine are prone to selection of resistant strains of parasites. But the absence of cross resistance between DDS resistant strain and pyrimethamine shows that the drugs act at different points in the metabolic processes of the parasites (Ramakrishnan, Basu *et al.*, 1961 *loc. cit.*). Therefore the combination of the two drugs is likely to reduce the chances of selection of resistance. Individually, both the drugs are excreted slowly. Whether the combination would actually prolong the duration of action, remains to be determined experimentally.

In the present investigation, the sporontocidal activity of the combination has not been determined due to practical difficulties. However, the dosage level of pyrimethamine, maintained in the combination, is expected to retain the sporontocidal activity which, if substantiated, will be an added advantage of significance.

SUMMARY.

250 and 200 mg. DDS, as a single dose, secured asexual parasite clearance as well as termination of fever in *falciparum* infections within 72 hours. These dosages of DDS were inadequate in completely clearing the asexual parasites in *vivax* infections.

Small doses of DDS, combined with a small dose of pyrimethamine, (100 and 50 mg. of the former and 12·5 mg. of latter), were adequate for treatment of both *vivax* and *falciparum* infections. The speed of clearance of asexual parasites as

* Chloroquine 600 mg. base— Rs. 0·144
Pyrimethamine 25 mg. — Rs. 0·035
DDS 100 mg. — Rs. 0·020

well as symptoms was comparable to that obtained with a single dose of 600 mg. of chloroquine base. A lower dosage of DDS in the combination was inadequate.

The advantages of DDS and pyrimethamine combination, in the treatment of human malaria, are discussed.

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SINGLE DOSE TREATMENT OF HUMAN MALARIA BY PAM-780* : A STUDY OF ITS EFFECTS IN *P. VIVAX* AND *P. FALCIPARUM* INFECTIONS IN RAJASTHAN, INDIA.

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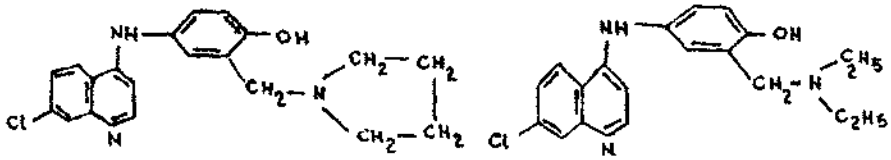
PAM-780, an analogue of amodiaquine, is chemically described as 4 – (7-chloro-4-quinolylamino)-a-1-pyrrolidyl-0-cresol. It differs from amodiaquine in the substitution of a-diethyl-aminomethyl group of 0-cresol side chain with a-pyrrolidylmethyl group :



PAM-780. **Amodiaquine.**

Pam-780 is obtained in the form of hydrochloride salt put up in capsules, each capsule containing 150 mg. of the drug base. It is a yellow crystalline compound with a very bitter taste.

Toxicity studies in monkeys and screening of the drug for antimalarial activity in avian and simian malaria were completed. The results indicated its clinical trial in human malaria (Basu *et al.*, 1962). The object was to find a single non-toxic dose of the drug and compare its efficacy in securing clinical cure.

A focal outbreak of malaria in Jodhpur and its environs (Rajasthan State) provided the requisite cases for the clinical trial. The trial was conducted in the Government Hospital at Chopasni, situated at the outskirts of Jodhpur City. The results of the clinical trial carried out in October, 1961, are set out in this paper.

PATIENTS, DOSAGES AND PROCEDURE.

All fever cases, reporting to the hospital, were examined microscopically. Only the cases found positive for malaria parasites were admitted into the hospital

*Synonyms : Amopyroquine ; Propoquin, received through the courtesy of Messrs Parke Davis and Company, U.S.A.

for treatment and observation for a period of 24—28 hours. All cases received a single dose treatment by mouth. Only two dosage schedules were tried, namely, 600 mg. (Schedule I) and 450 mg. (Schedule II) of the drug base. Only those persons who received 600 mg. dosage were hospitalised for close observation. The persons who received the lower dosage were allowed to leave the out-door clinic after taking the drug.

Proportionate fractional dosages of the capsule contents were carefully ensured by the authors before actual administration. The details of age-groupwise proportionate doses are mentioned in Table I.

TABLE I.

Single-dose schedules for adults, and proportionate dosages for different age-groups (Dose in mg.) of Pam-780.

Age-group.	Schedule I.	Schedule II.
A (Adults) ...	600	450
B (9 — 14 years) ...	450	350
C (5 — 8 years) ...	300	No cases available.
D (1 — 4 years) ...	150	112
E (Infants) ...	75	No cases available.

The cases were classified age-wise into five groups, *A* to *E* (Table II). Fever was present in all the cases when they were first seen except in one child in the age-group *D*. Parasitaemia was investigated by the use of both thick and thin blood smears. The degree of parasitaemia was ascertained by counting the number of parasitized cells per 10,000 erythrocytes. Asexual parasite density index (PDI) was calculated according to the method described by Bruce Chwatt (1951 : 1958) initially, and daily on the subsequent days of observation period, The total erythrocyte count was, however, not determined and was assumed to be 5 million per c. mm. in each case.

TABLE II.

Details of age, infection, PDI (Parasite density index) and pyrexia in respect of the five groups of subjects before treatment.

		AGE-GROUP, IN YEARS :				
		A (15 and above) adults	*B* (9 — 14)	*C* (5 — 8)	*D* (2 — 4)	*E* (Infants) (0 — 1)
		Number of cases :				
Number	Male ...	9	4	5	6	1
	Female ...	..	1	1	1	1
	Total ...	9	5	6	7	..
Age in years	Mean ...	26·75	11	6·08	2·6	0·5
	Range ...	16 to 41	10 to 12	4 to 8	1·25 to 4	0·5
Parasite density index ...		6·5	8·4	8·3	6·7	5·0
Pyrexia before treatment ...		Present in all cases except in one child in Group *D*.				

The first eighteen cases were treated under Schedule I (600 mg. drug base) and the subsequent ten cases under Schedule II (450 mg. drug base).

Each treated case was observed for five days (120 hours), following the treatment by daily clinical and parasitological examination which, besides those seen in the hospital, involved visits to the patients' homes or places of work. The cases, which were cleared of asexual parasites by the Schedules under investigation, were transferred to the NMEP* Surveillance Organisation for radical treatment with primaquine after the expiry of 5-day observation. Cases which were not cleared of asexual parasites by the fifth day of the trial dose, received the standard single dose treatment with chloroquine and were also transferred to the surveillance organisation for radical treatment.

RESULTS.

The results have been analysed schedule-wise with respect to the following criteria :

(*a*) Initial fever and its termination in hours,

(*b*) Complete clearance of asexual parasites in hours,

(*c*) Sensitivity of the two species of parasites (*P. vivax* and *P. falciparum*),

(*d*) Effect on gametocytes in the peripheral blood, and

(*e*) The parasite density index (PDI), its initial value and the amplitude of its decline.

The determination of the relapse pattern, after treatment by PAM-780, did not arise as radical treatment with primaquine was obligatory under the NMEP*.

SCHEDULE I.

Tables III, IV, V and VI show the results of treatment by Schedule I (600 mg. base of PAM-780 single adult dose). There were five cases of *P. vivax*, eleven cases of *P. falciparum* and two cases of mixed infection of both the species.

(*a*) *P. VIVAX* INFECTION.

Pyrexia as well as asexual parasites were cleared in all the five cases within 48 hours. The asexual parasite density index decreased from 6·4 on the D day to zero on the D +2 day. Gametocytes were not cleared in one out of four cases in which these were detected.

(*b*) *P. FALCIPARUM* INFECTION.

Pyrexia, present initially in ten cases, was cleared in each within 48 hours (Table IV). The asexual parasites were cleared in nine out of eleven cases within 72 hours. In two other cases (age-group D), these were not cleared even within 120 hours, and recourse had to be taken to chloroquine treatment. The PDI (Parasite density index) decreased from 8·0 on the D day to 1·4 on the D+3 day, but again increased to 1·7 on the D+5 day. Crescents were cleared within 96 hours in only one case out of six which showed such stages. In the other five cases, these continued to be present even up to 120 hours of drug administration.

*National Malaria Eradication Programme.

TABLE III.

Asexual parasite density index on the day of treatment and on the following 5 days (Schedule I).

	P. vivax.	*P. falciparum.*	*P. vivax* and *P. falciparum* (mixed).
On D day	6·4	8·00	9·0
On D+1 day	1·6	4·4	5·5
On D+2 day	0	2·7	0
On D+3 day	0	1·4	0
On D+4 day	0	1·4	0
On D+5 day	0	1·7	0

TABLE IV.

Effect of single adult dose of 600 mg. Pam-780 on the clearance of clinical symptoms (Schedule I).

Age-group.	*P. vivax* infection afebrile within hours :						*P. falciparum* infection afebrile within hours :						*P. vivax* and *P. falciparum* (mixed) infection afebrile within hours :					
	Number of cases.	24	48	72	96	Not cleared.	Number of cases.	24	48	72	96	Not cleared.	Number of cases.	24	48	72	96	Not cleared.
A (Adults)	1	1	..	..	..	Nil	1	..	1	..	..	Nil	..	..	..	..	..	..
B (9—14 years)	1	1	..	..	..	Nil	2	1	1	..	..	Nil	1	1	..	..	..	Nil
C (5— 8 years)	1	1	..	..	..	Nil	4	4	..	..	..	Nil	1	..	1	..	..	Nil
D (1— 4 years)	2	1	1	..	...	Nil	†2	1	1	..	..	Nil	..	..	..	..	..	..
E (Infants)	.	..	..	..	..	..	1	1	..	..	..	Nil	..	..	.	..	..	..
Total	5	4	1	..	..	Nil	10	7	3	..	..	Nil	2	1	1	..	..	Nil

† Afebrile in one.

TABLE V.

Effect of a single dose of 600 mg. PAM-780 on the clearance of asexual parasites (Schedule I).

Age-group.	*P. vivax* infection clearance within hours :						*P. falciparum* infection clearance within hours :							*P. vivax* and *P. falciparum* (mixed infection) clearance within hours :	
	Number of cases.	24	48	72	96	Not cleared.	Number of cases.	24	48	72	96	120	Not cleared.	Number of cases.	
A (Adults)	1	1	..	..	..	Nil	1	..	1	..	..	..	Nil	..	
B (9 — 14 years)	1	1	..	..	..	Nil	2	1	..	1	..	..	Nil	1	*P. vivax* asexual forms cleared within 24 hours. *P. falciparum* asexual forms cleared within 48 hours.
C (5 — 8 years)	1	..	1	..	..	Nil	4	..	2	2	..	..	Nil	1	*P. vivax* asexual forms cleared within 48 hours. *P. falciparum* asexual forms cleared within 24 hours.
D (1 — 4 years)	2	1	1	..	..	Nil	3	..	..	1	..	..	2	..	
E (Infants)	..	..	..	..	..	..	1	1	..	..	..	..	Nil		
Total	5	3	2	..	..	Nil	11	2	3	4	..	..	2	2	

TABLE VI.

Effect on gametocytes (Schedule I).

Type of infection.	Number of cases.	Gametocytes absent initially.	APPEARANCE WITHIN HOURS OF DRUG ADMINISTRATION :						CLEARANCE WITHIN HOURS OF DRUG ADMINISTRATION :					Total not cleared.
			24	48	72	96	120	Total	24	48	72	96	120	
P. vivax	5	1	..	..	..	..	..	4	2	..	1	..	..	1
P. falciparum	11	10	..	1	4	..	..	6	..	..	..	1	..	5
Mixed (*P. vivax* and *P. falciparum*)	2	2	..	..	1/0	..	..	1	..	..	0/0	..	..	Nil

Amongst the two cases in whom asexual parasite clearance was not obtained, one (aged 3 years) had initially a comparatively high parasite count of 450,000 per c.mm. on the D day. In this case, the count was reduced to 11,000 per c.mm. on the D+2 day but again increased to 200,000 per c.mm. on the D+3 day ; the count was 39,000 per c.mm. on the D+5 day when chloroquine was given. The second case (also aged 3 years) had 250 parasites per c.mm. on the D day. The parasite count in this case increased to 5,000 per c.mm. on the D+3 day and 12,500 per c.mm. on the D+5 day when chloroquine was administered as an alternative treatment.

(c) MIXED INFECTION.

Two cases of mixed infection were cleared of both fever and asexual parasites of both species within 48 hours. The PDI was reduced to zero on the D+2 day from the initial 9·0 on the D day. *P. vivax* gametocytes, seen in one case, were cleared within 72 hours.

SCHEDULE II.

Tables VII, VIII, IX and X show the results of treatment by Schedule II (450 mg. base of PAM-780 single adult dose). There were six cases of *P. vivax* and four cases of *P. falciparum*.

(a) *P. VIVAX* INFECTION.

All the six cases were free from both fever and asexual parasites within 48 hours. The PDI (Parasite density index) declined to zero on D+2 day from the initial 6·3 on the D day. Gametocytes could be detected in only one case and these were cleared within 120 hours.

(b) *P. FALCIPARUM* INFECTION.

Fever was cleared in all the four cases within 48 hours. The asexual parasites were cleared in three within 48 hours. In the fourth case, the clearance of asexual parasites was achieved only within 96 hours. The PDI declined to zero on the D+4 day from the initial 6·5 on the D day. Crescents were detected in only one case 72 hours after drug administration and these were cleared within 120 hours.

TOXICITY OF PAM-780.

No toxic symptoms were noted which could be attributed to the drug. Vomiting was noted in two cases (aged 11 years and 16 years) which had received equivalents of 600 mg. base of Pam-780 single adult dose. Another case of vomiting was noted in one (aged 25 years) who had received a single dose of 450 mg. of the drug base. All the three cases had *P. falciparum* infection and vomiting disappeared with the clearance of fever.

TABLE VII.

Effect of 450 mg. Pam-780 on the clearance of clinical symptoms (Schedule II).

Age-group.	*P. vivax* infection afebrile within hours :						*P. falciparum* infection afebrile within hours:					
	Number of cases.	24	48	72	96	Not cleared.	Number of cases.	24	48	72	96	Not cleared.
A (Adults)	5	4	1	..	..	Nil	2	1	1	..	..	Nil
B (9 — 14 years)	..	..	..	..	..	..	1	1	..	..	..	Nil
D (1 — 4 years)	1	..	1	..	..	Nil	1	..	1	..	..	Nil
Total ...	6	4	2	..	..	Nil	4	2	2	..	..	Nil

TABLE VIII.

Effect of a single dose of 450 mg. PAM-780 on the clearance of asexual parasite. (Schedule II).

Age-group	*P. vivax* infection clearance within hours:						*P. falciparum* infection clearance within hours :					
	Number of cases.	24	48	72	96	Not cleared.	Number of cases.	24	48	72	96	Not cleared.
A (Adults)	5	5	..	..	..	Nil	2	..	2	..	..	Nil
B (9—14 years)	..	..	..	..	..	..	1	1	..	..	..	Nil
D (1— 4 years)	1	..	1	..	..	Nil	1	..	..	..	1	Nil
Total	6	5	1	..	..	Nil	4	1	2	..	1	Nil

TABLE IX.

Asexual parasite index on the day of treatment and following 5 days. (Schedule II).

	P. vivax.	*P. falciparum.*
On D day	6·8	6·5
On D + 1 day	1·3	5·5
On D + 2 day	0	1·5
On D + 3 day	0	1·2
On D + 4 day	0	0
On D + 5 day	0	0

TABLE X.

Effect on gametocytes (Schedule II).

Type of infection.	Number of cases.	Gametocytes absent initially.	Appearance within hours of drug administration:						Clearance within hours of drug administration:			Total not cleared.
			24	48	72	96	120	Total	72	96	120	
P. vivax	6	6	1	..	..	..	..	1	..	..	1	Nil
P. falciparum	4	4	..	..	1	..	.	1	..	..	1	Nil

DISCUSSION.

A single dose of 600 mg. or 450 mg. base of Pam-780 achieved clearance of fever due to both *falciparum* and *vivax* malaria within 48 hours. This compared well with that obtained by a single dose of 0·5 g. of either chloroquine or amodiaquine (Chaudhuri *et al.*, 1950).

Both the dosage schedules used in this investigation were found adequate to clear the asexual parasites of *P. vivax* within 48 hours. The speed of clearance was the same as that obtained with a single dose of 600 mg. of either resochin or chloroquine and superior to that obtained by 600 mg. of amodiaquine which took a day longer (Jaswant Singh *et al.*, 1953). 600 mg. single dose of Pam-780, however, failed to clear the asexual parasites of *P. falciparum* in two cases out of eleven treated. The speed of clearance of asexual parasites in the other nine cases, where the treatment proved successful, was 72 hours. This was comparable to that obtained by the equivalent dosage of amodiaquine but inferior to that of Resochin or Nivaquine (Jaswant Singh *et al.*, 1953 *loc. cit.*). However, in the same treatment schedule, two cases—one boy and another girl, both aged three years—had 150 mg. of the drug base each. No vomiting was noted in either of them. The boy had very high parasitaemia at the time of drug administration. In both, the Schedule I (600 mg. Pam-780 single adult dose) failed to clear the asexual parasites of *P. falciparum*. Thus, a single dose of 600 mg. of Pam-780 was found inadequate for the routine treatment of *falciparum* malaria.

Only four cases of *falciparum* malaria were treated by a single dose of 450 mg. of Pam-780. Although the clearance of asexual parasites was obtained

in all, the speed of action was rather slow at least in one in whom this was obtained in 96 hours.

Thus, Pam-780 at the dosages used did not show the efficacy of the corresponding dosages of drugs like Resochin, Nivaquine or Amodiaquine. The drug appeared to be inactive in clearing the gametocytes of *P. falciparum* from the peripheral blood as these stages continued to be present up to 120 hours at least in five out of six cases in whom they were detected. The gametocytes of *P. vivax* were cleared within 72 hours of drug administration in four out of five cases in whom they were detected ; one continued to show the *vivax* gametocytes even up to 120 hours.

The use of 600 mg. or 450 mg. of Pam-780, as single dose routine therapy for the clinical cure of malaria, appeared to be unsuitable because of the hazards of failure in *falciparum* cases. The drug, however, did not give rise to any toxic symptoms in the dosages used.

SUMMARY.

Single dose of 600 mg. or 450 mg. base of Pam-780 was found effective in *vivax* malaria. Clearance of both fever and asexual parasite was obtained within 48 hours.

600 mg. single dose of Pam-780 failed to clear the asexual parasite of *P. falciparum* in two cases out of eleven treated.

ACKNOWLEDGEMENT.

The facilities extended by the Director of Medical and Health Services, Rajasthan State, and his local staff, are gratefully acknowledged.

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STUDIES ON THE TOXICITY AND THE ACTION OF PAM-780* IN AVIAN AND SIMIAN MALARIA.

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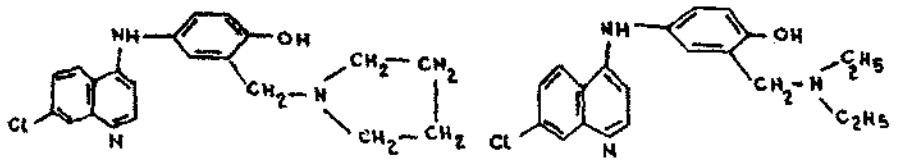
[March 5, 1962.]

PAM-780, an analogue of amodiaquine, is chemically 4-(7-chloro-4-quinolyl-amino)-α-1-pyrolidyl-0-cresol. The drug is available in the form of hydrochloride salt in capsules, each capsule containing 150 mg. of the drug base. It is a yellow crystalline compound with a very bitter taste and has the following structural formula :



PAM-780. **Amodiaquine.**

As the compound has a close structural relationship to the established antimalarial amodiaquine, laboratory studies on its antimalarial activity were indicated. This report deals with the investigations carried out against *P. gallinaceum*† in chicks, against *P. cynomolgi bastianelli*‡ and *P. knowlesi*§ in *Macaca mulatta* monkeys. The activity of the drug has been estimated in comparison with that of chloroquine and amodiaquine.

TOXICITY OF PAM-780.

Single dose of 150 mg./kg. Pam-780 by mouth to monkeys did not produce symptoms of poisoning. Doses of 200 mg./kg. and above were found toxic (Table I); the symptoms in the order of sequence were vomiting, convulsion and respiratory

* Synonyms: Amopyroquine; propoquin, received through the courtesy of Messrs Parke, Davis & Company, U. S. A.

† The strain of *P. gallinaceum* is the same as used by Jaswant Singh, Basu and Ray (1952).

‡ Strain of *P. cynomolgi bastianelli* was obtained through the courtesy of Dr. P. C. C. Garnham, London.

§ Nuri Strain of *P. knowlesi* as described by Jaswant Singh, Ray and Nair (1953).

failure followed by death. In toxic dosages, death occurred within a period of ½—2 hours of drug administration. Left ventricle in diastole, containing varying quantity of blood, was the usual post-mortem finding.

A single dose of chloroquine and amodiaquine respectively are more than two and three times as toxic as PAM-780. 75 mg./kg. of chloroquine and 45 mg./kg. of amodiaquine did not produce any symptom of poisoning in monkeys, whereas higher doses were found toxic (Malaria Institute of India, 1953).

TABLE I.

Toxicity of a single dose of PAM-780 in monkeys.

Dosage in mg./kg.	Number of animals.	Number survived.	Remarks.
900 mg.	1	Nil	Respiratory failure. Died within 30 minutes.
600 mg.	1	Nil	Respiratory failure. Died within 40 minutes.
300 mg.	2	Nil	Died within an hour. Vomiting and convulsion and respiratory failure. Left ventricle in diastole with fluid blood.
250 mg.	2	1	Died within an hour with vomiting and convulsion. Left ventricle in diastole with fluid blood.
200 mg.	5	4	Died within an hour with vomiting and convulsion. Left ventricle in diastole.
150 mg.	5	5	Nothing abnormal.

PARASITICIDAL ACTIVITY.

(I) SCHIZONTICIDAL PROPERTIES IN CHICK TEST.

Activity of Pam-780 was determined against *P. gallinaceum* by the technique routinely followed at the Institute (Jaswant Singh, Basu and Ray, 1952). Nine schedules of Pam-780 dosages, *viz.*, 1/32, 1/28, 1/24, 1/20, 1/16, 1/8, 1/4, 1 and 4 times the minimum effective dose (MED) of quinine*, were investigated.

The MED of Pam-780 was 0·066 mg. per 50 g. and its quinine equivalent was found to be 24 (Table II). The schizonticidal activity of the drug in *P. gallinaceum* was found slightly inferior to both chloroquine and amodiaquine, the quinine equivalents of the latter two drugs being 26 and 32 respectively (Jaswant Singh, Basu and Ray, 1952 ; Ray, Sharma and Misra, 1956).

(II) THERAPEUTIC PROPERTIES IN ACUTE *P. KNOWLESI* (NURI STRAIN) INFECTION.

Twenty-four monkeys were infected intravenously with the Nuri strain of *P. knowlesi*, each with the standard inoculum of five million parasitized erythrocytes per kg. body weight. Treatment commenced at the stage of 0·1 to 0·2

*Quinine equivalent $= \frac{\text{MED of quinine}}{\text{MED of X}}$, where MED of quinine is 1·6 mg. per 50 grammes.

per cent red cell infection. PAM-780 in doses from 2·0 to 4·0 mg./kg. was administered orally to each of the respective groups of animals while two monkeys were kept as untreated controls. Treatment continued for seven days. Criteria for assessment of results were based on the disappearance of parasites from the peripheral blood on the day following the last dose—Class II effect of Shannon (Wiselogle, 1946).

TABLE II.

Activity of PAM-780 against P. gallinaceum.

Dosage in terms of the quinine MED.	Number of chicks used.	Activity.
4 Q	5	Active.
1 Q	5	Active.
$\frac{1}{4}$ Q	5	Active.
$\frac{1}{8}$ Q	5	Active.
$\frac{1}{16}$ Q	5	Active.
$\frac{1}{20}$ Q	5	Active.
$\frac{1}{24}$ Q	5	Active.
$\frac{1}{28}$ Q	5	Inactive.
$\frac{1}{32}$ Q	5	Inactive.

TABLE III.

Therapeutic effect of PAM-780 in P. knowlesi *(Nuri strain) infection.*

Dose mg./kg. daily for 7 days.	Number of monkeys used.	EFFECTIVE (CLASS II):		INEFFECTIVE:	Recrudescences within 10 days of sub-patency.
		Number of animals.	Parasite clearance in hours.	Number of animals.	
2·0 mg.	2	Nil	Nil	2	..
2·25 mg.	2	Nil	Nil	2	..
2·5 mg.	5	Nil	Nil	5	..
2·75 mg.	3	Nil	Nil	3	..
3·0 mg.	4	2	72 to 168	2	..
3·5 mg.*	4	4	96 to 144	Nil	Nil
4·0 mg.	2	2	96	Nil	Nil
Control	2	Both animals died on the sixth day after inoculation due to 78 to 94 per cent cell infection.			

*MED (Minimum effective dose).

The above mentioned results showed that the minimum effective dose (MED) of PAM-780 in *P. knowlesi* infection was 3·5 mg./kg. and the parasite clearance was obtained within 96 - 144 hours of the commencement of treatment. Recrudescences were not observed during the following ten days. Dose for dose, PAM-780 was found slightly less active than both chloroquine and amodiaquine, the MED of the latter two drugs in *P. knowlesi* (Nuri strain) were 2·1 and 2·5 mg./kg. respectively (Nair and Ray, 1955). The speed of clearance of the parasites from the peripheral blood was comparable to that obtained by the above mentioned two members of the 4-aminoquinoline group of drug.

(III) THERAPEUTIC PROPERTIES IN ACUTE *P. CYNOMOLGI BASTIANELLI* INFECTION.

The technique, similar to that employed in the preceding investigation, was adopted to determine the activity of PAM-780 in *P. cynomolgi bastianelli* infection. Twenty-two monkeys were used for the purpose. The drug was used in doses of 1·5 to 7 mg./kg. daily for 7 days.

TABLE IV.

Therapeutic effect of PAM-780 in P. cynomolgi bastianelli *infection.*

Dose mg./kg. daily for 7 days.	Number of monkeys used.	EFFECTIVE (CLASS II):		INEFFECTIVE :	Recrudescences within 10 days of sub-patency.
		Number of animals.	Parasite clearance in hours.	Number of animals.	
1·5 mg.	3	Nil	Nil	3	..
2·5 mg.	5	Nil	Nil	5	..
3·5 mg.	4	Nil	Nil	4	..
5·0 mg.*	5	5	96 to 168	Nil	Nil
7·0 mg.	3	3	72 to 120	Nil	Nil
Control	2	Parasitaemia continued for 12 to 17 days and then passed on to chronic stage.			

*MED-(Minimum effective dose).

The minimum effective dose (MED) within the schedule was 5·0 mg./kg. daily for seven days (Table IV). Parasite clearance was obtained within 96 to 168 hours from the commencement of treatment. Recrudescences were not observed during 10 days of sub-patency. Thus in *P. cynomolgi bastianelli*, the MED of PAM-780, although comparatively massive in quantity, was found extremely slow in clearing the parasites in two out of five monkeys (168 hours). In contrast, the minimum effective dose of chloroquine in *P. cynomolgi bastianelli* was 1·5 mg./kg. daily for seven days (Malaria Institute of India, 1961).

DISCUSSION.

In monkeys, the toxicity of PAM-780 was found to be lower than that of both chloroquine and amodiaquine. Its antimalarial activity in *P. gallinaceum*, however, was lower than that of chloroquine or amodiaquine.

In *P. knowlesi* infection, the activity of the drug was found no better than either chloroquine or amodiaquine. In *P. cynomolgi bastianelli* infection, it was dose for dose, almost thrice less active than chloroquine. PAM-780 in avian and simian malaria did not show activity superior to that possessed by the other established members of the 4-aminoquinoline series.

In *vivax* malaria, the drug in single dose of 600 mg. or 450 mg. base was found effective. Clearance of both fever and asexual parasite was obtained within 48 hours. But 600 mg. single dose failed to clear the asexual parasites of *P. falciparum* in two out of eleven cases treated (Basu, Chakrabarty and Mondal, 1962).

The compound did not appear to hold any better claim than either chloroquine or amodiaquine.

SUMMARY.

PAM-780 in single dose of 150 mg./kg. base was well tolerated in monkeys. Doses of 200 mg./kg. and above were toxic.

Against *P. gallinaceum* in chicks, the quinine equivalent of PAM-780 was 24 which compared well with chloroquine but was inferior to amodiaquine.

The MED (minimum effective dose) of PAM-780 in *P. knowlesi* (Nuri strain) was 3·5 mg./kg. daily for seven days. The MED in *P. cynomolgi bastianelli* was 5·0 mg./kg. for seven days.

In avian and simian malaria, PAM-780 did not show any better activity than the other established members of the 4-aminoquinoline series.

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THE DURATION OF PATENT INFECTION OF *LITOMOSOIDES CARINII* IN THE ALBINO RAT.

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[February 27, 1962.]

INTRODUCTION.

OUT of 54 white rats exposed to infective tropical mites, *Ornithonyssus bacoti*, 46 showed microfilariae in their peripheral blood. The average period of patency was 69 days (Ramakrishnan *et al.*, 1961). The extrinsic incubation period for the parasites in mites was found to be identical whether the mites were infected from white rats or cotton rats. Detailed parasitological studies on the duration of *L. carinii* infection and microfilarial density in white rats are recorded in this paper.

MATERIAL AND METHODS.

The techniques for raising and infecting mites and for exposing the albino rats to the bites of infective mites were the same as described by Ramakrishnan *et al.* (1961 *loc. cit.*). Fifteen days after the mites were fed on an infected rat, sample dissections of the mites were made to ensure that a good proportion of them were infective.

Albino rats of different age-groups and both sexes were exposed to infected mites in cages for a period of about one week. The rats were demitted thereafter and kept in clean cages for further observations.

Blood-smears from the exposed rats were examined at weekly intervals for microfilariae, commencing from the 40th day after exposure to the infection. From the tail of each animal, 2 c.mm. of blood was taken with a graduated pippette and spread on a clean slide to make a thick smear. The air-dried smears were dehaemoglobinised in tap water, fixed in acid alcohol (2 per cent hydrochloric acid in methyl alcohol) and stained with J.S.B. Stain I (Jaswant Singh and Bhattacharji, 1944) for 30 seconds, dried and examined.

The animals were examined during the entire period of patent microfilaraemia and for a few weeks after the peripheral blood became negative. Autopsies were performed after a period of negativity to determine the state of the adult worms. In case any rat died during the period of patent parasitaemia, post-mortem was

resorted to find the adult worms. At autopsy, the thoracic cavity was opened and all visible worms removed carefully with a blunt hooked needle and transferred to petri dishes containing normal saline. The cavity was then flushed with saline solution to find any small worms that might have been missed.

RESULTS.

Eight groups of rats were exposed to infection at different periods of time (Table I). One hundred per cent of the rats in five of the groups became positive for microfilariae.

TABLE I.

Age-wise infection of white rats with L. carinii *through infective mites.*

Group number.	Age of rats at the time of exposure to infection.	Number of rats exposed.	Number of rats positive for microfilariae.	Percentage of rats positive.
1	20 days	30	30	100
2	25 days	20	17	85
3	27 days	6	6	100
4	30 days	20	20	100
5	3 months	4	1	25
6	3 months	8	6	75
7	6 months	2	2	100
8	6 months	8	8	100
	Total ...	98	90	91·8

Of the total of 98 rats exposed to bites of infective mites, 90 (91·8 per cent) showed microfilariae in their peripheral blood.

Table II depicts the course of microfilaraemia in rats of different ages. From Column 5 of Table II, it appeared that there was no correlation between the age

TABLE II.

Course of microfilaraemia in L. carinii *infection of albino rats — by age.*

Age.	Number of animals.	AVERAGE :			
		Prepatent period, in days.	Interval, in weeks, between the first patency and the peak microfilaraemia.	Number of *microfilariae in* 2 c.mm. blood at the peak period.	Duration of patency, in weeks.
20 days	30	60·23	6·5	364·66	17·13 (29)*
25 days	17	65·76	8·23	136·88	15·88 (17)*
27-30 days	26	61·08	6·77	363·3	17·81 (22)*
3 months	7	69·86	11·85	408·0	31·43 (7)*
6 months	10	66·5	7·2	398·8	15·30 (10)*
Total ...	90	62·97	7·40	328·41	18·02 (85)*

* Figures in brackets show the number of animals observed till their peripheral blood became negative for microfilariae.

of the animal and the microfilariae enumerated in 2 c.mm. blood at the peak period. The duration of patency was highest (about double) in animals of 3 months of age, as compared to other age-groups.

The prepatent period for the infection in the white rats was 62·97 days on an average. The highest density of microfilariae was observed on an average at 7·4 weeks from the first day of patency. The average number of microfilariae per 2 c.mm. of tail blood, at the peak period of infection, was 328·41. The average period of patent microfilaraemia worked out to be 18·02 weeks.

The method of "Analysis of Variance" was applied to the data (Table II, Column 6) to determine if the variation in the average patent period in animals of different ages was real or due to chance variation. Table III depicts results of the analysis.

TABLE III.

Results of "Analysis of Variance", applied to data in Column 6 of Table II.

Source of variation.	Degree of freedom.	Sum of squares.	Mean square.	F. ratio.
Between periods ...	4	1,445	361	4·3
Within periods ...	80	7,009	88	..

It was seen that the mean square for variation between periods is highly inflated and does not tend to be equal to the mean square for random variability. The value of the F. ratio exceeded the critical value at 1 per cent level of significance and this indicated a real difference in periods of patency in the different age-groups.

The '*t*' test, applied to the data for different pairs of ages, indicated that the period of patency of 3 months old animals (at exposure to infection) was significantly higher than the corresponding period for animals of other ages.

The course of patent microfilaraemia in white rats of different age-groups are set in Tables IV (*a*) and IV (*b*) :—

TABLE IV (*a*).

Duration, in weeks, of patent microfilaraemia in white rats.

Group number.	Number of animals.	Age at infection.	NUMBER OF RATS PATENT FOR MICROFILARIAE, BY WEEKS :										
			1	4	8	12	16	20	24	28	32	36	40
1	30	20 days	30	29	26	17	8	6	7	5	4	4	1
2	20	25 days	17	17	17	10	7	2	1	..	..	..	..
3	6	27 days	6	6	6	3	3	1	..	..	..	..	..
4	20	30 days	20	20	17	11	11	6	4	4	4	..	..
5	12	3 months	7	7	7	7	5	5	5	5	4	3	1
6	10	6 months	10	10	6	5	4	2	2	2	..	..	..
Total ...	98	..	90	89	79	53	38	22	19	16	12	7	2

TABLE IV (*b*).

Average number of microfilariae per 2 c.mm. blood of white-rats at 4-weekly intervals during the period of patency.

Group number.	Number of animals.	Age.	AVERAGE NUMBER OF MICROFILARIAE, BY WEEKS :										
			1	4	8	12	16	20	24	28	32	36	40
1	30	20 days	15	147	171	172	58	33	34	35	43	37	19
2	20	25 days	24	22	88	85	9	6	1	..	..	..	..
3	6	27 days	2	25	27	12	17	3	..	..	..	..	..
4	20	30 days	13	196	320	150	112	92	11	40	6	..	..
5	12	3 months	3	44	271	162	169	72	179	75	11	5	3
6	10	6 months	8	70	530	562	1,596	1	1	..	..	..	..

From Tables IV (*a*) and IV (*b*) it appeared that the number of microfilariae, per 2 c.mm. of blood, built up sufficiently high by the 4th week of its appearance in the peripheral blood and stayed high for another period of 12 weeks and thereafter gradually declined till the animals became negative for microfilariae.

Table IV (*a*) above indicates how gradually the patent infection in blood of the rats became latent. Out of the 90 rats with patent microfilaraemia, 38 (42·2 per cent) were still patent for microfilariae during the 16th week from the first appearance of microfilariae in their peripheral blood.

Table V below shows that sex did not appear to affect the course of microfilaraemia in the white rats. Male and female rats were equally susceptible to *L. carinii* infection.

TABLE V.

Course of microfilaraemia in L. Carinii *infection of albino rats – by sex.*

Sex.	Number of animals.	AVERAGE :			
		Prepatent period, in days.	Peak of microfilariae, at weeks.	Number of microfilariae in 2 c.mm. blood, at the peak period.	Number of weeks the peripheral blood (2 c.mm.) remained positive for microfilariae.
Male	44	63·14	7·4	301·48	17·3 (43)*
Female	46	62·8	7·39	354·17	18·7 (42)*

*Figures in brackets show the number of animals observed till their peripheral blood became negative for microfilariae.

The animals were allowed to run the full course of patent microfilaraemia. In most cases, they were sacrificed only when their peripheral blood (2 c.mm.) was negative for a number of weeks. Living adult worms were not found in the thoracic cavity of some of the rats (Table VI). Dead and fragmented worms were, however, found in many cases where living worms could not be recovered. In a few cases, after the peripheral blood had become negative, the 2 c.mm. blood samples from the heart at autopsy showed microfilariae.

TABLE VI.

Adult filarial worms recovered from white rats at autopsy.*

Group. number.	Total number of rats autopsied.	Number of rats with living worms only.	Number of rats with dead worms only.	Number of rats with living and dead worms.	Number of rats without worms.	Number of rats whose blood never showed microfilariae.
1	29	11	13	3	2	..
2	20	7	..	1	12	3†
3	6	2	1	..	3	..
4	16	9	..	7	..	..
5	4	1	..	..	3	3
6	8	1	1	3	3	2
7	2	1	..	..	1	..
8	7	6	..	1	..	..
Total	92	38	15	15	24	8

* Includes 6 rats from which worms were recovered at postmortem.

† Includes one rat from whose thoracic cavity, one male and one female worms were recovered alive.

The animals, which harboured living, dead or both living and dead worms in their thoracic cavities, were also positive for microfilariae in their peripheral blood except for one rat which, in spite of having one male and one female living worms, never showed microfilariae in its peripheral blood. Out of the 92 animals autopsied, 68 (73·9 per cent) showed living, dead or both living and dead worms in the thoracic cavities. Out of the 84 rats showing patent microfilaraemia, only 17 rats (20·2 per cent) did not show any adult worms in the thoracic cavities. Had these rats been autopsied during the patent period of microfilaraemia, adult worms would have certainly been recovered. Our experience shows that rats autopsied during the patent period of microfilaraemia, invariably show worms in their thoracic cavities. Eight rats were autopsied during patency and living worms were recovered from the thoracic cavities of all these animals. It was also observed (Table VII) that the peak of microfilaraemia was roughly proportional to the number of adult worms in the pleural cavity in light infections of up to 19 worms.

From Table VII, it appeared that the number of microfilariae was more or less proportional to the number of adult female worms recovered from the animals.

DISCUSSIONS.

A literature review of the attempts (which were mostly unsuccessful and inconsistent) made by various workers to establish the *L. carinii* infection of the cotton rat in the albino rat, a more convenient laboratory animal, has been made by Ramakrishnan *et al.* (1961 *loc. cit.*). The present studies are mainly concerned with the duration of infection and the microfilarial density of *L. carinii* infection in the white rat.

The attempts by Rohde (1959*a*, 1959*b*, 1960) and Ramakrishnan *et al.* (1961 *loc. cit.*) have, however, been consistently successful. Out of the 98 white

TABLE VII.

Total number of microfilariae in 2 c.mm. of tail-blood of white rats at the peak period and the number of adult female worms recovered alive at autopsy.*

Serial number of rats.	Number of living female worms recovered.	Total number of microfilariae in 2 c.mm. blood at the peak period.
30	1	Nil
36	1	2
46	1	58
62	1	237
38	2	31
93	2	40
19	3	12
34	3	54
97	4	45
25	4	82
15	4	436
7	5	71
64	5	447
90	6	332
65	7	244
69	7	251
104	8	80
73	8	565
23	9	111
63	9	208
54	12	306
85	15	298
74	17	415
53	19	325

* Out of 38 rats showing living adult worms only (Table VI), the worms were counted only in 24 rats. The worms in the remaining 14 animals were not counted as the numbers were large and the worms were transplanted intact into normal white rats for other observations, the results of which will be communicated in a separate paper.

rats exposed to the bites of infective mites by the latter workers, 90 rats (91·8 per cent) became positive for microfilariae in the peripheral blood, 49 days to 86 days (average 62·97 days) after exposure to infection (Tables I and II). The 98 white rats reported above include the 54 rats exposed earlier (Ramakrishnan *et al.*, 1961 *loc. cit.*). Among the 44 rats exposed later on, 100 per cent of the exposed animals became infected.

Our strain of mites showed preference for cotton rats over white rats. Colonies of mites, are, however, being maintained on 2 or 3 days old baby mice or white rat. Bertram *et al.* (1946) and Sen and Bhattacharya (1961) also recorded preference of mites in their colonies for the cotton rat rather over the white rat. Scott *et al.* (1947), however, stated that mites could be raised more easily on white rats than on cotton rats. They also stated that the tropical rat mite is more difficult to raise in the laboratory during the summer than during mild winter. We, on the contrary, found it easy to raise mites during the monsoon period (July

to September) but difficult during April, May and June, the hot and dry months. Next to the monsoon period, it was easier to raise mites during early winter.

In the mite-induced infection, the duration of patent microfilaraemia in white rats, after being infected, was studied by Rohde (1960 *loc. cit.*) up to a maximum period of 160 days only. The rats were sacrificed thereafter. Microfilariae could be detected in the peripheral blood in all cases where both sexes of the worms were found. He has also recorded that in two groups a large number of the animals were free from parasites because many of the mites used for transmission had died. This was in agreement with our observation that the percentage of rats infected was low when the mite colony was poor and *vice versa*. As an example, it may be stated that the number of mites was very low when the 4 rats of Group 5 (Table I) were exposed. Only 1, out of these 4 rats, became positive for microfilariae.

The maximum period for which patent microfilaraemia in the peripheral blood was noted in the present studies was 51 weeks in white Rat No. 43* and the minimum period 6 weeks in Rat No. 47. Both the above mentioned animals are from Group I (Table I). The average period of patent microfilaraemia for the 85 rats observed (Table II), up to latency, was 18·02 weeks. The highest number of microfilariae per 2 c.mm. peripheral blood, seen at the peak period, was 2,562 in Rat No. 105 and the lowest 3 in Rat No. 95, belonging to Group 8 and Group 3, respectively (Table I). The average number of microfilariae at the peak period, for all the other 90 positive animals observed, was 328·41 (Table II).

The period of patency of 3 months old animals (at the time of exposure to infection) was significantly higher than the corresponding period for animals of other ages (Table II). It would thus appear that 3 months old animals (at the time of exposure to infection) are more suitable for studies on *L. carinii* infection than animals of other age-groups. The two factors which are not much in favour of the use of three months old animals, however, are (*i*) only 7 out of 12 (58·3 per cent) animals of this age-group (Table I) were positive for infection, and (*ii*) animals more than one month old (at the time of exposure to infection) destroy large number of mites.

In view of the fact that (*i*) 85 to 100 per cent of animals of 20 to 30 days old (at the time of exposure to infection) become positive for infection (Table I), (*ii*) that the mite colonies are not destroyed by animals of young age, and (*iii*) that these animals become adults by the time the infection becomes patent in blood, we prefer to use 20 to 30 days old animals for studies on *L. carinii* infection in albino rat.

Rohde (1960 *loc. cit.*) reported that in all rats, the number of microfilariae in the peripheral blood remained constant or increased during the duration of experiment. Our observations showed that the microfilaraemia gradually rose and

* The rat continued to be positive for microfilariae at the time of writing. The mite-induced infection in this rat has lasted about a year or as long as mite-induced infection lasts in cotton rats which is said to be about a year (Bertram, 1957).

reached the peak in about 7½ weeks and stayed stationary for some time and then gradually declined and the blood became negative in about 18 weeks (Table II).

Rohde (1960 *loc. cit.*) stated that the left thoracic cavity was preferred by the adult worms whereas the right thoracic cavity, as a rule, was invaded only after the grade of infection had become more severe. In our series, it was not so. The right thoracic cavity was more often invaded than the left. In most cases, both the thoracic cavities were invaded, the number of worms being more in the right rather than in the left cavity.

Rohde's (1960 *loc. cit.*) observation that the length of the adult worms (*L. carinii*) in white rats was nearly the same as in cotton rats, was confirmed in our series. In most of the white rats, whose peripheral blood was positive for microfilariae, either living or dead and fragmented or both living and dead adult worms were found even when the autopsies were performed a number of weeks after the peripheral blood of these animals had become negative for microfilariae (Table VI). The dead and fragmented and encapsulated worms were generally found attached to the lower parts of the lungs and to the pericardium.

Bertram (1958) stated that if *L. carinii* infection was transmitted to cotton rats on a single occasion only, the peak of microfilaraemia was roughly proportional to the number of adult worms in the pleural cavity—but only with very light infection of up to 12 to 20 (female) worms. With greater numbers the microfilaraemia ceased to increase proportionately. In the present series, the infection was transmitted to the white rats on a single occasion only. It was seen (Table VII) that the number of microfilariae was more or less proportional to the number of adult worms recovered and that the number of adult worms was also light (less than 20 worms). When the worms in thoracic cavity of an animal were found to be in large numbers, the bunch of worms was removed and used in another experiment. The worms were not counted as too much handling was likely to damage them.

SUMMARY AND CONCLUSIONS.

The prepatent period for microfilarial patency in the peripheral blood was about 63 days after infection. The duration of patent microfilaraemia in the white rat was observed to be about 18 weeks.

The growth rate of filariae in white rats was nearly the same as in cotton rats.

The macrofilariae are found both in the right and left thoracic cavities, the numbers being more in the right than in the left cavity.

All the microfilaria positive rats, autopsied during the period of patent microfilaraemia, showed presence of both male and female adult worms in the thoracic cavity.

After the first appearance of microfilariae in the peripheral blood, their number builds up to an appreciable extent in about a month's time and stays constantly high for about 2 months and then gradually falls.

The period of patency of 3 months old animals (at the time of exposure to infection) was significantly higher than the corresponding period for animals of other ages. There was, however, no correlation between the age of the animal and the microfilariae enumerated in 2 c.mm. blood at the peak period.

Under suitable conditions, 90 to 100 per cent infection of the white rats with *L. carinii* is possible.

Large number of infective mites appear to produce a higher percentage of positive rats than small numbers.

Studies so far carried out show that our strain of the tropical rat mite, *Ornithonyssus bacoti*, thrives better on the cotton rat as compared to the white rat. The mite colony can, however, be maintained on 3 or 4 days old baby white rat or mouse offered every fifth day.

Based on the above mentioned results, the albino rat can be considered to be a suitable laboratory animal for routine screening of possible macro and microfilaricidal agents and for epidemiological studies.

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DDT SUSCEPTIBILITY AND SELECTION OF THE LARVAE OF A LABORATORY-COLONY OF *CULEX GELIDUS* THEOBALD*.

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INTRODUCTION.

VARIOUS degrees of resistance to insecticides have been found to develop in mosquitoes, houseflies and other insects as a result of wide scale use of insecticides against them. Resistance to DDT, dieldrin and BHC in *Culex pipiens fatigans* has been reported from Malaya (Reid, 1955 ; Collins, 1956 ; Wharton, 1958) and to these and other insecticides in other parts of the world (Pal *et al.*, 1952; Liu, 1958 ; Smith, 1958 ; Gentry and Hubert, 1957). *Culex tarsalis* has developed resistance to malathion in California, U.S.A. (Gjullin and Isaak, 1957) and resistance to DDT in Oakridge, U.S.A. (Eddy, Hopkins and Robbins, 1957) while *C. p. molestus* was found to be resistant to DDT in Italy (Mosna, 1948) and *C. p. piens* in Massachusetts, U.S.A. (Armstrong, 1955).

There are, however, no reports of the development of insecticide-resistance in *Culex gelidus* Theobald which is an important vector of Japanese *B* virus encephalitis (Traub, 1957). The only published data on this subject about the species is on the susceptibility of adults to DDT and dieldrin from Malaya (Wharton, 1955) and to DDT from India (Pal, 1958). Both reports indicated that the adults were very susceptible. There are no published data on larval susceptibility levels. The present study was, therefore, undertaken to determine the larval susceptibility of a laboratory-colony of *C. gelidus* to DDT. Attempts were also made to induce DDT resistance by repeated selection of fourth-stage larvae of this colony.

MATERIALS AND METHODS.

Larvae were obtained from a laboratory-colony of *C. gelidus* established in 1956 from a large number of females caught in Kuala Lumpur. The colony has since been maintained without any known contact with insecticides at the Institute for Medical Research. Adult mosquitoes were kept in large cages (2×2×2 feet) with 300-400 per cage, at temperatures between 24—28°C. and relative humidities of 77—88 per cent. Both sexes were given raisins and, in addition, the females received blood meals from guinea-pigs three times a week. Larvae for the initial

* The work reported on here, carried out at the Institute for Medical Research, was done in the course of studies towards the Ph.D. degree in the Department of Zoology, University of Malaya.

selections were obtained from the 39th generation of the colony and susceptibility tests were made with larvae from 37th to 40th generations.

Susceptibility levels were determined from tests on larvae raised from 10 or more egg-rafts (multiple raft tests). The range of variability among individuals of the colony was determined by testing the larvae from individual egg-rafts separately (single raft tests). Larvae for multiple raft tests were reared at a density of about 500 larvae per dish, in large (12×15×3 inches) dishes containing tap water, lightly infused with guinea-pig droppings. Larvae for single raft tests were reared in smaller (10×8×2 inches) dishes. Larval food, consisting of well-ground "Farex" and yeast in a 3 : 1 ratio, was given twice daily. Any scum, formed on the surface of the water, was removed each morning before fresh food was given.

TEST METHODS.

The test methods were those laid down by the World Health Organization (1960) in which batches of 20 larvae were exposed to different concentrations of insecticide in 250 ml. of tap water in 450 ml. porcelain bowls. Larvae used for all tests were early 4th-instar and four days old. Tests were run at temperatures that varied between 24—28°C. Where there was mortality among controls, the results were corrected by using Abott's formula (1925). Larvae which pupated were discarded and the percentage mortality was calculated after adjusting for this.

For selection, the number of larvae was increased to 50 per bowl but this probably had little influence on the rate of mortality. Shidrawi (1957) has shown that with *Aedes aegypti* and *Culex molestus* there was no change in percentage mortality due to large numbers of larvae until 80 or more larvae per bowl were exposed. Larvae, which survived 24 hours exposure to the inseticides, were removed with a fine muslin net. They were washed three times in large quantities of tap water and finally transferred to standard rearing dishes, containing infused tap water and given food normally. Those which pupated were transferred to small bowls of clean water and were placed in adult cages in the insectary in order to establish the next generation. Susceptibility to DDT of the larvae was determined at each generation. The selection dosage was aimed to give about a 50 per cent larval mortality. However, in some generations very few larvae survived and in order to maintain the colony, selection was missed.

The effect of repeated selection in successive generations on the size, fertility and fecundity of the adults was studied. Wing lengths of 10 females each of the normal colony, the control (adults reared from larvae which were starved for 24 hours without contact with insecticide) and those of successive selected generations were measured. The numbers of eggs were counted in each of 10 rafts laid by young females of the above mentioned series after the first blood meal.

ANALYSIS OF DATA.

The regression lines were drawn by plotting the percentage mortality against concentration on logarithmic dosage/probit paper and estimating the LC_{50} values

visually. The goodness of fit of the regression lines to the observed data and the upper and lower confidence limits, with 95 per cent confidence, were calculated by using the simplified methods of Swaroop (1957).

RESULTS.

SUSCEPTIBILITY LEVELS.

The results of tests on larvae, reared from single rafts, are summarised in Table I, which shows that the larvae were highly susceptible to DDT and that the LC_{50} varied from 0·012 to 0·018 p.p.m. Similarly, Table II shows that in the tests on larvae from multiple rafts the LC_{50} varied from 0·011 to 0·014 p.p.m. over several generations. The confidence limits overlap and clearly show that the differences are not significant.

TABLE I.

Susceptibility levels of a laboratory-colony of C. gelidus *to DDT as determined by tests on larvae obtained from single rafts. (The number of larvae tested at each concentration was 40).*

Raft number.	Corrected percentage mortality among larvae after exposure to different strengths of DDT for 24 hours :				LC_{50} (p.p.m.)
	0·01	0·02	0·03	0·04	
	39th generation				
1	37	75	90	95	0·013
2	35	75	90	95	0·013
3	30	70	88	95	0·014
	41st generation				
4	18	60	80	90	0·018
5	45	78	90	..	0·013
6	20	60	80	90	0·017
7	15	55	80	90	0·018
8	28	65	80	90	0·015
9	28	67	85	93	0·015
10	15	58	..	93	0·018
11	28	65	..	90	0·015
12	23	70	90	95	0·016
13	13	58	85	..	0·018
14	28	65	80	90	0·015
	42nd generation				
15	20	65	85	95	0·015
16	23	65	83	95	0·016
17	25	75	..	85	0·014
18	38	80	93	98	0·012
19	28	65	..	90	0·015
20	25	64	82	90	0·016

The multiple raft tests were carried out on different days on larvae from the same generation and also from different generations ; yet these gave very constant results. Further evidence of the absence of variability is indicated by the steep

regression lines in Graph I, which illustrates the data obtained from the multiple raft tests. The constant results obtained in the single rafts test also illustrate the lack of variability of the colony.

TABLE II.

Susceptibility levels of a laboratory colony of C. gelidus *to DDT as determined by tests on larvae reared from multiple rafts.*
(The minimum number of larvae used per concentration was 200.)

DDT concentration in p.p.m.	CORRECTED PERCENTAGE MORTALITY OF LARVAE IN SUCCESSIVE GENERATIONS AFTER 24 HOURS EXPOSURE PERIOD :			
	37th generation.	38th generation.	39th generation.	40th generation.
0·004	5	10	8	8
0·01	35	37	45	32
0·02	70	65	78	75
0·03	87	80	..	..
0·04	..	..	95	95
0·05	94	91	100	..
1·0	100	100	..	..
LC_{50} (p.p.m.)	0·014	0·014	0·011	0·012
95 per cent confidence limits	0·010—0·019	0·011—0·018	0·010—0·012	0·010—0·014

DDT SELECTION.

DDT selection experiments were performed on larvae of the susceptible stock colony of *C. gelidus* over 10 generations. Larvae were obtained from the F_{39} generation whose LC_{50} value was 0·014 p.p.m. Selection dosages varied from 0·01 – 0·025 p.p.m., giving mortalities ranging from 41-65 per cent.

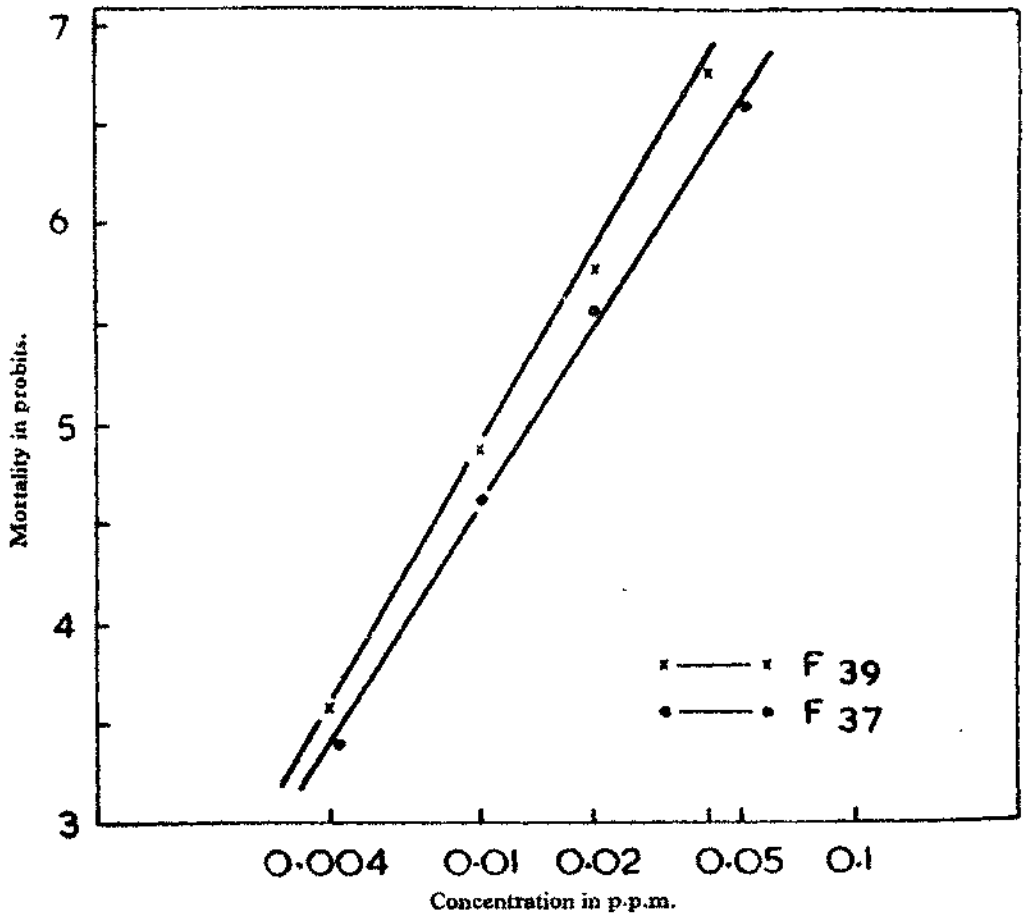
TABLE III.

DDT susceptibility levels of selected C. gelidus *larvae over successive generations.*
(The minimum number of larvae used per concentration was 160.)

Generation.	CORRECTED PERCENTAGE MORTALITY BY VARIOUS CONCENTRATIONS OF DDT (p.p.m.) AFTER 24 HOURS.						LC_{50} (p.p.m.).	95 per cent confidence limits.
	0·004	0·01	0·02	0·03	0·04	0·05		
F. 1	10	37	65	80	..	91	0·014	0·011—0·018
F. 2	6	31	63	77	86	..	0·016	0·014—0·018
F. 3	5	22	47	58	..	77	0·023	0·018—0·029
F. 4	..	18	47	69	90	..	0·020	0.014—0·023
F. 5	..	4	30	56	..	92	0·026	0·023—0·029
F. 6	..	11	45	78	91	..	0·020	0·018—0·023
F. 7	..	..	..	..	..	..	..	..
F. 8	..	10	61	89	100	..	0·018	0·016—0·020
F. 9	..	15	45	70	85	..	0·021	0·019—0·024
F. 10	..	12	61	90	100	..	0·017	0·016—0·019

GRAPH 1.

Dosage mortality regression lines for early fourth-instar larvae of 37th and 39th generation of a laboratory colony of Culex gelidus *to DDT.*



The percentage mortality of the larvae in successive generations by selection dosages and their LC_{50}'s are depicted in Table III, which shows that these selections did not produce any significant DDT resistance in the larvae. The maximum LC_{50} (0·026 p.p.m.) was reached in the 5th generation and this is only about twice the parental LC_{50} value. The LC_{50}, however, was 0·018 p.p.m. in F_8 and 0·017 p.p.m. in F_{10} which is of the same order as the highest LC_{50} recorded in single raft tests. The slight increase in LC_{50} was apparently the result of elimination of the more susceptible members of the population.

EFFECT OF DDT SELECTION ON THE POST-SELECTION DEVELOPMENT OF THE SURVIVORS.

Table IV depicts the percentage of larvae that survived the DDT selections and those which died later as larvae, pupae or adults. The post-treatment larval

mortality varied from 14 to 46 per cent, compared with an average of 3 per cent among the controls. The mortality was between 8 and 37 per cent among the pupae and 3 to 17 per cent among the adults of the selected strain, compared with 1 and 2 per cent among the pupae and adults of the controls. It is thus clear that there was a high post-selection mortality due to exposure to DDT, which was most pronounced in the larval stage.

TABLE IV.

Effect of exposure of C. gelidus *larvae to DDT on the subsequent larval, pupal and adult mortality.*

Generation.	LC_{50} (p.p.m.)	DDT dose used for selection.	Number of larvae used.	Percentage of larvae killed during each test.	Percentage of surviving larvae which died as:			Percentage of adults which left water.
					Larvae.	Pupae.	Adults.	
F. 1	0·014	0·01	1,500	43·3	21·1	14·1	5·3	59·5
F. 2	0·016	0·02	500	46·1	14·4	23·3	3·7	58·5
F. 3	0·023	0·025	1,000	58·7	34·6	13·3	2·9	49·1
F. 4	0·020	0·02	1,500	50·5	20·2	20·9	14·5	44·3
F. 5	0·026	0·025	1,500	41·7	22·6	16·2	6·5	54·9
F. 6	0·020	0·029	2,000	55·0	37·7	37·3	14·0	10·9
F. 7	..	..	..	..	..	..	..	..
F. 8	0·018	0·02	3,500	50·4	47·1	7·8	5·2	40·8
F. 9	0·021	0·02	3,000	45·4	24·3	16·1	17·0	42·4
F. 10	0·017	0·02	2,500	65·4	45·0	13·0	3·5	38·4
Control (mean)	..	..	100	1·0	3·0	1·0	2·0	93·0

EFFECT OF DDT SELECTIONS ON THE SIZE, FECUNDITY AND VIABILITY OF SURVIVING ADULTS.

Adults, which emerged from the survivors of DDT selection, were smaller than the parents. Data on wing lengths, and on the number of eggs per raft from 10 adults of various strains, are stated in Table V. The mean of these are shown in Graphs 2 and 3.

The mean wing length of the normally reared adults (3·16±0·03 mm.) was significantly greater ($P<0·001$) than that of DDT selected F_1 individuals (2·77± 0·05 mm.) (Graph 2). After the initial selection, a new low level was attained and later selections brought about only a slight variation. Thus in F_{10} generation, the mean was 2·70±0·05 mm., compared with 2·77±0·05 mm. of the F_1 generation and 3·16±0·03 mm. of the parents (Table V). Similarly the mean number of eggs, obtained from normal adults (225±11·06), was also significantly greater ($P<0·001$) than those from the selected F_1 individuals (148±6·53). The mean obtained for the subsequent generations of DDT selected individuals, however, showed only slight fluctuations (Graph 3).

In the 5th generation, the colony was allowed to continue without selection and the size and fecundity of the adults increased in the following generation. At the same time, fourth-stage larvae were starved for 24 hours but without DDT

selection and the adults which developed were also of normal size and fecundity. Similar results were also obtained on the F_2 generation (Table V). Thus the observed reduction in size and fecundity must have been due to exposure of the larvae to DDT.

TABLE V.

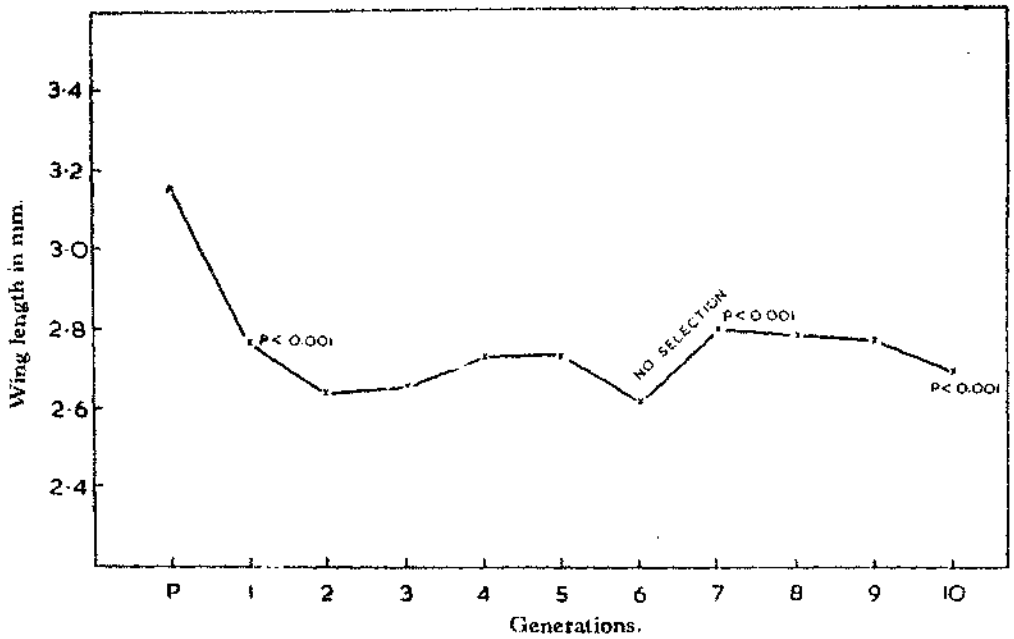
Effect of DDT larval selections on the size and fecundity of adults of C. gelidus.

Generation.	Mean wing lengths (10 females):		Mean Number of Eggs (10 rafts):	
	Mean in mm.	Coefficient of variance.	Mean.	Coefficient of variance.
Normal	3·16±0·03	2·85 per cent.	225±11·06	15·6 per cent.
Control	..	..	222± 7·91	11·3 per cent.
F. 1	2·77±0·05	5·41 per cent.	148± 6·53	14·0 per cent.
F. 2	2·64±0·08	10·00 per cent.	123± 8·76	22·5 per cent.
F. 3	2·66±0·08	9·85 per cent.	111±12·03	34·4 per cent.
F. 4	2·74±0·05	6·31 per cent.	115± 6·11	16·8 per cent.
F. 5	2·74±0·02	1·97 per cent.	121±11·77	30·8 per cent.
*F. 5u	2·93±0·02	2·30 per cent.	181± 8·30	14·5 per cent.
†F. 5s	..	..	199± 9·72	15·4 per cent.
F. 6	2·61±0·02	2·97 per cent.	110±10·06	28·9 per cent.
*F. 7u	2·81±0·03	3·42 per cent.	195± 7·92	12·5 per cent.
F. 8	2·79±0·04	4·66 per cent.	119± 6·52	17·3 per cent.
F. 9	2·78±0·05	5·21 per cent.	135± 8·00	21·1 per cent.
F. 10	2·70±0·05	5·09 per cent.	..	..

*These adults were reared normally from 4th-instar larvae without selection or starvation
†These adults were reared from larvae which were starved for 24 hours without DDT selection.

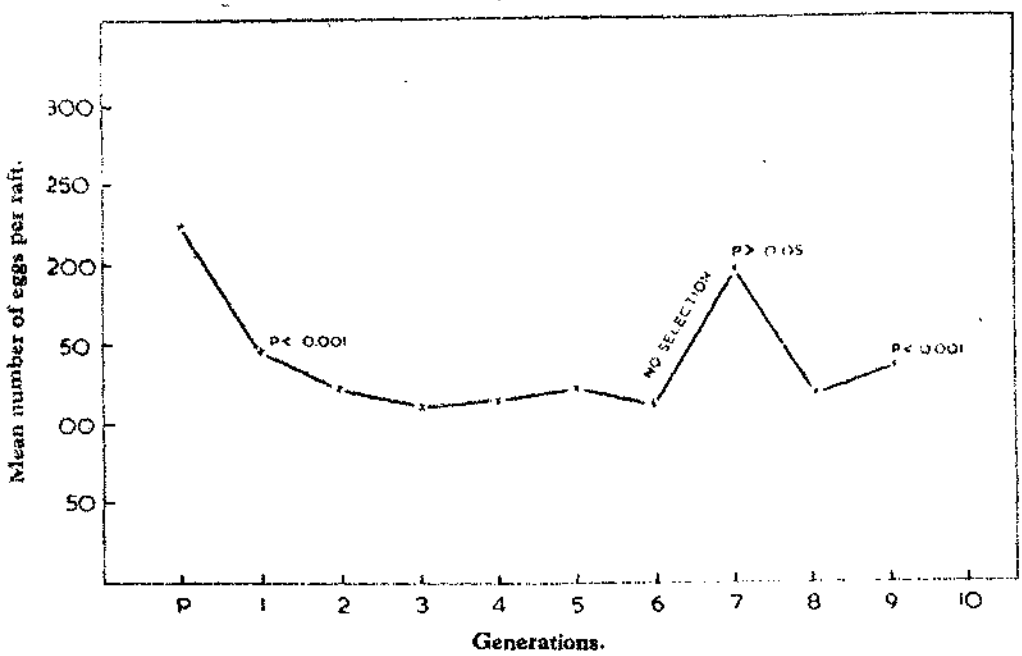
GRAPH 2.

Mean wing lengths (in mm.) of 10 parental females and of subsequent generations selected with DDT.



GRAPH 3.

Mean number of eggs collected from 10 young females of unselected parent and subsequent selected generation.



EFFECT OF DDT SELECTION ON THE LARVAE OF THE SUCCEEDING GENERATION.

The DDT larval selections in successive generations of C. *gelidus* produced an increased mortality among the 4th-instar larvae of the succeeding generation. This mortality was first noticed among the larvae of the F_3 generation which were never exposed to DDT but were reared normally from eggs laid by F_2 females which emerged from larvae exposed to DDT. It was observed that the mortality rate of untreated 4th-stage lavae increased after each selection generation until it reached enormous proportions in the F_7 generation, in which most of the larvae died. There were insufficient numbers for selection and it was, therefore, necessary to miss selection in this generation. The few available healthy larvae were reared normally without selection and there was no excessive mortality in the next generation.

The possibilities of contamination, and of defective feeding or rearing, were thoroughly investigated and eliminated. Batches of larvae of different ages and instars, from the normal laboratory-colony, were transferred into the same rearing dish and the same water (after removing the dead larvae) and given the same food. These larvae grew well and pupated normally without exhibiting any noticeable mortality among the 4th-instar larvae. It was, therefore, concluded that the high mortality among the larvae was due to DDT selection of their parents.

DISCUSSION.

C. gelidus is a difficult mosquito to colonise and the establishment of a colony involves a high degree of selection. Originally a very large number of adults were caught in Kuala Lumpur (Reid, 1957) and, due to the difficulties in colonising under normal laboratory conditions, F_1 and F_2 generations were reared in large ($6 \times 6 \times 7$ feet) "walk-in" cages. Even under these conditions, only a few adults survived and laid eggs. Later generations were reared in smaller ($2 \times 2 \times 2$ feet) cages. Only a very small proportion of *C. gelidus*, collected from the wild population, therefore, appear to be adaptable to laboratory colonisation. This results in a population which most probably is not a true representative of the wild population. This may apply to all colonies but more so in such species as *C. gelidus* which is difficult to colonise. As a result of the high mortality, much of the genetic variability is lost and its potential is considerably reduced. This may account for the constant LC_{50} values obtained and the failure to develop resistance in spite of repeated DDT selection.

The stock colony has been kept for more than 4 years over 35 generations. Each generation has been maintained by individuals derived from a few (5—6) egg-rafts. Therefore, some degree of inbreeding is inevitable. This again leads to homogeneity which is indicated by the constant LC_{50} values obtained from multiple and single raft tests over several generations. Similar tests on a laboratory colony of *C. fatigans* showed a high degree of variability in DDT larval susceptibility (Thomas, *unpublished data*). It has been suggested that the deciding factor in developing resistance is the potential genetic variability (Busvine and Harrison, 1953 ; King, 1954) and the lack of genetic potential would explain why the stock colony of *C. gelidus* failed to develop resistance.

Most confirmed cases of insecticide resistance have been detected in the field ; *Anopheles stephensi* developed high DDT resistance in the field but laboratory-colonies failed to develop DDT resistance (Brown, 1958). Larval selections of a laboratory-colony of *Aedes aegypti* induced only very slight DDT resistance ($\times 1{\cdot}8$), compared with a maximum of 13 times in a naturally resistant Malayan strain (Shidrawi, 1957). Exposure of laboratory stock colonies of inbred populations of *Drosophila melanogaster* to DDT failed to produce resistance whereas another stock which was not inbred developed DDT resistance (Merrell and Underhill, 1956). Similar observations have also been made in Japan by Oshima (1958) on the same species. Thus the failure to develop DDT resistance in the stock colony of *C. gelidus*, which was probably due to lack of genetic variability, does not indicate that wild populations of *C. gelidus* will not develop resistance.

Another point of interest is the delayed effect of DDT selection on the mortality rate of the survivors either as larvae, pupae or adults. Similar effects were noticed in *C. molestus* and *Aedes aegypti* after insecticidal selections on their larvae (Shidrawi, 1957). The adults showed reduced fecundity and a decrease in size (as indicated by wing lengths) as a result of larval DDT selection. In addition,

the effects of selection on the parents were carried over to the progeny for a single generation. Though the progeny were never exposed to DDT, these larvae died as 4th-stage in increasing proportions. Since these selected populations did not become DDT resistant, these features are not correlated with DDT resistance but were the after effects of larval DDT selection which disappeared as soon as selection ceased.

SUMMARY.

The larvae of a laboratory-colony of *C. gelidus* were found to be highly susceptible to DDT and had an LC_{50} of about 0·01 p.p.m. and there was little variation in the susceptibility from generation to generation or among individuals of a generation.

Attempts were made to induce DDT resistance in the larvae of the laboratory-colony of *C. gelidus*. It was found that selection with DDT at about the LC_{50} value for 10 generations produced only a two-fold increase in tolerance.

The effects of repeated DDT selections on fecundity, wing-lengths and survival rates were also studied. Selection with DDT increased the mortality and suppressed the subsequent development of the larvae and lowered the fecundity of the adults. The adults, which emerged, were found to be significantly smaller and laid smaller egg rafts than normal females.

ACKNOWLEDGEMENTS.

I wish to thank Dr. J.A. Reid and Dr. R.H. Wharton of the Institute for Medical Research, Kuala Lumpur, for providing laboratory facilities and for their constant guidance. I would also like to thank Professor J.R. Hendrickson, Dr. S.S. Dhaliwal and Mr. J.A. Bullock, Department of Zoology, University of Malaya, for their valuable suggestions and criticism. My thanks are also due to Mr. G.I.M. Martin of the College of Agriculture, Malaya, for providing facilities at the college.

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ANNOUNCEMENT

INDIAN COUNCIL OF MEDICAL RESEARCH.

(Colonel Amir Chand Trust Fund Prizes for Medical Research)

LIEUT.-COLONEL AMIR CHAND, lately Principal of Lady Hardinge Medical College, New Delhi, donated Rs. 50,000 to the Indian Council of Medical Research for creating a Prize Fund. From the interest earned by this sum, prizes are awarded for the best published research work in medical sciences. The Governing Body of the Council has constituted a Trust called the "COLONEL AMIR CHAND TRUST" for the administration and management of the Fund.

The prizes are awarded annually on an all-India basis for the best published research work in any subject in the field of medical sciences including clinical research. The term 'Clinical Research' covers research into the mechanism and causation of disease and its prevention and cure, and includes work on patients in hospitals, field studies in epidemiology and social medicine and observations in general practice.

It has been decided to award four prizes in 1962, each of Rs. 300 to Indian nationals of not more than 40 years of age on 1st January, 1962, for the best research papers in medical sciences published by them during 1961 in Indian or Foreign Journals as a result of work done in any Institution in India. These prizes will be known as 'SHAKUNTALA AMIR CHAND PRIZES'.

Those eligible for the prizes are MEDICAL OR NON-MEDICAL GRADUATES.

Selections for the award of the prizes will be made by a Selection Board.

In the case of a joint authorship of a publication, the prize shall be divided between the authors in such proportion as the Selection Board may decide. The role of the person who applies for the prize should be clearly indicated so as to make it easy to determine whether the major part of the work has been done by that person.

The award of the prizes will be announced at the meetings of the Council's Advisory Committees in November/December, 1962.

CANDIDATES for award of prizes are requried to submit TEN REPRINTS of their papers published during 1961. These should be sent to the DIRECTOR, INDIAN COUNCIL OF MEDICAL RESEARCH, P.O. BOX 494, NEW DELHI, so as to reach him NOT LATER THAN 1st SEPTEMBER, 1962. The PAPERS should be accompanied by a short biographical sketch and two copies of passport-size photographs of the candidate/candidates concerned.

Indian Journal of Malariology, **16**, 3, *September 1962*.

A PRE-MONSOONIC OUTBREAK OF MALARIA IN THE KHODIAR DAM SITE LABOUR COLONY, AMRELI DISTRICT, SAURASHTRA (GUJARAT STATE), INDIA.

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[February 19, 1962.]

A DAM is under construction across the River Setrunji, 3 miles away from the village, Dhari, about 24 miles to the east of Amreli and bordering the notoriously malarious Gir Forest in Saurashtra. This is meant to serve as a reservoir for feeding the irrigation system and has a second small receiving dam and tank about 12 miles downstream. This is called "Khodiar Dam", because of its location near Khodiar Mataji's (a Goddess) temple on the bank of this river. River Setrunji begins from the north-eastern part of Gir Hills, near the Village Dalkhania of Dhari Taluka and drains in the Gulf of Cambay near Talaja Village of Bhavnagar District. The soil is rocky. In summer, many small pools are formed in the bed of this river. Annual rainfall in this area is about 23 to 24 inches, mainly between June to September-October.

The construction of the dam commenced in December, 1959. The labour was drawn from Kutch and Ahmedabad districts and all the five districts of Saurashtra as well as from Rajasthan and Bombay. The labourers were housed in quarters made of galvanised iron sheets, built in a very irregular fashion, along the banks of the river. The labour strength at the beginning was 800, and reached up to 1,600 in December, 1960. The population came down to 922 in May, 1961, because many labourers had left due to an outbreak of malaria as well as smallpox in the colony. Details regarding the malaria outbreak are presented in this paper.

Prior to the institution of malaria control in 1952-53, the child spleen rate in the village (Dhari) was 6 per cent. This came down to 0·77 per cent in 1958-59 as a result of the control operations.

For the last 6 years, two rounds of DDT indoor residual spray have been carried out annually in this district to cover the malaria transmission season which was from July to September. The first round extended from June to middle of August and the second round from middle of August to October. In 1960-61, however, it was observed that transmission persisted longer in areas adjoining the

Gir Forest area where a third round of DDT was being carried out in November-December. As a measure of caution, therefore, a third round of DDT spray was similarly applied in the talukas of Jafrabad, Dhari, Rajula and Khambha in February, 1961. The Khodiar Dam site was also sprayed for the third time on February 6, 1961. The labour quarters at the dam site thus received three rounds of DDT during the year 1960-61, i.e., on June 18 and September 6, 1960, and February 6, 1961, respectively.

HISTORY OF THE PRESENT OUTBREAK OF MALARIA.

In the course of routine active surveillance in the colony, 10 blood smears were collected from fever cases on April 14, 1961, of which one turned out to be microscopically positive for malaria. During investigations of contacts of this case, twelve more smears were collected on April 24, two of these were found to be positive for plasmodia. A more detailed house-to-house survey, between April 24 and 27, yielded many malaria cases. A mass survey of all the residents of the dam site was organised from May 12, and mass treatment given with 600 mg*. chloroquine and 25 mg* daraprim. A weekly analysis of the recorded cases is shown in Table I.

Of the 774 fever cases examined during this outbreak, 135 were positive for malaria, of which epidemiological history could be obtained for 111. These latter cases were radically treated with an additional single dose of nivaquine (600 mg. base per adult) and primaquine for 5 days (14 mg./ day per adult and correspondingly lower doses for children). Those under 2 years were not given primaquine. The remaining 24 cases could not be treated immediately as their whereabouts could not be traced. Those who were later traced at their destination, were given radical treatment by the concerned malaria medical officers.

An attempt was made to get the chronological incidence of the first 77 malaria positive cases as they occurred. The first attack of fever in all these cases was after April 6, 1961. The first three cases were from the same village (Talgajarda) in Amreli Unit (Mahua Taluka), but the subsequent cases were among the persons drawn from all the districts mentioned above. It is not established that malaria has been completely eradicated from this area and the source of infection was in all probability indigenous. An analysis of these 77 cases, by age-groups, is depicted in Table II.

History of surveillance, prior to this outbreak, suggested that this was only of recent origin. The active surveillance between September, 1960, and early April, 1961, had revealed only one parasite-positive case out of 58 blood smears examined during the period. This was a case of *P. falciparum* recorded in the latter half of December, 1960.

Similar house-to-house surveillance in April-May, 1961, in 16 neighbouring villages did not yield any malaria-positive smear among the 27 smears collected

* Adult dose. Children were given proportionately smaller doses. Infants were excluded from treatment with daraprim.

TABLE I.

Result of blood-smears collected from Khodiar Dam area in Dhari Taluka of Amreli District, Gujarat State.

Week commencing.	Total smears.	Total positive.	Per cent positive.	Classification of positives*: Sporadic.	Indigenous	Imported.	Not treated.	Not traceable.	Gone to known destination.	Species of plasmodia: *P. vivax*	*P. falciparum.*	*P. malariae.*	Mixed.
April 4, 1961	12	1	8·33	..	1	..	..	..	..	..	..	..	1
April 21, 1961	60	20	33·33	2	16	..	..	..	2	12	8	..	..
April 28, 1961	74	35	47·3	4	20	2	..	4	5	22	13	..	..
May 6, 1961	296	35	11·8	5	21	1	2	2	4	29	6	..	..
May 13, 1961	281	26	9·3	3	20	..	2	1	..	24	1	..	1
May 20, 1961	51	18	33·4	1	15	..	2	..	..	18	..	..	..
Total	774	135	17·5	15	93	3	6	7	11	105	28	..	2

*There were no cases under the other group, *viz.*, Introduced and Induced cases.

TABLE II.

Analysis of the first 77 cases, by age-groups, carried out between April 14 and May 16, 1961.

	Age-group: 1 to 5 years.	5 to 15 years.	Above 15 years.	Total.
Radically treated	8	13	41	62
Untreated or could not be traced	2	4	9	15
Total	10	17	50	77
Indigenous	8	10	36	54
Imported	..	1	1	2
Induced	..	..	..	..
Introduced	..	..	..	..
Sporadic	..	2	4	6
Not investigated	..	..	..	15
Total	8	13	41	77
P. falciparum	5	3	17	25
P. vivax	4	14	33	51
P. malariae	..	..	..	..
Mixed infection	1	..	..	1
Total	10	17	50	77

from fever cases. This fact clearly indicated that the outbreak and transmission were stricty localised and restricted to the dam-site labour colony. Government servants' quarters, situated ½ mile away on the opposite bank on an elevated and a more breezy area, did not yield any case of malaria.

Report of a similar outbreak of malaria in another locality, Bhadar Dam construction site this year and at Setrunji Dam (near Palitana) during a previous year (both about 30–40 miles to north and east respectively), supported the view that the outbreak of malaria was indigenous.

Epidemiological investigations of the parasite-positive cases in the great majority of cases also showed that they were indigenous, and that autochthonous transmission was in progress. The Malaria Medical Officer, N.M.E.P.* Unit, Amreli, carried out a round of DDT indoor residual spray (100 mg./sq. foot) in all the quarters on the dam-site and field-houses in the vicinity on May 3 and 4, 1961.

ENTOMOLOGICAL INVESTIGATIONS.

An entomological survey was carried out prior to the insecticidal spray on May 3 and 4, 1961. The following species of mosquitoes were collected between 06·45 and 10·15 hours :—

Name of species.	Total time spent. Hrs. Mts.	Density per man-hour.
A. turkhudi	3—20	0·6
A. stephensi	3—20	0·9
A. subpictus	3—20	0·6
Total anophelines	3—20	2·1
Total culicines	3—20	0·6

The suspcted vectors (*A. culicifacies* and *A. fluviatilis*) were not encountered.

Hourly night collections were made between 20-30 and 00-30 hours on May 12, 1961 and between 18-30 and 22-30 hours on May 13, 1961. The collections did not yield any specimen of the suspected vector species.

On May 16, 1961, the first two authors made a special attempt to collect mosquitoes with two Insect Collectors. The results were gratifying. The following mosquitoes were collected from newly constructed and still unsprayed structures at the farthest end of the labour colony near the Khodiar Mataji's temple :

Species.	Time spent. Hrs. Mts.	Density per man-hour.
A. culicifacies	8—0	1·9
A. stephensi	8—0	1·1
A. turkhudi	8—0	0·6
Culex fatigans	8—0	4·1

The unsprayed structures were thereafter sprayed with DDT.

* National Malaria Eradication Programme.

The *A. culicifacies* (8 females) and *A. stephensi* (5 females) were dissected on the same evening. One specimen of *A. culicifacies* showed gland infection with sporozoites. This established autochthonous transmission beyond all doubt. This is the first time when a vector has been incriminated in Saurashtra.

The results of entomological collection along the river side in another place, Vekaria Para (of Dhari Town) on the next day (May 17, 1961) led to the collection of the species mentioned below. Pools formed in the river and one unused well showed breeding of anophelines.

SPECIES.	TOTAL TIME SPENT.	DENSITY PER MAN-HOUR.
	Hrs. Mts.	
A. culicifacies	4—0	2·7
A. stephensi	4—0	1·5
A. turkhudi	4—0	0·2
A. subpictus	4—0	0·5

Similarly, collections made at night at Kudba Village on May 18, 1961, for 10 minutes every hour between 18·00 and 12·00 hours, gave six specimens of *A. culicifacies* (♀♀) and one specimen of *A. stephensi* (♀♀). But in neither of these two places was there any incidence of fever, though *A. culicifacies* densities were more or less similar as at the colony on the dam site.

DISCUSSION.

Tropical aggregation of labour is one of the important factors in the genesis of epidemic diseases, particularly malaria. The development plans in the country, of which the multipurpose river valley projects have assumed an important place, attract large labour forces from widely scattered areas and often bring with them different strains of malaria parasite. The labour are generally housed under unsatisfactory condition and the river-bed pools provide ideal breeding facilities for the vector mosquitoes. The factors necessary for the establishment of autochthonous transmission thus exist in such places, and in the absence of suitable measures to interrupt transmission, an epidemic is inevitable.

Dispensary figures of Dhari (a place 2 miles away from the Khodiar Dam site), for the previous two years, are contained in Table III. A scrutiny thereof shows that there was a high incidence of malaria during some months of 1959, indicating a failure of the measures to check malaria transmission in that year.

The outbreak of malaria in this colony has been effectively brought under control by timely residual DDT spray, followed by effective surveillance. Mass blood survey was also carried out and positive cases given radical treatment as required under the National Malaria Eradication Programme.

TABLE III.

Dispensary figures of Dhari Village.

Month.	Year :					
	1959 :		1960* :		1961 :	
	All cases.	Malaria only.	All cases.	Malaria only.	All cases.	Malaria only.
Jaunary ...	787	63	945	22	1305	5
February ...	878	74	1070	17	954	5
March ...	936	110	1164	9	970	1
April ...	1045	92	1039	7	1106	4
May ...	930	47	1065	6	..	..
June ...	1050	66	1092	6	..	..
July ...	1239	119	1305	8	..	..
August ...	1262	166	1285	21	..	..
September ...	1362	79	1282	26	..	..
October ...	1091	79	930	13	..	..
November ...	1003	82	989	28	..	..
December ...	1024	42	1241	18	..	..

* Active surveillance has been established since September, 1960.

The following factors seem to have come into play in establishing unchecked autochthonous transmission leading to an outbreak of malaria in the labour-colony at Khodiar Dam site :—

(*i*) After the last round of DDT spray in 1960-61, many new labour structures were constructed and these were not protected by DDT. Material, dismantled from old sheds at the old site, is being used for making fresh shelters at new site. This practice is very common in such labour aggregations. In many such cases, DDT sprayed surfaces of sheets are turned inside out while erecting new structures.

(*ii*) The people's habit of sleeping out in open air in the hot summer months, probably makes effective transmission possible without the vectors ever coming in contact with the DDT sprayed surfaces.

(*iii*) Aggregation of labour from various parts of Gujarat, Rajasthan and Bombay appears to have introduced the reservoir of infection. The adjoining Gir Forest area is still notorious for its persistant transmission, and the incidence of malaria, in the labour colonies established for road construction there, has also been detected.

(*iv*) Pool formation in the river-bed formed suitable breeding grounds for the vector, *A. culicifacies*. Such low density of *A. culicifacies* was similarly observed in other adjoining villages also. The relative absence of any cattle population, helped to increase the man-mosquito contact, and *A. culicifacies*, though in low density, appears to have been effectively transmitting malaria in this colony. In view of the small numbers in which this species was available, no analysis of their blood-meals was undertaken to determine the anthropophilic

index. The finding of one gland-positive *A. culicifacies*, out of 8 dissected, makes one think of the likelihood of this species having turned highly anthropophilic, though *A. culicifacies* is known to be a highly zoophilic species.

SUMMARY.

An outbreak of malaria, due to autochthonous transmission, in a labour colony at Khodiar Dam site on River Setrunji in Dhari Taluka of Amreli District in Saurashtra Region of Gujarat State is described and its epidemiology discussed.

ACKNOWLEDGEMENT.

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Indian Journal of Malariology, **16**, 3, September 1962.

EFFECTS OF WIND AND ENVIRONMENTAL TEMPERATURE ON THE DISSIPATION OF MALATHION RESIDUES*.

BY

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[May 18, 1962.]

A VARIETY of factors influence disappearance of insecticidal residues from treated surfaces. The physical and chemical properties of the insecticide are obviously important. The characteristics of the substrate supporting the residue, together with a group of processes called weathering, generally determine the fate of a residue. Combinations of "weathering" factors have been isolated from the substrate effect in some investigations. Hopkins *et al.* (1952), Teotia and Dahm (1950), and Gaines and Dean (1949, 1950) investigated the effects of various combinations of temperature, humidity and sunlight on certain insecticidal residues on plant and artificial surfaces. This paper presents the first results of an attempt to simulate and control certain weathering factors in order to estimate the magnitudes of their independent effects on the dissipation of a pesticidal residue.

MATERIALS AND METHODS.

Glass plates 10×10 cm. were used as the inert substrate for residues to deposit technical malathion. Carbon tetrachloride solutions of malathion were carefully evaporated on one surface of the glass plate in order to provide a uniform film. After a predetermined exposure to a specific factor or combination of factors, the plates were thoroughly washed with carbon tetrachloride and the amount of malathion determined colorimetrically by the method of Norris *et al.* (1954). In some experiments aliquots of the washings were taken for chromatographic evaluation by the methods of Cook (1955), March *et al.* (1954) and Kaplanis (1954) for the presence of compounds derived from malathion.

*Contribution No. 768, Department of Entomology, Kansas Agricultural Experiment Station, Manhatten. Supported in part by funds from the NC-33 Regional Technical Committee on Pesticide Residues on or in food, feed, and forage crops (Their magnitude, character and persistence).

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‡Assistant Professor, and Professor of Entomology, respectively.

The factors investigated were air temperature, wind velocity and the amount of the inital malathion deposit. A small-scale, closed-circuit, wind tunnel with provisions for controlling and measuring the air speed and temperature was utilized. Its general characteristics are shown in Figure I.

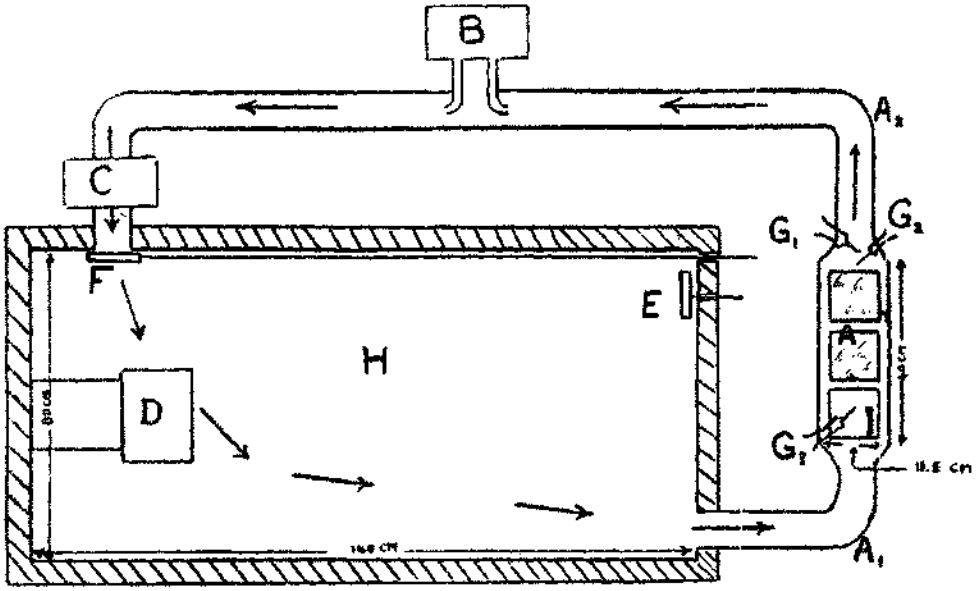

FIG. 1. **Wind tunnel sketch.** A_1=prechamber ; A_2=postchamber ; A=test chamber ; B=velometer ; C=circulation fan ; D=heater ; E=thermostat ; F=manual air velocity control ; H=constant temperature air reservoir ; I=glass plate ; G_1=thermocouple—wet bulb ; G_2=thermocouple—dry bulb ; G_3=thermocouple—temperature at bottom.

The glass plates were oriented in the air stream so that the treated surfaces were parallel to the flow of the air stream. All light was excluded from the exposure chamber. Three separate plates were run for each level of deposit in each experiment and each experiment was replicated at least three times. The relative humidity was not controlled and ranged from 40 to 80 per cent.

RESULTS AND DISCUSSION.

The results from the various experiments and replicates are averaged and summarized in Tables I and II. The effects of air movement were very pronounced during the first 24 hours ; the differences between the 6 and the 17 m.p.h. velocities were not so pronounced as those between the still-air condition and the 6 m.p.h. air movement. The initial amount of residue did not appear to have a major effect upon dissipation until it approached 100 per cent.

Under still-air conditions, the amounts dissipated during the first and second 24 hour periods were of the same magnitude (Table I).

The effects of temperature (Table II) under still-air conditions were very pronounced. An increase of 16°C. in the temperature doubled or tripled the

amount of loss in 24 hours. The combined action of rate of maximum air movement and relatively high temperatures, *i.e.*, 17 m.p.h. at 43°C., resulted in complete dissipation of residues at the one mg. per plate level and in nearly complete dissipation at twice that initial deposit.

TABLE I.

Effects of rate of air movement at 27°C. on the dissipation of malathion residues from glass plates.

Air speed.	PERCENTAGE OF RESIDUE LOST DURING EXPOSURE PERIODS : AIR MOVEMENT IN M.P.H.							
	0		6		11		17	
Initial residue, mg./plate (100 cm.²).	1	2	1	2	1	2	1	2
Exposure period (Hours)								
4	10	5	14	9	11	12	37	29
8	13	7	31	17	70	44	77	51
24	15	10	72	73	79	66	84	69
48	38	17	87	77	89	79	90	80

TABLE II.

Effects of temperature and rate of air movement on the dissipation of malathion residues from glass plates.

Air speed.	PERCENTAGE OF RESIDUE LOST DURING EXPOSURE PERIODS : AIR MOVEMENT IN M.P.H.							
	0				17			
Temperture	27°C.		43°C.		27°C.		43°C.	
Initial residue, mg./plate (100 cm.²).	1	2	1	2	1	2	1	2
Exposure period (Hours)								
4	10	5	29	15	37	20	86	77
8	13	7	33	18	77	51	92	84
24	15	10	44	26	84	69	100	96

Only malathion was detected in the residue at the end of the experimental periods. The chromatographic techniques employed for these analyses would be expected to detect the presence of malaoxon but could not be expected to indicate presence of more completely hydrolyzed or oxidized products.

SUMMARY.

Under still-air conditions, malathion residues on glass plates were rapidly dissipated at 43°C. Air moving at 6 m.p.h. at 27°C. dissipated 72 per cent of the residue in 24 hours in contrast with a 15 per cent dissipation in equal time under still conditions. An increase of velocity to 17 m.p.h. increased the dissipation to approximately 85 per cent in 24 hours. Amount of initial residue appeared to play a minor role in percentage until dissipation was nearly completed.

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A NOTE ON LABORATORY CULTURING OF BED-BUGS.

BY

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[June 6, 1962.]

SEVERAL methods have been used by different workers for maintaining laboratory cultures of bed-bugs for studies in toxicology and medical entomology (Adkins and Arant, 1959 ; Wattal and Kalra, 1961). The technique described by Adkins and Arant (1959 *loc. cit.*) was initially adopted in this laboratory. The method, however, proved to be cumbersome and great inconvenience was experienced in handling the various chambers. The bed-bugs were also able to crawl even over the well furbished inner walls of both the feeding and the rearing glass chambers. Attempts were, therefore, made to counteract these difficulties. The present note describes a modified technique for maintaining a laboratory colony of the bed-bug, *Cimex lectularius* Linn. on rabbits' blood.

CULTURING PROCEDURE.

Approximately 600-800 bed-bugs, including all the stages of the insect, were sorted out into three batches, *viz.*, the adults, the fourth to fifth instar nymphs and the first to third instar nymphs. Each batch, consisting of 200-250 insects, was kept in a separate glass lantern chimney, 10 cm. diameter × 10 cm. high which served both as a *feeding and rearing chamber*. One end of the chimney was covered with a single layer of fine nylon cloth, held stretched by means of a rubber band and adhesive tapes. The insects were not able to escape through the open end of the 'chamber' since as they crawl up they lose foothold due to the inward curvature of the chimney. To avoid even a remote possibility of a stray bed-bug crossing over the curvature of the chimney and spreading an infestation, the 'chamber' was mounted on 'island' made from petri dishes (Fig. 1). Each chamber was provided with an egg repository, fashioned from ordinary packing paper cut into strips, approximately 60 mm. × 30 mm. and folded longitudinally in zigzag manner. These also served as shelters for bed-bugs, and at the time of feeding enabled the insects in the chamber to take shorter time to have full blood-meal from the animal (Table I).

TABLE I.

Feeding Time.

Stage of bed-bug.	FEEDING TIME (IN MINUTES).	
	Chamber provided with a 'strip' of paper.	Chamber without the paper strip.
Adults.	7·0	9·0
>100	6·5	9·0
Nymphs.	8·0	14·0
>100	7·5	12·0
	7·5	11·0
Mixed population.	6·0	7·5
>100	7·0	9·0
Mixed population.	10·0	12·0
>300	11·0	12·5

The different stages of bed-bugs were fed on rabbits' blood every third day. This ensured continuous egg production, moulting of nymphal stages and thereby a continuous supply of adult bed-bugs. About four hours prior to feeding, the hair on the belly of the rabbit were removed by the application of a mild 'hair remover' and the exposed part washed with water.

The feeding was carried out by strapping the chamber on 'hair free' portion of the belly of the rabbit by means of a *holding device* (Fig. 2) developed in this laboratory. The device firmly secures the chamber and at the same time avoids undue pressure on its abdominal region. This has also been found to be a time-saving mechanism since it considerably reduces the laborious manipulations involved in feeding the insects. During the feeding, the animal was held immobile by fastening it dorsally on a wooden board (approx. 75 cm. × 20 cm.), using cords (Fig. 3). Each chamber was strapped for about 15 minutes and during this period the insects were able to have full blood-meal from the animal.

After the feeding, the chambers were inverted over a petri dish and gently tapped. This enabled the dead insects, the cast skin and the paper strips containing insects to fall in the petri dish. The paper strips were put back into the chambers by means of a forceps. The insects that fell in the petri dish during the process were also transferred into the 'chamber' by means of a camel hair brush. Adults that emerged in the chamber containing the fifth instar nymphs, were transferred into the 'adult' chamber. The corrugated paper strip from the adult chamber was gently tapped to free it from all the insects. Subsequently the strip which had the eggs glued to it, was introduced in a separate chamber for incubation and a fresh strip placed inside the adult chamber for the egg-laying. A continuous supply of a large number of bed-bugs (about 500 per week) was thus ensured for toxicological work in the laboratory. Since, however, the egg production is dependent on food supply, it was possible to adjust the population of bed-bugs by regulating the feeding intervals.

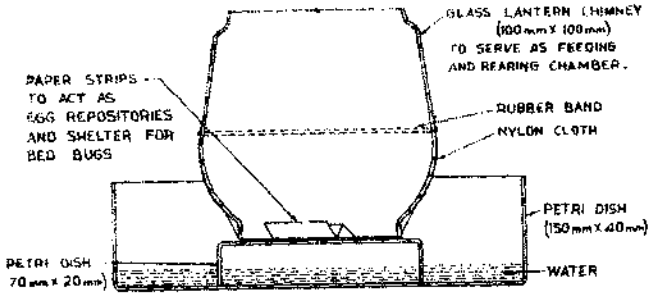

FIG. 1. Feeding and rearing chamber mounted on island.

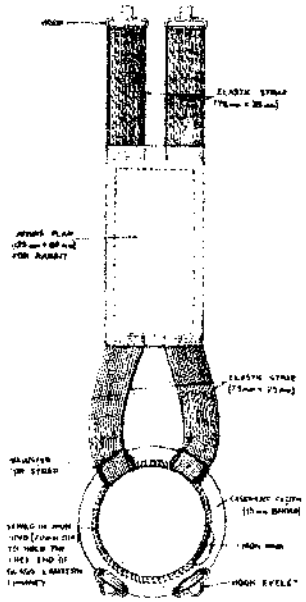

FIG. 2. Device for holding, feeding and rearing chamber on rabbit.

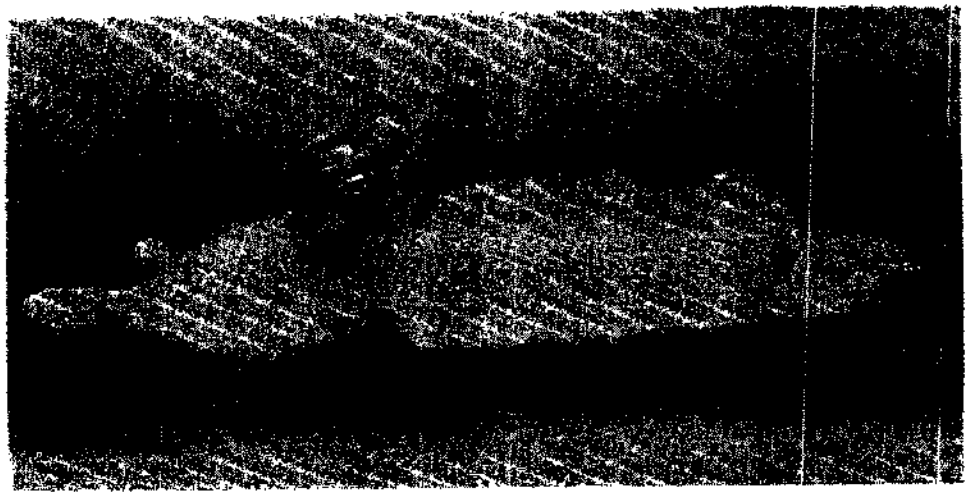

FIG. 3. Photograph showing assembly of feeding and rearing chamber, holding device for the chamber on rabbit mounted on wooden board for providing blood-meal to bed-bugs.

The culturing of bed-bugs in the laboratory was carried out in a room maintained at 27±1°C. temperature and 70±5 per cent relative humidity.

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THE RELATIVE EFFICIENCY OF INSECTICIDES AS SPACE SPRAYS.

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INTRODUCTION.

THE importance of newer synthetic contact insecticides for use in space sprays for vector control programmes is now well recognised (World Health Organization, 1960). Available information on the susceptibility of different species of insects to the insecticides has been reviewed by Negherbon (1959). It is, however, observed that no comprehensive data exists on grading the insecticides as space sprays against the housefly, *Musca nebulo* Linn., and the mosquito, *Culex fatigans* Wied. In an earlier paper, Wal *et al.* (1962) described the susceptibility of flies and mosquitoes to certain synthetic contact insecticides when topically applied on the insect or when the insects were exposed on residual film of the insecticide. This paper presents data on the relative efficiency of the insecticides as space sprays against the two vector species.

MATERIAL AND METHODS.

INSECTICIDES.

The insecticides used in these investigations were : aldrin, dieldrin, endrin, chlordane, toxaphene, Lethane 384, thanite, allethrin, pybuthrin, hexaethyl tetraphosphate (HETP), tetraethyl pyrophosphate (TEPP), Pestox 3, diazinon, malathion, DDT, lindane and pyrethrins. These insecticides were obtained from the trade in India or from manufacturers in the U.K. or U.S.A.

TEST INSECTS.

Houseflies, *Musca nebulo* Linn., and mosquitoes, *Culex fatigans* Wied., drawn from laboratory cultures were used as test insects. The flies were reared by the method described by Basden (1949) for *M. domestica* and the age of the insects used was four to five days. The mosquitoes were reared by the method described by Newman *et al.* (1949) and adult females, forty to sixty hours old, were used in the tests.

EXPERIMENTAL PROCEDURE.

The efficiency of the insecticides was assessed by the modified Peet Grady Chamber described by Parkin and Green (1944). Approximately 100 insects of each species were released in the test chamber (86 cm. × 86 cm. × 56 cm.) and exposed for ten minutes to the mist formed by the atomization of 5 ml. of solution of insecticide using a spray gun, at an air pressure of 50 cm. free mercury. After exposure, the number of insects knocked-down was recorded. All the insects in the chamber were collected and mortality count made 24 hours thereafter. There were two replicates in each assay. The insecticides were used as solutions in white spirit. Exceptions were HETP, TEPP and Pestox 3 which, on account of insolubility in white spirit, were dissolved in acetone. The temperature and relative humidity during the experiments were 27—30°C. and 55—85 per cent., respectively.

After each experiment, the residues of the insecticides used were removed by thoroughly cleaning the chamber with cotton wool saturated with alcohol containing ten per cent acetone. It was also ensured that there was no mortality in the unsprayed insects.

RESULTS AND DISCUSSION.

The results obtained in the various experiments are summarised in Tables I to IV. The data in respect of kill of insects was subjected to probit analysis

TABLE I.

Knockdown of M. nebulo.

(At dosages effecting high kill of insects).*

Insecticide.	Concentration: Per cent. (w/v).	Concentration: mg. per cu.m.	Knockdown in 10 minutes (per cent)
Thanite ...	1·2500	150·00	100
Lethane 384 ...	3·0000	360·00	100
Allethrin ...	0·1000	12·00	100
Pyrethrins ...	0·0080	0·96	100
Pybuthrin ...	0·1500	18·00	100
TEPP ...	0·0250	3·00	90
DDT (Tech.) ...	0·5000	60·00	86
Lindane (γ-BHC) ...	0·0020	0·35	22
Dieldrin ...	0·0030	0·36	11
Malathion ..	0·0150	1·80	5
Aldrin ...	0·0033	0·39	0
Endrin ...	0·0020	0·24	0
Chlordane ...	0·0300	3·60	0
Toxaphene ...	0·0600	7·20	0
HETP ...	0·0600	6·00	0
Pestox-3 ...	2·5000	300·00	0
Diazinon ...	0·0015	0·18	0

* Above eighty per cent.

Note: The solvents (white spirit or acetone), used for dissolving the insecticides for atomization, were not toxic to the insects, that is, there was no knockdown (in 10 minutes) or kill (in 24 hours) of the insects when exposed to the mist formed by the atomization of 5 ml. of the solvent alone.

TABLE II.

Relative toxicity of insecticides to M. nebulo.

Insecticide.	Regression equation.	Mg. per cu.m.:		Relative toxicity* compared to:		
		LD_{50}.	LD_{90}	DDT.	Lindane.	Pyrethrine.
Lindane	Y=2·1579x+ 4·2232	0·08	0·32	396·9	1·0000	6·806
Endrin	Y=3·5892x− 0·1597	0·10	0·22	332·0	0·8634	5·693
Dieldrin	Y=2·8111x+ 3·5128	0·12	0·34	268·9	0·6776	4·613
Diazinon	Y=5·3965x− 3·2718	0·12	0·21	266·7	0·6717	4·572
Aldrin	Y=4·4986x− 3·2811	0·25	0·47	131·2	0·3306	2·250
Pyrethrins	Y=3·3315x+ 1·0258	0·55	1·34	58·3	0·1469	1·000
Chlordane	Y=1·3858x+ 3·2902	0·61	5·09	53·1	0·1338	0·910
Malathion	Y=5·9980x− 3·7971	1·93	1·69	31·1	0·0782	0·532
TEPP	Y=3·0506x+ 0·2903	1·24	3·25	26·0	0·0655	0·446
Toxaphene	Y=1·8234x+ 1·9308	1·70	8·59	18·9	0·0475	0·323
HETP	Y=3·2526x− 1·2199	2·89	7·15	11·1	0·0280	0·191
Allethrin	Y=2·5100x− 0·1036	3·81	12·35	8·4	0·0212	0·144
Pybuthrin	Y=3·5715x− 0·1411	9·71	22·19	3·3	0·0083	0·057
DDT (Tech)	Y=2·7681x− 3·1899	32·10	93·19	1·0	0·0025	0·017
Thanite	Y=3·3547x− 6·3118	83·10	200·30	0·4	0·0001	0·007
Pestox 3	Y=4·2052x−10·3218	138·10	275·80	0·2	0·0006	0·004
Lethane 384	Y=2·6342x− 4·6796	166·90	511·50	0·2	0·0005	0·003

Y=Probit kill. x=Log concentration. * Based on LD_{50}.

TABLE III.

Knockdown of C. fatigans.

(At dosages effecting high kill of insects).*

Insecticide.	Concentration:		Knockdown in 10 minutes (per cent.).
	per cent. (w/v).	Mg. per cu.m.	
Thanite ...	0·500	60·00	100
Lethane 384 ...	1·000	120·00	100
Allethrin ...	0·150	18·00	100
Pybuthrin ...	0·100	12·00	100
DDT (Tech.) ...	0·500	60·00	100
Pyrethrins ...	0·008	0·96	100
Lindane (γ-BHC) ...	0·010	1·20	53
HETP ...	0·006	0·72	42
Diazinon ...	0·005	0·60	40
TEPP ...	0·002	0·24	18
Malathion ...	0·002	0·24	9
Dieldrin ...	0·004	0·48	7
Toxaphene ...	0·050	6·00	7
Aldrin ...	0·035	4·20	0
Endrin ...	0·004	0·48	0
Chlordane ...	0·070	8·40	0
Pestox-3 ...	0·750	90·00	0

* Above eighty per cent.

Note: The solvents (white spirit or acetone) used for dissolving insecticides for atomization were not toxic to the insects, that is, there was no knockdown (in 10 minutes) or kill (in 24 hours) of the insects when exposed to the mist formed by the atomization of 5 ml. of the solvent alone.

TABLE IV.

Relative toxicity of insecticides to C. fatigans.

Insecticide.	Regression equation.	Mg. per cu.m. :		Relative toxicity* compared to :		
		LD_{50}.	LD_{90}.	DDT.	Lindane.	Pyrethrins.
TEPP	Y=3·2104x+3·2732	0·12	0·31	177·6	4·089	4·249
Malathion	Y=4·4866x+2·5282	0·13	0·24	172·4	3·908	4·123
Endrin	Y=3·3391x−1·0287	0·22	0·53	98·3	2·263	2·352
Dieldrin	Y=2·9358x+2·5794	0·24	0·64	91·8	2·113	2·196
HETP	Y=4·4764x+0·5009	0·35	0·69	60·8	1·401	1·456
Diazinon	Y=5·5448x−0·6172	0·26	0·62	59·5	1·369	1·423
Lindane	Y=3·0232x+1·5249	0·50	1·32	43·4	1·000	1·039
Pyrethrins	Y=2·0650x+2·5259	0·52	1·20	41·8	0·963	1·000
Aldrin	Y=4·8242x−4·1515	2·81	5·36	7·7	0·177	0·184
Toxaphene	Y=3·3800x−1·5614	3·08	7·38	7·0	0·162	0·168
Chlordane	Y=5·0661x−5·9960	5·23	9·36	4·1	0·095	0·099
Pybuthrin	Y=3·3730x−2·4969	5·89	14·12	3·7	0·085	0·088
Allethrin	Y=2·0023x+0·3692	7·25	31·67	3·0	0·069	0·071
DDT (Tech)	Y=2·4103x−1·7182	21·60	73·57	1·0	0·023	0·024
Thanite	Y=2·8168x−3·1701	23·10	85·56	0·8	0·018	0·018
Pestox-3	Y=2·0861x−1·3041	37·10	152·80	0·6	0·013	0·014
Lethane 384	Y=3·3480x−5·6384	53·10	128·30	0·4	0·009	0·010

Y=Probit kill. x=Log concentration. * Based on LD_{50}.

TABLE V.

Grading of insecticides as space sprays.*

Insecticide.	*M. nebulo* :		*C. fatigans* :		Total number of marks.	Average§.
	KD†.	LD_{90}‡.	KD†.	LD_{90}‡.		
Pyrethrins ...	7	12	8	11	38	9·50
Diazinon ...	1	17	5	14	37	9·25
Lindane ...	4	15	7	10	36	9·00
TEPP ...	6	10	4	16	36	9·00
Endrin ...	1	16	1	15	33	8·25
Malathion ...	2	11	3	17	33	8·25
Dieldrin ..	3	14	2	13	32	8·00
HETP ...	1	8	6	12	27	6·75
Allethrin ...	7	6	8	5	26	6·50
Pybuthrin ...	7	5	8	6	26	6·50
Aldrin ...	1	13	1	9	24	6·00
Thanite ...	7	3	8	3	21	5·25
DDT ...	5	4	8	4	21	5·25
Lethane 384 ...	7	1	8	2	18	4·50
Chlordane ...	1	9	1	7	18	4·50
Toxaphene ...	1	7	2	8	18	4·50
Pestox-3 ...	1	2	1	1	5	1·25

* By the Method described by Wal *et al.* (1962).

† Marks obtained on the basis of knockdown (KD) of insects.

‡ Marks obtained on the basis of LD_{90} of insecticides.

§ Total number of marks scored divided by the number of techniques employed.

(Finney, 1952). The LD_{50} and LD_{90} of the insecticides have been estimated from probit log concentration curves. The relative toxicity of the insecticides to *M. nebulo* and *C. fatigans* has been determined based on LD_{50}. It will be noted from this data that the knockdown effect on the insects and the order of toxicity of the insecticides to *M. nebulo* and *C. fatigans* vary with the species of the insect.

The grading of efficiency of the insecticides has been based on both knockdown and kill of the insects. The results on the grading are depicted in Table V. It will be seen from these data that, in the overall assessment the most satisfactory insecticides are pyrethrins, diazinon, lindane and TEPP. These are followed by endrin, malathion, dieldrin, HETP, allethrin and pybuthrin. The remaining insecticides are much less effective when used as space sprays against both flies and mosquitoes.

SUMMARY.

The relative efficiency of certain synthetic contact insecticides, *viz.*, aldrin, dieldrin, endrin, chlordane, toxaphene, Lethane 384, thanite, allethrin, pybuthrin, hexaethyl tetraphosphate (HETP), tetraethyl pyrophosphate (TEPP), Pestox-3, diazinon and malathion as space sprays against the housefly, *Musca nebulo* Linn., and the mosquito, *Culex fatigans* Wied., has been investigated in relation to DDT, lindane and pyrethrins. The LD_{50} and LD_{90} have been estimated from probit/log concentration curves. It has been found that, considering both knockdown and mortality effects, the most satisfactory insecticides are pyrethrins, diazinon, lindane and TEPP followed by endrin, malathion, dieldrin, HETP, allethrin and pybuthrin. The remaining insecticides DDT, thanite and chlordane are much less effective when used as space sprays.

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MODE OF ACTION OF DDT ON THE HOUSEFLY, *MUSCA NEBULO* LINN.

Part II.

EVIDENCE FOR THE DISSOLUTION OF DDT IN THE CUTICULAR LIPOIDS OF THE INSECT.

BY

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[June 29, 1962.]

INTRODUCTION.

In an earlier paper, Damodar *et al.* (1961) have described the results of studies on the penetration of topically applied DDT through the cuticle of the housefly, *Musca nebulo* Linn., and its transference and distribution in various tissues of the insect. In this paper, experimental proof for the dissolution of DDT in the cuticular lipoids of the insect has been presented.

In order that contact insecticides may effect a response at the appropriate site of action, it is necessary that they enter the body of insects. Richards and Cutkomp (1946), in tracing the relationship between the possession of a chitinous exoskeleton and susceptibility to DDT, reported that the insecticide is absorbed by chitin. Lord (1948) also showed that DDT is absorbed by chitin. Dresden and Krijgsman (1948) claimed that the specificity of DDT as a contact insecticide is not so much due to its toxicity as due to its liposolubility and ability to penetrate the insect cuticle. Pradhan *et al.* (1952) demonstrated the solubility of DDT in the epicuticular waxes extracted from the larval exuviae of *Euproctis lunata* Wlk. and *Trogoderma granarium* Everts. Pal *et al.* (1954) have demonstrated the dissolution of DDT in the lipoid obtained by immersing whole insects in chloroform for 24 hours. Thus their lipoid preparation may not have been free from body fat. Bradbury *et al.* (1958), who followed a similar method of removing the epicuticular waxes, by immersing mosquitoes in petroleum ether for 60 seconds, have confirmed the possibility of extraction of fat from internal tissues if the insects are allowed to remain long enough in the solvent. The present paper describes the method of isolation of cuticular lipoids, as far as possible free from body fat, from a normal laboratory strain of *M. nebulo* and advances proof of the solubility of DDT in such a material.

MATERIAL AND METHODS.

Cuticular lipoids.—Adult files, *M. nebulo*, 4 to 5 days old, cultured in the laboratory (Basden, 1947) at 28±1°C. and 50±10 per cent R.H., were used. The cuticle from the thoracic region of 700 flies was dissected and muscle fibres and fat adhering to the cuticle were carefully removed. This cuticular preparation was initially extracted with absolute alcohol to remove non-lipoid matter and subsequently with chloroform to obtain the lipoids in a Soxhlet apparatus. The chloroform extract was filtered and the solvent removed by evaporation. The residual lipoids constituted three per cent of the weight of the cuticle initially started with.

A five per cent solution of the cuticular lipoids was prepared in chloroform. Films of the lipoids were prepared in the evacuated portion (surface area 1·76 sq. cm.) of the cavity glass slides by transferring 0·1 ml. of the lipoid preparation and removing the solvent by evaporation.

DDT.—DDT-(*pp'*-isomer) m.p. 108°C. was used. Fine crystals of the powdered insecticide, passing through 100 mesh sieve, were dusted uniformly on films of cuticular lipoids.

Rotenone and talc.—For comparison with DDT, the stomach poison rotenone and an inert material like talc were also similarly dusted on the films of cuticular lipoids.

RESULTS AND DISCUSSION.

The action of DDT, rotenone and talc on films of cuticular lipoids was studied under a microscope and also by taking microphotographs at different intervals as shown in Plates I, II, III. It is observed from Plate I, Figs. 1 to 6, that crystals of DDT show progressive dissolution in the films of cuticular lipoids and very few crystals of the insecticide are seen 24 hours after incorporation. The dissolution of rotenone in the films of cuticular lipoids was found to be very poor and even 24 hours after incorporation numerous particles of the insecticide could be seen on the films (Plate II, Figs. 1 to 4). Talc similarly did not undergo any dissolution (Plate III, Figs. 1 and 2). It is, therefore, evident that a contact insecticide, such as DDT, gets dissolved in the cuticular lipoids and then penetrates the insect body.

SUMMARY.

Cuticular lipoids of the housefly, *Musca nebulo* Linn., free from body fat have been extracted and dissolution of DDT in these lipoids has been demonstrated. The behaviour of a stomach poison and that of an inert material such as talc, when applied on films of cuticular lipoids, has also been studied. It has been shown that a contact poison, such as DDT, gets dissolved in the cuticular lipoids and then penetrates in the insect body.

PLATE I.

Action of DDT on the reticular lipoids of *M. nivula*.

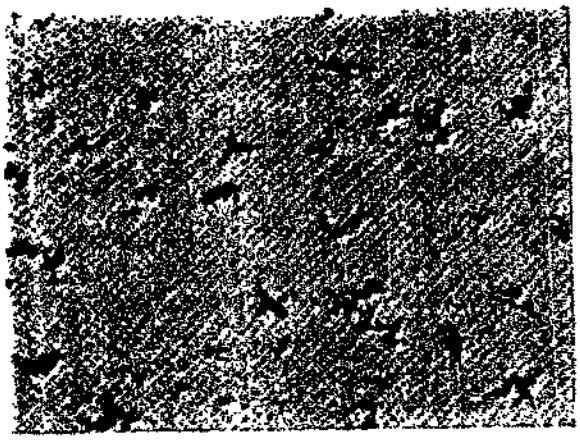

FIG. 1. DDT.

FIG. 2. Initial.

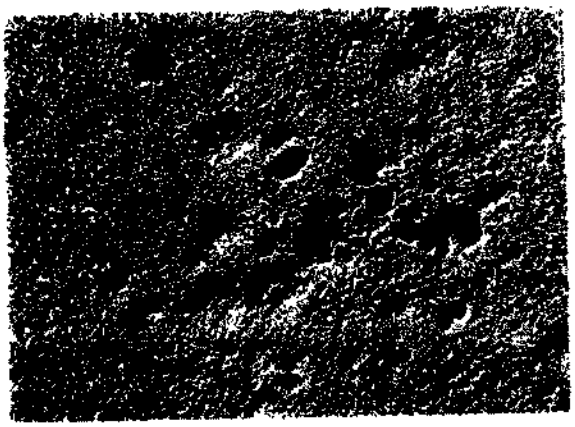

FIG. 3. 30 Minutes.

FIG. 4. 2 Hours.

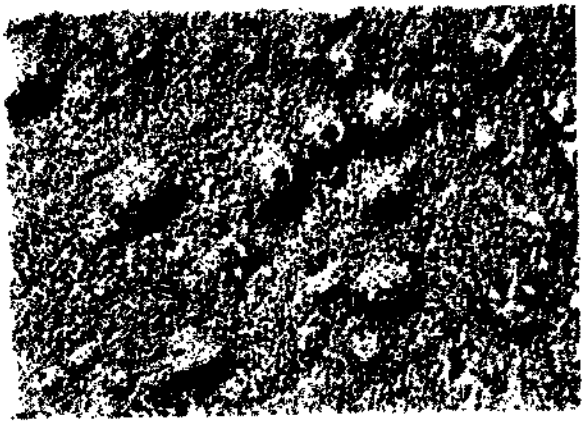

FIG. 5. 6 Hours.

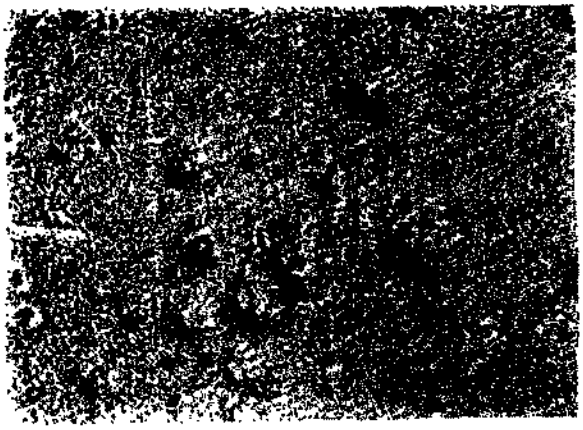

FIG. 6. 24 Hours.

PLATE II.

Action of rotenone on the cuticular lipoids of *M. nebulo*.

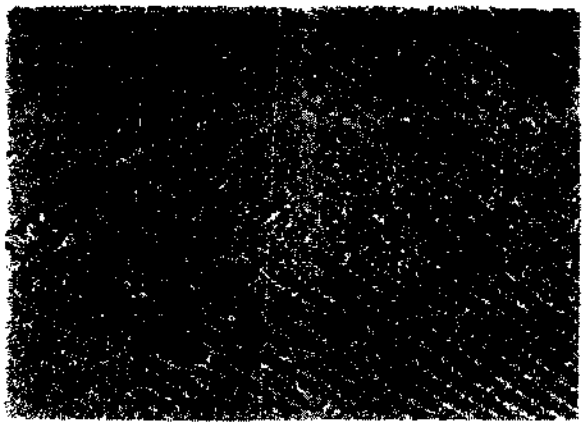

FIG. 1. Rotenone.

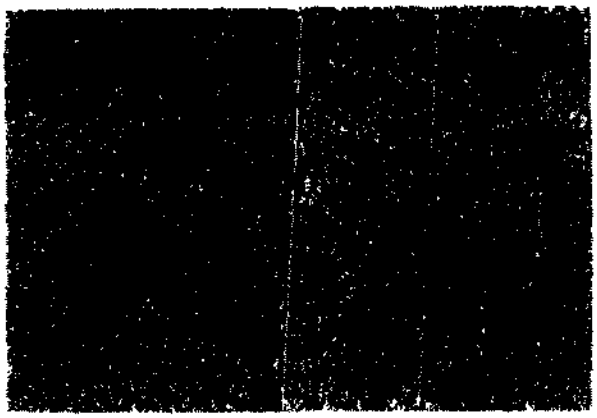

FIG. 2. 30 Minutes.

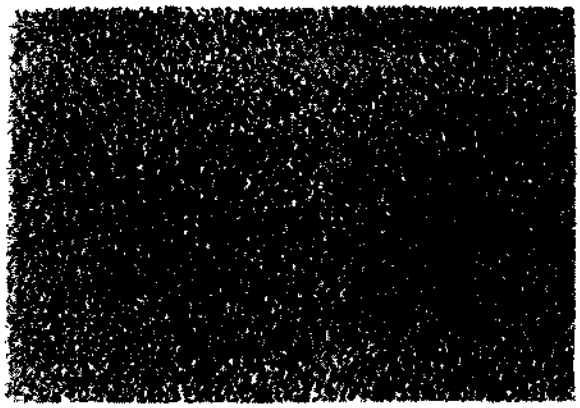

FIG. 3. 6 Hours.

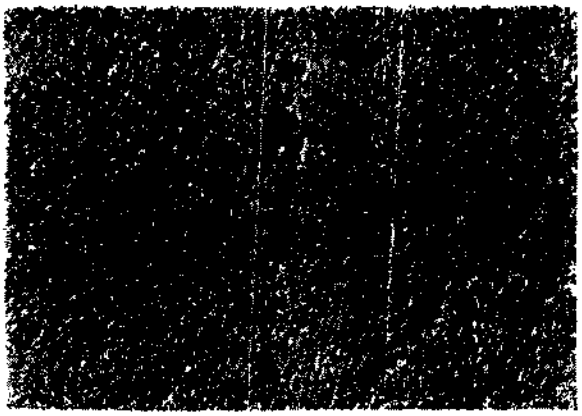

FIG. 4. 24 Hours.

PLATE III.

Action of Talc on cuticular lipoids of *M. sabula*.

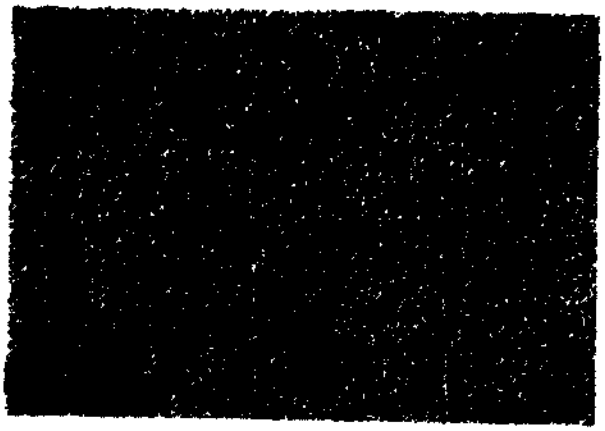

FIG. 1. Initial.

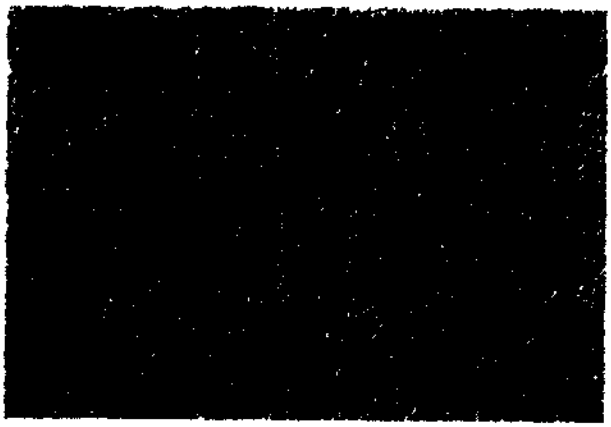

FIG. 2. 24 Hours.

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THE RELATIVE EFFICIENCY OF INSECTICIDES AS FLY AND MOSQUITO LARVICIDES.

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[August 13, 1962].

INTRODUCTION.

In an earlier paper from this laboratory, Dixit and Perti (1962) have described the relative efficiency of several newer synthetic contact insecticides as space sprays against the housefly, *Musca nebulo* Linn., and the mosquito, *Culex fatigans* Wied. In this paper, data on the relative efficiency of the above insecticides against the larvae of these two vector species have been presented.

MATERIALS AND METHODS.

The insecticides and laboratory cultures of the flies and mosquitoes were those employed by Wal *et al.* (1962) and Dixit and Perti (1962). Larvae of *Musca nebulo*, 30±6 hours old and third-instar larvae of *Culex fatigans* were used in the larvicidal tests. The efficiency of the insecticides was assessed by the procedures described below :—

Fly larvicide.—The assessment was carried out by introducing 25 larvae in glass jars, 12·7 cm.×7·6 cm., each containing 100 gm. of the medium made from milk powder (10 gm.), brewers' yeast (10 gm.), agar (2 gm.), and water (100 ml.) in which various concentrations of the insecticides under examination had been thoroughly dispersed. Observations on the per cent emergence of flies were recorded after seven days. There were two replicates in each assay. The temperature and relative humidity of the room were 27—32°C. and 55—85 per cent respectively.

Mosquito larvicide.—The assessment was carried out by liberating 50 larvae in glass dishes, 15·2 cm.×3·8 cm., containing varying concentrations of the insecticide in 200 ml. water. The per cent mortality of the larvae was determined after 48 hours. There were three replicates for each concentration investigated. The temperature and relative humidity of the room were 30—32°C. and 63—87 per cent respectively.

TABLE I.

Relative toxicity of insecticides to the larvae of M. nebulo.

Insecticides.	Heterogeneity.	Regression equation.	LC_{30} ppm.	LC_{50} ppm.	Log LC_{50} ± S.E.	Fiducial limits of log LC_{50}	Relative toxicity*. DDT.	Lindane.	Pyrethrins.
Diazinon	$\chi^2(2)=0.5929$	$Y=5.2091x+3.8184$	1.686	2.971	0.2268 ±0.0105	0.1817 0.2719	1391.861	3.243	44.538
Aldrin	$\chi^2(3)=0.7741$	$Y=4.3691x+2.7410$	3.289	6.463	0.5170 ±0.0155	0.4503 0.5837	713.495	1.662	22.831
Endrin	$\chi^2(3)=1.4605$	$Y=3.2404x+2.9445$	4.308	10.701	0.6343 ±0.0235	0.5596 0.7090	545.636	1.269	17.428
Dieldrin	$\chi^2(2)=1.2052$	$Y=4.6268x+1.7337$	5.082	9.613	0.7060 ±0.0182	0.6277 0.7843	461.744	1.076	14.775
Lindane	$\chi^2(2)=3.2194$	$Y=4.6813x+1.5463$	5.468	10.268	0.7376 ±0.0336	0.5933 0.8823	429.110	1.000	13.732
Malathion	$\chi^2(2)=4.6955$	$Y=3.0447x+2.7007$	5.691	15.001	0.7552 ±0.0494	0.5428 0.9676	412.286	0.961	13.193
HETP	$\chi^2(3)=1.7861$	$Y=1.9697x+2.4942$	18.715	83.720	1.2722 ±0.0336	1.1653 1.3791	125.375	0.292	4.012
Chlordane	$\chi^2(3)=0.2051$	$Y=2.4994x+1.8181$	18.753	61.060	1.2731 ±0.0100	1.2413 1.3049	125.121	0.292	4.004
TEPP	$\chi^2(3)=4.0916$	$Y=2.3086x+2.0578$	18.815	67.547	1.2745 ±0.0816	1.0150 1.5340	124.710	0.291	3.991
Toxaphene	$\chi^2(3)=0.3225$	$Y=3.9652x-1.0562$	32.674	70.876	1.5273 ±0.0104	1.4826 1.5720	69.680	0.162	2.230
Allethrin	$\chi^2(3)=0.2904$	$Y=1.9179x+0.8066$	153.630	715.600	2.1865 ±0.0186	2.1272 2.2458	15.273	0.036	0.489
Pyrethrins	$\chi^2(3)=0.4393$	$Y=3.0655x-0.7494$	75.082	196.622	2.5745 ±0.0105	2.5412 2.6078	31.249	0.076	1.000
Lethane 384	$\chi^2(2)=0.3184$	$Y=3.2361x-4.3002$	748.000	1861.700	2.8739 ±0.0126	2.8197 2.9281	3.137	0.007	0.100
Thanite	$\chi^2(3)=0.5679$	$Y=2.5056x-2.5530$	1249.000	4043.600	3.0943 ±0.0152	2.0461 2.1425	1.878	0.004	0.060
Pestox-3	$\chi^2(3)=1.1640$	$Y=5.7607x-13.1154$	1390.000	2714.000	3.1447 ±0.0090	2.1161 2.1733	1.688	0.004	0.054
DDT (Tech.)	$\chi^2(4)=0.7075$	$Y=2.4817x-3.7364$	2346.000	4624.400	3.5203 ±0.0130	0.3179 0.4229	1.000	0.002	0.032

Y = Probit kill. x = log concentration. *Based on LC_{50}

TABLE II.

Relative toxicity of insecticides to the larvae of C. fatigans.

Insecticide.	Heterogeneity.	Regression equation.	LC_{50} ppm.	LC_{90} ppm.	Log LC_{50} ± S.E.	Fiducial limits of log LC_{50}	Relative toxicity*.		
							DDT.	Lindane.	Pyrethrins
Aldrin	$\chi^2(3)=0.5526$	$Y=4.8824x+17.5503$	0.003	0.005	$\bar{3}.4295 \pm 0.0055$	$\bar{3}.4121$ $\bar{3}.4469$	14.370	30.111	4.667
Dieldrin	$\chi^2(3)=19.5270$	$Y=5.7585x+18.3725$	0.005	0.008	$\bar{3}.6778 \pm 0.0223$	$\bar{3}.6052$ $\bar{3}.7504$	8.083	16.938	2.625
Endrin	$\chi^2(3)=10.6108$	$Y=8.1308x+21.4346$	0.010	0.014	$\bar{3}.9787 \pm 0.0105$	$\bar{3}.9496$ $\bar{2}.0076$	4.084	8.558	1.326
Pyrethrins	$\chi^2(2)=0.0188$	$Y=5.9502x+16.3030$	0.013	0.021	$\bar{1}.6240 \pm 0.0094$	$\bar{1}.6200$ $\bar{1}.6280$	3.079	6.453	1.000
Malathion	$\chi^2(2)=17.9933$	$Y=6.0269x+21.2097$	0.020	0.033	$\bar{2}.3104 \pm 1.0285$	$\bar{2}.1878$ $\bar{2}.4330$	1.901	3.985	0.618
Diazinon	$\chi^2(4)=14.4901$	$Y=6.7603x+15.6836$	0.026	0.041	$\bar{2}.4197 \pm 0.1703$	$\bar{2}.3655$ $\bar{2}.4739$	1.475	3.091	0.479
Chlordane	$\chi^2(4)=4.7828$	$Y=6.9650x+15.9095$	0.027	0.041	$\bar{2}.4337 \pm 0.0098$	$\bar{2}.4025$ $\bar{2}.4649$	1.432	3.000	0.465
DDT (Tech.)	$\chi^2(4)=5.9116$	$Y=8.5049x+17.0047$	0.039	0.055	$\bar{2}.5885 \pm 0.0066$	$\bar{2}.5701$ $\bar{2}.6069$	1.000	2.095	0.325
Toxaphene	$\chi^2(3)=5.2334$	$Y=4.6866x+11.3701$	0.044	0.082	$\bar{2}.6408 \pm 0.0062$	$\bar{2}.6264$ $\bar{2}.6552$	0.888	1.860	0.288
Allethrin	$\chi^2(3)=2.2672$	$Y=13.9114x+21.4258$	0.066	0.082	$\bar{2}.8193 \pm 0.0045$	$\bar{2}.8057$ $\bar{2}.8335$	0.589	1.234	0.191
Lindane	$\chi^2(2)=7.9302$	$Y=1.9528x+4.5946$	0.081	0.372	$\bar{2}.9100 \pm 0.0637$	$\bar{2}.6401$ $\bar{2}.1798$	0.477	1.000	0.155
TEPP	$\chi^2(3)=1.5260$	$Y=8.9315x+4.1193$	1.255	1.746	0.0986 ± 0.0039	0.0862 0.1110	0.031	0.065	0.010
HETP	$\chi^2(3)=7.3055$	$Y=10.5440x+0.1946$	2.856	3.779	0.4560 ± 0.0071	0.4335 0.4785	0.014	0.029	0.004
Thanite	$\chi^2(3)=17.5955$	$Y=6.3956x-0.3136$	6.773	10.740	0.8308 ± 0.0202	0.7664 0.8952	0.006	0.012	0.002
Lethane 384	$\chi^2(2)=1.5265$	$Y=18.4796x-17.9965$	17.560	120.600	1.2444 ± 0.0028	1.2352 1.2566	0.002	0.005	0.007
Pestox-3	$\chi^2(2)=3.0496$	$Y=3.9336x-1.2678$	39.210	83.020	1.5934 ± 0.0179	1.5166 1.6702	0.001	0.003	0.0003

Y = Probit kill. x = log concentration. *Based on LC_{90}

RESULTS AND DISCUSSION.

The results obtained in the various experiments were subjected to probit analysis (Finney, 1952) and are summarized in Tables I and II. The LC_{50} and LC_{90} of the insecticides were estimated from probit-log concentration curves. The relative toxicity of the insecticides to the larvae of *Musca nebulo* and *Culex fatigans* was determined based on LC_{50}. It will be noted from these data that the order of toxicity of the insecticides to the larvae varies with the species of the insect. The fly larvae were found to be most susceptible to diazinon followed by aldrin, endrin, dieldrin, lindane and malathion. The mosquito larvae, however, were found to be most susceptible to aldrin followed by dieldrin, endrin, pyrethrins, malathion, diazinon, chlordane, DDT, toxaphene, allethrin and lindane.

The grading of the efficiency of the insecticides as fly and mosquito larvicides was carried out by the method described by Wal *et al.* (1962). The results on the grading are depicted in Table III. It will be seen from the data that in the overall assessment the most satisfactory larvicides are aldrin, dieldrin, diazinon and endrin in the order stated. These are followed by malathion, chlordane, lindane and pyrethrins, which are moderately effective. The remaining insecticides are much less effective when used as fly and mosquito larvicides.

TABLE III.

Grading of the efficiency of larvicides.

Insecticide.	*M. nebulo**.	*C. fatigans**.	Total number of marks.	Average†.	Insecticide.	*M. nebulo**.	*C. fatigans**.	Total number of marks.	Average†.
Aldrin	15	16	31	15·5	Toxaphene	8	7	15	7·5
Dieldrin	14	15	29	14·5	TEPP	9	5	14	7·0
Diazinon	16	11	27	13·5	Allethrin	5	8	13	6·5
Endrin	12	14	26	13·0	HETP	7	4	11	5·5
Malathion	11	12	23	11·5	DDT (Tech.)	1	9	10	5·0
Chlordane	10	10	20	10·0	Lethane 384	4	2	6	3·0
Lindane	13	6	10	9·5	Thanite	2	3	5	2·5
Pyrethrins	6	13	19	9·5	Pestox-3	3	1	4	2·0

* Marks obtained on the basis of LC_{90} of insecticides.
† Total number of marks scored divided by the number of techniques employed.

SUMMARY.

The relative efficiency of certain synthetic contact insecticides, *viz.*, aldrin, dieldrin, endrin, chlordane, toxaphene, Lethane 384, thanite, allethrin, hexaethyl tetraphosphate (HETP), tetraethyl pyrophosphate (TEPP), Pestox-3, diazinon and malathion has been investigated in relation to DDT, lindane and pyrethrins against the larvae of the housefly, *Musca nebulo* Linn., and the mosquito, *Culex fatigans* Wied. The LC_{50} and LC_{90} have been estimated from probit-log concentration

curves. It has been found that the most satisfactory larvicides are aldrin, dieldrin, diazinon and endrin followed by malathion, chlordane, lindane and pyrethrins. The remaining insecticides are much less effective as larvicides.

ACKNOWLEDGEMENTS.

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FILARIAL MANIFESTATIONS IN THE GENITAL ORGANS WITH A FEW INTERESTING OBSERVATIONS IN HISTOPATHOLOGY.

(Study of 7 cases)

BY

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AND

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[July 20, 1962.]

INTRODUCTION.

It is known that filarial cases present us with protean manifestations, not only clinically, but also in histopathologic appearances. The male genital organs like spermatic cords, epididymes and testes are the most frequent habitat of adult filarial worms. In these favourable sites, the adult worms lying in the lymphatic vessels, bring about the histopathological changes. We have observed certain interesting histopathological changes in six cases of filarial infection of the male genital tract where the orchidectomy specimens were available for detailed study. Besides these cases, an unusual and a rare finding of an adult female filarial worm from a specimen of ovary in another case has also been studied.

MATERIALS AND METHODS.

The materials presented in this paper consist of a study of a total of 6 testes along with the epididymes and spermatic cords and an ovary from another case. These specimens were available during routine histopathological examination of biopsy and autopsy materials in the Department of Pathology, Andhra Medical College, Visakhapatnam, South India.

Paraffin sections of formal-saline fixed tissue blocks were used for histopathological examination and haematoxylin and eosin stain has been used. A diagnosis of filarial infection was made in each case by finding of filarial worms and associated histological features. The results of histopathological examination, along with brief clinical notes of the cases, are presented in Table I and the significance of these histopathological findings are discussed in detail.

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† Post-graduate Student.

TABLE I.

Brief summary of the presenting symptoms and pathological findings in all the seven cases of filarial infection.

Case number.	Mode of presentation.	Specimen.	Histopathological findings.
1. (5284)	Swelling testis; clinically tumour testis.	Testis and epididymis.	Calcified materials in lymphatic vessels indicating previous existence of macrofilariae, and calcification in fibrous connective tissue developing into bone and live female macrofilariae in a dilated lymphatic vessel at a close proximity without cellular reaction.
2. (9137)	Scrotal swelling with orchitis.	Testis and epididymis	Dead and hyalinised macrofilaria in a dilated lymphatic vessel with eosinophilic abscess.
3. (3034)	Hydrocele and orchitis.	Testis and epididymis.	Calcified macrofilariae in a dilated lymphatic vessel encapsulated by concentric layers of hyaline fibrous connective tissue and severe eosinophilic reaction around (filarial grave-yard) lymph vessel.
4. (PM 2899)	Swelling scrotum and testis in a case of mitral stenosis with congestive cardiac failure.	Postmortem specimen of testis.	Dead, eosinophilic, macrofilariae encapsulated by accumulation of degenerated eosinophils in dilated lymphatic spaces.
5. (RFX)	Swelling scrotum and orchitis.	Testis and epididymis.	Female macrofilariae with well-defined ova and embryo at various stages of development in dilated lymphatic vessels. Surrounding tissues are infiltrated with inflammatory cells, mainly eosinophils and others being plasma cells, mononuclear cells and lymphocytes. Also, perilymphangitis and polypoid endolymphangitis, giant cells with formations of filarial pseudotubercle and filarial granuloma are conspicuous.
6. (PM 8314)	Swelling of right scrotum in an autopsy case of Algid malaria.	Testis and epididymis; spermatic cord.	A dead female macrofilarial containing basophilic ova, lying in the dilated lymphatic spaces surrounded by chronic inflammatory cells, eosinophils, plasma cells, mononuclear cells and fibroblasts enmeshed in newly-formed fibrous tissue.
7. (5307)	Irregular profuse uterine bleeding (Menorrhagia).	Uterus, tube and ovary.	In one section female macrofilariae with ova lying in dilated lymphatic vessels of ovary, without any inflammatory cellular reaction in the surrounding tissues. In the other sections, granulomatous endolymphangitis with infiltration of eosinophilic leucocytes around a hyaline structure lying in the centre of a dilated lymphatic vessel and surrounding tissue infiltrated with eosinophils, plasma cells, mononuclear cells and fibroblasts.

Bone formation in filarial infection is a very rare observation and we have come across one such case in one of the testes examined, where clinically it was mistaken for a tumour. The details of the case are recorded below :

CASE NUMBER I : (5284/61).

"T", a Hindu, male, 55 years of age, had elephantoid condition of the scrotum and penis of 20 years' duration. History of previous attacks of fever with chill and inflammation of scrotum was present. For the past few years he had no fever, but was noticing a gradual increase in size of the scrotum and penis due to the formation of blubbery tissue of elephantiasis. Testes were not palpable through the thickened scrotum. On operation one of the testes was hard to feel, as if it was calcified. A clinical diagnosis of ? tumour of the testis was made and an orchidectomy was done.

Gross specimen.—The specimen consisted of the testis together with epididymis and spermatic cord. In the upper part of the testis, one area showed a mass of varying consistency extremely hard and firm, cut section of the thickened mass showed hard areas of calcification and an area of softening with cavity formation (Fig. 1).

Histopathological examination.—Histopathological examination revealed that the tissue was made up of fibrous connective tissue, mainly with irregular strands of collagen fibres of varying thickness. In places, varying amounts of calcium were seen. This calcified connective tissue was transformed into bony lamellae, the interspaces of which showed blood vascular channels containing scant erythroid and myeloid series of cells (Fig. 2). One of the sections examined showed completely calcified remnants of a worm in a lymphatic vessel. Tissue spaces between the strands of fibrous tissue, appeared to be well-developed. These have no endothelial lining, as the lymph capillaries, but were merely crevices of tissue. These spaces were so numerous that the collagen bundles were split up into thin strands separated by fluid. At other places, lymph laking and dilated lymph capillaries were seen.

In such of those calcified areas no evidence of a live worm could be found, but peculiarly enough at a close proximity, where there was newly formed fibrous connective tissue, a live adult filarial worm was found lying in a dilated lymphatic channel lined by a single layer of endothelium and showing no inflammatory cellular reaction around it (Fig. 3).

Another interesting feature was that there was hardly any inflammatory cellular infiltration in this thickened tissue, except at places where only a few clumps of lymphocytes and histocytes were seen. One of the sections revealed seminiferous tubules which showed evidence of atrophy and inactive spermatogenesis.

OBSERVATIONS IN CASE NUMBERS TWO TO SIX.

Table I gives a short summary of the clinical and pathological findings in the other five specimens of testes. epididymes and spermatic cords. In all the cases live, dead or calcified adult filarial worms were found in the tissues.

The composite histopathological findings in these specimens were as follows:

1. Perfectly healthy macrofilariae, lying in dilated spaces, are seen to produce no cellular reaction in the surrounding tissues (Fig. 3). On the other hand, the tissue reactions associated with dead macrofilariae included usually a precipitation of a strongly eosinophilic barrier made up of degenerated eosinophils around the parasite in dilated lymphatic vessels (Fig. 4). The dead macrofilariae in some of the dilated lymph spaces are calcified with or without any remnants of the parasite, and with or without being encapsulated by the concentric hyaline fibrous connective tissue, the so-called "tomb stone in the filarial graveyard", or "mummy in the image of defunct parasite" (Fig. 5), and with or without any cellular infiltration around the lymph vessel containing calcified materials. It is also observed that in some cases female macrofilariae, containing well-defined ova and embryos in the uterine cavity, have produced cellular reactions in surrounding tissues even though there has been no evidence of death (Fig. 6).

2. Acute diffuse orchitis with inflammatory oedema in the inter-tubular spaces of testis and infiltration with clusters of eosinophilic leucocytes (acute eosinophilic orchitis) is a common finding. In some cases, the eosinophilic cells are diffuse and are separated by inflammatory oedema, with clusters of lymphoid and plasma cells here and there. The concentration of the cells at places is so marked as to suggest an eosinophilic abscess. In one of the sections, there was a hyaline structure of a dead adult filarial worm, situated in a dilated lymphatic space with formation of such an abscess (Fig. 7).

3. In the medium-sized lymph vessels histological changes vary. Irregular inflammatory masses of tissue cells project into the vessels, forming a type of polypoid endolymphangitis, resulting in obstruction and obliteration (Figs. 8, 9 and 10). At places, the whole wall of the lymph vessels is diffusely infiltrated with eosinophilic leucocytes, plasma cells and mono-nuclear cells appearing as perilymphangitis (Fig. 11). Another type of inflammatory change observed, is the filarial granulomatous reaction resembling a pseudotubercle which is found in the lymphatic vessels, due to marked cellular reaction in the subendothelial connective tissue and granulomatous reaction characterised by the presence of pale staining endothelioid cells (epithelioid) and giant cells (Figs. 9 and 12). This phenomenon is seen not only in the vicinity of defunct worms but also in adjacent areas in the same tissues.

4. Lymphatic channels contained a thin pink staining albuminoid fluid with the formation of lymph thrombus. Lymph stasis in lymphatic spaces of interstitial tissues was observed in a few sections.

FIG. 1. Photograph of testis, showing area of calcification and cavity formation.

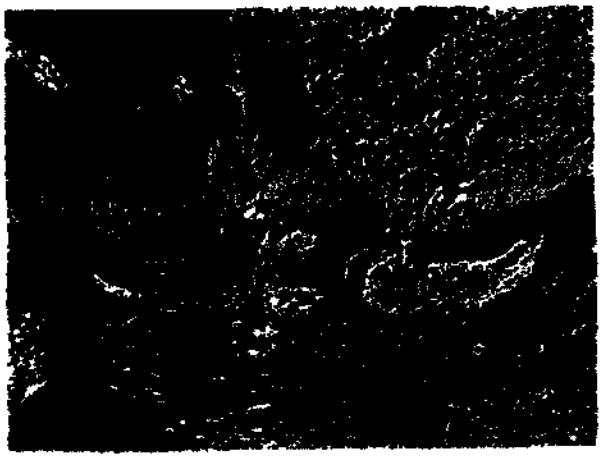

FIG. 2. Photomicrograph illustrates calcified filarial connective tissue with bony lamellae formation. Centrally some vascular channels are seen.

FIG. 3. Photomicrograph illustrates live female macrofilariae with ova, lying in a dilated lymphatic space with no cellular reaction around.

FIG. 4. Photomicrograph illustrates dead macrofilaria surrounded by strongly eosinophilic fringe.

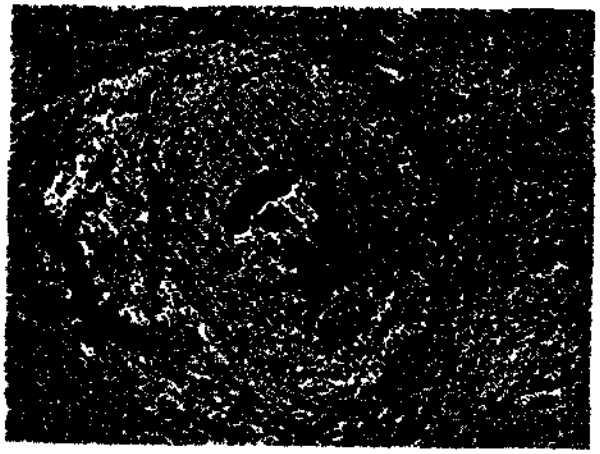

FIG. 5. Photomicrograph illustrates calcified remnants of filarial worm, surrounded by concentric layers of fibrous connective tissue in a dilated lymph space surrounded by cellular reaction (Mummy in the image of defunct parasite).

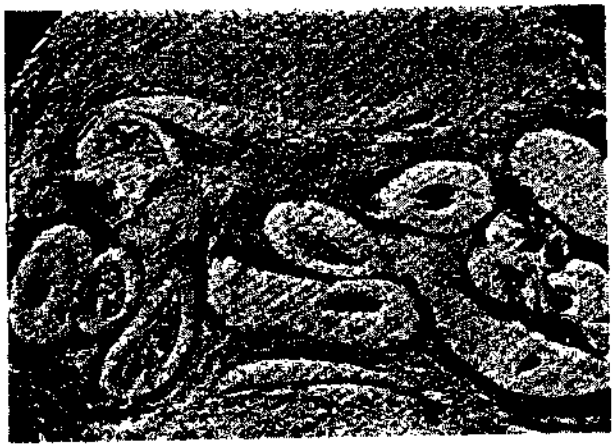

FIG. 6. Photomicrograph illustrates adult female worm embedded in a dilated lymphatic space, surrounded by cellular reactions.

FIG. 7. Photomicrograph illustrates a hyaline structure of a dead macrofilariae lying in a dilated lymphatic space surrounded by concentration of eosinophils in clumps forming eosinophilic abscess.

FIG. 8. Photomicrograph illustrates polypoid endolymphangitis with a part of worm inside in lymphatic vessel.

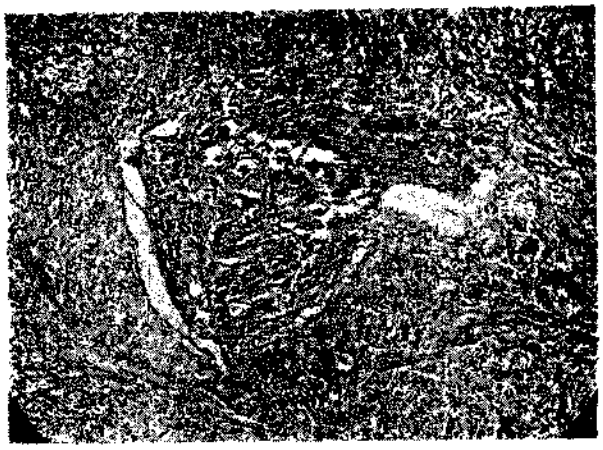

FIG. 9. Photomicrograph illustrates obliterative endolymphangitis with giant cells.

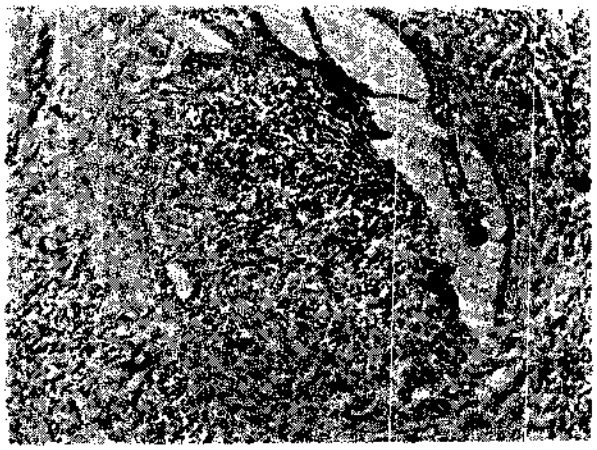

FIG. 10. Photomicrograph illustrates obstructive endolymphangitis.

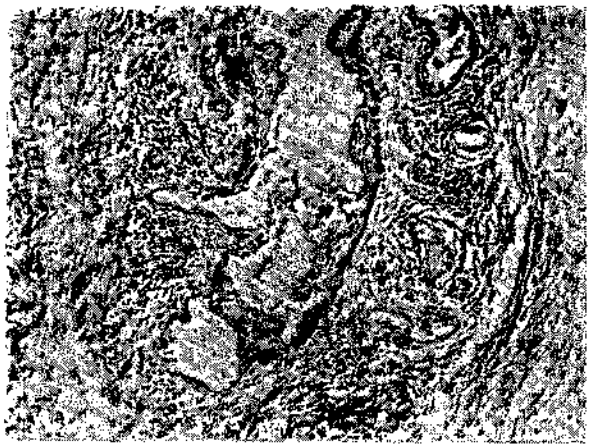

FIG. 11. Photomicrograph illustrates perilymphangitis and perivascular cuffing with lymph stasis.

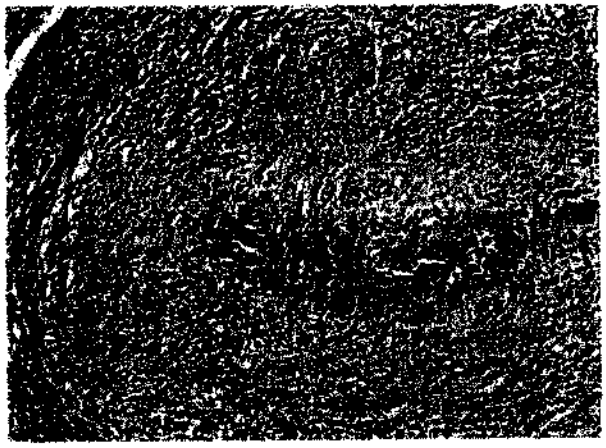

FIG. 12. Photomicrograph illustrates obliterative endolymphangitis with giant cell granuloma in ovary.

FIG. 13. Photomicrograph illustrates structure of ovary showing female macrofilariae without any cellular reaction in surrounding tissues.

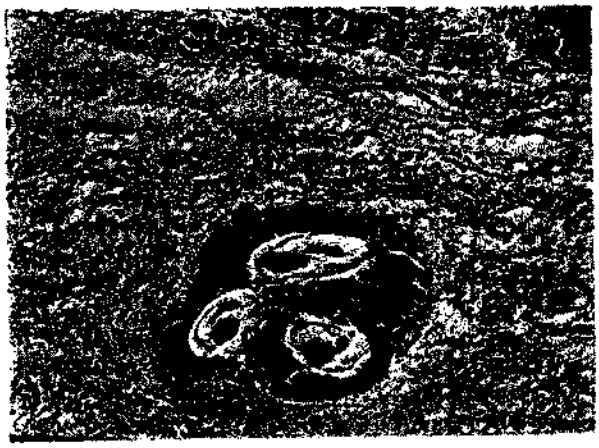

FIG. 14. Photomicrograph illustrates female macrofilariae in a dilated lymph space with fibrosis and cellular reaction around.

5. Proliferation of the lining endothelium of lymphatic vessels resulting in thickening of not only lymph vascular walls but also such phenomena as hypertrophy of the blood vascular walls of medium sized arteries and veins resulting in obstruction and obliteration were observed.

6. An extensive fibrosis superimposed upon inflammatory changes due to proliferation of fibroblasts laying down collagen fibrils which at many places appear only as eosinophilic homogenous hyaline material was almost a constant feature in the specimens studied.

7. Spermatogenesis was active in all the testicular specimens except in Case Number 1 where seminiferous tubules appeared to be undergoing atrophic degeneration with very few tubules containing sperm cells.

CASE NUMBER 7 : (5307/60).

"M" aged 30 years, a Hindu female of Orissa (India), was admitted for the treatment of irregular profuse uterine bleeding. The uterus, fallopian tubes and ovaries were removed at operation.

Histopathological examination.—One of the sections of the ovary showed female macrofilariae filled with ova, lying in dilated lymphatic vessels of the ovary. There was no inflammatory cellular reaction in the surrounding tissue (Fig. 13). The other sections showed granulomatous endolymphangitis and infiltration with inflammatory cells, chiefly eosinophilic leucocytes which formed an acidophilic fringe around a necrotic hyaline structure in the centre of a dilated lymphatic vessel. In other areas there were granulomatous endolymphangitis with giant cells, eosinophils, plasma cells, round cells, mononuclear cells and scattered fibroblastic. Obliterating the lymphatic vessels, similar cells were also seen in surrounding tissue.

DISCUSSION.

Filarial manifestations of male genital organs are frequently seen in Coastal Andhra Pradesh. Although the clinician in the tropics is mindful of filariasis as being the cause, a certain diagnosis is not always possible. It is of interest that one (Case Number 1) of the specimens of the male genital organs like testes, epididymes and spermatic cords produced such a hard swelling, as to be mistaken by the surgeon clinically to be a tumour, and orchidectomy was done ; and on histopathological examination it was found to be unusual manifestation of filariasis where the tissue is transformed into bone. Bahr (1912) traced the process of calcification in recently dead specimen including ova and microfilariae by the laying down of lamellar plates of calcium carbonate. Bhaskara Menon (1935) in his "*Curzon Lectures*" on the problem of filariasis described two cases of epididymo-orchitis in which radiological opacities, due to irregular calcification were presented and on histological sections, demonstrated calcified filarial worms in the lymphatic of testes. In many cases, he added, live worms were found to live in close proximity to those areas of calcification and this feature is of immense importance.

Cooray (1960) observed that when an adult filarial worm is alive there is little or no eosinophilic cell infiltration and no granulomatous reactions are seen ; and in case of recent death of adult worm, the uterine wall can still be identified and a large number of embryos formed ill-defined basophilic masses. It was postulated originally by Manson (1881) that lymph stasis could be brought about by dead filariae or by their calcified remains. Recorded instances of actual bone formation in these cases of filariasis have been scarce even though calcification has been very often demonstrated in many cases. In the first specimen there was intense proliferation of large amount of fibrous connective tissue leading to bone formation. This was probably due to the existance of the worms at an earlier stage at these sites or at its neighbourhood, having the defunct worm degenerated and calcium deposited in these areas. This has led to the formation of bony trabeculae in the interspaces of which previously existing blood vessels are discernible. It has been observed by Bhaskara Menon (1935 *loc. cit.*) and others that live adult filarial worms, lying in a dilated lymphatic vessel in the newly formed connective tissue near about the calcified area, the worm being alive, did not produce any cellular reaction around its situation. This has also been observed by us in Case Number 1 where, in addition to calcification, there was bone formation.

Tissue reactions appear to result from the presence of dead worms which appear in sections as an ill-defined structureless mass and it is quite clear that the living adult worms do not effectively block a lymphatic. It is also known at present that lymphatic obstruction results from granulomatous reaction (endolymphangitis) which develops after the death of the worms.

In Case Number 5, female macrofilariae with well-defined ova and embryo at various stages of development which were considered to be still alive, is seen to give the characteristic features of filarial granuloma formations with infiltration of eosionophilic cells, mononuclear cells, plasma cells and fibroblasts in the interstitial oedematous connective tissue. In Case Number 6, the basophilic mass of ova in a filarial worm, considered to be dying, produced similar tissue reactions (Fig. 14).

From this, it may be postulated that some kind of antigenic substance, toxigenic in nature, produced by the filarial worm, some stages either dead or alive, is responsible for such kind of allergic inflammation which is granulomatous in nature and bring about other manifestations such as necrosis or fibrosis.

In all cases, the dead macrofilariae were either encapsulated by degenerated eosinophilic cellular fringe, or a fibrous connective tissue barrier with chronic inflammatory cellular infiltration. Wartman (1947) described the tissue reactions associated with adults, the finding of precipitation of strongly acidophilic material about the worms with necrosis, proliferation of macrophages, eosinophils and plasma cells. Eosinophilic infiltration was found around living and dead adult *W. bancrofti* by Fulleborn (1929). Anderson (1962) suggested that the eosinophilic fringe is probably an indication of antigen-antibody reaction.

The perivascular cuffing, perilymphangitis and epithelioid or endothelioid endolymphangitis and with formation of giant cells (pseudotubercle) may mimic tuberculous or syphilitic infection in the absence of macro or microfilariae.

Bhaskara Menon (1935 *loc. cit.*) found this pseudotubercle at the tail end of female worms. Our study in the ovary has offered a similar appearance of reaction. This histological picture, which closely mimics tuberculosis and syphilis, has to be borne in mind in the diagnosis of filariasis particularly in filarial endemic areas.

The tissue reactions in formation of filarial abscess of acute eosinophilic orchitis, consists of the eosinophilic cells emigration and pouring out of an inflammatory exudate which shows varying degrees of coagulation necrosis. The concentration of these cells might be so marked as to suggest an eosinophilic abscess. Bahr (1912) observed concentration of eosinophilic cells in the pus of filarial abscess in the testis and epididymis.

Bhaskara Menon (1935 *loc. cit.*) found this reaction most marked at the anterior end of the parasite. In one of our cases (Case Number 2), an eosinophilic abscess was also observed in tissues having hyalinised remnants of the adult filarial worm.

In 1939 O'Connor and Hulse found that, of 162 mature *W. bancrofti* living or dead in male genitalia, 38 were in spermatic cord, 111 in the epididymis and its junction with testis, and 13 in the testis proper. In 13 cases in which *W. bancrofti* were found in male external genitalia, spermatogenesis was active in nine. This suggests that, generally speaking, the parasite does not interfere with fecundity. This is in conformity with our present study except in Case Number 1 where spermatogenesis was not active probably due to pressure atrophy.

Romiti (1935) found *W. bancrofti* in tangled masses of varicolymphoceles of the utero-ovarian plexus, but none of the obvious manifestations of filariasis could be observed.

Coorey (1960) reported an unusual case of an ectopic gestation which showed an adult filarial worm in an ovarian cyst. In our present study, Case Number 7 which presented as menorrhagia, macrofilariae in one of the ovaries, produced granulomatous lesions in dilated lymph vessels and surrounding tissues in some sections, and no cellular reaction in other sections.

SUMMARY AND CONCLUSIONS.

A detailed histologic study of six specimens of testes, epididymes and spermatic cords and one specimen of ovary, which showed changes due to filarial infection, is presented in this paper.

An unusual case of bone formation in the testis is one of the cases wherein adult macrofilaria was demonstrated. It has been recorded in detail.

Histopathologic appearances, although of varying nature, are characteristic and should help the pathologist in the diagnosis of a filarial infection.

A rare case of adult filarial worm in an ovary is also recorded and its significance discussed.

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EVIDENCE OF ACQUIRED IMMUNITY AGAINST MICROFILARIAE OF *LITOMOSOIDES CARINII* IN ALBINO RATS WITH MITE-INDUCED INFECTION.

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INTRODUCTION.

THE course of mite-induced, untreated infection of *Litomosoides carinii* in white rats was reported by Dalip Singh and Raghavan (1962). The salient features of the observations were that the incubation or the pre-patent period was constant (about 63 days) and the average duration of patent microfilaraemia was about 18 weeks. The microfilarial density in the peripheral blood increased gradually reaching a peak in about 4 weeks, which maintained itself for about 8 weeks and then gradually decreased till none was seen in the peripheral blood. This observation, together with the fact that the adult worms were alive in most of the animals, indicated the likelihood of an acquired immunity against microfilariae. This paper reports the experimental evidence for the same.

MATERIAL AND METHODS.

Each albino rat* was exposed to the bites of a single batch of infective mites*. Blood smears (2 c.mm. blood) from the tail of the rats were examined weekly and microfilariae were enumerated when found. The observations continued throughout the period of patency and for 5 days to 35 weeks after the microfilariae disappeared from the blood as revealed by the above mentioned procedure. All the infections referred to in this report were untreated ones.

At varying intervals (specified in the text), after the microfilariae were not detectable in the peripheral blood, the rats were sacrificed and autopsied. The adult worms, when found in the thoracic cavity, were transplanted into the abdominal cavity of clean adult rats and weekly blood smears from the recipient animals were examined from the third day following transplantation as long as microfilariae continued to appear in the peripheral blood.

* The rats and mites were obtained from the colony maintained at the Institute.

The details of the techniques adopted in the present study were according to those described by Ramakrishnan *et al.* (1961) and Dalip Singh and Raghavan (1962 *loc. cit.*).

RESULTS.

In all, 92 rats were sacrificed and autopsied ; 40 showed live adult worms. Worms from eleven of the latter were transplanted into eleven clean rats (Table I). With a single exception (Rat 97-T) of the recipient rats, all of them showed microfilariae in the peripheral blood. Rat 79-T showed only 2 microfilariae on one occassion two weeks after the transplantation during a total period of 14 weeks' observation. At the time of transplantation, it was noted that the worms put into the above mentioned two rats were sluggish. The maximum period during which microfilariae were patent in the blood of animals after transplantation was 27 weeks in Rat 80-T.

Although the peripheral blood was negative to microfilariae in all the 11 donor rats, 8 of them showed at autopsy microfilariae in the heart-blood. The number of microfilariae in 2 c.mm. of heart-blood showed a wide range. This seemed to be related to the interval between the commencement of latency to microfilaraemia and the autopsy, and showed a fall as the interval increased. The microfilarial density per 2 c.mm. of heart-blood was 302 in the animal which was autopsied after 5 days of negativity in the peripheral blood. When the interval was 11 and 25 weeks the density was 39 and 10 respectively. With longer intervals, namely 31 to 35 weeks, the density was still lower and varied between 1 and 4 microfilariae per 2 c.mm. of heart-blood. An exception to this general pattern was seen in one animal (Number 175-T) that was autopsied after 44 days of latency ; the density in this case was only 6 microfilariae per 2 c.mm. of heart-blood.

Neither the duration of patency of microfilariae nor their density after transplantation of the adult worms indicated any set pattern (Table I). The course of infection in the recipient rats did not apppear to be influenced by the latent interval allowed in the donor before autopsy and transplantation. Indeed, the longest duration and the highest level of parasitaemia were seen in Rat 80-T into which were transplanted worms recovered from a rat which was negative to microfilariae for as long as 31 weeks. A limitation to the experimental observations was that the number of adult worms transplanted in each case was not estimated as too much handling was likely to injure the worms. Because of this, no inference can be drawn.

DISCUSSION.

The data presented were mostly available in the course of routine maintenance of the mite-induced *L. carinii* infection in albino rats. The recovery of adult worms from untreated animals, which had become negative to microfilariae for varying periods, and their transplantation to clean hosts and the observation of the resulting parasitaemia was specifically undertaken to determine the factor(s)

TABLE I.

Results of transplantation of adult worms, L. carinii, into normal albino rats when the donor albino rats had become negative for microfilariae in peripheral blood without treatment.

Rat into which worms were transplanted.	Period for which the donor rat remained negative for microfilariae before transplantation of worms to normal rat in Column I.	Number of microfilariae in heartblood of donor rat at autopsy.	Number of microfilariae per 2 c.mm. blood, weeks after transplantation of worms into normal rat.													
			days		Weeks											
			3	5	1	2	3	4	5	6	7	8	9	10	11	14
40-T	16 weeks	N	7	8	15	27	32	60	3	N	N	N	N	N	N	N
76-T	33 weeks	N	..	1	11	15	12	8	6	6	1	5	5	1	N	N
79-T	32 weeks	1	..	N	N	2	N	N	N	N	N	N	N	N	N	..
80-T	31 weeks	1	...	706	1055	784	540	362	110	236	139	84	128	85	46	24
87-T	11 weeks	39	5	6	10	11	15	5	1	4	2	1	2	N	N	N
88-T	35 weeks	4	N	13	Rat died.											
89-T	35 weeks	4	2	3	2	5	4	2	2	3	2	N	N	N	N	N
97-T	23 weeks	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
103-T	25 weeks	10	6	9	11	36	60	72	72	20	29	12	2	2	2	2
175-T	44 days	6	3	5	10	19	23	6	24	13	7	4	9	4	4	8
232-T	5 days	302	1	3	3	6	7	4	4	6	4	5	5	1	1	3

N=Negative for microfilariae.

responsible for the clearance of the peripheral blood of microfilariae and its (their) effect on macrofilariae. The experimental limitations to the observations have been indicated earlier, namely, (1) the original exposures of infection were made with an undetermined number of infective mites ; therefore, a quantitative estimate of the infection was not available, and (2) the number of adult worms recovered and transplanted was not enumerated. Despite the limitations, the data lent themselves to certain inferences.

Logically, any of the following factors may be responsible for microfilariae disappearing from the peripheral blood after a period of patency : (1) the death of all the adult worms, (2) sterility of the macrofilariae after a period, or (3) an immune reaction of the host. An additional question posed was whether the last mentioned factor, responsible for clearance of the peripheral blood of microfilariae, had any action on the macrofilariae also.

It was obvious from the data (Table I) that for long periods after the disappearance of microfilariae (maximum 35 weeks), macrofilariae were still available and could be recovered from the hosts. Further, on being transplanted into clean rats, they were capable of continuous reproduction. Therefore, it seemed that the factor responsible for the clearance of microfilariae from the peripheral blood did not affect the macrofilariae, in any event at the same speed as the microfilariae. There was no evidence that the macrofilariae became sterile either spontaneously or as a result of the factor responsible for clearance of peripheral blood of microfilariae. The first two of the three factors mentioned earlier could, therefore, be dismissed as possible explanations for the absence of microfilariae in the peripheral blood. It was also obvious that the only possible factor leading to clearance of the peripheral blood of microfilariae in untreated infections was an immune host reaction.

Reports of previous work on immunity in filariasis have been scanty. Bertram (1958) stated that complete immunity to filarial worms has not been demonstrated in any laboratory animal. Macdonald and Scott (1953) reported that a second homologous re-infection superimposed on the primary infection resulted in retardation of growth and development of the worms of the second infection. The growth retardation was considered as an expression of immunity. Scott *et al.* (1958) extended their observations by transplanting into previously infected cotton-rats worms which had completed 7 days' growth following primary infection in cotton-rats and found that the transplanted worms were not retarded in growth during the subsequent 17 days. They concluded that the retarding effect is produced during the first 7 days of development.

Macdonald and Scott (1958) reported that challenging infection, given after long periods, still showed a significant retardation of growth even though the immunizing infection had in some cases died since long time. Immunity produced by primary *L. carinii* infection in cotton-rats persisted for one or two years after the introduction of primary infection. It also persisted for a considerable time after the death of the primary infecting worms.

Scott (1958) stated that the principal immunizing effect is produced by worms during their first 7 days of development. The question must be left open as to whether the immunity was produced by the original infection during the first 7 days and persisted without re-inforcement, or whether the presence of live mature worms or even dead worm tissue could have some reinforcing effect.

The present work shows that even worms, taken out of infected but untreated rats in which the microfilaraemia had been latent for 35 weeks, were viable and produced microfilariae when transplanted into normal animals. Some of the donor rats (Numbers 76, 79, 80, 87 and 88) contained living as well as dead worms; others (Numbers 40, 89, 97, 103, 175 and 232) had living worms only. This finding indicated the likelihood of the presence of acquired immunity against microfilariae but not necessarily against the adult worms.

In view of the above it is considerd likely that the infection became latent when the immunity in the infected animals was sufficient to destroy the microfilariae produced by the macrofilariae.

As already stated, 8 of the 11 animals showed microfilariae in the heart-blood at the time of autopsy. Blood smears obtained from the tail of some of these animals had been consistently negative to microfilariae for 35 weeks. This indicated that the female macrofilariae continued to give rise to embryos even in the presence of a demontrable immunity, which prevented their long survival and being seen in samples of blood drawn from the tail.

The microfilaria being seen in the heart-blood at autopsy seemed to be in the nature of an agonal phenomenon (Hawking and Thurston, 1951). Worms *et al.* (1961) reported that a Jird (a rodent), infected with *Dipetalonema witei*, contained 7 microfilariae per 20 c.mm. of peripheral blood, and showed 2,850 microfilariae per 20 c.mm. blood from the left ventricle of the heart immediately after death caused to the host by a blow on the head. This indicated that during the process of dying there was a massive liberation of microfilariae from the lungs and these migrated to the heart. Duke (1960) stated that densities of microfilariae taken from the right and left sides of the heart of monkeys infected with *Loa loa* immediately after death showed that those monkeys with suppressed microfilarial densities in the peripheral blood were still harbouring numerous microfilariae in the lung-blood. The lung of one monkey showed 1,545 microfilariae per 50 c.mm. of tissue at a time when the peripheral blood showed a density of only six microfilariae per 20 c.mm.

SUMMARY.

Adult *Litomosoides carinii*, taken out at autopsy from the thoracic cavities of untreated albino rats with latent microfilaraemia and transplanted into the peritoneal cavities of clean albino rats, resulted in the appearance of microfilariae in the peripheral blood of the latter up to a maximum of 27 weeks after the worm transplant.

Evidence of acquired immunity to microfilariae but not against adult worms of the mite-induced *L. carinii* infection in albino rats is presented.

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FILARIASIS IN GORAKHPUR DISTRICT, UTTAR PRADESH.

BY

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[August 5, 1961.]

INTRODUCTION.

THE present survey was conducted in Gorakhpur District, under the National Filaria Control Programme launched in the district during the year 1957, with a view to delimiting the extent of prevalence of filarial infection and disease and to determine the type of infection and vector.

DESCRIPTION.

The district of Gorakhpur is situated in the north-eastern corner of Uttar Pradesh. It is bounded by the districts of Basti in the west, Deoria in the east, Azamgarh in the south and the territories of Nepal in the north (Map I). The northern portion of the district is criss-crossed by small streams which originate in the Himalayas and saturate the whole area with water. The Rapti, Rohin and Ami rivers run across the district, the Kuwan River cuts the south-western portion and the southern boundary is formed by the Ghaghra River. The district has a number of lakes and ponds, some of which are quite big in area.

The population of the district is 22,38,190 (1951 census) living in an area of 3,437 sq. miles. The density of population is very high, being 918 persons per square mile. The urban population is 1,66,230 while the rural population is 20,71,960.

The climate of the district is far more equable. In the western tract, great extremes of heat and cold are rarely experienced. The average maximum and the minimum temperature is 31°C. and 19°C. respectively. The average annual rainfall is 1,408 and the relative humidity ranges from 55 to 96 per cent. The meteorological data of Gorakhpur District for the year 1959 is contained in Table I. This can be considered to be typical.

The water supply in the rural and most of the urban area is from wells and hand-pumps. Safe water supply has been provided in part of the Gorakhpur

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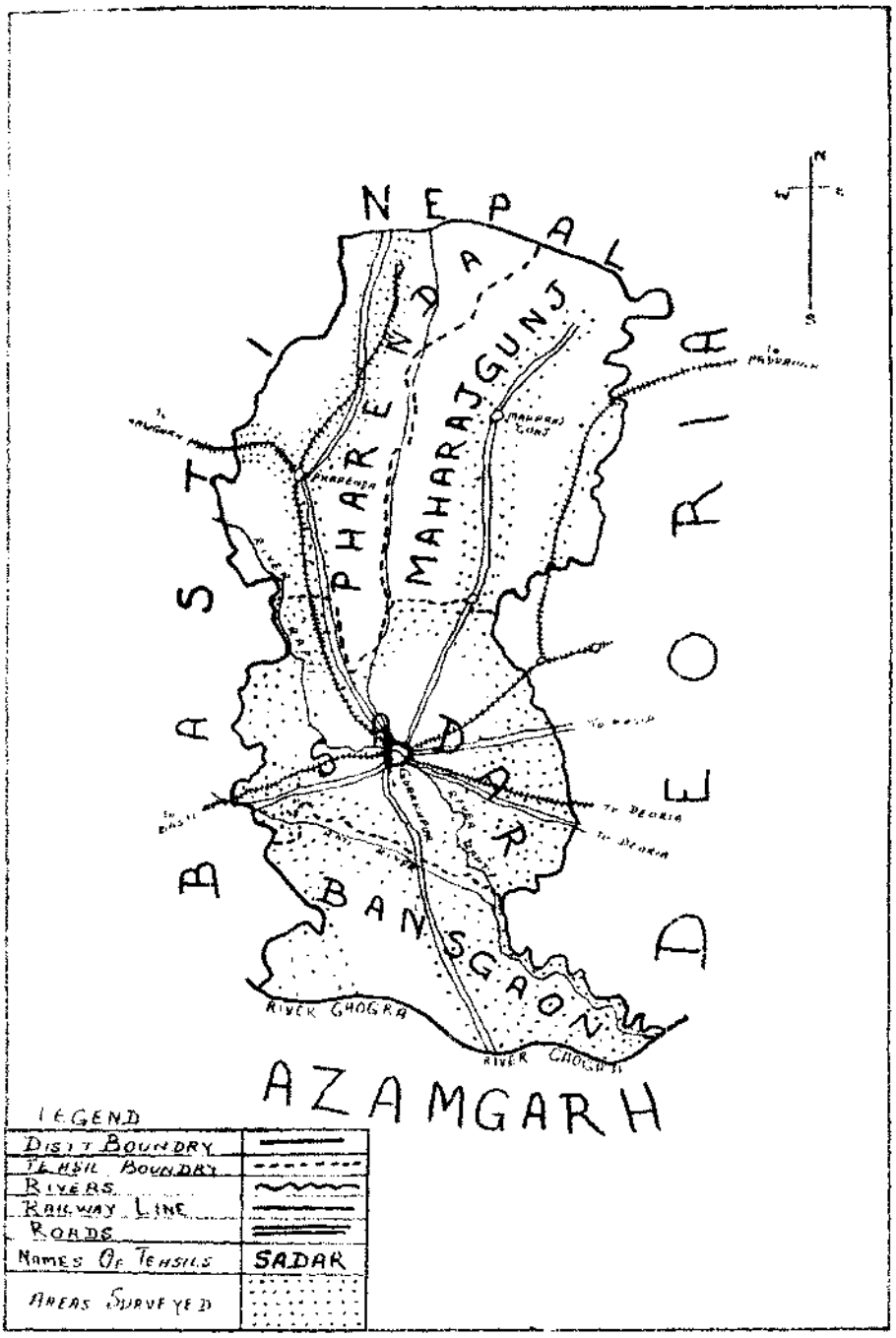
MAP 1.
GORAKHPUR DISTRICT.



Municipality. The drainage system in urban and rural areas is highly defective, leading to collection of sullage, and ablution water stagnating in pits and smallpools forming ideal breeding places of mosquitoes. In the Gorakhpur Municipality, there are no sewerage or proper surface drains. The existing drains and nullas, along with big ponds filled with water hyacinth, have intensive culicine breeding.

TABLE I.

Meteorological data of Gorakhpur District for the year 1959.

Months.	Mean maximum temperature.	Mean minimum temperature.	Mean relative humidity, per cent.		Rainfall (millimetres).
			08·00 hours.	14·00 hours.	
1959 :					
January ...	21·6°C	9·2°C	95	54	40·2
February ...	24·2°C	11·1°C	86	41	1·8
March ...	32·3°C	15·2°C	76	26	19·3
April ...	36·9°C	20·8°C	62	26	36·8
May ...	38·8°C	24·8°C	60	32	3·3
June ...	37·2°C	27·1°C	78	46	35·6
July ...	33·9°C	26·5°C	89	69	135·7
August ...	32·2°C	26·0°C	91	78	354·1
September ...	33·4°C	25·2°C	87	64	132·5
October ...	30·1°C	21·9°C	93	66	286·7
November ...	28·0°C	13·4°C	96	40	Nil
December ...	24·5°C	9·0°C	94	42	Nil

The communications inside the district are fairly good, the important places being connected by pucca roads. The economic condition of the people is of a low level, the main occupation being agriculture. Cottage industry in the district is handloom weaving. There are four sugar mills and one jute mill. Annually migration of labour takes place from this district to other industrialised centres.

HISTORY OF FILARIASIS.

Hydrocele and elephantiasis are reported to be prevalent in the district from a very long time. The prevalence of hydrocele and elephantiasis is mentioned in the District Gazetteer of Gorakhpur (Nevill, 1929) as a noticeable feature in the medical aspects of the district, being common everywhere and contracted not only by natives of the district but also by those whose business or occupation has necessiated residence there. The local belief regarding the causation of disease is drinking of bad water. Hydrocele and elephantiasis are also associated by local people with goitre which is also widely prevalent.

FILARIA SURVEY.

The survey of Gorakhpur District, comprising four tehsils (sub-divisions), namely, Sadar (Gorakhpur), Bansgaon, Mahrajganj and Pharenda was conducted during the period November, 1957, to May, 1960. Random, representative survey of all the parts of the district, including both the urban and rural areas, was carried out. Persons of all age-groups and both the sexes were examined by house-to-house visits in the night between 8 p.m. and mid-night. Approximately 20 c.mm. of blood, from a pricked finger, was obtained from each person and made into thick

smear which was examined after dehaemoglobinisation and staining for 4 minutes in J.S.B. Solution I only (Jaswant Singh and Bhattacharji, 1944). The total number of microfilariae in each positive slide was counted. The disease manifestations of these persons were also recorded during the night survey.

BLOOD SURVEY.

Out of 22,334 persons whose blood was examined, 2,944 persons were found positive for microfilaria. The infection rate worked out to 13·1 per cent. Table II shows the infection rate and the average infestation according to age-groups.

TABLE II.

Infection rate and average infestation, according to age-groups.

Age-groups (years).	Number of persons examined.	Number found positive for microfilaria.	Infection rate. per cent.	Average infestation per 20 c.mm. of blood.
0—1	11	Nil	Nil	Nil
2—5	1678	56	3·3	10
6—10	2867	236	8·2	15
11—20	4070	675	14·1	19
21—30	5212	778	14·9	20
31—40	3045	429	14·09	20
41—50	4051	590	14·5	18
Above 50	1400	180	12·8	19
Total	22,334	2,944	13·1	17·8

Table II shows that the infection rate increases as the age advances, the maximum being in the age-group of "21 to 30 years" after which it gradually starts declining. The lowest age and sex at which the infection was found was in a female child of two years. The type of infection detected was *W. bancrofti*.

The average infestation was found to be 17·8 microfilariae in 20 c. mm. of blood. The maximum number of microfilariae found in one blood-smear being 223.

DISEASE RATE

Out of 22,334 persons examined, 2,646 were found to have various disease processes in the form of hydrocele, elephantiasis of limbs etc., giving a disease rate of 11·9 per cent. Table III shows disease rate according to age-groups.

An analysis of Table III shows that the disease rate starts rising from the age-group "6-10 years" and reaches the peak at the age-group "above 50 years". The earliest age at which the disease manifestation was noted was in a male child of 2 years of age, who had hydrocele.

The diseased persons were further analysed for various disease manifestations which are contained in Table IV.

From Table IV, it is seen that the commonest manifestation was hydrocele and genital lesions and next comes the elephantiasis of the left lower limb.

TABLE III.

Disease processes according to various age-groups.

Age-groups (years).	Number of persons examined.	Number of persons showing disease processes.	Disease rate, per cent.
0—1	11	Nil	Nil
2—5	1,678	21	1·3
6—10	2,867	63	2·2
11—20	4,070	403	9·9
21—30	5,212	768	14·7
31—40	3,045	500	16·4
41—50	4,051	630	15·5
Above 50	1,400	261	18·6
Total	22,334	2,646	11·9

TABLE IV.

Percentage of disease processes.

Disease manifestations.	Total cases.	Percentage in relation to the total number of positive cases.
Hydrocele and other genital affections	1,882	71·5
Right lower limb	281	10·6
Right upper limb	116	4·3
Left lower limb	308	11·6
Left upper limb	117	4·3
Chyluria	Nil	Nil

ENDEMICITY RATE.

Out of 22,334 persons examined, 2,944 showed microfilaria in their blood and 2,646 were found showing disease manifestations, 358 persons showed both the infection in their blood and disease manifestations. The endemicity rate of Gorakhpur District thus works out to be 23·3 per cent which is highly endemic. The endemicity according to age-group is shown in Table V.

TABLE V.

Endemicity rates according to various age-groups.

Age-group (years).	Infection rate, per cent.	Disease rate, per cent.	Mixed rate, per cent.	Endemicity rate, per cent.
0—1	Nil	Nil	Nil	Nil
2—5	3·3	1·3	0·29	4·3
6—10	5·2	2·2	0·45	9·9
11—20	16·5	9·9	0·95	26·5
21—30	15·9	12·1	1·0	27·7
31—40	14·5	16·4	2·2	28·2
41—50	14·09	15·5	2·5	27·5
Above 50	12·8	18·6	2·6	28·8
Total	13·4	11·9	1·7	23·3

The data collected in the survey was further analysed for the epidemiological studies regarding sex and religion. Table VI shows the infection, disease and endemicity rates amongst the male and female population.

TABLE VI.

Infection, disease, mixed and endemicity rates according to sex.

Sex.	Number examined.	Infection rate, per cent.	Disease rate, per cent.	Mixed rate, per cent.	Endemicity rate, per cent.
Male ...	15,966	13·3	14·09	1·7	25·6
Female ...	6,368	12·8	6·2	1·3	17·8
Total	22,334	13·1	11·9	1·7	23·3

From Table VI, it is seen that the disease, infection and endemicity rates among the male population are higher than the female population.

In Table VII, disease, infection and endemicity rates have been shown according to the two main religions of the district, *viz.*, Hindus and Muslims.

TABLE VII.

Infection, disease, mixed and endemicity rates according to religion.

Religion.	Number examined.	Infection rate, per cent.	Disease rate, per cent.	Mixed rate, per cent.	Endemicity rate, per cent.
Hindus ...	18,458	12·8	11·6	1·5	22·3
Muslims ...	3,876	15·4	12·9	2·2	26·1
Total	22,334	13·1	11·9	1·7	23·3

It is seen from Table VII, that the disease, infection and endemicity rates are slightly higher amongst Muslims than in Hindus.

The infection, disease, and endemicity rates according to rural and urban areas in the district are shown in Table VIII.

TABLE VIII.

Infection, disease, mixed and endemicity rates according to rural and urban areas.

Area.	Number examined.	Infection rate, per cent.	Disease rate, per cent.	Mixed rate, per cent.	Endemicity rate, per cent.
Urban ...	6,396	11·2	12·3	0·9	22·6
Rural ...	15,938	13·9	11·6	1·8	23·
Total	22,334	13·1	11·9	1·7	23·3

From Table VIII, it is seen that the infection, disease, and endemicity rates are almost equal both in urban and rural areas.

In Table IX, the infection, disease and endemicity rates, as observed in the four tehsils (sub-divisions) of the district, are depicted.

TABLE IX.

Infection, disease, mixed and endemicity rates according to tehsils (sub-divisions) of the district.

Name of tehsil.	Infection rate, per cent.	Disease rate, per cent.	Mixed rate, per cent.	Endemicity rate, per cent.
1. Bansgaon ...	20·9	16·1	2·8	34·3
2. Pharenda ...	17·9	13·3	1·5	29·5
3. Sadar (Gorakhpur) ...	12·0	11·4	1·5	21·3
4. Mahrajganj ...	11·7	9·3	0·9	20·0

From Table IX, it is seen that the endemicity rate is highest in the Bansgaon Tehsil, followed by Pharenda, Sadar and Maharajganj. In all the four tehsils, there is high endemicity.

ENTOMOLOGICAL DATA.

Adult mosquito collection was carried out in this district from human dwellings, cattle-sheds and mixed dwellings.

A total number of 1,11,191 mosquitoes were collected. Out of this, 78,007 were culicines and 33,184 anophelines. 14,613 mosquitoes were dissected out of which 10,979 were culicines and 3,634 anophelines. The details of the dissection of mosquitoes are mentioned in Table X.

TABLE X.

Result of dissection of mosquitoes.

Name of species.	Number of mosquitoes captured.	Number dissected.	Number found positive.	Stage of development of larvae.			
				I	II	III	IV
Culex fatigans ...	70,188	10,277	771	100	306	235	133
Culex vishnui ...	4,617	124	Nil	..	Nil	..	..
Mansonioides ...	1,019	242	..	..	..	..	..
Anophelines ...	33,184	3,634	..	..	..	..	..

On a perusal of Table X, it is found that the developing forms were in *C. fatigans* only. Out of 10,277 *Culex fatigans* dissected, 771 were positive for infective larvae, the infection and the infectivity rates being 7·5 and 3·5 per cent respectively. It is, therefore, evident that the vector species of filariasis in Gorakhpur District is *C. fatigans*.

Breeding places of mosquitoes were searched out thoroughly in both the urban and rural areas, which consist of :—

1. Domestic cesspools.
2. Stagnant pools.
3. Ponds, specially those with water hyacinth.
4. Kacha nullas.
5. Septic tanks.
6. Crevices in side-walls of small running drains.
7. Effluent discharged from sugar mills in open land.

SUMMARY.

Filaria survey was carried out in all the four tehsils (sub-divisions) of Gorakhpur District from November, 1957 to May, 1960, when 22,334 persons were examined, out of which 2,944 were positive for microfilaria, the infection rate being 13·1 per cent. The lowest age and sex at which the infection was found was in a female child of two years. The average infestation was found to be 17·8 microfilariae in 20 c.mm. of blood. The type of infection was *W. bancrofti*.

Out of 22,334 persons examined, 2,646 were found to have various disease processes, the disease rate being 11·9 per cent. The earliest age at which disease manifestation was noted was in a male child of two years who had hydrocele. The chief disease manifestation was hydrocele.

The endemicity rate in the district comes to 23·3 per cent which shows high endemicity.

The endemicity rate amongst male and female population was found to be 25·6 and 17·8 per cent, while that amongst Hindus and Muslims was 22·8 and 26·1 per cent respectively. In rural and urban areas the endemicity was 23·07 and 22·6 per cent respectively.

The vector of filaria in Gorakhpur District is *Culex fatigans*.

ACKNOWLEDGMENT.

The authors are grateful to Dr. I.S. Tyagi, Research Officer and Sri Brij Bhushan Nath, Assistant Entomologist, for their active work in the field. They are also grateful to the staff of the National Filaria Control Unit, Gorakhpur, for their help in the preparation of this paper.

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SUSCEPTIBILITY STATUS OF RAT-FLEAS, *X. CHEOPIS* AND *X. ASTIA*, TO CHLORINATED HYDROCARBON INSECTICIDES IN SOME AREAS OF MADRAS AND MYSORE STATES*.

BY

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[March 15, 1962.]

THE wide-spread application of insecticides for the control and eradication of malaria has helped in reducing the incidence of certain other insects and insect-borne diseases. The decline in the flea densities and the occurrence of plague cases, the latter almost to the point of total disappearance, has been so marked that the anti-plague activities have been discontinued in some States of India. Due to their continued use, the possibility of selection of insecticide-resistant strains has increased. Patel *et al.* (1960) Mohan (1960), Sharma and Joshi (1961*a*) and Ramakrishnan *et al.* (1961) reported resistance of rat-fleas to insecticides in various parts of India. Results, obtained during studies to determine the status of susceptibility of *X. cheopis* and *X. astia* in some parts of Madras and Mysore States to chlorinated hydrocarbon insecticides, are presented in this paper.

The investigations were carried out during 1961 in (*a*) Ootacamund and Coonoor Towns of Madras State, and (*b*) in Gundlupet Town, Masti and Challaganahalli villages (Kolar District) of Mysore State. Both Ootacamund and Coonoor Towns, being above 1707 meters altitude, did not get sprayed under the National Malaria Control/Eradication Programmes. Nevertheless, insecticides have been in use in these areas since 1948 for insufflation of rat-burrows with dusting powder or spraying of houses with DDT (2·5 per cent) suspension. Gundlupet, Masti and Challaganahalli were being sprayed under the National Malaria Control/Eradication Programmes since 1954. In addition to the routine DDT spraying under the National Malaria Eradication Programme, Gundlupet Town was also sprayed with gamma BHC in 1958 and 1959 ; Masti and Challaganahalli villages were sprayed with BHC instead of DDT in 1955 and 1957 for anti-plague purposes.

MATERIAL AND METHODS.

The fleas for test of susceptibility status were collected from rodents trapped alive in the various investigation areas†.

*These studies were carried out under the auspices of the enquiry, "*Assessment of susceptibility status of insects of public health Importance,*" financed by the Indian Council of Medical Research.

†A note on the flea fauna of Nilgiris is under publication separately.

The World Health Organization provisional technique for rat-fleas [World Health Organisation Tech. Rep. Series (1960) No. 191] was employed for determining the susceptibility of fleas to insecticides. For tests against DDT and dieldrin, impregnated filter papers supplied with the WHO adult mosquito test-kit were used. Filter papers, impregnated with gamma BHC (Lindane), were prepared in the laboratory by the Busvine-Nash (1954) method. The treated papers were cut into strips measuring 5 cm. × 2·5 cm. and folded in 'Z' form longitudinally for exposure. After exposure for one hour, the fleas were transferred to clean tubes and kept for 24 hours following which the mortality in them was recorded. In cases where the mortality amongst fleas was low with one hour exposure, they were exposed to the treated papers continuously for 24 hours at the end of which the mortality was recorded without any further holding period. The dead and live fleas were then mounted and identified species and sex-wise. The data obtained was analysed using the probit method (Finney, 1952). LC_{50} was visually estimated from the *lc-pr* graph.

RESULTS.

(A) MADRAS STATE.

(i) *Ootacamund.*—Only a limited number of rat-flleas, *X. astia* and *X. cheopis*, could be collected from this area.

Susceptibility tests were carried out with *X. astia* exposing them to 1, 2 and 4 per cent DDT papers for one hour. Only 2 per cent mortality was observed even with the highest concentration used (4 per cent DDT), indicating that the *X. astia* was highly resistant to DDT in Ootacamund. Details of tests are summarised below :—

Results of susceptibility tests to DDT with X. astia *collected from Ootacamund Town. (Exposure period—One hour).*

DDT. Concentration, per cent.	Number of fleas exposed :			Percentage of fleas dead :			LC_{50} per cent.
	Total	Male	Female	Total	Male	Female	
1·0	15	3	12	0·0	0	0·0	>4
2·0	20	6	14	0·0	0	0·0	
4·0	41	14	27	2·0	0	4·0	

There was 100 per cent survival in the control groups of fleas.

Susceptibility to gamma BHC and dieldrin could not be estimated due to the low density of fleas.

X. cheopis collected, when tested, also indicated tolerance to DDT. Only seven specimens (5 females and 2 males) of *X. cheopis* were collected. Following one hour exposure, 4 females survived 2 per cent while 1 female survived 4 per cent concentration. Both the males died with 2 per cent DDT.

(ii) *Coonoor.*—*X. cheopis* and *X. astia* were both obtained from the rodents trapped but the prevailing species of fleas in the area was *X. astia.*

Susceptibility tests were carried out with DDT, gamma BHC and dieldrin. Details of the tests are shown in Table I. *X. astia* in this area was highly resistant to DDT, with LC_{50} for DDT more than 4·0 per cent with one hour exposure. Even 24 hours exposure of 219 fleas to 4·0 per cent DDT produced only 52·5 per cent mortality.

Susceptibility to gamma BHC and dieldrin was also estimated (Table I). The LC_{50} value of *X. astia** to gamma BHC was estimated to be 0·02 and for dieldrin 0·18 per cent. Increased tolerance of *X. astia* to gamma BHC was recorded in this area.

The density of *X. cheopis* was low. Consequently testing with all the three insecticides could not be as complete as with *X. astia*. The results of tests (Table I) indicated that one hour exposure to 4·0 per cent DDT gave no mortalities among the test insects. Continuous exposure to 4·0 per cent for 24 hours resulted in 77·5 per cent mortality in the fleas exposed. Exposure to gamma BHC and dieldrin yielded LC_{50} values of 0·018 and less then 0·1 per cent respectively, indicating slight tolerance to gamma BHC and normal susceptibility to dieldrin.

(B) MYSORE STATE

(i) *Gundlupet Town.*—The susceptibility tests were carried out in Gundlupet Town with both *X. astia* and *X. cheopis*. Results of the tests carried out with DDT, BHC and dieldrin are summarised in Table II. LC_{50} of *X. astia* to DDT, was more than four per cent following one hour exposure. When the exposure period was extended to 24 hours, the mortality obtained was 100 and 96·2 per cent with 2 and 4 per cent respectively.

The MLC of *X. cheopis* for DDT was estimated to be 3·5 with one hour exposure. When the exposure to 2 and 4 per cent DDT was extended to 24 hours, the results indicated that the level of tolerance to DDT was almost similar with both the species of fleas.

Susceptibility tests to gamma BHC and dieldrin revealed that the LC_{50} of *X. astia* and *X. cheopis* to gamma BHC was less than 0·0625 and 0·102 per cent respectively. The LC_{50} of dieldrin for the two species was determined to be 0·132 and less than 0·2 per cent respectively.

(ii) *Masti and Challaganhalli villages, Kolar District.*—In Masti and Challaganahalli villages, both *X. cheopis* and *X. astia* were encountered. The susceptibility tests were carried out using DDT and dieldrin papers. The results obtained in both the villages were almost identical and are depicted in Table III. From the data, it is apparent that *X. cheopis* and *X. astia* with one hour exposure gave a MLC value of more than 4 per cent DDT. But when the exposure was extended to 2 hours to 2 per cent DDT, *X. cheopis* was found to be more tolerant than *X. astia*.

* Sharma and Joshi (1961*b*) estimated the LC_{50} of a susceptible strain of *X. astia* females to be 0·001 and 0·01 per cent respectively for gamma BHC and dieldrin.

TABLE I.

Results of susceptibility tests with X. astia *and* X. cheopis *collected from Coonoor (Nilgiri District), Madras State.*

Insecticide.	Concentration (per cent).	Number of fleas exposed: Total.	Male.	Female.	Percentage of dead fleas: Total.	Male.	Female.	LC_{50} (per cent).
			(i) *Susceptibility tests with* X. astia.					
DDT (one hour exposure)	1·0	58	29	29	0	0	0	>4·0
	2·0	133	69	65	7·5	11·8	3·1	
	4·0	89	39	50	29·2	28·2	30·0	
				C=0				
Gamma B.H.C. (one hour exposure)	0·005	41	16	25	10·0	0·0	16·0	0·02
	0·01	31	13	18	23·0	24·0	22·0	
	0·02	51	22	29	41·0	50·0	35·0	
	0·04	60	27	33	88·0	85·0	91·0	
				C=0				
Dieldrin (one hour exposure)	0·1	52	18	34	32·7	38·9	29·4	0·18
	0·2	61	33	28	63·9	72·7	53·6	
	0·4	43	20	23	72·1	80·0	65·2	
	0·8	22	9	13	100·0	100·0	100·0	
	1·6	21	12	9	90·5	91·7	88·9	
				C=0				
			(ii) *Susceptibility tests with* X. cheopis.					
DDT (one hour exposure)	1·0	3	1	2	0	0	0	>4·0
	2·0	5	4	1	0	0	0	
	4·0	4	2	2	0	0	0	
				C=0				
DDT (24 hour exposure)	4·0	58	24	34	77·5	87·5	67·6	
				C=0				
Gamma B.H.C. (Lindane) (one hour exposure)	0·005	8	7	1	13·0	14·0	0	0·018
	0·01	11	3	8	18·0	33·0	13	
	0·02	9	3	6	56·0	33·0	67	
	0·04	5	3	2	100·0	100·0	100·0	
				C=0				
Dieldrin (one hour exposure)	0·1	6	4	2	66·7	100·0	0	<0·1
	0·2	22	10	12	63·6	70·0	58·3	
	0·4	12	10	2	100·0	100·0	100·0	
	0·8	2	..	2	100·0	..	100·0	
	1·6	8	7	1	100·0	100·0	100·0	
				C=0				

C=percentage mortality in control.

Tests with both *X. astia* and *X. cheopis* to dieldrin were carried out at a single dosage of 0·1 per cent with one hour exposure (Table III). As the exposure was done to one single dosage which produced mortalities less than 50 per cent, LC_{50} could not be assessed.

TABLE II.

Results of susceptibility tests with X. astia *and* X. cheopis *collected from Gundlupet Town, Mysore State.*

Insecticide.	Concentration (per cent).	Number of fleas exposed: Total.	Male.	Female.	Percentage of fleas dead: Total.	Male.	Female.	LC_{50} (per cent).
		(i) *Susceptibility tests with* X. astia.						
DDT (one hour exposure)	2·0	32	9	23	12·5	0	17·4	>4·0
	4·0	79	12	67	43·0	33·3	44·8	
				C=0				
DDT (24 hours exposure)	2·0	4	3	1	100·0	100·0	100·0	<2·0
	4·0	53	24	29	96·2	100·0	93·1	
				C=0				
Gamma B.H.C. (one hour exposure)	0·0625	25	9	16	88·0	100·0	81·3	<0·0625
	0·125	83	27	56	81·9	85·2	80·4	
	0·25	37	16	21	89·2	100·0	81·0	
	0·5	18	6	12	88·9	100·0	83·3	
	1·0			C=0				
Dieldrin (one hour exposure)	0·05	4	2	2	0·0	0·0	0·0	0·132
	0·1	48	12	36	25·0	41·6	19·4	
	0·2	36	9	27	80·6	100·0	74·1	
	0·4	19	1	18	89·5	100·0	88·9	
	0·8	23	11	12	100·0	100·0	100·0	
				C=0				
		(ii) *Susceptibility tests with* X. cheopis.						
DDT (one hour exposure)	2·0	18	10	8	38·9	60·0	12·5	3·5
	4·0	25	9	16	56·0	55·6	56·3	
				C=0				
DDT (24 hours exposure)	2·0	11	4	7	72·7	100·0	57·1	<2·0
	4·0	26	12	14	84·6	100·0	71·4	
				C=0				
Gamma B.H.C. (one hour exposure)	0·0625	15	3	12	20·0	33·3	16·7	0·102
	0·125	29	14	15	65·5	78·6	53·3	
	0·25	28	13	15	89·3	84·6	93·3	
	0·5	76	36	40	94·7	100·0	90·0	
	1·0	34	24	10	100·0	100·0	100·0	
				C=0				
Dieldrin (one hour exposure)	0·05	8	2	6	0·0	0·0	0·0	<0·2
	0·1	11	..	11	0·0	..	0·0	
	0·2	60	30	30	51·7	70·0	51·7	
	0·4	40	19	21	97·5	100·0	97·5	
	0·8	17	8	9	100·0	100·0	100·0	
				C=0				

C=percentage mortality in controls.

SUMMARY AND CONCLUSION.

Susceptibility tests against DDT, gamma BHC and dieldrin were carried out with *X. cheopis* and *X. astia* at Coonoor, and Ootacamund Towns of Madras State and in Gundlupet Town, Masti and Challaganahalli villages of Mysore State.

The observations recorded revealed that both the species of these areas were resistant to DDT and tolerant to BHC and dieldrin. All these localities had been under insecticide spraying either under the National Malaria Control/Eradication Programme or anti-plague measures for varying periods.

TABLE III.

Results of susceptibility tests with X. astia *and* X. cheopis *collected from Masti and Challaganahalli villages, Mysore State.*

Insecticide.	Concentration (per cent.)	Number of fleas exposed: Total.	Male.	Female.	Percentage of fleas dead: Total.	Male.	Female.	LC50 (per cent.)
(i) Susceptibility tests with X. astia.								
DDT (one hour exposure)	1·0	23	12	11	39·1	75·0	0·0	>4·0
	2·0	52	22	30	25·0	45·5	10·0	
	4·0	53	15	38	30·2	40·0	26·3	
				C=0				
DDT (2 hour exposure)	2·0	10	6	4	70·0	83·0	50·0	<2·0
	4·0	7	..	7	85·7	..	85·7	
				C=0				
DDT (24 hours exposure)	1·0	8	4	4	0·0	0·0	0·0	< 0
	2·0	21	6	15	100·0	100·0	100·0	
				C=0				
Dieldrin (one hour exposure)	0·1	19	8	11	36·8	75·0	9·1	
(ii) Susceptibility tests with X. cheopis.								
DDT (one hour exposure)*	1·0	42	29	13	21·4	24·1	15·4	>4·0
	2·0	67	33	34	26·9	30·3	23·5	
	4·0	82	39	43	42·7	51·3	34·9	
				C=0				
DDT (24 hours exposure)	2·0	33	18	15	84·8	100·0	66·7	<2·0
	4·0	6	2	4	100·0	100·0	100·0	
				C=0				
Dieldrin (one hour exposure)	0·1	39	23	16	46·2	60·9	25·0	
				C=0				

C=percentage mortality in controls.

*26 fleas of *X. cheopis* were exposed to 2 per cent DDT for 2 hours. The mortality obtained was 26·9 per cent compared to 70·0 per cent mortality with *X. astia* with similar exposure.

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A NOTE ON THE FLEA FAUNA OF COONOOR AND OOTACAMUND TOWNS (NILGIRIS), MADRAS STATE.

BY

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[July 15, 1962.]

KING and PANDIT (1931) have discussed the distribution of fleas in Madras Presidency during which a detailed survey of Ootacamund, Nilgiris, was made by them. Ootacamund and Coonoor towns in Madras State are situated in the Nilgiri range of mountains in South India (Lat. 21°-35′ and Long. 86°-57′) with a general slope of the land towards south-east. In the course of work undertaken to determine the susceptibility of fleas to insecticides (Mohan, 1962) the flea collections were identified sex-wise and recorded. Their monthly densities in 1961 were determined.

OOTACAMUND.

Flea collections were made periodically during 1961 ; only a limited number of rat-fleas could be collected from this area details of which are summarised in Table I. *X. astia, X. cheopis* and *C. nilgiriensis* were the three species obtained.

COONOOR.

The incidence of fleas (all species) was recorded for the year 1961 month-wise. Details of flea collection are summarised in Table II. Apart from *X. cheopis* and *X. astia, Leptopsylla segnis, Cerotopsyllus nilgiriensis* and *Stivalius ahale* were also obtained in the collections. *Ctenocephalis canis, Echidnophaga gallinaceus* and *Pulex irritans* recorded by King and Pandit (1931 *loc. cit.*) were either not encountered or not identified in the present collections.

The first two species, *X. astia* and *X. cheopis*, occurred during all the twelve months. *L. segnis* was obtained only during the winter months, December to February. *C. nilgiriensis* and *S. ahale* appeared to be absent during the summer months, May and June.

The prevailing species of flea in the Nilgiris was *X. astia*.

SUMMARY.

The flea fauna of Nilgiris was found to be rich. The collections included *X. cheopis, X. astia, C. nilgiriensis, S. ahale* and *L. segnis*.

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TABLE I.

Incidence of rat-flea species and flea index in Ootacamund Town and some villages.

Area.	Period of collection.	Number of traps set.	Number of rodents captured.	Number and species of fleas captured: *X. cheopis.*	*X. astia*	*C. nilgiriensis.*	*Unidentified.*	Total all fleas.	Total fleas Index.
Ootacamund Town	August 28 to 31, 1961.	15	26	.	2		..	2	0·08
	September 1 to 3, 1961.	25	52	..	8	..	..	8	0·15
	October 11 to 23, 1961.	43	92	1	34	12	4	51	0·55
Villages	October 9 to 23, 1961.	40	151	6	54	5	..	65	0·43

TABLE II.

Monthly incidence of rat-flea species and flea index in Coonoor (Nilgiri District) during the year 1961.

Month.	Number of traps set.	Number of rodents captured.	Number and species of flea captured: *X. cheopis.*	*X. astia.*	*L. segnis.*	*C. nilgiriensis.*	*S. ahale.*	Unidentified.	Total all fleas.	Total flea index.
1961—										
January ...	..	52	3	3	9	..	..	1	16	0·31
February ...	121	416	42	169	3	26	18	70	328	0·79
March ...	112	413	44	278	..	1	5	12	340	0·82
April ...	88	271	26	79	..	..	1	11	117	0·43
May ...	30	106	6	35	..	3	..	..	44	0·42
June ...	90	413	38	152	..	..	..	17	207	0·50
July ...	68	274	22	77	..	..	1	13	113	0·41
August ...	95	324	182	235	..	10	19	3	449	1·77
September ...	81	328	13	181	..	4	5	..	203	0·62
October ...	86	304	15	153	..	1	1	9	179	0·59
November ...	107	485	33	78	..	1	7	2	121	0·25
December ...	101	490	26	161	2	19	3	12	223	0·46

X=xenopsylla.
L. segnis=Leptopsylla segnis (=Ctenopsylla musculi).
C=Ceratopsyllus (=Nosopsyllus).
S=Stivalius.

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THE MALARIA PARASITES OF THE PIG-TAILED MACAQUE, *MACACA NEMESTRINA NEMESTRINA* (LINNAEUS), IN MALAYA.

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[August 27, 1962.]

THE pig-tailed macaque (*Macaca nemestrina nemestrina*) is an easily recognized, distinct species of macaque occurring in its various sub-species from easternmost India and East Pakistan through Burma and Thailand and the Malay peninsula to Sumatra, Borneo and certain nearby smaller islands (Ellerman and Morrison-Scott, 1931 ; Pocock, 1939). Although it is frequently kept as a pet and exhibited in zoos, little is known of its habits in nature. It is one of the few primates used as a working animal. Malays frequently train these animals to harvest coconuts, and a botanist has been known to train animals to assist in the collection of jungle plants. Occasionally, at least, this animal is closely associated with man.

The pig-tailed macaque (hereafter pig-tail) coexists over much of its range with the long-tailed macaque (*Macaca irus*), but ecologically is probably less diverse in its choice of habitats. It is less common than the long-tail and during the period spent by the authors in Malaya, was seen less frequently in nature. There have been no published records of which we are aware of malaria in the pig-tail except the very early report of Halberstadter and Prowazek (1907) that *Plasmodium inui* occurs in this species in Borneo.

This paper reports detailed studies of the prevalence of malarias in *Macaca nemestrina nemestrina* in Malaya, and represents a part of a cooperative study between the U.S. Public Health Service and the Institute for Medical Research of the Federation of Malaya.

METHODS.

Most of the monkeys studied were obtained from commercial animal trappers in Kuala Lumpur, Malaya, and originated from nearby localities as shown in Table I. Some smears were obtained from animals kept as pets by aborigines in

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the jungle in Selangor. Another part of the series was obtained in conjunction with the filariasis studies of the Institute for Medical Research (I.M.R.) in Kuantan, Pahang. Altogether 84 monkeys were studied.

Weight and sex data and information as to origin of the monkeys were recorded. Most were young animals, from two to five pounds in weight. The localities as given by the trappers could only be considered as approximate.

Single original thick and thin blood films were made from each monkey. These were stained with Giemsa stain and examined for parasites. In the majority of instances no further studies were made; however, 17 of the monkeys were studied more intensively.

In the intensive study, repeated smears were examined. Blood was inoculated intravenously into uninfected rhesus monkeys *(Macaca mulatta)* and splenectomy was performed on the pig-tails. At the same time the splenectomy was done, the liver was examined and a biopsy was done if lesions resembling those of *Hepatocystis* were seen.

Following splenectomy, the monkeys were observed for a minimum of six weeks to determine whether or not a latent malaria infection became reactivated. In two monkeys, it was not possible to carry out all of the above mentioned procedures.

Other procedures were followed whenever necessary and are described in the text. Selected strains of parasites were studied through additional passages in rhesus monkeys.

TABLE I.

Prevalence of malarias in the pig-tailed macaque. Results of original single smear examinations.

Origin of monkeys.	Number examined.	*P. cynomolgi* type parasite.	*P. inui* type parasite.	*Hepatocystis semnopitheci.*	Species undetermined.	Total positive *	Per cent positive.
Kuala Selangor District, Selangor State	48	2	10	6	4	18	38
Nr. Port Dickson, Negri Sembilan State	8	0	1	6	0	6	75
IMR series, mostly Pahang State.	16	1†	1	1	0	2	13
Kept as pets by aborigines, Selangor State	12	0	1	1	0	2	17
All monkeys.	84	3	13	14	4	28	33

* Some monkeys had more than one parasite species; total as given is the number of monkeys with one or more types of malaria.

† This monkey was from the Rembau District in Negri Sembilan,

The field work and part of the original examinations were done in the Federation of Malaya, and the monkeys to be studied intensively were shipped by air to our laboratories in the United States where facilities were available.

Some studies of mosquito infection were done utilizing standard techniques and insectary-reared *Anopheles quadrimaculatus* and *Anopheles freeborni* mosquitoes.

OBSERVATIONS.

Table I summarizes the prevalence information obtained from the original smears of the series of monkeys. Identifications were attempted, but most infections were low-grade and reservations are necessary regarding the diagnoses. Overall, 33 per cent of the pig-tails showed infection with one or more species of malaria parasites. Parasites of the *cynomolgi (vivax)* type; the *inui (malariae)* type; and of the *Hepatocystis* type were present. No infection of the *knowlesi* type was determined with certainty, but two strains were recovered after splenectomy and subinoculation. An infection with the newly described *Plasmodium fieldi* was also detected after splenectomy (Eyles *et al.*, 1962). The species could not be determined in several instances.

In Table I, prevalence is given as related to source of the monkeys, and it is seen that infections were found in each series. The numbers are so small that no significant differences can be detected, except that the Port Dickson sample was characterized by a high prevalence of *Hepatocystis* infection.

The species as tentatively identified were : *Plasmodium cynomolgi* type infection, four per cent; *P. inui* type infections, 15 per cent ; and *Hepatocystis* infections, 17 per cent.

As has been stated there was some uncertainty as to diagnosis of the type of malaria seen in single smears from some of the naturally infected pig-tails. This was due to the fact that (*1*) most of the parasitemias were scanty, (*2*) it was not certain at the start of the study that this macaque carried parasites known to science, and (*3*) the only malaria previously seen in this species was not studied in detail (Halberstadter and Prowazek, 1907).

For these reasons detailed studies were made of 17 of these macaques using the following procedures ; (*1*) Repeated smear examination ; (*2*) subinoculation of blood to rhesus monkeys; (*3*) splenectomy followed by observation to determine if latent or low-grade infections were reactivated ; and (*4*) liver examination, followed by biopsy if *Hepatocystis*-like lesions were seen.

The observations of this series of monkeys follow :—

MONKEY NUMBER N3.

Acquisition data.—From Kuala Selangor District, Selangor State. Female weighing about five pounds.

Original diagnosis.—Positive, species undetermined.

Results of repeated examinations.—Monkey was intermittently positive through a three month period prior to splenectomy. The parasite count varied from negative to 33 per c. mm.

Course of infection after splenectomy.—Following splenectomy, the parasite count of pig-tail N3 rose to 9,000 per c.mm. on the fifth day, following which the count diminished. Low level parasitemia then persisted for weeks.

It was immediately recognized that the malaria of this monkey, while resembling *P. cynomolgi*, was distinctly different. The trophozoites were compact. The infected erythrocytes were extremely heavily stippled and were frequently oval in shape and fimbriate. This parasite has been separately described (Eyles *et al.*, 1962) as *Plasmodium fieldi* and further details are given in the original description.

Results of rhesus inoculation.—The results of rhesus inoculation are given fully with the original description (Eyles *et al.*, 1962).

Liver examination.—No lesions resembling those of *Hepatocystis* were seen on the surface of the liver at the time of splenectomy.

Final evaluation.—The monkey was infected with *P. fieldi.*

MONKEY NUMBER N6.

Acquisition data.—From Kuala Selangor District, Selangor State, Malaya. Female weighing about five pounds.

Original diagnosis.—Positive, *P. cynomolgi* and *P. inui.*

Course of infection after splenectomy.—Not done.

Results of rhesus inoculation.—Blood was shipped from Malaya by air to the U.S.A. where it was injected intravenously into rhesus V345 which, after an extended incubation period, developed a low grade *inui* type infection, reaching a peak parasite density of only 1,250 per c.mm. Blood from this monkey was passed to rhesus X169 which developed an *inui* type infection with peak parasite density of 46,200 on the 17th day of the infection. It is considered that the low density of parasitemia in the first passage may have been due to attenuation due to damage in transit to the United States.

Liver examination.—Not done.

Final evaluation.—Monkey was infected with a *P. inui* type parasite. Morphologically the parasites were indistinguishable from established strains of *P. inui.* Since the intensity of infection in the second passage was similar to that usually associated with *P. inui* in rhesus monkeys, we are considering that the parasite from this monkey is typical *P. inui.* This strain is being maintained and has been designated the N6 strain of *P. inui.*

MONKEY NUMBER N9.

Acquisition data.—From Kuala Selangor District, Selangor State, Malaya. Male weighing about seven pounds.

Original diagnosis.—Positive, *P. cynomolgi* and *P. inui.*

Course of infection after splenectomy.—Not done.

Results of rhesus inoculation.—Blood from this monkey was shipped by air to the U.S.A. where it was injected intravenously into rhesus V371. After an extended incubation period, an *inui* type infection was developed, reaching a peak parasite density of 23,100 per c.mm. Rhesus X162, inoculated from V371, developed an infection with a high parasite density of 282,800 per c.mm. This strain is being maintained and has been designated the N9 strain of *P. inui.*

Liver examination.—Not done.

Final evaluation.—This monkey was infected with *P. inui.*

MONKEY NUMBER N34.

Acquisition data.—From Port Dickson area, State of Negri Sembilan, Malaya. Male weighing about five pounds.

Original diagnosis.—Positive, *Hepatocystis.*

Results of repeated examinations.—This monkey was consistently positive for several weeks prior to splenectomy with parasite count from 58 to 241 per c.mm. Most parasites appeared to represent *Hepatocystis.*

Course of infection after splenectomy.—A *P. inui* type infection developed which reached a peak parasite density of 658,400 per c.mm. on the 21st day after splenectomy. *Anopheles quadrimaculatus* mosquitoes were fed and a moderate mosquito infection resulted. Growth of the oocysts was slow as is characteristic of *P. inui.* The passage of this strain to rhesus by mosquito from the naturally infected host demonstrated the practicability of isolating strains in this natural manner ; however, it was not possible to continue passage with this strain.

Morphological studies made on monkey N34 and on rhesus monkey X535 showed parasites of the *inui* type, characterized by quartan periodicity, lack of enlargement of erythrocyte, occasional band-form trophozoites and segmenters with eight to twelve merozoites, arranged in rosette form around a clumped pigment mass. Light stippling of the erythrocytes was prominent both in the original pig-tail host and in the rhesus, this phenomenon being more marked than in strains of *inui* with which the authors were familiar. This strain is being maintained and has been designated the N34 strain of *P. inui.*

Results of rhesus inoculation.—Blood inoculation to intact rhesus X261 produced *inui* type infection, peaking at 5,500 parasites per c.mm. on the 26th day of the infection. Blood inoculation to splenectomized rhesus X535 produced *inui* type infection, peaking at 620,000 per c.mm. on the 20th day of the infection. Salivary glands from *Anopheles quadrimaculatus* mosquitoes fed on N34 were injected into splenectomized rhesus X403 which reached a parasite density of 868,000 per c.mm. on the 32nd day after gland injection, at which time it was treated with chloroquine. Parasites were of the *inui* type.

Liver examination.—Twenty to thirty *Hepatocystis* type lesions were seen on the surface of the liver at the time of splenectomy. Biopsy and histological procedures confirmed the identification.

Final evaluation.—Monkey had a mixed infection of *Hepatocystis semnopitheci* and *P. inui*. Only the former was evident prior to splenectomy.

MONKEY NUMBER N56.

Acquisition data.—From Port Dickson area, State of Negri Sembilan, Malaya. Female weighing about five pounds.

Original diagnosis.—Positive, *Hepatocystis* and *P. inui*.

Results of repeated examinations.—Four smears were consistently positive with from 50 to 300 parasites per c.mm. Mostly *Hepatocystis* type parasites were seen.

Course of infection after splenectomy.—Parasite count rose to 6,625 per c.mm. on the 19th day after splenectomy but all parasites appeared to be *Hepatocystis* and no segmenting forms were seen.

Results of rhesus inoculation.—Blood inoculation to rhesus X918 did not produce patent infection.

Liver examination.—Numerous *Hepatocystis* like lesions were seen on the surface of the liver at the time of splenectomy, and *Hepatocystis* nature of the lesions was confirmed histologically.

Final evaluatian.—Monkey was infected with *Hepatocystis semnopitheci*. *P. inui* did not grow out in rhesus monkey or in the splenectomized pig-tail and the original diagnosis may have been in error.

MONKEY NUMBER N57.

Acquisition data.—From Port Dickson area, State of Negri Sembilan, Malaya. Male weighing about four pounds.

Original diagnosis.—Positive, *Hepatocystis*.

Results of repeated examinations.—Five smears from the monkey were consistently positive during the three months prior to splenectomy. Only *Hepatocystis* type parasites were identified, and the parasite count ranged from 67 to 863 per c.mm.

Course of infection after splenectomy.—Monkey continued to show *Hepatocystis* parasites with densities up to 847 per c.mm. No segmenting forms were seen at any time.

Result of rhesus inoculation.—Blood inoculation on two occasions to rhesus R49 did not produce patent infection.

Liver examination.—Twenty or more *Hepatocystis* type lesions were seen on surface of liver at time of splenectomy. Biopsy and histological procedures confirmed identification.

Final evaluation.—Monkey was infected with *Hepatocystis semnopitheci*.

MONKEY NUMBER N58.

Acquisition data.—From Port Dickson area, State of Negri Sembilan, Malaya. Female weighing about five pounds.

Original diagnosis.—Positive, *Hepatocystis.*

Results of repeated examinations.—Five smears, examined during the three months preceding splenectomy, were all positive for *Hepatocystis* ; parasite densities ranging from 58 to 2,808 per c.mm. No segmenting forms were seen at any time.

Results of rhesus inoculation.—Blood, inoculated into intact rhesus X874 prior to splenectomy of N58, did not produce patent infection. Blood, injected into splenectomized rhesus X760 after the splenectomy of N58, produced a *cynomolgi* type infection, peaking at 600,000 parasites per c.mm.

Subinoculation from rhesus X760 to intact rhesus X202 produced a severe infection, reaching a parasite density of 724,800 per c.mm. From the above, it is seen that this strain produces infections similar in intensity to those produced by established strains of *P. cynomolgi*. Morphologically the parasite closely resembled the established strains.

Course of infection following splenectomy.—The monkey continued to show *Hepatocystis* parasites at densities up to 1,088 per c.mm. Nineteen days after splenectomy, a *cynomolgi* type malaria infection became apparent, which reached a peak parasitemia of 34,800 per c.mm. on the 21st day. The count then diminished rapidly and a few days later only *Hepatocystis* parasites could be recognized in the blood smears.

The *cynomolgi* type parasites closely resembled those of *P. cynomolgi bastianellii* and typical *P. cynomolgi* previously studied by us and did not resemble the new *P. fieldi* (*see* monkey N3). The parasites were amoeboid in form and the erythrocytes tended to be round. The stippling, though evident, was not heavy. The mature segmenters usually contained 10 or more merozoites. We consider that this strain represents true *P. cynomolgi*. It has been preserved by freezing for future study, and has been designated the N58 strain.

Liver examination.—Twenty-five or more *Hepatocystis* type lesions were seen on the surface of the liver at the time of splenectomy. Histological study, following biopsy, confirmed the identification.

Final evaluation.—This monkey was infected with *Hepatocystis semnopitheci* and with *P. cynomolgi.*

MONKEY NUMBER N59.

Acquisition data.—From Port Dickson area, State of Negri Sembilan, Malaya. Female weighing about four pounds.

Original diagnosis.—Positive, *Hepatocystis.*

Results of repeated examinations.—The monkey was intermittently positive for *Hepatocystis* during three months prior to splenectomy (five smears). Parasite densities ranged from negative to 2,425 per c.mm.

Course of infection after splenectomy.—Monkey continued to show *Hepatocystis* parasites with densities up to 8,600 per c.mm.

Results of rhesus inoculation.—Blood injected into rhesus R50 on three occasions failed to produce patent infection.

Liver examination.—About 10 *Hepatocystis* type lesions were seen on the surface of the liver at the time of splenectomy. Identity of lesions confirmed histologically following biopsy.

Final evaluation.—Monkey was infected with *Hepatocystis semnopitheci.*

MONKEY NUMBER N60.

Acquisition data.—From Port Dickson area, State of Negri Sembilan, Malaya. Male weighing about three and one-half pounds.

Original diagnosis.—Positive, *Hepatocystis.*

Results of repeated examinations.—Monkey was consistently positive during three months preceding splenectomy (six smears). Most of the parasites appeared to be *Hepatocystis* and no segmenting forms were seen. Parasite densities ranged from 41 to 564 per c.mm.

Course of infection following splenectomy.—Monkey continued to show *Hepatocystis* parasites and density increased to as high as 2,000 per c.mm. on the 25th day after splenectomy.

Results of rhesus inoculation.—Blood inoculated into rhesus X960 did not produce patent infection.

Liver examination.—About eight lesions, resembling *Hepatocystis*, were seen on surface of liver at time of splenectomy. Histological study, following biopsy, confirmed the identification.

Final evaluation.—Monkey was infected with *Hepatocystis semnopitheci.*

MONKEY NUMBER N62.

Acquisition data.—From Kuala Selangor area, Selangor State, Malaya. Female weighing about four pounds and five ounces.

Original diagnosis.—Positive, *P. inui.*

Results of repeated examinations.—Only two examinations were made prior to splenectomy, both positive, parasite density 921 per c.mm.

Course of infection after splenectomy.—An infection developed reaching a peak parasite density of 496,000 per c.mm. on the ninth day after splenectomy. Parasites showed moderate stippling but otherwise were typical of *P. inui.*

Although subinoculation demonstrated the presence of *P. knowlesi*, it was not recognized in pig-tail N62.

Results of rhesus inoculation.—Blood inoculated into rhesus X524 produced a *knowlesi* type infection, peaking at 1,240,000 parasites per c.mm. on the 7th day of the infection and the monkey died later that day. Unsuccessful treatment with sulfamerazine had been instituted on the sixth day.

Liver examination.—Examination of the surface of the liver at time of splenectomy revealed no lesions.

Final evaluation.—Monkey was infected with *P. inui* and *P. knowlesi*.

MONKEY NUMBER N65.

Acquisition data.—From Kuala Selangor District, Selangor State, Malaya. Female weighing about four pounds.

Original diagnosis.—Positive, *P. inui*.

Results of repeated examinations.—Monkey intermittently positive with parasite densities up to 125 per c.mm.

Course of infection after splenectomy.—An infection with *inui* type parasites developed, peaking at 250,800 parasites per c.mm. on the 29th day after splenectomy.

Results of rhesus inoculation.—Blood inoculated into rhesus V328 produced an *inui* type infection, peaking at 62,800 parasites per c.mm. on the 13th day of the infection.

Liver examination.—No lesions seen on the surface of the liver at the time of splenectomy.

Final evaluation.—Monkey was infected with *P. inui*.

MONKEY NUMBER N73.

Acquisition data.—From Kuala Selangor District, Selangor State, Malaya. Female weighing about four pounds.

Original diagnosis.—Negative.

Results of repeated examinations.—Nine smears prior to splenectomy were all negative.

Course of infection after splenectomy.—Monkey remained negative.

Results of rhesus inoculation.—Blood inoculated into rhesus X509 did not produce patent infection.

Liver examination.—No lesions seen on surface of liver at time of splenectomy.

Final evaluation.—Monkey was not infected with parasites which could be demonstrated by methods employed.

MONKEY NUMBER N74.

Acquisition data.—From Kuala Selangor District, Selangor State, Malaya. Female weighing about four pounds.

Original diagnosis.—Positive, species undetermined.

Results of repeated examinations.—Nine smears prior to splenectomy were all negative except original.

Course of infection after splenectomy.—Monkey showed parasitemia eight days after splenectomy, and infection of the *knowlesi* type ensued which killed the monkey on the 14th day. Parasite count just before death could not be done accurately but was estimated to be about 800,000 per c.mm.

Two unmistakable male gametocytes of *Hepatocystis* were seen on thin smears from this monkey and a few other suspected *Hepatocystis* parasites were seen in thick films.

Results of rhesus inoculation.—Blood inoculated to rhesus X818 on the 12th day after splenectomy produced a fatal infection, monkey dying on the fifth day after inoculation. Parasites were typical of *P. knowlesi*.

Liver examination.—No lesions were seen on the surface of the liver at the time of splenectomy, but two small lesions were seen post-mortem. Histological study demonstrated these to be due to *Hepatocystis*.

Final evaluation.—Monkey was infected with *P. knowlesi* and *Hepatocystis semnopitheci*.

MONKEY NUMBER N79.

Acquisition data.—From Kuala Selangor District, Selangor State, Malaya. Female weighing about six pounds.

Original diagnosis.—Positive, *Hepatocystis*.

Results of repeated examinations.—Monkey was intermittently positive throughout period of one month prior to splenectomy. Count varied from negative to 2,500 per c.mm.

Course of infection after splenectomy.—Parasite count rose to 52,000 per c.mm. on 20th day after splenectomy, but all parasites seen appeared to be *Hepatocystis* and no segmenting forms were seen at any time.

Results of rhesus inoculation.—Blood injected intravenously into rhesus R27 did not produce patent infection.

Liver examination.—Examination of the surface of the liver at the time of splenectomy revealed 10 to 20 *Hepatocystis*-like lesions, which were confirmed histologically as *Hepatocystis*.

Final evaluation.—Monkey was infected with *Hepatocystis semnopitheci*.

MONKEY NUMBER N80.

Acquisition data.—From Kuala Selangor District, Selangor State, Malaya. Male weighing about seven pounds.

Original diagnosis.—Positive, *Hepatocystis* and *P. inui.*

Results of repeated examinations.—Monkey intermittently positive during the month preceding splenectomy with peak parasite density of 166 per c.mm.

Course of infection after splenectomy.—An *inui* type infection developed, reaching a peak parasite density of 682,000 per c.mm. on the 8th day after splenectomy. The monkey died on the ninth day probably from the malaria infection.

Results of rhesus inoculation.—Not done.

Liver examination.—Only a single lesion about three mm. in diameter was seen on the liver. This lesion did not resemble lesions of *Hepatocystis.*

Final evaluation.—Monkey was infected with *P. inui.*

MONKEY NUMBER N85.

Acquisition data.—From Kuala Selangor District, Selangor State, Malaya. Male weighing about three and one-half pounds.

Original diagnosis.—Negative.

Results of repeated examinations.—Four smears during three weeks prior to splenectomy, all negative.

Course of infection following splenectomy.—Monkey remained negative.

Results of rhesus inoculation.—Blood inoculation into rhesus X780 did not produce patent infection.

Liver examination.—No lesion seen on surface of liver when examined at time of splenectomy.

Final evaluation.—Monkey had no parasites demonstrable by methods employed.

MONKEY NUMBER N86.

Acquisition data.—From Kuala Selangor District, Selangor State, Malaya. Male weighing about three pounds.

Original diagnosis.—Negative.

Results of repeated examinations.—Three smears during three weeks preceding splenectomy, all negative.

Course of infection following splenectomy.—Monkey remained negative.

Results of rhesus inoculation.—Blood inoculated into rhesus X173 did not produce patent infection.

Liver examination.—No lesions seen on surface of liver at time of splenectomy.

Final evaluation.—Monkey had no parasites demonstrable by methods employed.

From the intensive study of the 17 pig-tails, it was determined that five species of hematozoa are present in this species in Malaya.

Plasmodium fieldi, a new species which is being described separately (Eyles et al., 1962), was found in a single monkey.

Plasmodium cynomolgi was also found in a single monkey. The blood forms seemed identical to those of *P. cynomolgi bastianellii* and to typical *P. cynomolgi*. Studies of the exoerythrocytic parasites will be necessary to determine which subspecies was represented.

Plasmodium inui was recovered from five of the 17 monkeys. Although showing stippling to a moderate degree this parasite was otherwise similar to the established strains of *P. inui* from the long-tailed macaque, and the rhesus monkey was similarly susceptible to it.

Plasmodium knowlesi was found in two monkeys, and would probably have been overlooked in one had not a subinoculation to a rhesus monkey, which produced a fatal infection, revealed its presence. In the other monkey a fatal *knowlesi* infection in the pig-tail followed splenectomy.

Hepatocystis semnopitheci was observed in the blood of eight monkeys and in seven cases liver lesions, studied histologically, confirmed the identity of the parasite. This is a new host record for *Hepatocystis*. We are considering it as species *semnopitheci*, since evidence to the contrary is lacking. As more information on the basic biology of this parasite becomes available, it may be necessary to revise this opinion.

The blood forms of this parasite closely resemble those of *H. kochi* described by Garnham (1948). The liver cysts resemble the cysts described in *Macaca irus* by Edeson (1953) which were at first attributed to *Plasmodium knowlesi*. Since the original publication, these authors have acknowledged that the parasites were probably *Hepatocystis*, and the recent finding of this parasite in *Macaca irus* in Thailand by Garnham (1961) adds confirmation.

Chance probably was responsible for the high prevalence of *Hepatocystis* in the intensive study sample, since six of the eight infected monkeys (N34 and N56 through N60) were obtained from the same source. We are attempting to pinpoint the origin of these animals for possible future study.

Comparison of the information derived from the intensive study with that derived from the original smears on the same monkeys, summarized in Table II, gives information on the reliability of the survey method. Several facts stand out.

All monkeys, originally found negative, remained negative after splenectomy, and rhesus monkeys inoculated from them did not develop infections. Admittedly the number of negative animals was too small to allow a sweeping conclusion but

so far as the study goes it indicates a higher degree of reliability than expected for the original smear examination.

TABLE II.

Comparison of the results of the original examination with findings after intensive study of 17 pig-tail macaques.

Monkey number.	Original diagnosis.	Final diagnosis.
N3	*P. inui*	*P. fieldi*
N6	*P. inui, P. cynomolgi*	*P. inui*
N9	*P. inui, P. cynomolgi*	*P. inui*
N34	*H. semnopitheci*	*P. inui, H. semnopitheci*
N56	*H. semnopitheci. P. inui*	*H. semnopitheci*
N57	*H. semnopitheci*	*H. semuopitheci*
N58	*H. semnopitheci*	*H. semnopitheci, P. cynomolgi*
N59	*H. semnopitheci*	*H. semnopitheci*
N60	*H. semnopitheci*	*H. semnopitheci*
N62	*P. inui*	*P. inui, P. knowlesi*
N65	*P. inui*	*P. inui*
N73	Negative	Negative
N74	Positive, species undetemined	*P. knowlesi, H. semnopitheci*
N79	*H. semnopitheci*	*H. semnopitheci*
N80	*P. inui, H. semnopitheci*	*P. inui*
N85	Negative	Negative
N86	Negative	Negative

Identifications from the original survey smears were mostly correct, but a number of mixed infections were not detected originally. Some probable errors of original identification were detected. *Plasmodium knowlesi*, which was unsuspected from the original survey, became evident after splenectomy and when subinoculation was made to rhesus monkeys.

SUMMARY.

Blood films from 84 pig-tailed macaques (*Macaca nemestrina nemestrina*) from the Federation of Malaya were examined for malaria parasites. One-third of the monkeys showed infection and the parasites were identified as *Plasmodium cynomolgi*, *P. inui*, and *Hepatocystis semnopitheci*. Some parasites could not be identified and it was realized that many of the identifications were uncertain.

In order to determine with more certainty the parasites present in this species in Malaya, intensive studies involving repeated smears, splenectomy, and subinoculation to rhesus monkeys were done with 17 animals, most of which had shown some type of parasite on original examination. These intensive studies confirmed the existence in this species of monkey of *P. cynomolgi*, *P. inui*, and *H. semnopitheci*. They also revealed the unsuspected presence of *P. knowlesi* and a new species separately described as *Plasmodium fieldi*.

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HOST PREFERENCE OF *C. FATIGANS* WIED., 1828, IN SOME STATES OF INDIA : RESULTS OF PRECIPITIN TESTS CARRIED OUT IN 1960 AND 1961.

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[July 22, 1962.]

CULEX FATIGANS is the primary vector of *W. bancrofti* infection which is widely prevalent in India (Jaswant Singh and Raghavan, 1953). The host preference of the vector in some of the States participating in the National Filaria Control Programme (Raghavan, 1955) was determined between 1956-1961 and the analysis of the observations are recorded here. Due to reasons explained later, the observations between 1956 to 1959 have been considered separately and not included in the analysis.

A total of 19,549 blood meals of *C. fatigans* received from Andhra Pradesh, Assam, Bihar, Gujarat, Kerala, Madras, Madhya Pradesh, Maharashtra, Mysore, Orissa and Uttar Pradesh were tested during 1956-1959. About half the total number of blood meals tested gave negative reaction to all the five antisera (man, buffalo, dog, goat and pig) in use. The anthropophilic index varied from a minimum of 21·2 per cent in Madras to a maximum of 64·7 per cent in Kerala. The mean was 49·8 per cent.

There were probably more than one reason for the high proportion of negative reactions recorded above. The blood-meals, some of which were poor in quality and partially digested, were also included in the tests. The technique adopted was that of Barber and Rice (1935), using capillary tubes ; this also appears to have been one of the factors responsible for the high percentage of negative results.

Trials were carried out to study the utility of Durham's fermentation tubes (50 mm. long and 5—6 mm. diameter) for carrying out the tests. It was observed that this change in the technique yielded a higher proportion of positive results as compared to the method of Barber and Rice (1935 *loc. cit.*). The change was necessitated and tried as an experimental measure due to shortage in supplies of capillary tubes of the required calibre and has, since 1960, been used as a routine procedure at this Institute. The advantage of the Durham's tubes are : (i) the ease with which they could be cleaned, and (ii) the chances of diffusion of the antigen and antisera are reduced, and even very fine rings can be easily discerned.

The antisera for testing the source of blood-meals* were prepared at the Institute† from the various antigens. Partially digested blood-meals and samples that were poor in quality or quantity were discarded as these were considered to be partly responsible for the high percentage of negative results during the previous years.

Out of 8,986 samples received and tested during 1960 and 1961, 8,228 (91·6 per cent) yielded positive results. Of these 77·4 per cent were positive for human blood. State-wise analysis of results of tests, according to the types of the habitations from which the mosquitoes were collected, is depicted in Table I. The percentage of negative reactions in various States and from different resting places was low, ranging from nil to 17·0 per cent. The percentage positive reaction with human antiserum did not show wide variations irrespective of the types of shelters‡ or the State of origin.

CONCLUSIONS.

The most preferred host of *C. fatigans* is man ; cattle, dog and pig follow in that order. The nature of the shelter does not indicate that the mosquitoes have a preference to feed on the host available in that shelter.

*The specimens of mosquito blood-meals collected in the field were received at the Malaria Institute of India, Delhi, within about a month after collection. These specimens were stored at the Institute in an air-tight dessicator placed inside a refrigerator. The mosquito blood-meals were tested with the least delay after their arrival, the time lag between their arrival and testing being 1 to 6 weeks. After reconstitution, the blood-meal was allowed to stand for 4 hours to ensure proper extraction. Every sample was tested against four types of antisera, those of man, cattle, dog and pig.

†The blood of buffalo, dog and pig was collected from the local butchery and Veterinary Hospital. Human serum was obtained from the blood bank of a local hospital. The serum was separated, centrifuged and deactivated in water bath at 56°C. for half an hour and then filtered through Seitz filter so as to make it sterile. A single injection of the sterilized antigen was given intravenously to the fowls. Six fowls were generally immunized at a time for each antigen, using an immunizing doze 3 c.c. for a fowl weighing 3 lbs. or less and an additional c.c. for every pound beyond this weight. The immunized fowls, after being starved on the 10th day, were bled to death on the 11th day. The blood was collected in sterile bottles and the serum allowed to separate. The antiserum was then centrifuged and tested for its titre and specificity with the help of homologous test sera and two other sera of different animals. If found specific and positive in dilution of 1 to 2000 or above, the antiserum was transferred to sterilised glass ampoules, sealed and stored in the freezing chamber of a refrigerator.

‡The collections were made only from indoor shelters.

TABLE I.

Results of testing of Culex fatigans *blood-meals by precipitin test, resting places and years of collection.*

State of origin of the specimens tested.	Year of collection.	Total:			Break-up of the resting places: Human habitations.			Animal sheds.			Mixed dwellings. Human and animal dwellings.			Unspecified.		
		Blood-meals tested.	Giving positive reaction.	Percentage positive for human blood.	Total giving positive reaction.	Percentage positive for human blood.	Percentage giving no positive reaction.	Total giving positive reaction.	Percentage positive for human blood.	Percentage giving no positive reaction.	Total giving positive reaction.	Percentage positive for human blood.	Percentage giving no positive reaction.	Total giving positive reaction.	Percentage positive for human blood.	Percentage giving no positive reaction.
Andhra Pradesh ...	1960	2,255	2,020	68·8	1,004	69·1	14·0	876	66·6	6·3	140	79·6	7·9	..	..	..
	1961	8	8	100·0*	5	100·0	0·0	3	100·0	0·0	..	..	..	..	..	..
Bihar ...	1960	3,985	3,805	81·6	2,307	82·9	4·2	1,243	81·1	4·6	230	75·2	8·0	25	48·0	0·0
	1961	127	124	87·4	124	87·4	2·4	..	..	..	..	..	..	..	..	.
Gujarat ...	1960	100	88	71·0	31	62·9	11·4	..	..	..	57	75·3	12·3	..	.	..
	1961	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Maharashtra ...	1960	209	187	77·0	175	77·6	10·7	..	..	..	12	69·2	7·7	..	..	..
	1961	303	280	76·5	280	87·5	7·6	..	..	.	..	..	..	..	..	..
Mysore ...	1960	402	371	87·3	362	87·5	7·4	..	..	..	9	81·8	18·2	..	..	..
	1961	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Uttar Pradesh ...	1960	1,300	1,099	73·1	574	73·3	13·8	253	74·0	15·7	269	71·6	18·7	3	100·0	0·0
	1961	297	246	80·5	225	80·4	17·0	21	80·8	19·2	..	..	..	..	..	..

* Samples inadequate in number.

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THE ISOLATION AND DESCRIPTION OF *PLASMODIUM CYNOMOLGI* (MAYER, 1907) AND *PLASMODIUM INUI* (HALBERSTADTER AND PROWAZEK, 1907) FROM NATURALLY OCCURRING MIXED INFECTIONS IN *MACACA RADIATA RADIATA* MONKEYS OF THE NILGIRIS, MADRAS STATE, INDIA.

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[March 13, 1962.]

THE finding of an enzootic focus of simian malaria in *Macaca radiata radiata* of the Nilgiris, Madras State, India, has been described by Ramakrishnan and Mohan (1962). Samples of blood from the naturally infected monkeys were despatched from the Nilgiris to the Malaria Institute of India at Delhi, where clean *Macaca mulatta mulatta** monkeys are regularly available in sufficient numbers.

Each of two blood-samples from two monkeys captured in the Nilgiris with patent mixed infection of *P. cynomolgi* and *P. inui* was inoculated intravenously into *mulatta* monkeys numbers 1990 and 2199 respectively. The resulting patent infection in the recipients showed a predominance of *P. cynomolgi* in the former and *P. inui* in the latter. Two separate lines of rapid serial blood passage from the two monkeys were made when there was a predominance of *P. cynomolgi* in one line and *P. inui* in the other as revealed by daily blood examinations. The procedure was in accordance with that of Mulligan (1935). The isolation of pure *cynomolgi* was achieved in 6 passages and that of pure *inui* in 5 passages. The pure lines have since been maintained and confirmed to be pure by daily blood examinations.

The morphological description, periodicity of the asexual cycle in the erythrocytes, and the course of single, untreated infections in *Macaca mulatta* monkeys are reported here separately in respect of each of the two species.

PLASMODIUM CYNOMOLGI (NILGIRI STRAIN)

Morphology (Plate I).—The parasites were as a rule seen in the normocytes; in the peak of the infection ; however, they were sometimes seen in polychromatophilic erythrocytes. Except when infested with very early forms of the para-

* In the course of the last nearly 30 years, no natural malarial infection in the *mulatta* monkeys of North India has ever been encountered in 600 to 2,500 monkeys used every year at the Institute.

sites, the infested host cell was invariably enlarged. Such enlarged erythrocytes showed well-marked acidophilic stippling which seemed finer and more abundant than that in erythrocytes infested by the Mulligan* or *bastianelli* strains of the same species.

Usually, the infection of the erythrocyte was by a single parasite. Occasionally, multiple infections of the same cell occurred. Sometimes the outline of the host cell was distorted, oval, pear shaped or even irregular. (Plate I, Figs. 13 to 15).

When the parasitaemia was high, one or more parasites engulfed by monocytes could be seen. Monocytes containing golden-yellow, dark-brown or dark pigment, were also present. (Plate I, Figs. 16 and 17).

The earliest forms of the parasite seen in the peripheral blood were solid, small bodies measuring about $\frac{1}{8}$ to $\frac{1}{16}$ of the diameter of the red cell. The ring forms were typical—signet rings—with the vacuole. The chromatin mass was usually single with a circular outline. Occasionally, it seemed oval. It was as a rule typically situated on the cytoplasmic ring. At times it appeared more elongated than usual and located within the vacuole. Ring forms with two chromatin masses were also seen at times. In such cases the two masses were close together as well as widely separated from each other. When separated, the sizes of the masses were unequal. The location of the ring forms within the erythrocyte varied and occasionally was marginal. As a rule, erythrocytes with the ring forms were not enlarged; some, however, were enlarged and stippled.

Erythrocytes with trophozoites (Plate I, Figs. 2 to 6) were always enlarged and stippled. The younger forms were about a quarter to half and the older about two-thirds the size of the erythrocyte. The outline of the parasite was irregular. Frequently long irregular strands of the protoplasm were seen. The trophozoites showed pigment which varied from golden yellow granules to dark-brown masses.

Prior to nuclear division the parasite was found to be compact and occupied a greater portion of the erythrocyte. The vacuole was very much reduced and generally absent. The chromatin mass was seen to elongate and segment into four, six, eight masses irregularly distributed in the cytoplasm. Pigment was distributed in the form of golden-brown and dark granules.

The number of merozoites per mature schizont varied from 16 to 20 with an average of 16. The pigment was generally aggregated into a single dark-brown mass with cytoplasm situated either centrally or peripherally.

The microgametocyte (Plate I, Fig. 11) was usually found to have a circular outline occupying about three-quarters of the enlarged erythrocyte. In some instances it appeared elongated or oval. The chromatin mass was diffused and stained lightly. The pigment granules were scattered.

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* The Mulligan strain of *P. cynomolgi* was isolated by Sinton and Mulligan (1933) from *Sillenus irus* (Synonym *Macaca radiata radiata* Geoffroy) from Malaya. This strain has since been maintained in *Macaca mulatta mulatta* at the Institute by blood passage.

PLATE I.

Plasmodium cynomolgi (Nilgiri strain).

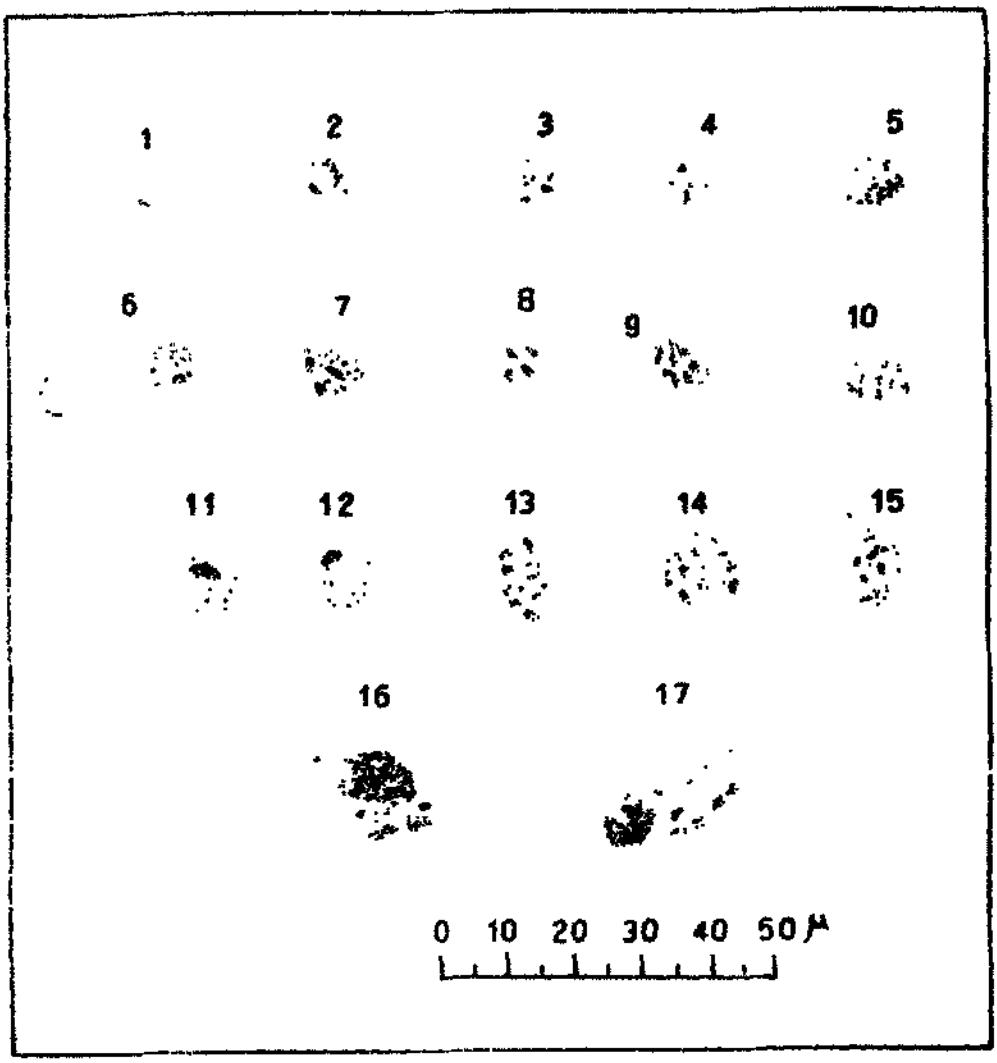


FIG. 1. Ring. FIGS. 2 to 6. Trophozoites. FIGS. 7 to 10. Schizonts. FIG. 11. Microgametocyte. FIG. 12. Macrogametocyte; FIGS. 13 to 15. Multiple infections; FIGS. 16 and 17. Monocytes with parasites and pigment.

The macrogametocyte (Plate I, Fig. 12) was also usually circular in outline, occupying a little more than three-fourths of the enlarged erythrocyte. At times the outline was elongated or oval. The compact chromatin mass stained deeply and was situated in the centre or periphery of the parasite. The dark and, at times, black pigment granules were scattered in the cytoplasm.

The average diameter of normal and infested erythrocytes were measured in the blood of monkeys infected with the Nilgiri, bastianelli and Mulligan strains of *P. cynomolgi* respectively. The measurements are tabulated in Table I.

TABLE I.

Diameters of normal erythrocytes and those infected with different growth stages of* P. cynomologi, *Nilgiri, bastianelli and Mulligan strains respectively.*

Types of erythrocytes measured.	NILGIRI STRAIN :		BASTIANELLI STRAIN :		MULLIGAN STRAIN :	
	Number measured.	Average diameter in microns.	Number measured.	Average diameter in microns.	Number measured.	Average diameter in microns
Uninfected.	343	7·6	372	7·7	274	7·6
Infected with :						
rings	305	7·7	278	8·2	237	8·1
trophozoites	365	8·6	304	8·9	278	8·6
mature schizonts	305	9·2	214	9·4	184	9·2
micro-gametocytes	133	8·6	173	9·4	144	9·2
macrogametocytes	245	9·0	188	9·6	160	9·4

*Only one diameter, right to left, was measured.

It appeared that the enlargement of the erythrocytes, infested with all the three strains of the parasite, was similar.

PLASMODIUM INUI (NILGIRI STRAIN)

The infested host cell was not found enlarged at any stage of the infection (Plate II). Occasionally, however, a slight enlargement of the cell was noticeable in the case of multiple infection of the same cell (Plate II, Figs. 6 and 7). In general, only the normocytes were found infected; occasionally, however, at the peak of infection a few polychromatophilic cells were found infected too. The host cells were devoid of any recognizable stippling; rarely, very scanty fine dots were seen.

The earliest erythrocytic stage of the parasite was seen to be solid—not unlike a merozoite—without a vacuole. The mass of chromatin, relatively large, was situated on one side of the cytoplasm. The vacuole appeared within a few hours of growth. The ring stage of the parasite occupied about a fourth of the erythrocyte. The chromatin of the ring stage was often double, and the lobes of unequal size. Multiple infections of the same cell were not uncommon (Plate II, Figs. 1 to 9).

Amoeboidicity of the early trophozoites seemed to be more as compared to that of the Mulligan strain of *P. inui*. The pigment, golden-yellow or brown, appeared

in the early trophozoites. In the older forms of the parasite the pigment was abundant and dark. Some of the trophozoites (Plate II, Fig. 9) were seen as typical band forms.

The early schizont (Plate II, Figs. 11 and 12) showed the commencement of the division of chromatin. The vacuole in these forms was small, if present. The number of merozoites varied from eight to sixteen per schizont with an average of twelve. The pigment in the mature schizont was seen to be compact in one or two irregular masses and dark in colour (Plate II, Figs. 15 and 16)

The microgametocyte (Plate II, Figs. 17 and 18) occupied the major portion of the erythrocyte. The faintly pink chromatin was dispersed and occupied most of the cytoplasm which was seen at the periphery, stained light-blue. The brownish dark pigment was found scattered all over the parasite.

The macrogametocyte (Plate II, Figs. 19 and 20) occupied practically the whole of the erythrocyte which very occasionally appeared slightly enlarged. The brick-red chromatin was compact and the cytoplasm deep blue. The pigment granules were scattered in the cytoplasm.

Comparative measurements were made of the diameter of the normal erythrocytes and those infested with the Nilgiri and Mulligan* strains of *P. inui*, and the results are shown in Table II.

TABLE II.

Diameter of normal erythrocytes and those infested with different growth stages of P. inui, *Nilgiri and Mulligan strains respectively.*

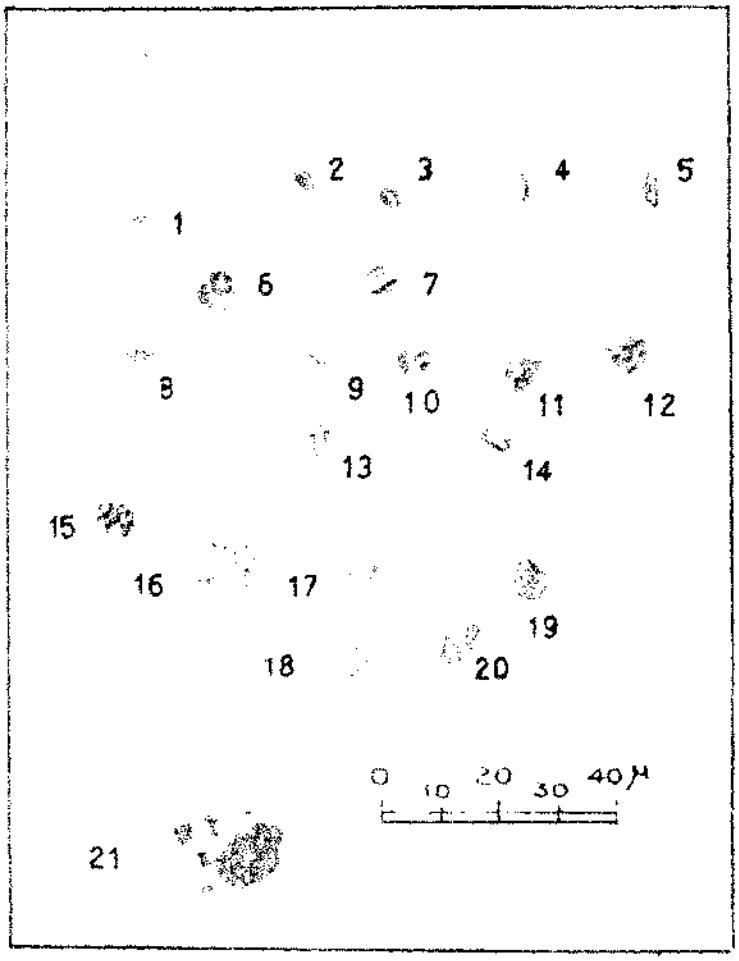
Types of erythrocytes measured.		NILGIRI STRAINS :		MULLIGAN STRAINS :	
		Number measured.	Average diameter, in microns.	Number measured.	Average diameter, in microns.
Uninfected.	...	466	7·5	387	7·8
Infected with :					
rings	...	423	7·5	274	7·8
trophozoites	...	349	7·7	321	8·1
early schizonts	...	421	7·5	318	8·3
mature schizonts	...	319	7·8	316	8·7
microgametocytes	...	246	7·7	228	8·5
macrogametocytes	...	263	7·3	251	8·5

It appeared that the enlargement, if any, of the erythrocytes infested with the two strains of the species was minimal.

* The Mulligan strain of *P. inui* was isolated from *M. irus* from Malaya by Sinton and Mulligan (1933). This strain has since been maintained in *M. mulatta mulatta* at the Institute by blood passage.

PLATE II.

Plasmodium inui (Nilgiri strain).



FIGS. 1 to 9. Rings, trophozoites and multiple infections. FIGS. 10 to 16. Schizonts; FIGS. 17 and 18. Microgametocytes; FIGS. 19 and 20. Macrogametocytes; FIG. 21. Monocyte with pigment and a parasite.

PERIODICITY.

The periodicity of the asexual forms of the Nilgiri strain of the two species was determined in accordance with the technique described by Mulligan (1935). Four-hourly blood smears were examined through 5·5 days in four monkeys infected with *cynomolgi* and through 15 days from 2 monkeys infected with *P. inui*. The following six stages of development were enumerated:

1. Early non-vacuolated forms with chromatin.
2. Rings.
3. Trophozoites.
4. Early dividing forms (2 chromatin divisions).
5. Developing schizonts (3 to 5 chromatin divisions), and
6. Mature schizonts (with more than 5 chromatin divisions).

A total of 250 parasites were enumerated at random in every slide and classified as belonging to one or the other stages listed above. The data were graphed. The occurrence of maximum number of mature schizonts as well as the early forms showed a clear peak every 48 hours in the case of *P. cynomolgi*. In the case of *P. inui* maximum number of mature schizonts showed a peak every 72 hours. Such a clear periodicity was, however, not apparent in the intermediate stages as a certain degree of overlap was inevitable in the arbitrary classification of the growing stages. No periodicity could be discerned in respect of the earliest forms in the case of *P. inui*, perhaps due to the generally lower number of parasites and the presence of secondary broods. Graphs I and II depict the data in respect of monkey numbers 2291 and 2481 in respect of *P. cynomolgi* and *P. inui* respectively.

THE COURSE OF INFECTION.

P. CYNOMOLGI (NILGIRI STRAIN).

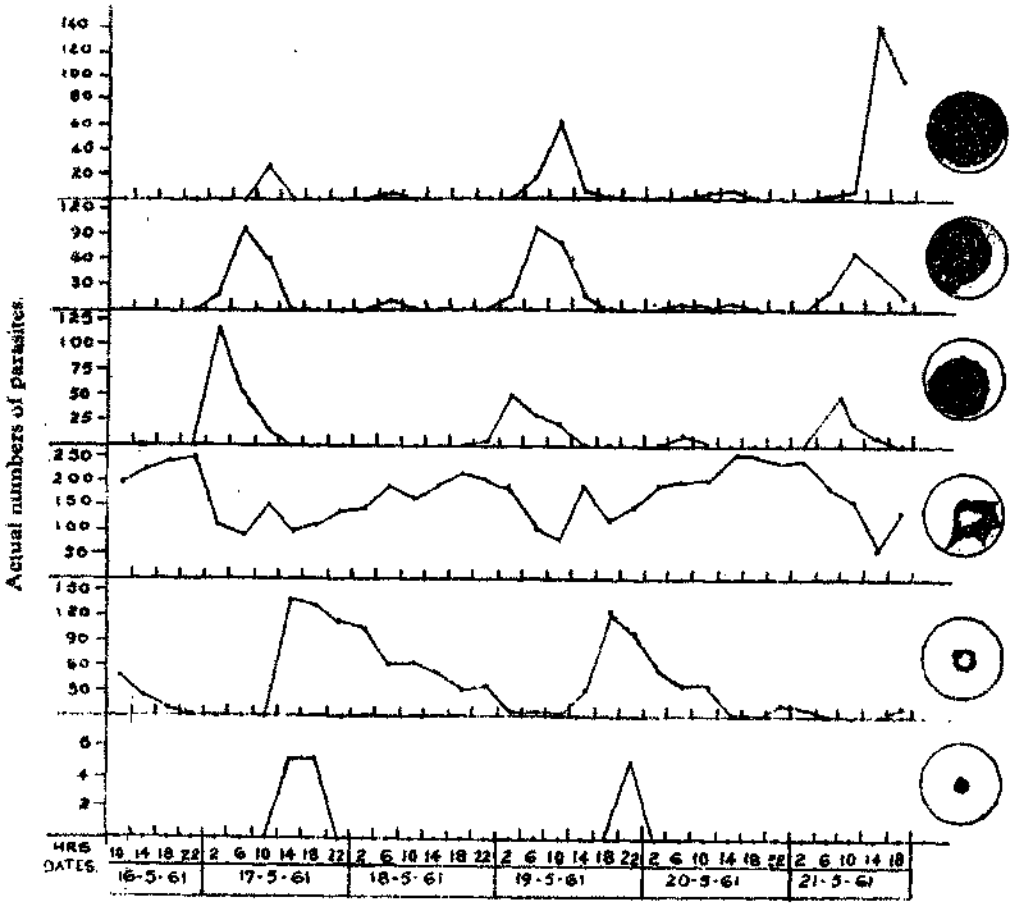
Of the 124 monkeys (*M. mulatta*) inoculated with *P. cynomolgi* during a part of the studies reported here, 106 died at different periods within 50 days of inoculation. During a certain period of the observations a considerable number died within a few days of the inoculation due to an intercurrent infection of diarrhoea. A few died of a pulmonary infection during the coldest part of the winter. Course of a single untreated infection in 6 monkeys that lived for 75 days or more from the date of inoculation, was scrutinised. The analysis of the data is shown in Table III.

The weight of four of the monkeys varied from 1·47 to 2 kg. and other two weighed 3 and 4 kg. respectively. The prepatent period in five of them was one day while in one it was two days. The course of the infection was essentially the same in all of them. The period of initial uninterrupted parasitaemia varied from 5 days in one to 26, 33, 43, 50 and 53 days. In the case of monkey number 2385, however, the short uninterrupted initial parasitaemia of 5 days was made up

GRAPH 1.

Periodicity of erythrocytic schizogony of P. cynomolgi *isolated from naturally infected monkeys.* (Macaca radiata radiata Geoffroy) *in Coonoor (Nilgiris).*

Monkey . 2291



GRAPH 2.

Periodicity of erythrocytic schizogony of P. inui *isolated from naturally infected monkeys.* (Macaca radiata radiata *Geoffroy*) *in Coonoor (Nilgaris).*

Monkey No. 2481

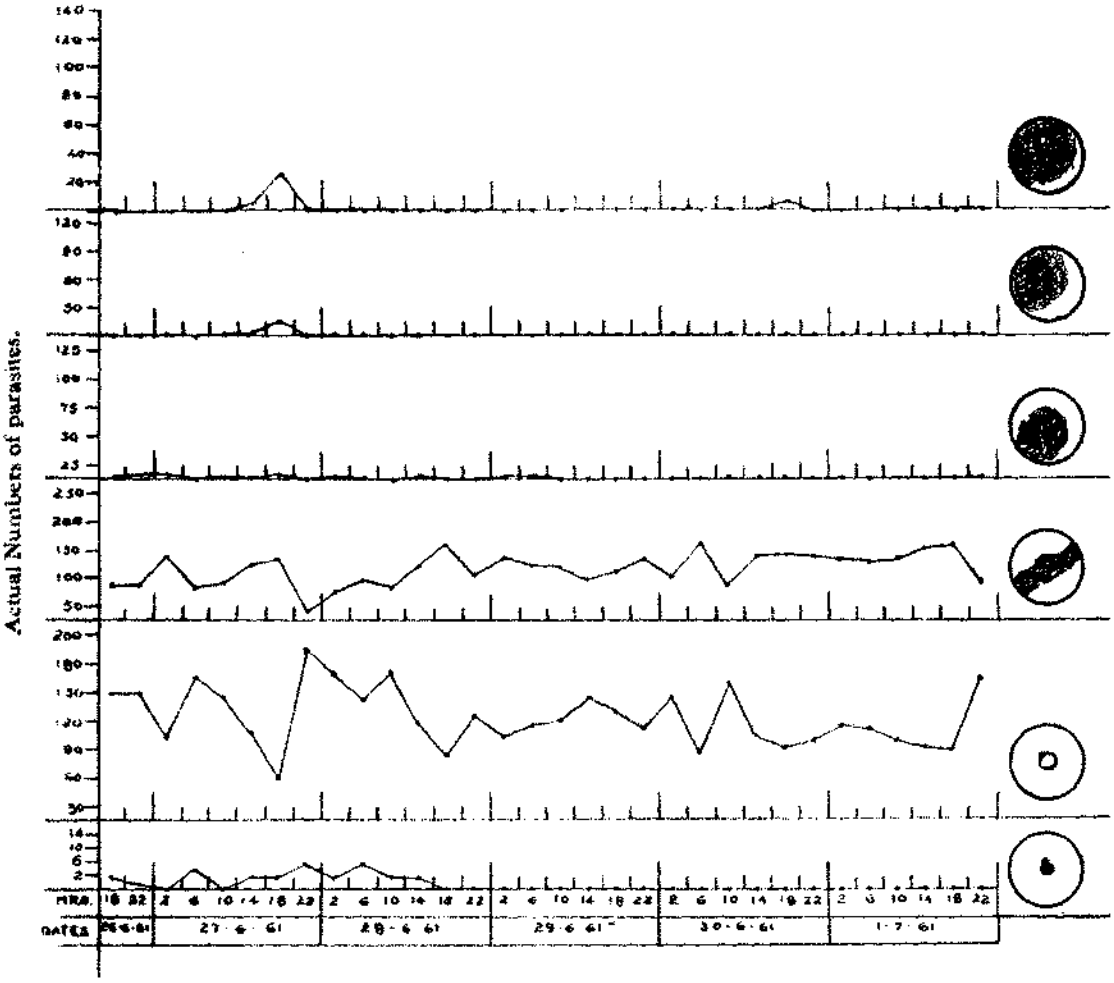


TABLE III.

The course of a single untreated infection of P. cynomolgi (*Nilgiri strain*) in Macaca mulatta mulatta *monkey that survived 75 days of the infection. Dose of inoculation* 5×10^6 *parasites, intravenously.*

Monkey number, sex and weight.	Pre-patent period, in days.	Number of episodes of patent parasitaemia.	Number of latent episodes.	Period (in days) of successive episodes of patent parasitaemia								Period (in days) of successive latent episodes :								Total period of observation, in days.
				I	II	III	IV	V	VI	VII	VIII	I	II	III	IV	V	VI	VII	VIII	
Male 2282, 2·0 kg.	2	8	8	33	8	7	3	7	7	7	7	10	4	5	4	20	20	29	19	192
Male 2385, 1·47 kg.	1	3	3	5	61	15	..	..	..	..	..	13	10	13	..	..	..	..	..	118
Male 2388, 1·47 kg.	1	2	2	53	11	..	..	..	..	..	..	13	38	..	..	..	..	..	..	116
Male 2533, 1·8 kg.	1	3	3	50	12	11	..	..	..	..	..	12	18	49	..	..	..	..	..	153
Male 3304, 3 kg.	1	3	3	43	14	11	..	..	..	..	..	6	13	16	..	..	..	..	..	104
Female 3401, 4 kg.	1	3	3	26	15	6	..	..	..	..	..	6	7	17	..	..	..	..	..	78

TABLE IV.

The course of a single untreated infection of P. inui (*Nilgiri strain*) *in 3* Macaca mulatta mulatta *monkeys that survived 75 days or more. Dose of inoculation* 5×10^6 *parasites intravenously.*

Monkey number, sex and weight.	Prepatent period, in days.	Number of episodes of patent parasitæmia.	Number of latent episodes.	Period, in days, of successive episodes of patent parasitæmia.				Period, in days, of successive latent episodes.				Total period of observation, in days.
				I	II	III	IV	I	II	III	IV	
Female 2528, 2·0 kg.	5	4	4	147	6	4	12	11	5	10	16	216
Female 3095, 6·6 kg.	6	3	2	15	15	46	..	4	7	..	..	93
Female, 3348, 5·5 kg.	4	2	2	49	11	..	..	4	8	..	..	76

after a latent period of 13 days by a second period of uninterrupted patent parasitaemia of 62 days. Even allowing for individual variation, no specific pattern of relapse could be made out. Except in the case of monkey number 2385 where as short a period as of 5 days of initial patent parasitaemia was followed by a latent period of 13 days, the patent episodes, following the first, were uniformly shorter in duration.

P. INUI

A total of 117 monkeys infected with *P. inui* were observed in the course of the studies reported here. Of them, 115 died at different periods of similar causes as mentioned in respect of monkeys infected with *P. cynomolgi*. The results of the daily examination of blood smears were scrutinised in respect of the 3 monkeys that could be observed for a minimum period of 75 days from inoculation. The analysis of the data is shown in Table IV.

The monkey with lowest weight (Number 2528) had the largest initial period of uninterrupted patent parasitaemia. The monkey with the highest weight (Number 3095) had the shortest period of uninterrupted initial patent parasitaemia. No inferences of pattern of alternating patency and latency could be drawn from the small numbers of monkeys that survived a sufficient period of observation.

SUMMARY.

The isolation was effected in *Macaca mulatta* monkeys of *P. cynomolgi* and *P. inui* from naturally occurring mixed infections in *Macaca radiata radiata*. The morphological description, periodicity of the asexual cycle, and the course of single untreated infections in *Macaca mulatta* are described.

Periodicity studies of the asexual erythrocytic cycle was found to be 48 hours in the case of *P. cynomolgi*. That of *P. inui* seemed to be 72 hours. The periodicity was not as clearly seen in the latter as in the former.

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STUDY OF THE EFFECT OF CLIMATE ON THE DENSITY OF *C. FATIGANS* AND THE DEVELOPMENT OF FILARIAL PARASITE IN IT.

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[September 16, 1961.]

THE existence of a correlation between the climatic conditions and the filarial endemicity of an area was suggested by Acton and Rao (1930) and Iyengar (1938). Acton and Rao (1930 *loc. cit.*) in their study of the degree of endemicity of a number of important towns in India, in relation to their meteorological conditions, came to the conclusion that filarial endemicity was largely determined by the extent of the period in the year during which favourable climatic conditions and the prevalence of vector mosquitoes coincided in these places. The effect of climate on the vector, the parasite in the vector or the vertebrate host, and consequently on the endemicity of filariasis in any locality, has been reviewed by Raghavan (1958).

The present paper is limited to the observations of the effect of climate on the vector *C. fatigans* and the development of parasite in it, as carried out for three years from January, 1957, to December, 1959, by the Filaria Control Unit, Barabanki, Uttar Pradesh.

TOPOGRAPHY.

Barabanki District, a component of Faizabad Division, lies at the very heart of Uttar Pradesh and forms a portion of the tract lying between the rivers Gomti and Ghagra. It is considered to be one of the most fertile and densely populated district in the State. The district may generally be described as a level plain with poor natural drainage. The water level is very close to the surface and during rains many parts of the district get water-logged.

METEOROLOGICAL DATA.

For the present study, climatological data of Barabanki District from January, 1957, to December, 1959, have been obtained from the Meteorological Department of the Government of India, New Delhi (Tables I, II and III). The district can be stated to have a moderate climate.

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TABLE I.

Average monthly maximum, minimum and mean temperature (in centrigade) in Barabanki from 1957 to 1959.

Months.	Years: 1957			1958			1959			Average for 3 years.		
	Maximum.	Minimum.	Mean.	Maximum.	Minimum.	Mean.	Maximum.	Minimum.	Mean.	Maximum.	Minimum.	Mean.
January	21·3	10·5	15·9	24·3	10·1	17·2	21·9	9·9	15·9	22·5	10·1	16·3
February	25·4	9·9	17·65	26·6	11·3	18·95	24·4	11·1	17·75	25·46	10·7	18·0
March	30·8	15·5	23·15	33·3	17·0	25·15	32·7	16·7	24·7	32·26	16·4	24·3
April	36·7	21·0	28·85	38·2	23·0	30·4	38·1	22·2	30·15	37·66	22·06	29·7
May	40·7	25·9	33·3	41·3	25·6	33·45	40·1	26·1	33·1	54·03	25·8	33·2
June	40·4	27·5	33·95	41·7	28·4	35·05	38·3	27·3	32·8	40·13	27·7	33·9
July	34·2	26·3	30·25	34·3	26·0	30·15	33·5	26·1	29·8	34·0	26·1	30·0
August	38·4	25·5	29·45	32·00	24·8	28·4	32·8	25·6	29·2	32·7	25·3	29·0
September	32·7	24·2	28·45	32·9	24·9	28·9	33·7	25·2	29·45	33·1	24·7	28·9
October	33·3	19·5	26·4	31·9	20·9	26·4	32·1	21·4	26·75	32·43	20·6	26·5
November	29·2	13·2	21·2	29·1	13·4	21·2	N.A.*	N.A.	N.A.	29·15	13·3	21·2
December	23·4	10·1	16·75	24·5	10·7	17·6	N.A.	N.A.	N.A.	23·95	10·4	17·1

*N.A. = Data not available.

TABLE II.

Relative humidity (per cent) in Barabanki from 1957 to 1959.

Months.	Years: 1957			1958			1959			Average for 3 years.		
	8.30 a.m.	5.30 p.m.	Mean.	8.30 a.m.	5.30 p.m.	Mean.	8.30 a.m.	5.30 p.m.	Mean.	8.30 a.m.	5.30 p.m.	Mean.
January ...	84	66	75·0	84	58	71·0	85	62	73·5	84·33	62·0	73·16
February ...	60	45	52·5	60	41	50·5	73	47	60·0	64·33	44·33	54·33
March ...	58	33	45·5	48	22	35·0	56	30	43·0	54·00	28·33	41·16
April ...	39	16	27·5	37	25	31·0	39	24	31·5	38·33	21·66	30·0
May ...	39	25	32·0	36	14	25·0	44	28	36·0	39·66	22·33	31·0
June ...	53	36	44·5	47	29	38·0	66	48	57·0	55·33	37·66	46·83
July ...	81	68	74·5	79	72	75·5	84	71	77·5	81·33	70·33	75·83
August ...	86	79	82·5	87	79	83·0	87	78	82·5	86·66	78·66	82·66
September ..	81	68	74·5	82	75	78·5	81	64	72·5	81·33	69·0	75·16
October ...	71	53	62·0	73	69	71·0	79	64	71·5	74·33	62·0	68·16
November ...	80	57	68·5	67	56	61·5	N.A.*	N.A.	N.A.	73·5	36·5	63·5
December ...	85	64	74·5	82	65	73·5	N.A.	N.A.	N.A.	83·5	64·5	74·6

* N.A. = Data not available.

TABLE III.

Monthly average rainfall (in millimetres) in Barabanki from 1957 to 1959.

Month.	Years :			Average (3 years).
	1957	1958	1959	
January ...	51·3 mm.	10·7 mm.	42·7 mm.	34·7 mm.
February ...	0·0 ,,	2·3 ,,	3·3 ,,	1·7 ,,
March ...	4·6 ,,	3·5 ,,	2·0 ,,	3·0 ,,
April ...	0·8 ,,	0·0 ,,	0·3 ,,	0·3 ,,
May ...	0·0 ,,	0·0 ,,	42·2 ,,	14·0 ,,
June ...	46·5 ,,	100·8 ,,	36·3 ,,	61·2 ,,
July ...	340·9 ,,	185·9 ,,	218·9 ,,	247·6 ,,
August ...	305·3 ,,	372·1 ,,	222·7 ,,	300·0 ,,
September ...	191·0 ,,	165·9 ,,	139·1 ,,	165·0 ,,
October ...	0·0 ,,	146·3 ,,	28·7 ,,	60·9 ,,
November ...	0·0 ,,	0·0 ,,	1·0 ,,	0·3 ,,
December ...	5·1 ,,	6·8 ,,	0·0 ,,	3·8 ,,
Total annual rainfall (in millimetres)	945·5 ,,	994·3 ,,	737·2 ,,	892·5 ,,

From Tables I, II and III, it will be seen that Barabanki District has a mean relative humidity above 60 per cent for 7 to 8 months in a year, and the mean monthly temperature lies within the 15°C. to 32°C. range almost the whole year—conditions condsidered optimum both for the breeding of *C. fatigans* and the development of parasite in it (Basu and Rao, 1939).

METHODS AND MATERIAL.

For the above mentioned study, entomological data were collected from the special study and check areas of the Filaria Control Unit, Barabanki. These areas have a total population of about 60,000 comprising both urban and rural components. The area selected has representative physiographical conditions of the entire district.

Each area had sixty catching stations, distributed equally in human dwellings and cattle-sheds. Mosquito collections were made simultaneously from both the areas, every catching station being visited weekly by an Insect Collector. Catches were made for 10 minutes at each station between 06·00 and 11·00 hours.

The mosquitoes were collected by suction tube. The specimens collected in the field were brought to the laboratory for identification and dissection. The per man-hour density of *C. fatigans* and the infection and infectivity rates were worked out and the results are shown in Table IV (*i.e.*, per man-hour density of *C. fatigans* in the Special Study Area and in the Comparison Area) and Tables V and VI [*i.e.*, infection and infectivity rates in *C. fatigans* in the (*a*) Special Study Area, and (*b*) in the Comparison Area, respectively].

TABLE IV.

Monthly average per man-hour density of Culex fatigans, *(i) in the Special Study Area (relating to the period January, 1957, to December, 1959), and (ii) in the Comparison Area (relating to the period January, 1958, to December, 1959).*

Month.	Special study area: Average per man-hour density of *C. fatigans* (relating to the period January, 1957 to December, 1959).	Comparison area: Average per man-hour density of *C. fatigans* (relating to the period January, 1958 to December, 1959).
January	30·50	18·00
February	18·30	14·00
March	37·40	37·50
April	38·30	41·00
May	28·80	21·00
June	11·30	10·00
July	12·00	15·75
August	7·00	9·50
September	7·00	5·75
October	13·00	11·60
November	22·00	10·50
December ...	23·00	10·60

OBSERVATIONS.

Tables I, II and III represent the maximum, minimum and mean monthly temperature; mean monthly rainfall and relative humidity respectively for the period January, 1957, to December, 1959, while in Tables IV, V and VI, the per man-hour density of *C. fatigans*, and the infection and the infectivity rates have been shown for the corresponding period. By super-imposing the above mentioned climatological and entomological data in Graphs I and II, an attempt has been made to study the correlation between the climatic conditions and *(a)* density of *C. fatigans* and *(b)* development of parasite in *C. fatigans, i.e.*, the infection and infectivity rates.

(A) DENSITY OF *C. FATIGANS*.

Table IV shows that the monthly average per man-hour density of *C. fatigans* is highest during the months of March and April both in the Special Study Area (37·4 and 38·3 per man-hour) and Comparion Areas (37·5 and 41·0 per man-hour). During these months the temperature ranges between 25°C. and 30°C, the relative humidity is 30 to 41 per cent and the rainfall is negligible. With the onset of hot weather (from the month of May), the density starts declining and remains low during the monsoon period (July to September), touching the lowest 7 and 5·75 per man-hour in the Special Study Area and in the Comparison Area respectively in September when the rains are heavy.

During the monsoon period, though the temperature (25°C. to 30°C.) and relative humidity (over 60 per cent) are favourable for the longevity of the mosquitoes (Basu and Rao, 1939 *loc. cit.*), the heavy rainfall seems to be inimical to the breeding of *C. fatigans* due to its flushing action.

TABLE V.

Infection and infectivity rate in C. fatigans, *relating to the period March, 1957 to December, 1959, in the Special Study Area.*

Month.	Years: 1957			1958			1959			Average from March, 1957 to December, 1959.	
	Number of *C. fatigans* dissected.	Infection rate, per cent.	Infectivity rate, per cent.	Number of *C. fatigans* dissected.	Infection rate, per cent.	Infectivity rate, per cent.	Number of *C. fatigans* dissected.	Infection rate, per cent.	Infectivity rate, per cent.	Infection rate, per cent.	Infectivity rate, per cent.
January	..	..	..	339	0·8	0·5	830	0	0	0·4	0·25
February	..	..	..	162	0	0	750	0·1	0	0·05	0
March	58	0	0	852	0·6	0	770	0·3	0	0·3	0
April	1,001	1·6	0·4	1,053	0·7	0·2	980	0·2	0·1	0·5	0·2
May	451	3·5	1·3	1,015	1·5	0·1	421	1·4	0	2·1	0·4
June	398	4·5	2·0	359	0·6	0	476	1·4	0·4	2·2	0·8
July	770	3·0	0·8	520	1·0	0·4	610	4·5	1·5	2·8	0·9
August	240	1·2	Nil	238	0·8	0·4	485	7·2	1·8	3·0	0·7
September	Nil	Nil	Nil	351	0·5	0·3	493	7·4	1·8	4·0	1·0
October	131	1·5	Nil	393	0·7	0	537	6·0	0·7	2·7	1·2
November	278	1·4	Nil	350	0	0	790	3·0	0·6	1·5	0·2
December	554	0·5	Nil	681	0	0	980	2·5	0·6	1·0	0·2

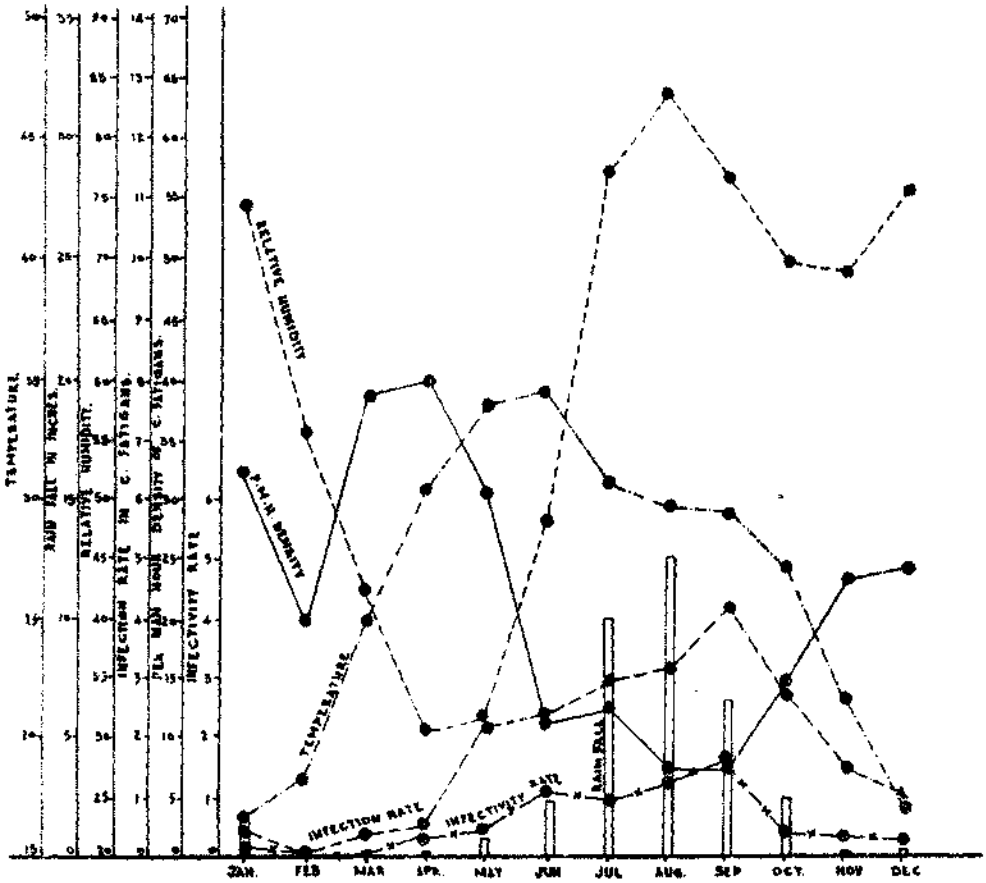
TABLE VI.

Infection and infectivity rate in C. fatigans, *relating to the period January, 1958 to December, 1959), in the Comparison Area.*

Month.	Years: 1958.			1959.			Average from January, 1958 to December, 1959.	
	Number of *C. fatigans* dissected.	Infection rate, per cent.	Infectivity rate, per cent.	Number of *C. fatigans* dissected.	Infection rate, per cent.	Infectivity rate, per cent.	Infection rate, per cent.	Infectivity rate, per cent.
January	273	0·7	0	419	0	0	0·3	0
February	272	0·4	0	451	0·4	0	0·4	0
March	979	1·2	0·1	615	0·5	0	0·8	0·05
April	830	0·5	0	606	1·0	0	0·7	0
May	151	0·6	0	370	1·6	0	1·1	0
June	343	0·6	0	238	2·5	1·3	1·6	0·6
July	431	1·0	0·2	390	2·5	1·0	1·8	0·6
August	187	0·5	0	450	2	0·9	1·3	0·4
September	276	0·9	0·3	96	9	4·0	4·95	2·1
October	303	1·0	0·3	246	4·9	0·4	2·95	0·3
November	151	0·9	0	391	3·3	1·2	1·95	0·6
December	219	0	0	548	3·0	1·5	1·5	0·7

GRAPH 1.

Meteorological data (monthly means), infection, rate, infectivity rate and per-man hour density (monthly means) of C. fatigans *from January, 1957 to December, 1959. Special Study Area.*



From October, when the monsoon is over, the mosquito density again starts rising till it reaches its peak in the months of March and April.

(*B*) DEVELOPMENT OF PARASITE IN *C. FATIGANS* (INFECTION AND INFECTIVITY RATES).

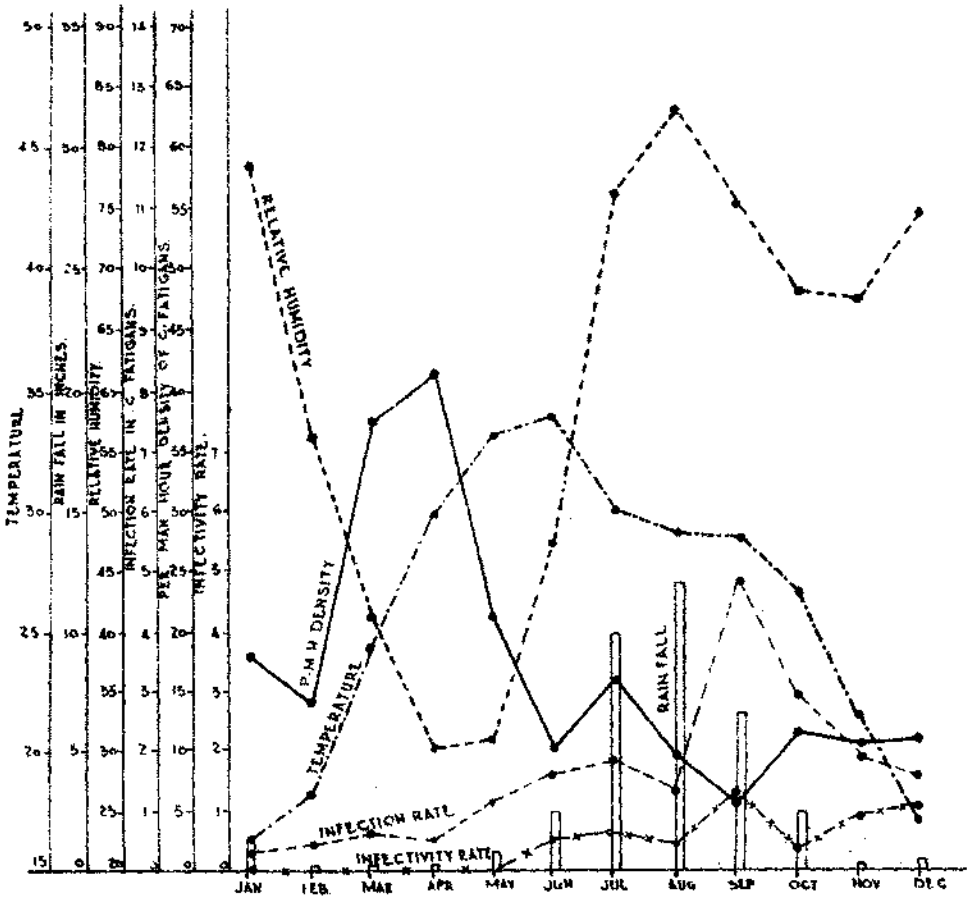
It can be observed from the Graphs 1 and 2 that the filarial infection and infectivity rates in *C. fatigans* start rising from the month of May and reach their peak in September, after which they gradually decline. This observation is similar in respect of the Special Study Area and the Comparison Area. It can be seen that during these months (May to September) the temperature ranges between 29°C. and 34°C., the relative humidity is above 60 per cent and the rainfall is also heavy. September, the peak month for the infection and infectivity rates in both the areas of study, has a temperature of 29°C. and relative humidity 75 per cent. During

the months of April, October and November, both infection and infectivity rates in the mosquito are noted, though at much lower levels.

Thus it will be seen that for the development of filarial infection in the vector mosquito, favourable climatic conditions exist in this district for about 6 months in the year.

GRAPH 2.

Meteorological data (monthly means), infection rate, infectivity rate, and per man-hour density (monthly means) of C. fatigans *from January, 1958 to December, 1959. Comparison area.*



SUMMARY AND CONCLUSION.

An analysis of three years' observation on the effect of climatic conditions on the density of *C. fatigans* and infection in the vector has been set out.

The data show that the optimum climatic conditions for the breeding of *C. fatigans* exist in this district during the months of March and April when the

vector density reaches its peak. During these months, the weather is warm (temperature 25°C. to 30°C.), humidity 35-40 per cent., and the rainfall is scanty. The infection rate in the mosquito, however, is low.

For the development of parasite in the vector, the most favourable climatic conditions exist from June to September when the weather is hot and humid and the rainfall is heavy.

From the analysis of the data mentioned above, it is evident that there is a lack of correlation between the optimum breeding conditions for *C. fatigans* and the development of parasite in it. It is seen that the month of September, which is the peak period for the development of infection in *C. fatigans*, has the lowest density. Again, during the months of March and April the mosquito density is at its peak while the infection in them is the lowest. Similar observations have been made in the past by Knowles and Basu (1934) in their study in Calcutta where they have demonstrated that the peak periods of density of *C. fatigans* and the infection in them do not coincide. This has been attributed to the fact that during monsoon, though the climatic conditions are favourable both for breeding of the mosquitoes and development of infection in them, the flushing of the breeding places due to heavy rains keeps the mosquito density at a low level. They had further stated that unfavourable climate during winter in Calcutta has also its obvious ill-effects for development of parasite in *C. fatigans*. These factors tended to make Calcutta low endemic for filaria.

In such an analysis, one has to remember the "micro-climate" in line as opposed to "macro-climate". While considering with this limitation, it may be stated that the district of Barabanki has favourable climatic conditions both for the breeding of *C. fatigans* and development of infection in them for over 6 months in the year. This would make it a moderately endemic area as per the criteria of Acton and Rao (1930 *loc. cit.*). The infection, disease and endemicity rates are 10·00, 3·10 and 13·00 per cent (Rehman *et al.*, 1959).

ACKNOWLEDGEMENT.

The authors are grateful to Shri N. Singh, Assistant Entomologist and other staff of the unit for their whole-hearted cooperation in the above mentioned studies.

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*THE COURSE OF PARASITAEMIA OF *P.C. BASTIANELLI*† IN *MACACA MULATTA* MONKEYS : ITS SENSITIVITY TO DIFFERENT ANTIMALARIALS.

BY

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AND

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[May 31, 1962.]

SINTON (1939) drew attention to the frequent occurrence of several different strains or races within each morphological species of *plasmodium* which differed not only in their immunological properties but, in some instances, in their reaction to drugs. Garnham (1959) described the third form of *Plasmodium cynomolgi* isolated from a *Macaca irus* monkey originating in Malaya. This new sub-species was named by him as *P.c. bastianelli* in honour of the celebrated Italian malariologist. The strain was shown to possess five distinct characters, namely, shorter prepatent period, completion of the erythrocytic schizogonic cycle just before mid-day, smaller size of the cryptozoic schizonts with small vacuoles in the liver, earlier appearance of deoxyribonuclic acid in the nuclei of the cryptozoic schizonts and absence‡ of cross-immunity with type species.

The present investigation deals with (*i*) the course of *P.c. bastianelli* in *M. mulatta* monkeys, and (*ii*) the sensitivity of the strain to different antimalarials as compared to that of the type ('*M*') strain**.

METHODS AND MATERIALS.

Macaca mulatta monkeys, weighing between 2·5 and 5·0 kg., obtained from Northern India, were utilized for these investigations.

The course of infection was studied in blood-induced infections††. The route

* This work was partly financed from a grant received from the World Health Organization on the study of mechanism and dynamics of resistance of malaria parasites to drugs.

† Strain of *P. cynomolgi bastianelli* was obtained through the courtesy of Prof. P.C.C. Garnham, London, and maintained at this Institute in *M. mulatta* monkeys by blood inoculation since 1961.

‡ The evidence for this was, however, based on 2 animals.

** '*M*' strain of *P. cynomolgi* isolated by Sinton and Mulligan (1933) and maintained in this Institute through blood passages.

†† *Sporozoite induced infection* :—Inocula of one, three and ten-mosquito-equivalent of sporozoites, obtained by dissection of infective salivary glands of *A. stephensi*, were given intravenously to 2, 1 and 1 monkeys respectively. Samples of inocula were invariably confirmed by detection of stained sporozoites. The animals, which had received one and three mosquito-equivalent of sporozoites, did not develop patent parasitaemia for periods of 31 to 51 days at the end of which they died from intercurrent disease. The monkey which had received ten mosquito-equivalent of sporozoites intravenously, showed parasites in the peripheral blood on the 13th day of inoculation. Only a low degree of parasitaemia, not exceeding one parasitized cell per 10^4 r.b.c. was observed during the subsequent 3 days. The monkey died from lobar pneumonia at this time. The study of the course in sporozoite-induced infection, therefore, was not possible.

of inoculation was intravenous and a standard inoculum of 5×10^6 parasitized cells per kg. body weight was given. Daily blood smears were examined and when found positive the parasites were enumerated against 10,000 erythrocytes.

Sensitivity to antimalarials was ascertained in blood-induced infection produced by the standard inoculum described above. Treatment was commenced in each individual monkey when parasitaemia was between 0·1 and 0·2 per cent cell infection. The dose of the antimalarial was calculated in mg. of the drug base per kg. of body weight and administered orally once a day for a period of seven days. The sensitivity was determined in respect of quinine, sulphadiazine, DDS (diaminodiphenyl-sulphone), primaquine, chloroquine, amodiaquine, proguanil and pyrimethamine.

The minimum dose of an antimalarial which produced complete parasite clearance (at least one hundred fields of thick film were screened to declare complete clearance) by the day following the last dose, *viz.*, Class II effect of Shannon (Wiselogle, 1946), was taken as the MED (minimum effective dose).

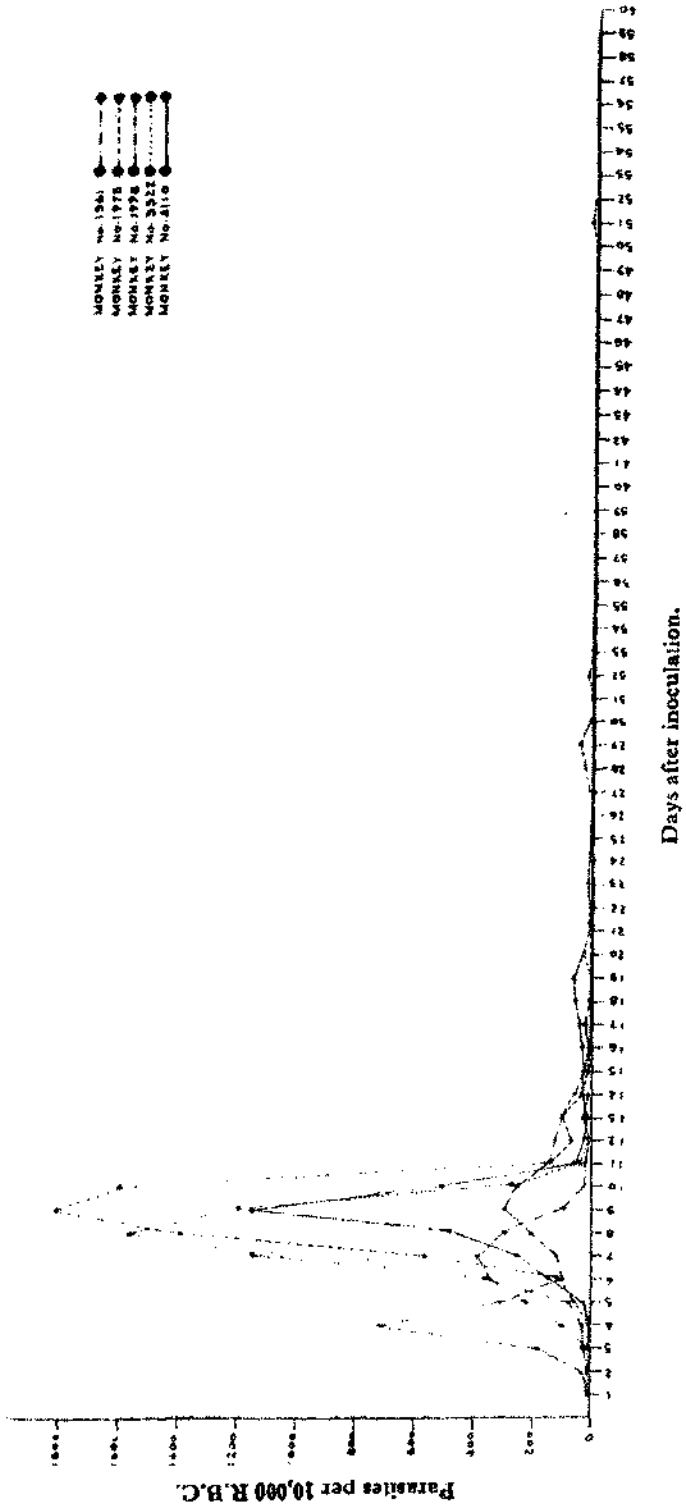
RESULTS.

(A) COURSE OF PARASITAEMIA IN BLOOD-INDUCED INFECTIONS OF *P. c. BASTIANELLI* AND THE '*M*' STRAIN.

The course of parasitaemia in untreated infection in five monkeys is shown in Graph 1. The infection became patent within 2 to 4 days of inoculation. The duration of primary patency varied from 16 to 21 days. The peak parasitaemia varied widely from about 4 to 18 per cent cell involvement. In three monkeys out of five, the peak parasitaemia varied from 11 to 18 per cent cell infection. The peaks occurred between 7th and 9th day of inoculation in four monkeys while in one, it occurred on the 4th day. In one monkey, parasite recrudescence occurred, after periods of 7 to 20 days of subpatency, as late as 50 days after inoculation. The other monkeys did not survive long enough for observation of the relapse pattern due to intercurrent disease (usually lobar pneumonia).

With the same standard inoculum 5×10^6 parasitized cells per kg. weight, given intravenously, the type (*M*) strain also showed a prepatent period of 2 to 4 days (Jaswant Singh, Nair and Ray, 1953). The duration of primary patency in the type strain was usually 13 days, although in one monkey out of five, this extended to a longer period of at least 29 days. The peak parasitaemia varied from 13 to 17 per cent cell involvement. The peaks occurred between 7th and 10th day of inoculation. Subsequent recrudescences of parasitaemia were more common in the type strain than in the other.

Thus, the primary patency periods and the extent of parasitaemia caused by the two strains did not indicate any appreciable difference except the tendency to recrudescence in the type ('*M*') strain. A limitation is, however, that the observations pertained to five monkeys in each case.



GRAPH 1.

Uncontrolled infection of P. cynomolgi bastianelli : Inoculum 5×10^5 parasites per kg. body weight.

(B) SENSITIVITY OF *P. C. BASTIANELLI* TO ANTIMALARIALS.

The sensitivity of the strain to antimalarials is indicated in Table I. The results showed that the MED (minimum effective doses) of quinine, sulphadiazine, DDS, primaquine, chloroquine, amodiaquine, proguanil and pyrimethamine were 15·0, 0·2, 0·15, 0·2, 1·5, 2·0, 0·1 and 0·05 mg./kg. daily of the respective drugs for 7 days. The speed of action in the clearance of parasite was fastest with chloroquine and slowest with sulphadiazine, DDS and pyrimethamine when the MED of the respective drugs were used. The speed of action of other drugs was of the intermediate range.

TABLE I.

Sensitivity of P.c. bastianelli *to antimalarials.*

Antimalarial.	Dose mg./kg. daily for 7 days.	Number of monkeys used.	Effective (Class II): Number of animals.	Effective (Class II): Parasite clearance, in hours.	Ineffective Number of animals.	Recrudescence within 10 days of sub-patency.
Quinine	20·0	1	1	96	..	
	15·0*	5	5	96 to 144		One; 7th day.
	10·0	5	3	120 to 168	2	
	5·0	1	Nil		1	
Sulphadiazine	0·2*	5	5	144 to 168		
	0·15	5	3	120 to 168	2	
	0·05	1	..		1	
DDS (diamino-diphenyl-sulphone)	0·5	5	5	96 to 168		
	0·25	5	5	120 to 168		One; 13th day.
	0·15*	5	5	120 to 168		
	0·05	2	Nil		2	
Primaquine	2·0	1	1	96		
	1·0	1	1	96		
	0·5	1	1	96		
	0·2*	5	5	96 to 168		
	0·1	5	Nil		5	
Chloroquine	2·0	3	3	72 to 96		
	1·5*	5	5	96 to 120		
	1·0	5	Nil		5	
	0·5	2	Nil		2	
Amodiaquine	2·0*	6	6	120 to 144		
	1·0	3	2	120 to 168	1	
Proguanil	1·5	3	3	96		
	1·0	3	3	72 to 144		
	0·5	5	5	72 to 168		
	0·2	5	5	72 to 144		
	0·1*	5	5	96 to 144		
	0·05	3	2	120	1	
Pyrimethamine	0·5	1	1	120		
	0·25	2	2	96 to 120		
	0·05*	5	5	120 to 144		One; 7th day.
	0·025	5	4	120 to 144	1	

* MED (Minimum effective dose) of respective drugs.

(C) COMPARISON OF THE SENSITIVITY TO ANTIMALARIALS IN THE *BASTIANELLI* AND THE TYPE ('M') STRAINS.

The MED (minimum effective doses) of the drugs in *bastianelli* and type strains are shown in Table II.

TABLE II.

Comparison of senitivity to antimalarials. (Oral administration).

Antimalarial.	*P. cynomolgi bastianelli.* Minimum effective dose in mg. base/kg. once a day for 7 days.	*P. cynomolgi* (Type strain). Minimum effective dose in mg. base/kg. once a day for 7 days.	References.
Quinine ...	15	20	Jaswant Singh *et al.*, 1953.
Proguanil ...	0·1	1·0	Nair *et al.*, 1953.
Pyrimethamine ...	0·05	1·1	Nair *et al.*, 1953.
Chloroquine ...	1·5	1·5	Jaswant Singh *et al.*, 1953.
Primaquine ...	0·2	0·5	(1961) Unpublished. (*Malaria Institute of India*).
Sulphadiazine ...	0·2	0·15	Jaswant Singh *et al.*, 1956.
Sulphone (DDS)	0·15	0·5	Ramakrishnan *et al.*, 1962.
Amodiaquine ...	2·0	3·5	Jaswant Singh *et al.*, 1953.

The results indicated that the two strains differed considerably in their drug sensitivity except in respect of chloroquine and also sulphadiazine which had the same MED in both the strains. The *bastianelli* strain was more sensitive to all the other antimalarials than the type strain. The difference in the sensitivity was marked ; dose for dose it ranged from 33 per cent to 2,100 per cent the dose required in *bastianelli* strain to produce the same effect in the type strain in respect of various antimalarials where such differences were observed. Thus, the *bastianelli* strain lent itself to differentiation from the type strain by its characteristic sensitivity to certain antimalarials.

DISCUSSION.

In blood-induced infection, both *bastianelli* and type ('M') strains of *P. cynomolgi* gave almost a similar pattern of parasitaemia and could not be distinguished from each other. Within the limitations of the observations (already referred to) being confined to 5 monkeys in each case, the recrudescence patterns, however, appeared to be different—such episodes being more frequent in the type (*M*) strain.

The two strains were found to be differently sensitive to antimalarials, namely, quinine, proguanil, pyrimethamine, primaquine, DDS and amodiaquine. This characteristic could be utilised to distinguish the strains as stated by Sinton (1939 *loc. cit.*). Chloroquine and sulphadiazine, however, could not be utilized for the purpose as both the strains were equally sensitive to these drugs.

Finally, considering the small period of patency in both the species (13 to 21 days), a long period of drug administration (7 days) appeared to be unsuitable for testing of drugs. The suitability of single dose treatment for comparison of schizonticidal activity of drugs needs investigation. In human infection also, except in the primary attack, the duration of treatment is short.

SUMMARY.

The course of blood-induced infection of *P. cynomolgi bastianelli* and that of type ('*M*') strain in *Macaca mulatta* monkeys were compared and no appreciable difference was noted.

In *P. cynomolgi bastianelli* the MED (minimum effective doses) of quinine, sulphadiazine, DDS, primaquine, chloroquine, amodiaquine, proguanil and pyrimethamine were determined as 15·0, 0·2, 0·15, 0·2, 1·5, 2·0, 0·1, and 0·05 mg./kg. daily for 7 days for the respective drug bases.

The sensitivity of *P. cynomolgi bastianelli* to a number of antimalarials was found markedly different from the type strain. The difference in sensitivity to antimalarials could be used as a characteristic for distinguishing the strains.

ACCREDITION.

The technical help rendered by Messrs Puran Singh and S. Venkatesan, Technicians of the Institute, is accredited.

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STUDIES ON THE JOINT ACTION OF PP'-DDT AND OP'-DDT, AND EFFECTIVENESS OF VARIOUS GRADES OF TECHNICAL DDT AGAINST MOSQUITO LARVAE.

BY

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[August 6, 1962.]

INTRODUCTION.

THE subject of joint action of drugs and insecticides has become important in view of the problem of resistance of microbes and insects to drugs and insecticides respectively. Bliss (1939) discussed the problem of predicting the toxicity of a mixture of poisons from a knowledge of the toxicity of the individual constituents. He distinguished three types of joint action, namely, independent, similar and synergistic. His idea was further developed by Finney (1947), Plackett and Hewlett (1948 : 1952) and Hewlett and Plackett (1950) with detailed mathematical representation. The joint action was defined as similar if the primary sites of action of the poisons in the organism were the same ; and dissimilar if the sites of action were different. Interaction of two poisons is said to occur if the presence of one influences the biological action of the other.

The joint action of stereoisomers of BHC has been studied by Van Asperen (1954 : 1956) in mice and cockroaches. It was shown that the action was of an antagonistic type in the former, and independent type in the latter. The joint action of pp' and op'-DDT was found to be synergistic against house-flies and body-lice (Yasutomi, 1956). Further, Reimschneider (1950), Ascher (1958) and Yasutomi (1956 *loc. cit.*) found that technical DDT was more toxic than pure pp'-DDT against insects even though pp'-DDT is known to be the most toxic of its isomers and related compounds which are present in technical DDT. Yasutomi (1956 *loc. cit.*) also found that technical DDT, containing lower amount of pp'-DDT, was more toxic than technical DDT containing higher amount of pp'-DDT.

The present studies were, therefore, carried out to elucidate the type of joint action of pp' and op'-DDT against the larvae of various species of mosquitoes. The effectiveness of various grades of technical DDT was also determined. Mosquito larvae of varying tolerance levels to DDT were used to understand some of the basic mechanisms of resistance.

MATERIALS AND METHODS.

TEST INSECTS.

Late third or early fourth-instar larvae of different strains of *C. fatigans*, *Ae. aegypti*, *A. stephensi* and *A. subpictus* were used. The details of the strains are tabulated below :

Name of the strain used.	Place of collection and nature of strain.	Period of collection
Laboratory reared *C. fatigans* (L_1).	Strain maintained at the Malaria Institute since 1948 without any insecticidal pressure.	...
Resistant *C. fatigans* (L_2).	A DDT-resistant strain of *C. fatigans* obtained from the Coonoor branch of the Institute (Mohan, 1960) and maintained at Delhi.	...
C. fatigans February strain.	Larvae collected from the area around Delhi. This area is being sprayed with DDT since 1947. Dieldrin has also been sprayed in this area. *C. fatigans* from this area has been reported to be DDT-resistant (Pal *et al.*, 1952 ; and Pal and Kalra, 1958).	February, 1959.
C. fatigans April strain.	Larvae collected from the area around Delhi.	April, 1959.
Ae. aegypti.	Strain maintained at the Malaria Institute of India without any insecticidal pressure.	...
A. stephensi.	A susceptible strain obtained from the Coonoor Branch of the Institute and maintained at Delhi.	...
A. subpictus June strain.	Larvae collected from the sprayed area around Delhi. The species was found to be DDT-resistant (Sharma *et al.*, 1957 ; and Sharma and Kalra, 1958).	June, 1959.
A. subpictus November strain.	Larvae collected from the sprayed area around Delhi.	November, 1959.

INSECTICIDES USED.

pp'-DDT ; op'-DDT ; Mixture I consisting of 80 per cent pp'-DDT and 20 per cent op'-DDT ; Mixture II consisting of 20 per cent pp'-DDT and 80 per cent op'-DDT ; DDT technical—A Grade (pp'-DDT content 80 per cent) ; DDT technical—B Grade (pp'-DDT content 69 per cent) ; DDT technical—C Grade (pp'-DDT content 63 per cent).

TEST METHODS.

The toxicity of the insecticides was determined by the method recommended by the World Health Organization for assessing the susceptibility of larvae to insecticides (World Health Organization, 1959). Late third or early fourth-instar larvae were exposed to various concentrations of solutions/suspensions of each insecticide in water prepared by mixing 250 c.c. of water and 1 c.c. of the alcoholic solution of insecticide. The mortality of the larvae was recorded after 24 hours of continuous exposure to insecticide in the case of *C. fatigans*, *A. subpictus* and *A. stephensi*. In the case of *Ae. aegypti*, a large proportion of the larvae were found to be moribund at the end of the 24 hours of continuous exposure. Therefore these larvae, after the 24 hours exposure, were washed and kept under observation for another 24 hours when mortality in them was recorded. The temperature of water was found to vary between 22-26°C.

To avoid a daily variation in mortality etc., parallel tests were carried out with the chemicals where a comparison has been made. The data of the experiments, where parallel tests were carried out, have been discussed under a single table.

STATISTICAL ANALYSIS.

To assess the relative effectiveness of the insecticides, the results obtained were analysed by the probit method as outlined by Finney (1947 *loc. cit.*). To elucidate the type of joint action of pp' and op'-DDT, the hypothetical models of different physiological mechanisms of joint action, *i.e.*, similar and dissimilar, were set up and tested for their agreement with the experimental data. The statistical analysis of the joint action was made by the method recommended by Bliss (1939 *loc. cit.*) ; Finney (1947 *loc. cit.*) ; Plackett and Hewlett (1952 *loc. cit.*) and Sakai (1960).

RESULTS.

RELATIVE EFFECTIVENESS OF PP' AND OP'-DDT AGAINST LABORATORY-REARED *C. FATIGANS* LARVAE (L_1).

The percentage mortality of *C. fatigans* larvae at different concentrations of pp'-DDT, op'-DDT, Mixture I and Mixture II is shown in Table I. The results of the probit analysis of the data are depicted in Table II. The LC_{50} of pp'-DDT was found to be 0·56 p.p.m. with fiducial limits 0·39—0·81. The LC_{50} of op'-DDT, when tested under similar conditions, was found to be 0·096 p.p.m. with fiducial limits 0·091—0·102. op'-DDT was, therefore, significantly more toxic than pp'-isomer against *C. fatigans* larvae.

JOINT ACTION OF PP'-DDT AND OP'-DDT AGAINST LABORATORY REARED *C. FATIGANS LARVAE* (L_1).

The similar action, as defined by Bliss (19 9 *loc. cit.*), implied that two poisons could act similarly only if the respective regressions of the probits on log dosage were parallel for the poisons applied singly. The regression coefficient of pp' and

op'-DDT were found to be 2·17 and 4·20 respectively (Table II). These values were found to differ significantly $\left\{ \chi^2_{[1]} = \frac{(2.17-4.20)^2}{0.069873+0.044864} = 37.46 > \chi^2_{[1]} \right.$ at $P=0.05$ level $=3.6$ $\left.\right\}$. The results, therefore, suggested that the compounds do not act similarly. The action of the combination of pp' and op'-DDT was, therefore, tested for dissimilar action. The values of expected probits at different

TABLE I.

Percentage mortality in C. fatigans *larvae when exposed to different concentrations of pp'-DDT, op'-DDT, Mixture I and Mixture II.*

Insecticide.	Concentration of insecticide in parts per million :					
	0·03	0·06	0·125	0·25	0·5	1·0
pp'-DDT		0·0 (240)	11·7 (240)	24·1 (240)	36·7 (180)	73·8 (240)
op'-DDT	0·8 (240)	23·8 (240)	70·4 (240)	94·8 (210)		
Mixture I (80 per cent pp'-DDT plus 20 per cent op'-DDT).	0·0 (240)	7·0 (240)	27·5 (240)	56·2 (240)	88·9 (180)	
Mixture II (20 per cent pp'-DDT plus 80 per cent op'-DDT).	5·0 (180)	33·3 (240)	71·7 (240)	93·3 (240)		

Mortality in controls was nil.

Figures in parenthesis indicate the number of larvae tested at the concentrations of insecticides.

TABLE II.

Equation of fitted pr-lc regression lines and the parameters with standard errors of pp'-DDT, op'-DDT Mixture I and Mixture II against laboratory-reared C. fatigans *larvae.*

Insecticide.	Equation of the fitted pr-lc regression line ($x=\log 100\, z^*$).	χ^2 (dF).	m ($\log 100z$).	Variance of m.	MLC (p.p.m.) (with fiducial limits for 95 per cent probability).	Variance of b.
pp'-DDT	$Y=1.2116+2.17x$	14·97 (3)	1·7458	0·0025475	0·56 (0·39–0·81)	0·069873
op'-DDT	$Y=0.8731+4.20x$	2·15 (2)	0·9826	0·000164	0·096 (0·091–0·102)	0·044864
Mixture I.	$Y=1.0598+2.99x$	4·38 (3)	1·3160	0·002802	0·21 (0·16–0·26)	0·024327
Mixture II.	$Y=1.8046+3.40x$	1·69 (2)	0·9412	0·0002231	0·087 (0·082–0·093)	0·03468

z^* = The dosage of insecticide in p.p.m.

concentrations of Mixture I for the various types of dissimilar joint action, *i.e.*, independent action with positive, 0 and negative correlation and dependent joint action were estimated from the equations of pr-lc regression lines of pp′ and op′-DDT. The values thus calculated are mentioned in Table III. A test of agreement between the values thus obtained and observed was made by finding the weights to be associated with the expected probits and multiplying these by the square of the corresponding discrepancies (Table III). The value of Chi^2 obtained by adding the values of Chi^2 obtained at each dosage for independent action with positive correlation, independent action with 0 correlation, independent action with negative correlation and dependent joint action were found to be highly significant at $P=0{\cdot}05$ level. However, at the concentration of 0·5 p.p.m., the value of expected probit for independent action with negative correlation and dependent joint action was in agreement with the observed value. Similar results were also obtained with the Mixture II (Table IV).

The results, therefore, indicated that the joint action of pp′ and op′-DDT was not of dissimilar type against the larvae of *C. fatigans*.

Plackett and Hewlett (1952 *loc. cit.*) quoted examples of closely related insecticides which were expected to act similarly having non-parallel probit-log concentration lines. The difference in regression coefficients, as suggested by them, may arise from difference in transmission from the site of application to the common site of action and a model of similar joint action for such drugs was developed. This was based on the assumption that parallelism exists in probits to the logarithm of the dose acting.

The equation of the mixture of two components taken to have the different slopes is given by :

$$Ym=\theta \log\left(h^{a_1/\theta} D_1^{b_1/\theta} + h^{a_2/\theta} D_2^{b_2/\theta}\right)$$

where h is the base of the logarithm and θ is a similar to a slope of mixture. pp′ and op′-DDT are quite alike chemically and it was, therefore, expected that they would act similarly. The analysis of the joint action of pp′ and op′-DDT was, therefore, attempted by following Plackett and Hewlett (1952 *loc. cit.*).

The results of the analysis of the Mixture I are shown in Table V. The average value of θ was found to be 4·5813 and using this value the equation of the simple similar action of pp′ and op′-DDT was represented as :

$$Ym = 4{\cdot}5813 \left(h^{0{\cdot}2645} D_{pp'\text{-}DDT}^{0{\cdot}4737} + h^{0{\cdot}1902} D_{op'\text{-}DDT}^{0{\cdot}9168} \right).$$

The values of expected probits at the various dosages of the Mixture I, as calculated from the above mentioned equation, are shown in Table V. A test of agreement between the observed and expected probits was made and the value of Chi^2 was found to be 1·963 which was not significant and could occur as a result of chance deviation.

TABLE III.

Expected probits for dissimilar joint action exhibited by pp'-DDT and op'-DDT against the laboratory-reared C. fatigans *larvae when mixed in the ratio of 80 and 20 per cent respectively.*

Dosage of mixture (p.p.m.).	Observed probit.	Expected probit for independent action with correlation: Positive. ($P=P_1$ or P_2)	Zero. ($P=P_1+P_2-P_1P_2$)	Negative. ($P=P_1+P_2$)	Expected probit for dependent joint action (Interaction) ($P=P_1+2P_2-2P_1P_2$)	X^2 value for independent action with correlation: Positive.	Zero.	Negative.	X^2 value for dependent joint action.
0·06	3·5	2·7	2·7	2·7	2·7	11·62	11·62	11·62	11·62
0·125	4·4	3·4	3·4	3·5	3·4	57·01	57·01	46·18	57·01
0·25	5·2	4·0	4·4	4·4	4·7	151·59	85·69	85·69	36·97
0·5	6·2	5·0	5·5	6·3	6·2	165·01	51·24	0·60	0·00
						385·23	205·56	144·09	105·60

X^2 = (Observed probit — Expected probit)^2 × weight.

TABLE IV.

Expected probits for dissimilar joint action exhibited by pp'-DDT and op'-DDT against the laboratory-reared C. fatigans *larvae when mixed in the ratio of 20 per cent and 80 per cent respectively.*

Dosage of mixture (p.p.m.)	Observed probit.	Expected probit for independent action with correlation. Positive.	Zero.	Negative.	Expected probit for dependent joint action (Interaction)	X^2 value for independent action with correlation: Positive.	Zero.	Negative.	X^2 value for dependent joint action.
0·03	3·4	2·6	2·6	2·6	2·6	9·47	9·47	9·47	9·47
0·06	4·6	3·8	3·8	3·8	3·8	56·88	56·88	56·88	56·88
0·125	5·6	5·0	5·0	5·0	5·0	55·00	55·00	55·00	55·00
0·25	6·5	6·3	6·3	6·4	6·3	3·22	3·22	0·72	3·22
						124·57	124·57	122·07	124·57

X^2 = (Observed probit — Expected probit)^2 × weight.

TABLE V.

Summarised analysis of the mixture of pp'-DDT and op'-DDT when mixed in the ratio of 80 per cent and 20 per cent as a simple similar action against laboratory-reared C. fatigans *larvae.*

Dosage of mixture (p.p.m.)	Observed probit.	Preliminary value of θ*.	Expected probit when θ is 4·5813.	(Observed probit—Expected probit)².	Weight for simple similar action.	χ^2
0·06	3·5	4·9407	3·55	0·0025	68·18	0·17
0·125	4·4	4·7564	4·39	0·0001	133·89	0·013
0·25	5·2	4·2401	5·30	0·01	147·86	1·48
0·5	6·2	4·3879	6·27	0·0049	60·46	0·30
						$1·963 = \chi^2_{(4)} < \chi^2_{(4)} = 9·37$ at P=0·05 level

* The value of 'θ' was calculated from the equation :

$$\gamma m = \tfrac{1}{2}(\gamma_1 + \gamma_2) + \theta \log \left(2 \operatorname{Cosh} \frac{(\gamma_1 - \gamma_2)}{2\theta \log e}\right) \ldots\ldots (i)$$

At the concentrations, 0·125, 0·25 and 0·5 p.p.m. of the Mixture I tested, the difference between the values of probits, γ_1 and γ_2, of the individual components, i.e., pp'-DDT and op'-DDT, as calculated from their regression equations, was found to be less than 1. So equation (I) is reduced to :

$$\gamma m = \tfrac{1}{2}(\gamma_1 + \gamma_2) + \theta \log 2 \ldots\ldots (ii).$$

By putting the value of γm, the observed probit at a particular concentration of Mixture I, and γ_1 and γ_2 the probits of the individual components, as calculated from their regression equation, the preliminary estimate of θ was made.

It was considered likely that this equation could also account for the Mixture II. The expected probits from this equation were, therefore, calculated for the different concentrations of Mixture II and are mentioned in Table VI. The difference between the observed and expected probits was, however, found significant (Table VI).

TABLE VI.

Expected probits of simple similar joint action with θ = 4·5813 exhibited by the mixture of pp'-DDT and op'-DDT in the ratio of 20 per cent and 80 per cent respectively against the laboratory-reared C. fatigans *larvae.*

Dosage of mixture (p.p.m.)	Observed probit.	Expected probit.	Weight.	χ^2
0·03	3·4	3·1	37·0464	3·33
0·06	4·6	4·3	127·5816	11·48
0·125	5·6	5·5	139·4376	1·39
0·25	6·5	6·6	57·0072	0·57
				$16·77 = \chi^2_{(4)} > \chi^2_{(4)} = 9·37$ at P=0·05 level.

The value of $\theta = 4{\cdot}5813$ was, therefore, not satisfactory for the general equation of the simple similar action of the mixture of these isomers against *C. fatigans.* For a better estimation of θ, therefore, its preliminary estimate was made also at the dosages of the Mixture II (Table VII). The value of θ was found to vary between 3·5 and 5·5 and using an average value of θ as 4·875, the equation of the simple similar action was found to be :

$$Ym = 4{\cdot}875 \log \left(h^{0{\cdot}2485} \; D^{0{\cdot}4451}_{pp'\text{-}DDT} + h^{0{\cdot}1791} \; D^{0{\cdot}8615}_{op'\text{-}DDT} \right).$$

The expected probits, as calculated from the equation, were found to differ significantly from the observed probits

$$\left\{ \chi^2_{(4)} = 9{\cdot}49 > \chi^2_{(4)} = 9{\cdot}37 \text{ at } P = 0{\cdot}05 \text{ level} \right\}.$$

TABLE VII.

Summarised analysis of the mixture of pp'-DDT and op'-DDT when mixed in the ratio of 20 per cent and 80 per cent as simple similar action against the laboratory reared C. fatigans *larvae.*

Dose of mixture (p.p.m.)	Observed probit.	Preliminary value of θ *	Expected probit when θ is 4·875	(Observed probit — Expected probit)²	Weight for simple similar action.	χ^2
0·03	3·4	5·2	3·12	0·0784	27·7848	2·178
0·06	4·6	5·5	4·41	0·0361	133·8912	4·833
0·125	5·6	5·3	5·53	0·0049	139·4376	0·683
0·25	6·5	3·5	6·69	0·0361	49·8576	1·800
						9·494

*The equation given in Table V for the calculation of θ could not be used as the difference between γ_1 and γ_2 was much more than 1. In this case, the value of θ was estimated from the equation :

$$\gamma m/\theta = \gamma_1/\theta + \gamma_2/\theta.$$

By putting different values of θ, the equation given above was equated and the preliminary estimate of θ was thus made at different concentrations of the mixture.

However, the value of $\theta = 4{\cdot}9$ was found to be fairly satisfactory and the equation of the simple similar action using this value was represented as :

$$Ym = 4{\cdot}9 \log \left(h^{0{\cdot}2473} \; D^{0{\cdot}4429}_{pp'\text{-}DDT} + h^{0{\cdot}1778} \; D^{0{\cdot}8571}_{op'\text{-}DDT} \right).$$

The value of expected probit for the different concentrations of the Mixture I and Mixture II, as estimated from this equation, were found to agree fairly well with the observed probits (Table VIII). The joint action of pp' and op'-DDT against laboratory-reared *C. fatigans* larvae (L_1) was, therefore, simple similar. It is interesting to note that Mixture II was even more toxic than the most active constituent (op'-DDT).

TABLE VIII.

Summary of the analysis as simple similar action of the mixture of pp'-DDT and op'-DDT both in the ratio of 80 per cent and 20 per cent as well as 20 per cent and 80 per cent respectively, using the value of θ as 4·9.

Mixture.	Dosage of insecticide (p.p.m.)	Observed probit.	Expected probit with θ=4·9.	Weight for simple similar action.	χ^2 for simple similar action.
Mixture I (pp'-DDT 80 per cent + op'-DDT 20 per cent).	0·06	3·5	3·7	80·6136	3·22
	0·125	4·4	4·5	139·4376	1·39
	0·25	5·2	5·3	147·8616	1·48
	0·5	6·2	6·4	54·36	2·17
					$8·26 < \chi^2_{(4)} = 9·37$ at P=0·05 level.
Mixture II (pp'-DDT 20 per cent + op'-DDT 80 per cent).	0·03	3·4	3·3	37·3972	0·37
	0·06	4·6	4·4	133·8912	5·36
	0·125	5·6	5·5	139·4376	1·39
	0·25	6·5	6·7	49·8576	1·99
					$9·11 < \chi^2_{(4)} = 9·37$ at P=0·05 level.

EFFECTIVENESS OF VARIOUS GRADES OF TECHNICAL DDT AGAINST LABORATORY-REARED *C. FATIGANS* LARVAE (L_1).

The percentage mortality at different concentrations of pp'-DDT and various grades of technical DDT are mentioned in Table IX. The results, as analysed by the probit analysis method, are depicted in Table X.

TABLE IX.

Percentage mortality in the laboratory-reared C. fatigans *larvae when exposed to different concentrations of pp'-DDT and various grades of technical DDT.*

Insecticide.	Concentration of insecticide in p.p.m.				
	0·0625	0·125	0·18	0·25	0·5
pp'-DDT ...	1·4 (360)	15·8 (480)	..	43·1 (480)	60·8 (480)
DDT-technical A grade. ...	3·3 (360)	22·9 (480)	..	64·4 (480)	88·1 (480)
DDT-technical B grade. ...	3·3 (360)	37·4 (420)	63·6 (239)	84·8 (420)	..
DDT-technical C grade. ...	26·9 (420)	66·0 (420)	79·0 (238)	90·6 (375)	..

Note: Mortality in control was nil.

Figures in parenthesis denote the number of larvae tested at each concentration of the insecticide.

The MLC's of DDT technical *A* grade, DDT technical *B* grade and DDT technical *C* grade were 0·21, 0·15 and 0·096 parts per million respectively. The

MLC of pp'-DDT was found to be 0·34 p.p.m. The order of toxicity of the different grades of technical DDT and pp'-DDT, therefore, was DDT technical *C* grade > DDT technical B-grade > DDT technical A-grade > pp'-DDT. The results, therefore, indicated that DDT technical, containing the lowest amount of pp'-DDT, was the most toxic of the three grades of technical DDT tested against the laboratory-reared *C. fatigans* larvae. Further, technical DDT was found to be more toxic than pp'-DDT.

EFFECTIVENESS OF PP'-DDT, OP'-DDT AND VARIOUS GRADES OF TECHNICAL DDT AGAINST THE FIELD-COLLECTED AND RESISTANT *C. FATIGANS* LARVAE.

The basic data showing the toxicity of pp'-DDT, op'-DDT and different grades of technical DDT to various strains of *C. fatigans*, differing in their susceptibility to DDT, are presented in Table XI. The results, as analysed by the probit analysis method, are mentioned in Table XII.

From Table XII it may be seen that the MLC of pp'-DDT for the larvae of *C. fatigans* Feb. strain was 1·75 p.p.m. with fiducial limits 1·50—2·04. The MLC of DDT technical *A* grade against this strain was found to be 0·90 p.p.m. with fiducial limits 0·80—1·00. The results, therefore, indicated that DDT technical *A* grade was also more toxic than pp'-DDT against the larvae of *C. fatigans* collected during the month of February.

C. fatigans April strain was found to be quite resistant, the MLC of pp'-DDT being 6·16 p.p.m. The LC_{50} of op'-DDT against this strain was found to be 0·60 p.p.m. The MLC's of different grades of technical DDT grades *A*, *B* and *C* were found to be 1·25, 0·96 and 0·56 p.p.m., respectively ; thereby showing that the order of toxicity of various grades of technical DDT against this strain of *C. fatigans* was the same as has been observed against laboratory-reared *C. fatigans* larvae (L_1). op'-DDT (MLC 1·84 p.p.m.) was also found to be more toxic than pp'-DDT (MLC > 10·0 p.p.m.) against highly resistant laboratory-reared strain of *C. fatigans* (L_2).

RELATIVE TOXICITY OF PP'-DDT AND OP'-DDT AGAINST *Ae. AEGYPTI* LARVAE.

The percentage mortality in *Ae. aegypti* larvae at different concentrations of pp'-DDT, op'-DDT, Mixture I and Mixture II are mentioned in Table XIII. The summary of the analysis by the probit method is contained in Table XIV.

From Table XIV it may be seen that the MLC of pp'-DDT for *Ae. aegipti* larvae was 0·045 p.p.m., which was significantly less than the MLC value of op'-DDT (0·14 p.p.m.). The results, therefore, indicated that pp'-DDT was more toxic than op'-DDT against *Ae. aegypti* larvae.

JOINT ACTION OF PP'-DDT AND OP'-DDT AGAINST *Ae. AEGYPTI* LARVAE.

Bliss (1939 *loc. cit.*), Finney (1947 *loc. cit.*) and Plackett and Hewlett (1952 *loc. cit.*) implied that the mixtures of insecticides, having parallel regression lines,

TABLE X.

Equations of the fitted pr-lc regression lines and the parameters with standard errors of pp'-DDT and various grades of technical DDT against laboratory-reared C. fatigans *larvae.*

Insecticide.	Equation of the fitted pr-lc regression line (x=log 100z)	χ^2 (df)	m (log 100z)	Variance of m	MLC with fiducial limits with 90 per cent probability.	Variance of b
pp'-DDT	Y=1·3112+2·40x	18·38 (2)	1·5344	0·002344	0·34 (0·21—0·60)	0·12896
DDT technical A grade.	Y=0·5802+3·35x	5·98 (2)	1·3193	0·0001134	0·21 (0·20—0·22)	0·017434
DDT technical B grade.	Y=—0·6229+4·78x	0·40 (2)	1·1763	0·00006787	0·15 (0·14—0·16)	0·048626
DDT technical C grade.	Y=1·8303+3·22x	0·05 (2)	0·9844	0·0001588	0·096 (0·090—0·103)	0·029136

TABLE XI.

Percentage mortality in larvae of different strains of C. fatigans *exposed to various concentrations of pp'-DDT, op'-DDT and different grades of technical DDT.*

Strain of C. *fatigans*.	Insecticide.	Concentration of insecticide (p.p.m.): 0·25	0·31	0·5	0·62	1·0	1·25	2·5	5·0	10·0	Percentage mortality in controls.
February strain (Larvae collected in February)	pp'-DDT	7·9 (210)	..	16·5 (210)	..	36·2 (210)	..	59·5 (210)	..	..	3·4
	DDT technical A grade	13·9 (210)	..	31·0 (210)	..	55·2 (210)	..	80·4 (210)	..	..	3·4
April Strain (Larvae collected in April)	pp'-DDT	..	12·0 (141)	..	21·1 (147)	..	35·1 (148)	..	43·8 (146)	..	0·0
	op'-DDT	..	31·3 (150)	..	51·4 (142)	..	70·9 (147)	..	96·5 (145)	..	0·0
	DDT technical A grade	..	20·4 (147)	..	36·2 (149)	..	38·8 (147)	..	87·0 (147)	..	0·0
	DDT technical B grade	..	35·3 (147)	..	42·9 (147)	..	53·8 (143)	..	72·6 (150)	..	0·0
	DDT technical C grade	..	28·0 (150)	..	55·8 (147)	..	7[illegible] (149)	..	89·7 (147)	..	0·0
Resistant C. *fatigans* (½)	pp'-DDT	..	..	0·0 (60)	..	1·7 (60)	..	5·0 (60)	8·3 (60)	10·0 (60)	0·0
	op'-DDT	9·3 (120)	..	12·5 (120)	..	42·5 (120)	..	59·1 (120)	71·7 (120)	..	0·0

Figures in parenthesis indicate the number of larvae tested at each concentration of the insecticide.

TABLE XII.

Summary of the statistical analysis showing the toxicity of pp'-DDT, op'-DDT and different grades of technical DDT against the larvae of various strains of C. fatigans.

Strain of C. *fatigans*.	Insecticide.	Equation of the fitted probit line.	m (log 10z)	Variance of m	MLC (ppm) (with fiducial limits for 95 per cent probability).	X^2 (df)	Variance of b
Feb. strain	pp'—DDT	Y=2·9133+1·68x	1·2401	0·00113022	1·75 (1·50—2·04)	0·68 (2)	0·023818
	DDT technical A grade	Y=3·1605+1·93x	0·9531	0·000643499	0·90 (0·80—1·00)	1·06 (2)	0·0200833
April strain	pp'—DDT	Y=3·5681+0·81x	1·7899	0·0188669	6·16 (3·28—11·58)	4·41 (2)	0·0171100
	op'—DDT	Y=3·5054+1·92x	0·7784	0·0010852	0·60 (0·52—0·70)	0·71 (2)	0·0273483
	DDT technical A grade	Y=3·3102+1·54x	1·0973	0·0068254	1·25 (0·55—2·83)	10·9 (2)	0·0924487
	DDT technical B grade	Y=4·1838+0·83x	0·9834	0·004424	0·96 (0·71—1·31)	0·09 (2)	0·014796
	DDT technical C grade	Y=3·7954+1·61x	0·7482	0·0090439	0·56 (0·23—1·36)	12·37 (2)	0·150724
Resistant strain	pp'—DDT	..	..	..	>10·0	..	..
	op'—DDT	Y=3·0402+1·55x	1·2644	0·0015316	1·84 (1·54—2·19)	7·7 (3)	0·017491

TABLE XIII.

Percentage mortality in Aedes aegypti *larvae exposed to different concentrations of pp'-DDT, op'-DDT, Mixture-I and Mixture-II.*

Insecticide.	Concentrations of insecticides in parts per million :							
	0·007	0·015	0·03	0·06	0·125	0·25	0·5	1·0
pp'-DDT	3·3 (180)	16·1 (180)	34·4 (180)	63·8 (180)	..	..	..	..
op'-DDT	..	..	..	17·8 (180)	..	76·1 (180)	..	96·7 (180)
Mixture-I (80 per cent pp'-DDT+ 20 per cent op'-DDT).	4·4 (180)	31·7 (180)	65·5 (180)	89·4 (180)	..	..	..	..
Mixture-II (20 per cent pp'-DDT+ 80 per cent op'-DDT).	..	..	11·7 (180)	..	76·1 (180)	95·0 (60)	100·0 (120)	..

Note : Mortality in control was nil.
Figures in parenthesis indicate the number of larvae tested at each concentration of the insecticide.

should also give slopes parallel to the individual components if their joint action is simple similar. The regression coefficient values of pp′ and op′-DDT were not found to differ significantly from each other. Therefore, parallel lines were fitted through the individual components and the Mixture I and Mixture II (Graph I) and the data were tested for parallelism. The summary of the analysis is contained in Table XV. The value of F, the variance ratio, was found to be 4·414 which is more than 4·347 with $n_1=3$ and $n_2=7$ significant at the level P : 0·05,

GRAPH I.

The fitted pr-ic regression lines of pp′-D.D.T., op′-D.D.T., Mixture I and Mixture II against A. aegypti *larvae.*

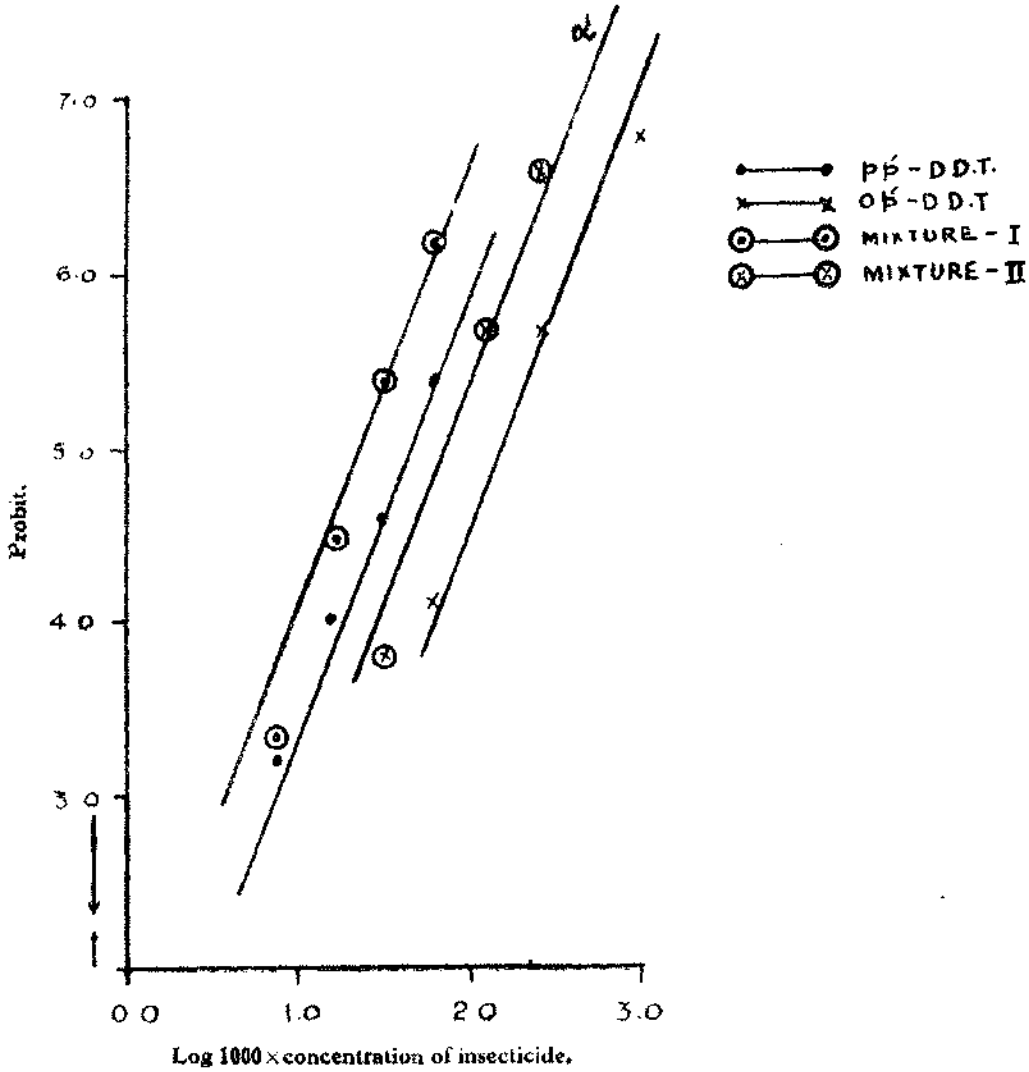

TABLE XIV.

Equations of the fitted pr-lc regression lines and the parameters with standard errors of pp'-DDT, op'-DDT, Mixture-I and Mixture-II against Aedes aegypti *larvae.*

Insecticide.	Equations of the fitted pr-lc line (x=log 1000xZ)	χ^2(df)	m (log 1000xZ)	Variance of m	MLC (p.p.m.) (with fiducial limits for 95 per cent probability)	Variance of b
pp'-DDT	Y= 1·1140+2·35x	1·04(2)	1·6536	0·00075722	0·045 (0·040—0·051)	0·033538
op'-DDT	Y=—0·2237+2·43x	2·07(1)	2·1497	0·00080785	0·14 (0·12 —0·16)	0·0298175
Mixture-I	Y= 0·6351+3·16x	4·53(2)	1·3813	0·0003281	0·024 (0·022—0·026)	0·041316
Mixture-II	Y=—0·9785+3·18x	0·45(2)	1·8800	0·0005505	0·076 (0·068—0·084)	0·046004

TABLE XV.

Summarised analysis for testing parallelism between pp'-DDT and op'-DDT and Mixture-I and Mixture-II.

pp'-DDT	$\frac{1}{Snw}$ = 0·0032633 ;	$\bar{x}$ = 1·4944 ;	$\bar{y}$ = 4·6328
op'-DDT	$\frac{1}{Snw}$ = 0·0052502 ;	$\bar{x}$ = 2·1863 ;	$\bar{y}$ = 5·0753
Mixture I	$\frac{1}{Snw}$ = 0·0028612 ;	$\bar{x}$ = 1·3500 ;	$\bar{y}$ = 4·9146
Mixture II	$\frac{1}{Snw}$ = 0·0048598 ;	$\bar{x}$ = 1·9025 ;	$\bar{y}$ = 5·1060

	$Snwx^2$	$Snwxy$	$Snwy^2$	
pp'-DDT	708·5356 684·3415 24·1941	2177·4315 2121·5687 55·8628	6707·0432 6577·0903 129·9529	—128·9816=0·9713
op'-DDT	934·1407 910·4354 23·7053	2169·1439 2113·4803 55·6636	5042·3617 4906·2555 136·1062	—130·6981=5·4081
Mixture I	668·8213 636·9615 31·8598	2416·8212 2318·8228 97·9984	8744·8776 8441·7047 303·1729	—301·4333=1·7396
Mixture II	772·0699 744·7970 27·2729	2083·8110 1998·9006 84·9104	5629·1505 5364·7236 264·4269	—264·3515=0·0754

Analysis of χ^2

	D.F.	*Sum of squares*	*Mean squares*	*F.*
Parallelism of regression	3	15·5027	5·1676	4·414
Residual heterogeneity	7	8·1944	1·1706	
Total	10	23·6971		

thereby indicating that the parallelism does not exist between the probit-log dosage regression lines of the mixtures and individual components. The results, therefore, suggested that probably the joint action of pp′ and op′-DDT was not of the similar type against *Ae. aegypti* larvae.

The joint action of the isomers of DDT against *Ae. aegypti* larvae was, therefore, tested for dissimilar joint action. The expected probits for different types of dissimilar joint action at the various concentrations of Mixture I and Mixture II were estimated from the probit-log concentration regression lines of the individual components and are stated in Table XVI. A test of agreement between the observed and expected probit was made (Table XVI).

The value of Chi^2 was found to be quite high in case of Mixture I for all the types of dissimilar joint action tested. In the case of Mixture II, the value of Chi^2 was found to be significant for independent action with positive, zero and negative correlation. However, the value of Chi^2 for dependent joint action for the Mixture II was found to be less than the value significant at P=0·05 level. The results, therefore, suggested that the joint action of pp′ and op′-DDT, when mixed in the ratio of 20 per cent and 80 per cent, was of a dependent type. The joint action of the two when mixed in the ratio of 80 per cent and 20 per cent respectively, however, did not at all agree with the dependent joint action. It is likely that the agreement between the expected probits for dependent joint action with the observed probits in case of Mixture II may be just a coincidence.

To further understand the type of joint action exhibited by the mixture of pp′ and op′-DDT against *Ae. aegypti* the expected probits at all the concentrations of both the mixtures were estimated assuming that the action was simple similar*. A test of agreement between the expected probits thus obtained and observed probits was made (Table XVII). The value of Chi^2 at the dosage 0·007 p.p.m. of Mixture I was not significant at P : 0·05 level ; thereby indicating an agreement between the observed and expected value. However, at other concentrations of Mixture I there was no agreement between the observed and expected probits.

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* The probit-log concentration regression lines of pp′ and op′-DDT were found to be parallel. The common value of the slope 'b' was, therefore, estimated as 2·393. The equations of the fitted pr-lc regression lines of pp′-DDT and op′-DDT were estimated as :

$$Y_{pp'\text{-DDT}} = 1{\cdot}0513 + 2{\cdot}393X$$

$$\text{and } Y_{op'\text{-DDT}} = -0{\cdot}1418 + 2{\cdot}393X \text{ respectively}$$

$$\log K = \frac{-0{\cdot}1418 - 1{\cdot}0513}{2{\cdot}393} = -0{\cdot}4985.$$

Hence K_2 was estimated as 0·3176

The equations for the Mixture I and Mixture II as estimated from the general equation $Y_m = a_1 + b \log (Q_1 + K_2 Q_2) D_m$ are :

$$Y_{m\text{-I}} = 0{\cdot}8998 + 2{\cdot}393 \log D_{m\text{-I}} \text{ or } 0{\cdot}8998 + 2{\cdot}393 X_{m\text{-I}}.$$

$$Y_{m\text{-II}} = 0{\cdot}2305 + 2{\cdot}393 \log D_{m\text{-II}} \text{ or } 0{\cdot}2305 + 2{\cdot}393 X_{m\text{-II}}.$$

The expected probits at different concentrations of the Mixture I and Mixture II were estimated from the above mentioned equations (Table XVII).

TABLE XVI.

Expected probits for dissimilar joint action exhibited by the Mixture I (80 per cent pp'-DDT and 20 per cent op'-DDT) and Mixture II (20 per cent pp'-DDT and 80 per cent op'-DDT) against Ae. aegypti *larvae.*

Mixture.	Dosage of mixture (p.p.m.)	Observed probit.	Expected probits for independent action with correlation: Positive ($P=P_1$ or P_2)	Zero ($P=P_1+P_2-P_1P_2$)	Negative ($P=P_1+P_2$)	Expected probit for dependent joint action ($P=P_1+2P_2-2P_1P_2$)	X^2 value for independent action with correlation: Positive.	Zero.	Negative.	X^2 value for dependent joint action.
Mixture-I (80 per cent pp'-DDT plus 20 per cent op'-DDT)	0·007	3·3	3·0	3·0	3·0	3·0	2·12	2·12	2·12	2·12
	0·015	4·5	3·7	3·7	3·7	3·7	38·69	38·69	38·69	38·69
	0·03	5·4	4·4	4·4	4·4	4·4	100·42	100·42	100·42	100·42
	0·06	6·2	5·1	5·1	5·1	5·1	138·15	138·15	138·15	138·15
Mixture II (20 per cent pp'-DDT plus 80 per cent op'-DDT)	0·03	3·8	3·2	3·4	3·4	3·6	11·66	6·84	6·84	2·17
	0·125	5·7	4·6	5·1	5·3	5·6	130·79	41·10	17·74	1·00
	0·25	6·6	5·4	5·7	∞	∞	51·88	25·83	..	..

TABLE XVII.

Expected probits for a simple similar action for the Mixture I and Mixture II of pp'-DDT and op'-DDT against Ae. aegypti *larvae.*

Mixture.	Dosage (p.p.m.)	Observed probit.	Expected probit for a simple similar action.	X^2 value for simple similar action.
Mixture I	0·007	3·3	3·1	1·11
	0·015	4·5	3·8	32·66
	0·03	5·4	4·5	84·71
	0·06	6·2	5·2	112·94
Mixture II	0·03	3·8	3·8	0·0
	0·125	5·7	5·3	17·74
	0·25	6·6	6·0	9·47

In the case of Mixture II, the expected probit was completely in agreement with the observed value of probit at the dosage of 0·03 p.p.m., whereas at other concentrations of the mixture there was no agreement. The value of the observed probits at all other concentrations were higher than the expected probits. From these results it appears that at lower concentrations of the mixtures the joint action of pp'and op'-DDT was just simple similar. It is likely that the joint action of pp' and op'-DDT at other concentrations of the mixture is complex similar, *i.e.*, similar action with interaction as the observed probits were higher than the expected probits. The absence of interaction at the lower dosage of the insecticides may be due to the availability of small amount of the constituents at the site of action for the effect (interaction) to be produced. The data were, therefore, further analysed to confirm it. The lowest concentrations of the Mixture I and Mixture II were deducted from the observations, and the analysis for testing parallelism between pp'-DDT, op'-DDT, Mixture I and Mixture II was made. The details of the analysis are mentioned in Table XVIII. The results showed that the probit-log concentration regression lines of pp'-DDT, op'-DDT and their mixtures were parallel.

TABLE XVIII.

Summary of analysis for parallelism between pp'-DDT, op'-DDT, Mixture I and Mixture II after deducting the observation at lower dosage.

Analysis of χ^2

	D.F.	Sum of squares.	Mean squares.	F.
Parallelism of regression	3	4·68	1·56	1·19
Residual heterogeneity	5	6·53	1·31	
Total	8	11·21		

The common value of the slope 'b' was estimated as 2·4948 and the equations of the pr-log concentration regression lines of pp'-DDT, op'-DDT, Mixture I and Mixture II, using the common value of 'b', were estimated as :

$$Y \text{ pp'-DDT} = 0{\cdot}9046 + 2{\cdot}4948x$$
$$Y \text{ op'-DDT} = -0{\cdot}3791 + 2{\cdot}4948x$$
$$Y \text{ Mixture I} = 1{\cdot}6319 + 2{\cdot}4948x$$
$$Y \text{ Mixture II} = 0{\cdot}5295 + 2{\cdot}4948x$$

From the probit-log concentration regression equations of pp'-DDT and op'-DDT, the value of K_3 was estimated as 0·3058. The hypothetical equation for the Mixture I was estimated to be $Y_{m-I} = 0{\cdot}7436 + 2{\cdot}4948x_{m-I}$ assuming, of course, that the joint action of pp' and op'-DDT was simple similar. Similarly the expected equation for the Mixture II was estimated to be $Y_{m-2} = 0{\cdot}0264 + 2{\cdot}4948x_{m-II}$.

A test of agreement between the observed regression equation and expected equation was thus made by following Finney (1952 *loc. cit.*)*.

The results obtained indicated that the joint action of pp'-DDT and op'-DDT was complex similar except at the low concentrations where the action was just simple similar.

The above mentioned analysis was made by deducting the observations at the lower concentrations of the Mixture I and Mixture II. The data were also analysed inclusive of all observations and necessary adjustment was made by taking into consideration the heterogeneity factor. In this case also the difference was found highly significant.

The result obtained, therefore, indicated that the joint action of pp' and op'-DDT in general was complex similar or in other words synergistic against *Ae. aegypti* larvae.

RELATIVE EFFECTIVENESS OF VARIOUS GRADES OF TECHNICAL DDT AGAINST *AE. AEGYPTI* LARVAE.

The basic data showing the toxicity of pp'-DDT and different grades of technical DDT against *Ae. aegypti* larvae are contained in Table XIX. The results of the probit analysis are depicted in Table XX. The MLC's of DDT technical grades *A*, *B* and *C* were 0·052, 0·097 and 0·16 p.p.m. respectively. The

TABLE XIX.

Percentage mortality in Aedes aegypti *larvae when exposed to various concentrations of pp'-DDT and different grades of technical DDT.*

Insecticide.	Concentration of insecticide (p.p.m.) :						
	0·031	0·046	0·062	0·094	0·125	0·25	0·5
pp'-DDT	11·7 (358)	28·7 (179)	53·8 (344)	82·2 (180)	94·8 (356)	99·1 (120)	..
DDT Technical A grade	15·5 (360)	40·5 (180)	65·2 (360)	86·1 (180)	95·2 (360)	99·1 (120)	..
DDT Technical B grade	3·9 (360)	..	33·3 (360)	..	60·0 (360)	85·3 (360)	95·3 (360)
DDT Technical C grade	4·3 (349)	..	13·2 (348)	..	42·2 (348)	69·8 (351)	88·9 (360)

Note :—Mortality in control was nil.

Figures in parenthesis indicate the number of larvae tested at each concentration of insecticide.

* For Mixture I, the Δs_1 was estimated to be 1·6319−0·7436=0·8883 and its variance $V(\Delta s_1)$ as 0·005693. The value of χ^2 was found to be highly significant ($\chi^2_{(1)} = 134{\cdot}7 > \chi^2_{(1)} = 3{\cdot}6$ at $P=0{\cdot}05$ level), thereby indicating that the observed mortalities were higher than what could be expected as chance deviation in a simple similar action. In case of Mixture II, the value of Δs_2 was estimated to be 0·5031 and its variance $V(\Delta s_2)$ as 0·016844. In this case also, the value of χ^2 was found to be highly significant ($\chi^2_{(1)} = 14{\cdot}7 > \chi^2_{(1)} = 3{\cdot}6$ at $P=0{\cdot}05$ level).

TABLE XX.

Equation of the fitted pr-lc regression line and the parameters with variance of pp'-DDT and different grades of technical DDT against Ae. aegypti *larvae.*

Insecticide.	Equations of the fitted pr-lc regression line (x = log 100z)	χ^2(df)	*m* (log 100z)	Variance of *m*	MLC (p.p.m.) (with fiducial limits for 95 per cent probability)	Variance of *b*
pp'-DDT	Y=1·4929+4·59x	5·73 (4)	0·7646	0·00007648	0·058 (0·056-0·060)	0·040855
DDT Technical A Grade	Y=1·8235+4·44x	0·91 (3)	0·7154	0·00008330	0·052 (0·050-0·054)	0·0447617
DDT Technical B grade	Y=2·6533+2·38x	1·16 (2)	0·9860	0·00034002	0·097 (0·089-1·05)	0·016806
DDT Technical C grade	Y=1·0622+2·53x	4·01 (3)	1·2007	0·00019822	0·16 (0·15-0·17)	0·009197

results obtained, therefore, showed that technical DDT, containing the highest amount of pp'-DDT, was the most toxic of all the grades of technical DDT tested. Technical DDT, containing the least amount of pp'-DDT, was the least toxic. The MLC of pp'-DDT against *Ae. aegypti* larvae was 0·058 p.p.m. Technical DDT, containing 80 per cent pp'-DDT, was found to be more toxic than pure pp'-DDT. The other grades of technical DDT, containing lower amount of pp'-DDT, were, however, found to be less toxic than pp'-DDT.

RELATIVE EFFECTIVENESS OF PP'-DDT AND OP'-DDT AGAINST *A. STEPHENSI* LARVAE.

The percentage mortality in *A. stephensi* larvae exposed to different concentrations of pp'-DDT and op'-DDT is shown in Table XXI. The summary of the results, as analysed by probit method, is contained in Table XXII. The LC_{50} of pp'-DDT against *A stephensi* larvae was 0·039 p.p.m., with fiducial limits 0·036—0·043. The LC_{50} of op'-DDT, when tested under identical conditions, was found to be 0·13 p.p.m., with fiducial limits 0·12—0·15. The regression coefficient values of pp' and

TABLE XXI.

Percentage mortality in A. stephensi *larvae exposed to different concentrations of pp'-DDT and op'-DDT.*

Insecticide.	Concentration of insecticide (p.p.m.):							
	0·0125	0·025	0·05	0·0625	0·125	0·25	0·5	1·0
pp'-DDT	9·4 (180)	30·6 (180)	61·1 (180)	...	90·8 (120)	...	...	...
op'-DDT	...	...	...	17·2 (180)	52·2 (180)	76·2 (177)	88·0 (175)	100·0 (60)

Note :—Mortality in control was nil.
Figures in parenthesis indicate the number of larvae tested at each concentration of the insecticide.

op'-DDT did not differ significantly from each other. pp'-DDT was found to be significantly more toxic than op'-DDT against *A. stephensi* larvae and the relative effectiveness of pp'-DDT to op'-DDT (*Prd*) was estimated to be 3·48 with standard error ± 0·25.

TABLE XXII.

Equations of the fitted pr-lc regression line and the parameters with variance of pp'-DDT and op'-DDT against A. stephensi *larvae.*

Insecticide	Equation of the fitted pr-lc regression line (x=log 100z)	X^2 (df)	m (log 100z)	Variance of m	MLC (p.p.m.) (with fiducial limits for 95 per cent probability)	Variance of b
pp'-DDT	Y=3·4172+2·66x	0·25 (2)	0·5950	0·0004422	0·039 (0·036-0·043)	0·031464
op'-DDT	Y=2·2522+2·42x	9·27 (3)	1·1355	0·000156	0·13 (0·12-0·15)	0·09045

RELATIVE EFFECTIVENESS OF PP'-DDT AND OP'-DDT AGAINST *A. SUBPICTUS* LARVAE (NOVEMBER STRAIN).

The percentage mortality in *A. subpictus*, collected from field in November, exposed to different concentrations of pp'-DDT, op'-DDT, Mixture I and Mixture II, is mentioned in Table XXIII. The results of probit analysis are contained in Table XXIV. The LC_{50} of pp'-DDT was found to be 27·54 p.p.m., with fiducial limits 20·05—38·55. The LC_{50} of op'-DDT was found to be 3·61 p p.m., with fiducial limits of 3·18—4·09. op'-DDT was, therefore, found to be significantly more toxic than pp'-DDT against *A. subpictus* larvae. The response in case of *A. subpictus* larvae to the mixtures of pp' and op'-DDT was found to be highly

TABLE XXIII.

Percentage mortality in A. subpictus *larvae (collected during November) when exposed to pp'-DDT, op'-DDT, Mixture-I and Mixture-II.*

Insecticide	CONCENTRATION OF INSECTICIDE in p p.m.				
	1·25	2·5	5·0	10·0	20·0
pp'-DDT	..	3·3 (120)	11·7 (120)	15·7 (120)	43·3 (120)
op'-DDT	20·8 (240)	..	57·5 (240)	77·5 (240)	94·2 (240)
Mixture I	6·7 (120)	..	11·7 (120)	30·0 (120)	46·7 (120)
Mixture II	15·4 (240)	..	37·6 (240)	67·5 (240)	91·7 (240)

Note :—Mortality in control was nil.

Figures in parenthesis indicate the number of larvae tested at each concentration of the insecticide.

heterogeneous, *i.e.*, the probits of the mortality were not found to be linearly related to log concentration of the insecticides. Therefore the LC_{50} and other parameters were not estimated for the mixtures.

TABLE XXIV.

Equation of the fitted pr-lc regression lines and the parameters with standard errors of pp'-DDT and op'-DDT against A. subpictus *larvae (November strain.)*

Insecticide.	Equations of the fitted pr-lc regression line (10z)	χ^2 (df)	m (log 10z)	Variance of m	MLC (p.p.m.) with fiducial limits for 95 per cent probability.	Variance of b
pp'-DDT	Y=0·6740+1·77x	3·91 (2)	2·4441	0·00524279	27·54 (20·05-38·55)	0·063909
op'-DDT	Y=2·0826+1·84x	3·20 (2)	1·5568	0·00081667	3·61 (3·18-4·09)	0·0134733

JOINT ACTION OF PP'-DDT AND OP'-DDT AGAINST *A. SUBPICTUS* LARVAE (NOVEMBER STRAIN).

The regression coefficient values of pp'-DDT and op'-DDT against *A. subpictus* larvae were not found to differ significantly from each other*. The mixture of pp' and op'-DDT was, therefore, likely to exhibit simple similar action against *A. subpictus* larvae. The expected regression equations for the Mixture I and Mixture II were computed from the dose response equation of the individual components†, assuming that the joint action was simple similar. From these equations, the expected probits at the various concentrations of the Mixture I and Mixture II were estimated and are shown in Table XXV. A test of agreement between the observed and expected probits was made and the value of Chi^2 was found to be 91·42 and 29·01 respectively. These values of Chi^2 were found to be highly significant at P : 0·05 level, thereby suggesting that the joint action of pp' and op'-DDT was not a simple similar. The observed probits in the case of both the mixtures were found to be less than the expected probits. The action, however, cannot be antagonistic, *i.e.*, similar action with negative interaction as the response produced by the mixtures in general was not less than what would have been produced by the component applied singly.

* $\chi^2_{(b)} = 0{\cdot}14 < \chi^2_{(1)} = 3{\cdot}6$ at $P = 0{\cdot}05$ level.

† The value of the common slope 'b' was estimated to be, 1·856 and by using this value the regression equations for pp'-DDT and op'-DDT were estimated to be

$$Y_{pp'\text{-DDT}} = 0{\cdot}5010 + 1{\cdot}856x \text{ and}$$

$$Y_{op'\text{-DDT}} = 2{\cdot}1139 + 1{\cdot}856x$$

respectively. From these, the value of K_2 was estimated as 0·1352. Therefore the expected equation of the simple similar equation for the Mixture I was $Y_{m\text{-I}} = 1{\cdot}1651 + 1{\cdot}856X_{m\text{-I}}$. Similarly the equation for the Mixture II was

$$Y_{m\text{-II}} = 1{\cdot}9608 + 1{\cdot}856X_{m\text{-II}}.$$

The expected probits for different types of dissimilar joint action at various concentrations of the Mixture I and Mixture II were, therefore, estimated from the probit-log concentration regression lines of the individual components. The results obtained are depicted in Table XXV. A test of agreement between the observed and expected probits was made (Table XXV).

The values of Chi^2 for independent action with zero correlation, independent action with negative correlation and dependent joint action in case of Mixture I were found to be significant at P=0·05 level. The results, however, suggest that the observation agreed more with the independent action with positive correlation of the various types of joint action; and at the concentration at 5·0, 10·0 and 20·0 p.p.m., the agreement between the expected and observed probits was fairly satisfactory.

In the case of Mixture II, the agreement between the observed and expected probits for all types of joint action was poor. The difference between the observed probits and expected probits for independent action with positive correlation, however, was not found to be significant at P=0·05 level except at the dosage of 5·0 p.p.m.

The results discussed above, therefore, indicated that there was no complete agreement of the observed value with any type of joint action postulated, *i.e.*, similar or dissimilar. The data obtained, however, tend to suggest that the joint action of pp' and op'-DDT against *A. subpictus* larvae collected during the month of November was of an independent type with positive correlation.

RELATIVE EFFECTIVENESS OF PP'-DDT, OP'-DDT AND VARIOUS TYPES OF TECHNICAL DDT AGAINST *A. SUBPICTUS* LARVAE (JULY AND NOVEMBER STRAIN).

The basic data showing the toxicity of pp'-DDT, op'-DDT and different grades of technical DDT against the larvae of A. *subpictus* collected during the months of November and July are contained in Table XXVI. The results of the analysis by probit method are presented in Table XXVII.

The LC_{50} of pp'-DDT against *A. subpictus* larvae, collected during the month of July, was 0·21 p.p.m., with fiducial limits 0·19—0·23. The LC_{50} of op'-DDT, when tested under similar conditions against these larvae, was found to be 1·05 with fiducial limits 0·80 to 1·38. The results, therefore, indicated that pp'-DDT was about five times more toxic than op'-DDT. It is quite interesting as op'-DDT was observed to be more toxic than pp'-DDT against the larvae of *A. subpictus* collected during the month of November (Tables XXIII and XXIV). As regards the relative toxicity of different grades of technical DDT against *A. subpictus* (collected during the month of July), technical DDT containing the highest amount of pp'-DDT was more toxic than the other grades of technical DDT. Technical DDT grades *B* and *C* were found to be equi toxic against *A. subpictus* larvae. Technical DDT grade 'A' was also found to be as toxic as pure pp'-DDT.

TABLE XXV.

Summarised analysis of the mixture of pp'-DDT and op'-DDT when mixed both in the ratio of 80 per cent and 20 per cent and 20 per cent and 80 per cent respectively against A. subpictus *larvae (Nov. strain) as similar and dissimilar joint action.*

Mixture.	Dosage of the mixture (p.p.m.)	Observed probit.	Expected probit for a simple similar action.	Expected probit for independent action with correlation: Positive.	Zero.	Negative.	Expected probit for dependent joint action.	χ^2 value for simple similar action.	χ^2 value for independent action with correlation: Positive.	Zero.	Negative.	Dependent joint action.
Mixture-I	1·25	3·5	3·21	2·9	3·0	3·0	3·0	1·94	4·76	3·93	3·93	3·93
	5·0	3·8	4·5	4·0	4·2	4·2	4·4	34·16	2·10	9·65	9·65	24·10
	10·0	4·4	4·9	4·5	4·8	5·0	5·1	37·30	1·27	27·10	48·89	61·65
	20·0	4·9	5·4	5·1	5·5	6·1	6·0	18·02	3·04	25·10	69·94	63·69
								91·42	11·17	65·78	132·41	153·37
Mixture-II	1·25	4·0	4·0	4·0	4·0	4·0	4·0	0·0	0·0	0·0	0·0	0·0
	5·0	4·7	5·1	5·1	5·1	5·1	5·1	24·36	24·36	24·36	24·36	24·36
	10·0	5·5	5·7	5·6	5·7	5·7	5·7	5·10	1·27	5·10	5·10	5·10
	20·0	6·4	6·2	6·2	6·3	6·6	6·3	3·55	8·55	2·28	3·55	0·8
								33·01	29·18	31·74	33·01	30·26

TABLE XXVI.

Percentage mortality in the larvae of different strains of A. subpictus *exposed to various concentrations of pp'-DDT, op'-DDT and different grades of technical DDT.*

Strain of *A. subpictus*.	Insecticide.	Concentration of insecticides in p.p.m.								
		0·0624	0·125	0·25	0·5	1·0	1·25	2·0	5·0	10·0
July	pp'-DDT	..	27·9 (240)	59·6 (240)	80·8 (240)	93·8 (240)	..	..	..	..
	op'-DDT	..	..	8·3 (60)	23·3 (60)	..	56·7 (60)	..	..	..
	DDT technical A grade	14·0 (178)	40·6 (180)	63·3 (180)	81·1 (180)	93·3 (180)	..	..	..	..
	DDT technical B grade	0·8 (240)	13·1 (237)	38·8 (237)	60·2 (236)	75·4 (240)	..	..	..	..
	DDT technical C grade	2·9 (240)	15·8 (240)	30·4 (240)	55·4 (240)	68·8 (240)	..	..	..	..
Nov.	DDT technical A grade	..	..	..	..	..	..	23·3 (60)	28·3 (60)	41·7 (60)
	DDT technical B grade	..	..	..	..	..	..	20·0 (60)	21·7 (60)	40·0 (60)
	DDT technical C grade	..	..	..	..	..	..	43·3 (60)	41·7 (60)	71·7 (60)

Note :—Mortality in control was nil.
Figures in parenthesis indicate the number of larvae tested at each concentration of the insecticides.

TABLE XXVII.

Summary of the statistical analysis showing the toxicity of pp'-DDT, op'-DDT and different grades of technical DDT against the larvae of A. subpictus *(July strain).*

Strain.	Insecticide.	Equation of the fitted probit regression line (x=log 100 z)	χ^2 (df)	m (log 100z)	Variance of m	MLC (ppm) with fiducial limits for 95 per cent probability.	Variance of b
July	pp'-DDT	Y=1·8608+2·37x	1·24 (2)	1·3246	0·000446958	0·21 (0·19—0·23)	0·023956
	op'-DDT	Y=0·4103+2·27x	0·02 (1)	2·0219	0·003623	1·05 (0·80—1·38)	0·0150951
	DDT technical A grade	Y=2·3866+2·08x	2·9 (3)	1·2625	0·00055449	0·18 (0·16—0·21)	0·0137877
	DDT technical B grade	Y=1·5039+2·16x	16·3 (3)	1·6186	0·00240806	0·42 (0·29—0·59)	0·0770742
	DDT technical C grade	Y=1·8575+1·87x	6·03 (3)	1·6605	0·0005786	0·48 (0·43—0·53)	0·0110102

The response in case of *A. subpictus* larvae, collected during November, was found to be very heterogeneous to the different grades of Technical DDT and, therefore, the LC_{50} and other parameters could not be estimated. The data obtained, however, clearly indicated that technical DDT, containing the least amount of pp'-DDT, was the most toxic against the larvae of this strain of *A. subpictus*. The toxicity of other grades of technical DDT, *i.e.*, grades *B* and *C*, however, appears to be the same.

DISCUSSIONS AND CONCLUSIONS.

pp'-DDT is known to be generally the most toxic of its isomers against insects. It was, however, found to be less toxic than op'-DDT against resistant lice (Yasutmi, 1956 *loc. cit.*). Cristol and Haller (1945) and Jones *et al.* (1952) studied the relative effectiveness of pp' and op'-DDT against the larvae of *A. quadrimaculatus* and *Ae. aegypti* respectively and observed that pp'-DDT was more toxic. Present investigations have indicated that pp'-DDT was more toxic than op'-DDT against the larvae of *Ae. aegypti*, *A. stephensi* and the July strain of *A. subpictus*. pp'-DDT was, however, found to be less toxic than op'-DDT against the larvae of the November strain of *A. subpictus* and laboratory-reared and field-collected strains of *C. fatigans*.

pp'-DDT which was found to be about 5 times more toxic than op'-DDT against the July strain of *A. subpictus* was about 8 times less toxic against the November strain of *A. subpictus*. The MLC's of pp'-DDT for the July and November strains of *A. subpictus* were found to be 0·21 p.p.m. and 27·54 p.p.m., respectively, thereby showing that the November strain of *A. subpictus* was 130 times as tolerant as the July strain of *A. subpictus*. *A. subpictus* (November strain), on the other hand, showed only 3 times increased tolerance to op'-DDT as compared to the July strain. The data obtained, therefore, indicated that the major factor responsible for the increased tolerance of the November strain was not common with pp' and op'-DDT.

The metabolism of DDT to non-toxic metabolite has been found to be the major factor responsible for resistance of house-flies to DDT. The enzyme, DDT dehydrochlorinase, responsible for this metabolism, was found to have the specificity for pp'-DDT and its analogues. *In vitro* conversion of op'-DDT by this enzyme could not be demonstrated (Stearnburg *et al.*, 1954). The enzyme responsible for metabolism of pp'-DDT in body-lice also did not detoxify op'-DDT (Perry *et al.*, 1958). Dehydrochlorination of DDT to DDE has been found to occur in DDT-resistant *Anopheles*, *Culex* and *Aedes* (Perry, 1960 ; Bami *et al.*, 1957 ; Hoskins *et al.*, 1958 ; Brown and Perry, 1956). Kalra and Pal (1959) also found the metabolism of DDT to DDE in resistant *A. subpictus*. The change in order of toxicity of pp'-DDT and op'-DDT against the November strain of *A. subpictus* may, therefore, be due to the presence of enzyme (s) in this strain which has (have) got specificity to detoxify pp'-DDT. In case of mosquitoes, so

far no correlation has been found between metabolism and DDT resistance (Chattoroj and Brown, 1960). Further it has not been possible to demonstrate DDT dehydrochlorinase *in vitro*. It is, therefore, not certain whether the metabolism is the cause or consequence of resistance. The data obtained in case of *A. subpictus*, however, suggest that the defence mechanisms of resistance involve the sites of loss having specificity for pp'-DDT (most probably the enzyme (s) to detoxify pp'-DDT). The LC_{50} of op'-DDT against this species was found to be very high as compared to the MLC's values obtained against *A. stephensi*, *Ae. aegypti* and laboratory-reared *C. fatigans*. This suggests that this species also manifests certain degree of resistance to op'-DDT. The resistance in this species may, therefore, also involve certain unspecific type of defence mechanisms as regards to the isomers of DDT. Further physiological studies are, however, needed to elucidate the mechanism of resistance.

Sharma and Kalra (1958 *loc. cit.*) reported that the degree of DDT-resistance exhibited by *A. subpictus* larvae was of a much lower order as compared to adults. During the present investigations the LC_{50} of pp'-DDT, obtained with the November strain of *A. subpictus*, was very high (27·54 p.p.m.) ; thereby indicating that the larvae of *A. subpictus* also manifested fairly high degree of resistance. The LC_{50} of pp'-DDT with the July strain was, however, found to be low. It is interesting to note that adults, collected both during the months of July and November, were highly resistant to DDT (LC_{50}> 4·0 per cent with 24 hours exposure by the Busvine-Nash method). The change in the susceptibility as observed in the case of *A. subpictus* may be just a seasonal variation in the susceptibility of larvae. Singh and Gad (1958) observed the MLC of DDT as low as 0·001 p.p.m. and as high as 0·2 p.p.m. against the larvae of *A. sergentii* collected during different months. The seasonal variation in tolerance is generally considered an unspecific type of tolerance but in case of *A. subpictus* larvae this was found to be more of a specific type. The larvae, however, showed some increased tolerance to op'-DDT. It is also likely that further spraying of insecticide has selected a strain which showed higher tolerance. Singh (1961) estimated the frequency of different genotypes, *i.e.*, susceptible, hybrid and resistant in *A. subpictus* during the year 1959 in areas around Delhi and found no change in their frequency with insecticidal spraying. May be that the insecticidal spraying has resulted in the selection of a population which manifest fairly high degree of resistance in the larval stage as well. Further work is needed to elucidate this point.

op'-DDT was found to be about 6 times more toxic than pp'-DDT even against the laboratory-reared *C. fatigans* larvae (L_1) (susceptible strain). The higher toxicity of op'-DDT than pp'-DDT in this case may be due to species specificity. The LC_{50} of pp'-DDT was found to be 0·63 p.p.m., which was much higher than the LC_{50} value obtained by various workers for the susceptible *C. fatigans* populations (Hoskins, 1962). The LC_{50} obtained for the susceptible *C. fatigans* larvae was about 0·06 p.p.m. This shows that the laboratory-reared *C. fatigans* strain,

used in the present investigations, was about 10 times more tolerant than the susceptible strain. The increased tolerance, as exhibited, may be due to the nature of the strain itself or there might have been some inadvertent selection during the process of colonization for DDT resistance. The higher toxicity of op'-DDT than pp'-DDT can also be explained if it is assumed that inadvertent selection has resulted in the selection of specific resistance to pp'-DDT. The relative toxicity of pp'-DDT and op'-DDT, if determined against the strains having higher susceptibility to pp'-DDT, will elucidate this point.

It was further observed during the course of present investigations that unlike *A. subpictus* there has been corresponding increase in tolerance of all strains of *C. fatigans* tested to op'-DDT. The mechanism of resistance in case of *C. fatigans*, therefore, appears to be different from that of *A. subpictus*. Bami *et al.* (1957 *loc. cit*), Hoskins *et al.* (1958 *loc. cit.*) and Perry (1960 *loc. cit.*) found the metabolism of DDT to DDE in case of resistant *C. fatigans*. The metabolism of DDT to DDE, as observed, may be due to certain unspecific type of enzymes and may be just the consequence of resistance.

However, it is now being more and more recognised that resistance is a multicomponent phenomenon (Spiller, 1958, Reiff and Beye, 1960). The defence mechanisms involve both specific as well as unspecific sites of loss. The relative toxicity of op'-DDT and pp'-DDT against *C. fatigans* could be explained if it is assumed that the resistance is of this type in this species. The increased tolerance, as exhibited by the laboratory-reared strain of *C. fatigans* (L_1), may be due to certain specific mechanisms which were common with all the strains tested. In other strains of *C. fatigans*, selection is assumed to select only the defence mechanisms which are unspecific for the isomers of DDT.

Studies on the joint action of pp' and op'-DDT indicated that the action was simple similar against *C. fatigans*, and complex similar against *Ae. aegypti*. The action, though not conclusive, was found to be of an independent type against *A. subpictus*. This implies that the site of action of the two insecticides is the same in case of *C. fatigans* and *Ae. aegypti* and different in the case of *A. subpictus* which appears to be a strange anomaly. Plackett and Hewlett (1959) suggested that apart from "sites of action", the "sites of loss" also play an important role in the type of joint action exhibited by the mixture. When a dose of drug is introduced into the complex of system forming an organism, only a part of the dose reaches the site of action. This part produces the biochemical and physiological changes which, if great enough, leads to the particular quantal response under consideration. The remainder goes to the "sites of loss". Thus, of the part of dose not acting, some may remain stored in the tissues without contributing to the action ; some may be metabolized enzymatically to a less active or inactive substance and some may be excreted either as unchanged or as metabolites. The metabolic modification is generally highly specific to drug, the occurrence of it will depend on the detailed molecular structure of the drug. The joint action of drugs is completely simple similar only if they act in the same way at the

same site of action and be stored, excreted and metabolised in the same way. However, two drugs could be similar chemically in such respects that they had the same site of action but could differ in such way, that one, but not the other, was metabolised to an inactive substance at the site of loss ; the type of joint action would then be between simple similar and independent action, and the correlation would depend on the relative capacity of the site of loss and site of action. The action can be almost independent if the tolerance depends on the capacities of specific sites of loss despite identical sites of action.

The insects manifest resistance to insecticides because most of the material applied goes to the site of loss. In the case of *A. subpictus* larvae (November strain) it has already been shown that the resistance manifested is probably due to sites of loss having specificity for pp'-DDT. The manifestation of an independent action, therefore, appears to be due to site of loss rather than the site of action. The joint action between similar and dissimilar was probably not detected due to the limitation of bioassay technique. The positive correlation was obtained as *A. subpictus* (November strain) showed some increased tolerance to op'-DDT.

The joint action of pp' and op'-DDT against *C. fatigans* was found to be simple similar, thereby showing that the site of action as well as the site of loss was identical for both of these insecticides. The results, therefore, suggested that there was no specific site of loss for pp'-DDT. The higher toxicity of op'-DDT than pp'-DDT, as observed against *C. fatigans*, appears to be due to species specificity. The defence mechanisms involved in resistance also appear to be unspecific in regard to the isomers of DDT. It will, therefore, be interesting to study the mechanism by which DDT is dehydrochlorinated.

Yasutomi (1956 *loc. cit.*) found the mixture of pp' and op'-DDT as synergistic against all strains of house-flies and body-lice. During the present study, the interaction (synergism) was found only against *Ae. aegypti* larvae.

The data collected on the relative toxicity of various grades of technical DDT indicated that DDT, containing lower amount of pp'-DDT, was the most toxic against the larvae of *C. fatigans* and *A. subpictus* (November strain) whereas it was least toxic against the larvae of *Ae. aegypti* and *A. subpictus* (July strain). These observations are similar to what could be expected due to the joint action of pp' and op'-DDT against a particular species. The results obtained, therefore, suggest that the proportion of different constituents for the best grade of technical DDT depends upon the relative effectiveness of the constituents separately against a particular species and it varies from species to species. The suggestion by Yasutomi (1956 *loc. cit.*) that the best grade of technical DDT should contain lower amount of pp'-DDT, does not seem to have a general application.

SUMMARY.

The relative toxicity of pp'-DDT and op'-DDT was studied against the larvae of various species of mosquitoes. pp'-DDT was found to be more toxic

than op'-DDT against *Ae. aegypti*, *A. stephensi* and the July strain of *A. subpictus*. It was, however, found to be less toxic against *A. subpictus* (November strain) and *C. fatigans*. The change in order of toxicity of op'-DDT and pp'-DDT against the November strain of *A. subpictus* was considered due to defence mechanisms having specificity for pp'-DDT. The higher toxicity of op'-DDT than pp'-DDT against *C. fatigans* was probably due to species specificity.

The joint action of pp'-DDT and op'-DDT was found to be simple similar, complex similar and independent against *C. fatigans*, *Ae. aegypti* and *A. subpictus* (November strain) respectively. The manifestation of an independent action in case of *A. subpictus* was considered due to specific site of loss rather than the site of action.

Studies on the relative toxicity of different grades of technical DDT against various species of mosquitoes larvae has been reported. The order of toxicity of different products was not found to be same against all the strains tested.

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LABORATORY STUDIES ON THE USE OF IRRADIATED STERILE MALES TO REDUCE *C. FATIGANS* WIED. POPULATIONS.

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RUNNER (1916) was the first to demonstrate that insects could be rendered sterile by exposure to irradiation. Muller (1928), while inducing mutations with *X*-ray irradiation, showed that part of the insects exposed became sterile. Bushland and Hopkins (1951) demonstrated that irradiated immature stages of screw worm (pupae) gave rise to sterile adults. Levon and Stahler (1958) and Davis *et al.* (1959) showed that different developmental stages of mosquitoes (*Ae. aegypti*, *A. quadrimaculatus*), on irradiation, produced sterile adults. Ghosh *et al.* (1961) obtained similar results with adult *Ae. aegypti*.

The following investigations were undertaken in the laboratory to determine the effect of radiation of *X*-ray and gamma ray (cobalt 60) radiation on the pupae of *C. fatigans* :—

(*a*) the effect of varying dosages of *X*-ray and gamma ray on the mortality of the exposed pupae and on reproductive capacity of the males hatched from them ;

(*b*) the effect of mating normal unmated females with irradiated and normal males ;

(*c*) the effect of radiation on the longevity of the male mosquitoes ;

(*d*) the effect on the viability of eggs of females mated initially with irradiated males and subsequently given the opportunity to mate with normal males.

MATERIAL AND METHODS.

The strain of *C. fatigans* (adults and pupae), used in the experiments, was obtained in 1960 from the Coonoor Branch of the Institute. Since then it has been maintained as an inbred colony† in the Delhi laboratory where the temperature

* Formerly Malaria Institute of India.

† The batches of egg-rafts obtained were reared under identical conditions of larval space, food and water temperature.

and relative humidity are maintained between 26 to 28°C. and 75 to 80 per cent., respectively.

Irradiation of the pupae with *X*-rays was carried out using the contact *X*-ray therapy unit* in which the pupae were confined in specimen tubes 5 cm. × 2·5 cm. (approximately 100 in each tube). The time of exposure was predetermined to obtain the desired dosage. Irradiation with gamma rays was carried out using cobalt 60† source at different dosages with a view to determining the optimum dose of radiation which would produce maximum sterility with minimum mortality. The dosage was calculated taking the distance from the source to surface and the rate of irradiation. About 1,000-1,200 pupae were exposed in a petri-dish with minimum quantity of water.

The effects of varying dosages of irradiation on pupae were determined with *X*-rays at 30,000 and 14,000 *r*, and with gamma rays at 11,550 ; 7,700 ; 5,700 and 2,850 *r*. The male mosquitoes hatched from these pupae were separated within 12 hours of emergence and were kept in nylon mosquito-net cage (30 cm. × 25 cm. × 25 cm.). These were allowed to mate with normal unmated‡ females from the parent colony. The male (irradiated) and female (normal) mosquitoes were kept together for 4 to 5 days to ensure mating after which the males were removed and the females were then allowed to feed on chicks. The mortality in the irradiated pupae and the adults hatched from these was also recorded. Normal males and females were used as control.

RESULTS.

(a) THE EFFECT OF *X*-RAY AND GAMMA RADIATION ON MOSQUITO PUPAE.

Details of the results obtained with different dosages of irradiation with *X*-rays and gamma-rays, the percentage mortality of the irradiated pupae, number of rafts and their viability are tabulated in Table I.

From the data, it was seen that *X*-rays and gamma-rays were both effective in causing sterility in *C. fatigans* males. The dosage of 7,700 gamma-rays was found to cause more or less complete sterility with minimum mortality in the insects exposed. It was also observed that males exposed to high dosages of irradiation had no effect on egg production of the normal females with which they were mated.

(b) EFFECT ON THE VIABILITY OF EGGS OF NORMAL FEMALES MATING WITH IRRADIATED AND NORMAL MALES.

Male mosquitoes hatched from irradiated pupae (7,700 *r*) were mixed with normal freshly hatched males and these were given the opportunity to mate with normal unmated females. The results of the preliminary tests are summarised in Table II.

* 50 K.V., 50 m. amp., F.O.D. 2 cm. with aluminium filter 0·1 mm.

† Theratron C II from Atomic Energy Canada Ltd. with a loading of 3,000 curies.

‡ Female pupae were isolated singly in specimen-tubes 7·5 cm. × 2·5 cm. to ensure against chance mating.

TABLE I.

Details regarding dosage of irradiation, percentage mortality of the irradiated pupae, number of irradiated males mated with normal females and viability of egg-rafts obtained from these females.

Nature of rays.	Dosage (r)	Mortality in irradiated pupae (per cent.)	Number of irradiated males crossed with normal females		Number of egg-rafts from females mated with irradiated males.	Viability of egg-rafts obtained from the females mated with irradiated males.	Remarks.
			Males.	Females.			
X-rays	30,000	47	10	10	4	Non-viable.*	Complete sterility.
	14,000	28	8	8	6	5 rafts non-viable, 3 larvae hatched only from one raft.	Almost complete sterility.
Gamma-rays	11,550	46	78	89	71	All non-viable.	Complete sterility.
	7,700	23	340	125	119	50 per cent rafts completely non-viable. In the rest there was hatching ranging from 1 to 6 larvae per raft.	Almost complete sterility.
	5,750	29	342	160	115	33 per cent egg-rafts non-viable. In 15 per cent rafts more than 10 larvae hatched out. Rest viable.	Partial sterility.
	2,850	23	320	160	106	Only 3 per cent of the rafts were non-viable.	Partial sterility.
Control	..	0	100	24	20	All viable.	..

*Rafts having embryonated eggs which did not batch.

TABLE II.

Number and ratio of irradiated to normal males mated with virgin female C. fatigans *and percentage of non-viable* egg-rafts obtained.*

Number of irradiated males.	Number of normal males.	Number of normal females.	Proportion of irradiated to normal males to virgin females.	Percentage of non-viable* egg-rafts.
50	100	100	1 : 2 : 2	20
100	100	100	1 : 1 : 1	30
150	100	100	3 : 2 : 2	37
200	100	100	2 : 1 : 1	40
100	..	100	1 : 0 : 1	98
..	100	100	0 : 1 : 1	0

*The rafts had a number of embryonated eggs which did not hatch.

As would be expected, the data showed that increase in the proportion of irradiated to normal males mated with the females increased the percentage of embryonated but non-viable egg-rafts. Therefore, these trials were enlarged by varying the proportion of only irradiated males while the normal males and females were kept constant as in the preliminary trials. Results obtained are summarised in Table II (*a*).

TABLE II(*a*).

Number and ratio of irradiated to normal males mated with normal unmated females and percentage of non-viable egg-rafts obtained.

Cage.	Number of irradiated males.	Number of normal males.	Number of normal females.	Proportion of irradiated to normal males to virgin females.	Percentage of non-viable* egg-rafts.
A	100	10	10	10 : 1 : 1	100
B	90	10	10	9 : 1 : 1	100
C	80	10	10	8 : 1 : 1	0†
D	70	10	10	7 : 1 : 1	80
E	60	10	10	6 : 1 : 1	43
F	50	10	10	5 : 1 : 1	50
G	40	10	10	4 : 1 : 1	100†
H	30	10	10	3 : 1 : 1	43
I	20	10	10	2 : 1 : 1	38
J	10	10	10	1 : 1 : 1	27
K (Control)	10	0	10	1 : 0 : 1	100
L (Control)	0	10	10	0 : 1 : 1	0

*The rafts had a number of embryonated eggs which did not hatch.

†Discrepancies were observed in the viability of rafts produced in cages C and G. In the former, 3 rafts obtained were viable whereas 5 rafts obtained in the latter were all non-viable.

The data, as expected, showed that the number of non-viable egg-rafts increased when the proportion of sterile to normal males increased. The maximum percentage of non-viable rafts were obtained when the proportion of irradiated to normal males to unmated females was 9 : 1 : 1. The proportion of 2 : 1 : 1 in both the trials gave about 40 per cent non-viability in the rafts obtained; thus indicating that reasonable reduction in the output of the mosquitoes could be obtained by using these ratios.

(*c*) LONGEVITY OF IRRADIATED AND NORMAL MALES IN THE LABORATORY.

Daily mortality was recorded (Table III) amongst the irradiated and normal males (100 each) held under identical conditions in the laboratory (where optimal temperature and humidity were maintained).

The data indicated that mortality amongst irradiated males was considerably more than that of the normal males when held under identical conditions.

(*d*) PRELIMINARY OBSERVATION ON THE IMPACT OF SUBSEQUENT MATINGS OF FEMALE *C. FATIGANS*, ONCE MATED WITH IRRADIATED MALES, ON THE VIABILITY OF EGGS PRODUCED.

A batch of normal unmated female mosquitoes (hatched from pupae isolated singly in specimen-tubes) from the colony were given the opportunity to mate with

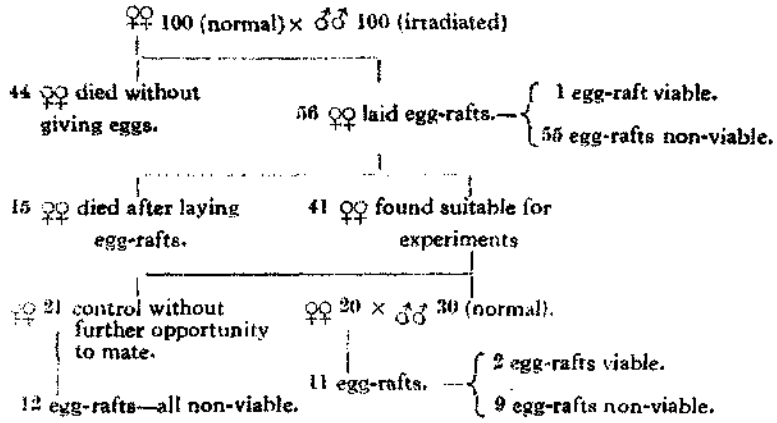
TABLE III.

Details of the comparative daily mortality-rate amongst irradiated and normal male C. fatigans.

Days.		Daily percentage mortality amongst mosquitoes: Irradiated males.	Normal males.
1— 4 days.	...	1·5	2·0
5— 8 days.	...	2·12	0·52
9—12 days.	...	3·19	0·29
13—16 days.	...	11·43	4·2
17—20 days.	...	4·68	0·0
21—24 days.	...	12·5	2·02
25—28 days.	...	13·46	1·1
29—31 days.	...	8·32	0·38

irradiated males (7,700 *r*) by keeping both in the same cage for five days. Then, after feeding on blood, the females were separated in specimen-tubes for egg-laying. The surviving females, which laid embryonated egg-rafts, were divided into two batches of which one batch of females was given the opportunity to mate with normal males. The second batch was held as control without any chance of further matings. Later the females from both the batches were given blood feed and kept separately in specimen-tubes for egg-laying. All the egg-rafts obtained were analysed.

Experiments carried out to determine multiple fertilization are diagramatically represented below :



The data showed that when the females, initially fertilised* with irradiated males, were provided with full opportunity to mate with normal males again, the viability of the rafts produced did not increase significantly.

* Only females which laid embryonated unhatched eggs were used in the subsequent mating so as to avoid any doubts regarding their having been fertilized by irradiated males.

(e) TO DETERMINE THE FREQUENCY OF UNMATED FEMALES LAYING EGG-RAFTS.

Females, singly hatched in specimen-tubes, were transferred to cages without any males and they were given blood feed. The percentage of females taking blood feed varied (65 to 88 per cent) in two replicates. A number of these females gave egg-rafts. Details of the females fed and the number of rafts obtained are given below :

Serial number of experiment.	Number of female *C. fatigans* used for observation.	Number of females which took blood feed.	Number of egg-rafts obtained.	Percentage of unmated females laying eggs.*
1	161	142	28	17·1
2	85	56	23	27·0

*The percentage of rafts obtained was calculated using the total number of females initially used in the experiment.

DISCUSSION.

Comparative efficacy of the *X*-rays and gamma rays in inducing sterility in insects was evaluated with a view to determining the maximum sterility produced with minimum mortality in the mosquito pupae exposed to them. The observations indicated that with gamma rays a dosage of 7,700 r produced almost complete sterility (98 per cent) with minimum mortality-rate (23 per cent) and hence was preferred to *X*-rays.

Experiments mixing irradiated and normal males in different proportions and giving them equal opportunity to mate with unmated females were carried out to estimate the optimum proportion which would give complete reduction. The results illustrated that 9 : 1 : 1 would be the ideal ratio (100 per cent reduction). However, it should be recognised that under field conditions it is impracticable to achieve this proportion. The results also highlighted the fact that 2 : 1 : 1 ratio yielded 38-40 per cent non-viability of egg-rafts which, when projected in the field, would depress the natural populations at its theoretical maximum. Computation of actual proportions of sterile to normal males would be possible only when a practicable and reliable method for determining the natural population in the field is evolved.

The data indicated that the viability of the egg-rafts obtained from females, once mated with sterile males and subsequently given the opportunity to mate with normal males, did not significantly rise. Kitzmiller and Laven (1958), in their experiments with markers, concluded that most probably there was no multiple fertilization. But the slight increase (18 per cent) in the fertility observed in the experiments requires further investigations on this aspect.

The observation on the longevity of irradiated males indicated the adverse effect of heavy dosages of radiation.

The high percentage of egg-rafts from unmated females indicated that the female mosquitoes, if given the opportunity to feed on blood irrespective of fertilization, would lay a batch of eggs. This contingency serves as a caution against the possibility of confusing such rafts (if encountered in nature) with embryonated but unhatched or partially hatched rafts which result from fertilization of the female by sterile males.

SUMMARY AND CONCLUSIONS.

The studies indicated that sterile *C. fatigans* males could be produced by irradiation of the pupae with *X*-rays or gamma rays. These studies also showed that when the mosquito pupae were exposed to varying dosages of irradiation (2,850 r to 30,000 r) and the resultant males given the opportunity to mate with normal females, the viability of eggs produced by them varied.

It was observed that the irradiation of the males or the dosage at which they were radiated did not have any effect on the egg production of the females.

A minimum of 7,700 roentgens direct irradiation with gamma rays was necessary to sterilize males with minimum mortality.

When sterile males were mixed with normal males in different proportions and were given the opportunity to mate with virgin normal females, in the laboratory it was seen that the number of non-viable egg-rafts increased with the increase in the proportion of sterile males. Maximum non-viability of eggs was obtained when the proportion of sterile to normal males to females was 9 : 1 : 1. It was also observed that the 2 : 1 : 1 proportion, which is more practicable, produced 38 to 40 per cent non-viability. It is reasonable to expect sizable reduction in population if this can be achieved in the field.

The results showed that irradiation of pupae affected longevity of the mosquitoes.

The percentage of egg-rafts from unfertilised females ranged from 17 to 27 per cent when they were allowed to feed on blood without mating, indicating the possibility of obtaining such rafts in nature.

ACKNOWLEDGEMENT.

Thanks are due to Dr. B.L. Taneja, Medical Superintendent and Dr. O.P. Bhardwaj, Radiologist, Irwin Hospital, New Delhi, for their help in radiating pupae with *X*-rays.

Grateful acknowledgement is made to Col. R.D. Ayyar, Medical Superintendent, Safdarjung Hospital, New Delhi, for permission to use the cobalt source for irradiation ; to Dr. A K. Bhattacharjee (Radio Therapist) and Dr. A.K. Das (Registrar), Radio Therapy Department of the same hospital, for their help and cooperation.

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A NOTE ON PRELIMINARY FIELD STUDIES OF THE USE OF IRRADIATED MALES FOR REDUCTION OF *C. FATIGANS* WIED. POPULATIONS.

BY

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[September 15, 1962.]

KNIPPLING (1938) was one of the earliest to suggest the possibility of insect control through the use of sexually sterile males. Lindquist (1955) described a field investigation† carried out to explore the possibility of reducing the natural screw-worm population by releasing sterile males on Sanible Island (about 15 square miles area) off the West Coast of Florida. Bushland *et al.* (1955) conducted preliminary field trials at Curacao (a Netherlands possession) in the Caribbean sea off the coast of Venezuela, by releasing sterilised males. The subsequent reduction in the screw-worm population was determined daily by oviposition records on experimental goats located over the island. Baumhover *et al.* (1955) described successful effort at eradication from the same island by releasing sterilized screw-worm males. In 1959 they also observed the effect of releasing large numbers of sterile screw-worms in Florida. Knippling (1960) reviewed the sequence of events of the screw-worm eradication campaign.

Ramakrishnan *et al.* (1962) found that irradiation of male pupae of *C. fatiagns* with gamma rays (at 7,700 *r*) rendered the adults sterile. The results obtained by them also indicated that the proportion of non-viable egg-rafts (embryonated eggs which did not hatch into larvae) increased with a rise in the proportion of sterile males to normal males. Studies were, therefore, undertaken to determine the possibility of the use of the sterile male mosquitoes to achieve reduction in the field in *C. fatigans* population.

The investigations consisted of the following steps :

1. Rearing of large number of mosquitoes in the laboratory.
2. Separation of large number of pupae from the larval cultures and sexing them.

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† Conducted by the personnel of the Laboratory, Kerrville, Texas, under the supervision of Dr. A.H. Baumhover.

3. Transport of the pupae from the laboratory to the source of irradiation and back.
4. Exposure of these pupae to gamma radiation in batches of 1000-1200 pupae.
5. Release of the irradiated sterile male mosquitoes in the experimental village.
6. Collection of data and evaluation of the results.

MATERIAL AND METHODS.

Experimental and comparison villages.—Village Aslatnagar alias Christ Nagar (Map I) of Meerut District, Uttar Pradesh, was selected for the release of irradiated male mosquitoes as this village was found to be almost isolated from the rest of the surrounding villages by at least a mile and had a limited number of breeding places. This village consists of 38—40 huts, with an approximate population of 150. The villagers are mostly agricultural labourers. The land adjoining the village is often flooded during the monsoon season and is rendered barren by deposits of sodium nitrate which results from the seepages of the nearby river. *C. fatigans* was seldom found breeding in these water collections.

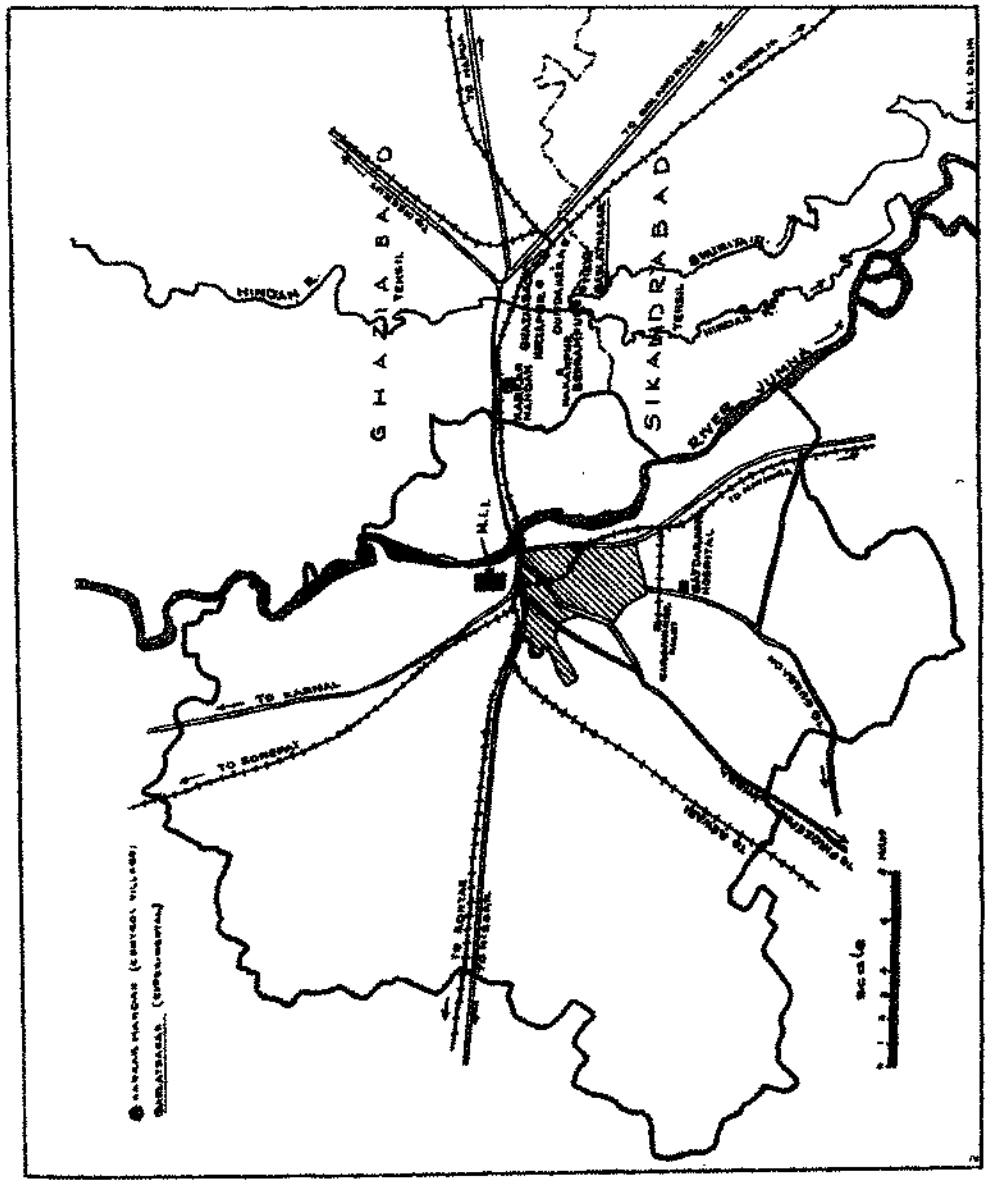
The density of *C. fatigans* mosquitoes in the village was found to be high. These were seen to breed chiefly in water collections near the two tube wells which were the chief source of water supply to the villagers. These wells are provided with hand operated pumps. The resultant water from these hand pumps collected into two small pools where *C. fatigans* breed profusely. The village has only a fair weather road across the fields.

Another village, Karkarmandan (Meerut District, Uttar Pradesh) is situated 8 miles south-west of the experimental village on the Grand Trunk Road (Map I). This was taken as the comparison village which has approximately 150 to 160 dwellings with a population of about 800. There were four breeding places near the water sources which consisted chiefly of bore wells.

The strain of *C. fatigans* used in these studies was cultured in the laboratory on a large scale. The temperature and humidity were maintained at 26° to 28°C. and 70—75 per cent, respectively. Approximately 300 enamel basins (40 cm. diameter) were used for culturing the larvae. In each tray, 5—6 egg-rafts (160—200 eggs per raft) were cultured. Initially the male pupae obtained from these cultures were separated manually by hand picking. But this method was found impracticable when thousands of pupae had to be separated daily. A technique*, using iced water, for easy and rapid separation of pupae from larval cultures was developed.

These pupae were irradiated with gamma rays using the technique and dosage of radiation employed by Ramakrishnan *et al.* (1962 *loc. cit.*). There was high mortality in the irradiated pupae due to repeated handling while separating and transporting them over long distance to the source of radiation and back to laboratory. Efforts to minimise pupal mortality were not successful.

* Details of the technique are under publication separately.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TO BAGHPAT
TO KARNAL
TO SAHARANPUR
TO MEERUT
TO BULANDSHAHR
TO ALIGARH
TO AGRA
TO BHARATPUR
TO KOTA
TO BOMBAY
TO DELHI
TO KANPUR
TO ALLAHABAD
TO CALCUTTA
TO MADRAS
TO BHOPAL
TO NAGPUR
TO JAIPUR
TO AHMEDABAD
TO BANGALORE
TO TRIVANDRUM
TO COCHIN
TO MADURAI
TO GWALIOR
TO JODHPUR
TO BOMBAY
TO DELHI
TO AGRA

EXPERIMENT.

Male mosquitoes, hatched from the irradiated pupae, were transported to the site of release in the evening and released bi-weekly near the breeding places. First release of sterile males was carried out on March 18, 1962, and last release on April 23, 1962.

It was intended to release a large number of sterile male mosquitoes in the village Aslatnagar during the peak period of culicine breeding. But it was only possible to release a total of 24,000 irradiated sterile males during the period of observation.

COLLECTION OF DATA AND EVALUATION OF THE RESULT.

The following criteria were employed to assess the results obtained after the release of the sterile males in the experimental as well as comparison village.

1. The density of mosquitoes was estimated by per man-hour collections. Details regarding the collection of adult mosquitoes are tabulated in Table I.

2. Estimation of the larval density, using dippers (7·5 cm. diameter), in the breeding places was carried out. The larval densities in these two villages are summarised in Table II.

3. Daily collection of 40-50 egg-rafts from the breeding areas of both the villages was made to determine the percentage of rafts having embryonated but unhatched or partially hatched and completely unfertilised eggs. The number of egg-rafts collected and the details regarding their viability are presented in Table III.

From the data (Tables I and II) it was seen that the larval, pupal and adult density in the experimental village showed increase similar to that of the comparison village during the observation period. The observations also revealed (Table III) that loss of fertility amongst the females, determined by the non-viability of the embryonated rafts, showed an increase of 6 per cent in the experimental village over the comparison village. It was observed that in nature completely unfertilised rafts are also obtained from the females. This was recorded to be 1·5 and 2·0 per cent in the experimental and control villages respectively.

DISCUSSION.

Knippling (1955) considered the following factors necessary for studies on the evaluation of the effects of release of sterile males for achieving reduction in the natural population of any insect.

(*a*) An economical method of rearing, sterilizing and releasing millions of insects.

(*b*) Suitability of the insect for easy dispersion.

TABLE I.

Per man-hour density of C. fatigans mosquitoes in the experimental village where irradiated males were released, and in the comparison village.

ASLATNAGAR (Experimental village) (Average of 4 catching stations) :					KARKARMANDAN (Comparison village) (Average of 4 catching stations) :			
Number of weeks.	Total number of mosquitoes collected.	Males.	Females.	Remarks.	Number of weeks.	Total number of mosquitoes collected.	Males.	Females.
1	29·0	13·5	15·5	Pre-release collections	1	Nil	Nil	Nil
2	73·5	33·0	40·5		2	39·5	13·0	26·5
3	266·0	96·5	169·5	Bi-weekly release of gamma irradiated sterile males was started from March 18, 1962.	3	194·0	132·5	127·5
4	373·0	149·0	224·0		4	414·0	165·0	249·0
5	380·0	175·0	205·0		5	280·0	121·0	159·0
6	387·0	210·0	177·0		6	389·5	184·5	205·0
7	380·5	237·0	143·5		7	294·0	139·0	155·0
8	334·0	213·0	121·0		8	264·0	114·0	150·0
9	252·5	153·0	99·5		9	Nil	Nil	Nil

N.B. :—The observations were recorded from March 9, 1962 to May 4, 1962.

TABLE II.

Density of aquatic stages of C. fatigans *(larvae and pupae) in the breeding places in villages Aslatnagar* and Karkarmandan†.*

Date.	Breeding place No. 1 (W.S.)‡ (Average of 2 dips): Larval stages.					Breeding place No. 2 (E.S.)** (Average of 2 dips). Larval stages.				
	I	II	III	IV	Pupae.	I	II	III	IV	Pupae.
Aslatnagar										
March 15, 1962	40·0	70·0	105·0	125·0	55·0	20·0	50·0	75·0	135·0	75·0
March 17, 1962	..	..	..	2·0	..	40·0	25·0	15·0	..	..
March 18, 1962	20·0	17·5	22·5	45·0	5·5	15·0	40·0	20·0	30·0	45·5
March 19, 1962	20·0	27·5	25·0	40·0	16·5	25·0	25·0	25·0	37·5	13·0
March 23, 1962	15·0	30·0	45·0	45·0	25·0	17·5	30·0	30·0	50·0	16·5
March 24, 1962	35·0	40·0	55·0	55·0	13·5	25·0	35·0	45·0	40·0	11·0
March 26, 1962	35·0	40·0	55·0	55·0	18·0	45·0	55·0	60·0	65·0	15·5
March 27, 1962	25·0	35·0	65·0	75·0	45·0	35·0	55·0	75·0	70·0	27·5
March 30, 1962	35·0	45·0	45·0	60·0	12·5	45·0	85·0	95·0	115·0	21·0
March 31, 1962	..	..	..	..	..	135·0	165·0	120·0	145·0	13·5
April 2, 1962	50·0	65·0	85·0	115·0	13·5	←—DRIED—→				
April 3, 1962	30·0	75·0	70·0	80·0	27·5					
April 6, 1962	105·0	155·0	65·0	37·5	16·5	85·0	90·0	110·0	135·0	55·0
April 7, 1962	107·5	125·0	60·0	42·5	27·5	82·5	95·0	122·0	115·0	57·5
April 9, 1962	39·5	97·5	77·5	52·5	27·5	107·5	106·0	104·0	74·0	47·5
April 10, 1962	80·0	70·0	82·5	77·5	27·5	127·5	39·5	115·0	61·5	47·5
April 16, 1962	..	..	..	.	..	200·0	180·0	130·0	130·0	117·5
April 17, 1962	215·0	175·0	110·0	82·5	87·5	..	..	..	..	..
April 21, 1962	70·0	135·0	105·0	162·5	75·0	67·5	82·5	92·5	117·5	90·0
April 23, 1962	90·0	60·0	55·0	20·0	10·0	85·0	87·5	97·5	107·5	65·0
April 24, 1962	50·0	30·0	37·5	50·0	7·0	52·5	55·0	160·0	180·0	22·5
April 25, 1962	15·0	21·0	37·5	7·5	0·0	125·0	175·0	140·0	82·5	53·0
April 28, 1962	55·0	47·5	45·0	50·0	17·5	115·0	190·0	75·0	59·5	36·5
April 30, 1962	20·0	37·5	27·5	26·0	2·5	47·5	67·5	80·0	42·5	37·5
May 1, 1962	45·0	47·5	57·5	67·5	25·0	55·0	57·5	92·5	175·0	90·0
May 2, 1962	12·5	15·0	50·0	60·0	12·0	←—DRIED—→				
May 3, 1962	37·0	25·0	37·5	30·0	15·0					
May 4, 1962	9·5	12·5	17·5	22·5	2·0					
Karkarmandan										
March 17, 1962	..	..	30·0	25·0	..	..	..	5·0	6·0	..
March 20, 1962	45·0	65·0	95·0	80·0	35·0	35·0	25·0	30·0	40·0	12·5
March 28, 1962	35·0	40·0	90·0	115·0	45·0	40·0	50·0	95·0	137·5	32·5
March 29, 1962	45·0	55·0	75·0	115·0	21·5	35·0	55·0	90·0	110·0	16·5
April 4, 1962	22·5	35·0	40·0	55·0	13·0	35·0	40·0	75·0	85·0	32·5
April 5, 1962	22·5	22·5	25·0	12·5	0·0	105·0	135·0	145·0	160·0	35·0
April 11, 1962	12·5	40·0	65·0	92·5	30·0	35·0	47·5	65·0	77·5	40·0
April 12, 1962	37·5	54·0	65·5	92·5	42·5	77·5	72·5	70·0	87·5	55·0
April 18, 1962	65·0	57·5	70·0	95·0	100·0	87·5	97·5	67·5	90·0	122·5
April 19, 1962	47·5	67·5	37·5	42·5	32·5	67·5	70·0	47·5	83·0	48·0
April 27, 1962	20·0	20·0	14·0	7·5	3·5	17·5	17·5	32·5	52·5	17·5
April 28, 1962	27·5	22·5	27·5	27·5	6·5	40·0	37·5	27·5	12·5	12·5

* Experimental village where irradiated sterile males were released.

† Comparison village.

‡ Western side of the village.

** Eastern side of the village.

TABLE III.

Details of collections of egg-rafts of C. fatigans from the breeding places in the experimental village where irradiated steriles male were released and from the comparison village in respect of total number of rafts collected during the week, number of rafts hatched, number of rafts with embryonated, unhatched or partially hatched eggs and completely unfertilised rafts.

ASLATNAGAR (Experimental village) :					KARKARMANDAN (Control village) :				
Number of weeks.	Total number of egg rafts collected during the week.	Number of egg-rafts completely hatched.	Number of egg-rafts unfertilised.	Number of egg rafts where eggs were embryonated but not hatched or only partially hatched.	Number of weeks.	Total number of egg rafts colleted during the week.	Number of egg-rafts completely hatched.	Number of egg-rafts unfertilised.	Number of egg rafts where eggs were embryonated but not hatched or only partially hatched.
1	75	73	Nil	2	1	Nil	Nil	Nil	Nil
2	74	71	3	Nil	2	88	83	5	Nil
3	95	88	2	5	3	83	80	3	Nil
4	92	86	2	4	4	Nil	Nil	Nil	Nil
5	97	93	2	2	5	91	90	Nil	1*
6	130	120	1	9	6	100	100	Nil	Nil
7	129	109	3	17	7	29	29	Nil	Nil
8	168	156	Nil	12	8	Nil	Nil	Nil	Nil
Total :	860	796	13	51	Total :	391	382	8	1

N.B.—The egg-raft collections were made for 4 days in the experimental village and 2 days in the comparison village per week during the observation period.

* Only one egg-raft with mostly embryonated and partially hatched larvae was recorded.

(*c*) The irradiating or other sterilising methods should produce sterility without interfering in the mating behaviour.

(*d*) Females of the species must normally mate only once, or if subsequent matings take place the fertility of the eggs should be low.

The results obtained by Ramakrishnan *et al.* (1962 *loc. cit.*) showed that *C. fatigans* conformed to most of the above requirements. Information provided by them was considered sufficient to venture a projection of those into the field to determine the extent to which sterile *C. fatigans* males could possibly be used to effect reduction in the natural population of this mosquito. They showed that a proportion of 2 : 1 : 1 of sterile to normal males and unmated normal females gave 38—40 per cent reduction in the laboratory. Knippling (*loc. cit.*) also opined that a proportion of 9 : 1 : 1 should be released to effect a reasonable assurance of a downward trend in population of most insects but a low ratio of sterile to fertile insects may depress an insect population near its theoretical maximum.

The non-availability of an easy and practicable method to estimate the natural populations did not permit computation of the results with limited release of sterile males in terms of ratios of sterile to fertile males. Nevertheless the reduction of 6 per cent in the viability of egg-rafts samples collected from the experimental village (Table III), as compared to the comparison village, was encouraging. The increase of unhatched or partially hatched embryonated egg rafts in the experimental village pointed out that with sufficient increase in the number of sterile males, if built up adequately from the beginning of culicine season, would materially affect the production of *C. fatigans* populations in the field and thus result in significant reduction amongst the adults.

Several practical problems regarding different aspects of the investigations mentioned earlier were encountered and were solved as best as cirumstances permitted. These problems were highly instructive. They provided experience in matters of rearing and handling large number of *C. fatigans* in the laboratory, separation of pupae, theirs exing, radiation, transport and release of the sterile males. These factors, however, restricted the release of irradiated males to 24,000 only. Davis *et al.* (1959) emphasised that the handicaps of shortage of suitable equipment and techniques in rearing and irradiating insects, cause serious interference in the progress as well as the success of these projects. This was well illustrated by the present trials.

The problem of public relations in the village, where the sterile mosquitoes were released, proved most serious. This was chiefly due to lack of understanding and cooperation on the part of the villagers. The initiative gained in the earlier stages, in the release of mosquitoes, were practically annulled by refusal of the villagers to allow further release of the sterile males near their village. However, observations in respect of the collection of data were continued for 2 weeks subsequent to the withdrawal of release of the sterile mosquitoes.

SUMMARY AND CONCLUSIONS.

Small scale preliminary field trials on the release of irradiated sterile male *C. fatigans*, with a view to assessing its effect on the field population, was carried out by the Central Institute for Communicable Diseases. The results showed that there was no reduction either in the adult or larval densities. But it was observed that with the increase of sterile males in the *C. fatigans* population, there was an increase in the percentage of embryonated unhatched egg-rafts. This indicated the possibility of the use of irradiated sterile males to effect reduction of *C. fatigans* population in the field.

ACKNOWLEDGEMENT.

The help rendered by the authorities of the Safdarjung Hospital in making available the radiation equipment, specially that of Col. R.D. Ayyar (Medical Superintendent), Dr. A.K. Bhattacharya (Radio Therapist) and Dr. A.K. Das (Registrar), is gratefully acknowledged.

Assistance rendered during the investigations by all the staff members of the Insecticide Laboratory of this Institute is accredited.

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DDT RESISTANCE IN *A. CULICIFACIES* GILES, 1901, AND *A. ANNULARIS* VAN DER WULP, 1884, IN A VILLAGE OF MEERUT DISTRICT, UTTAR PRADESH.

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[September 10, 1962.]

RESISTANCE of insect vectors to insecticides is of great significance to vector control or disease eradication programmes. Hence, the determination of the susceptibility of vector mosquitoes to the insecticides currently in use has become a routine entomological investigation under these programmes. As a result of such tests, Bhatia *et al.* (1958) and Rehman *et al.* (1959) have reported DDT resistance in *A. annularis* and *A. culicifacies*, respectively, in Panchmahals, Gujarat State.

Studies were carried out on the susceptibility to DDT of *A. culicifacies* and *A. annularis* found in significant numbers in the village Arthala (District Meerut, Uttar Pradesh) during July to September, 1962. This village has been sprayed with DDT under the National Malaria Control/Eradication Programme since 1958 with 100 mg. DDT twice a year. With reference to the present observations, it was last sprayed in August, 1962.

Susceptibility tests were carried out with blood-fed female *A. culicifacies* and *A. annularis* collected from village Arthala. Technique, recommended by the World Health Organization (*Tech. Rep. Ser.* No. 80, 1954) for assessing the susceptibility, was used with the following modification in respect of DDT-impregnated papers. The DDT papers supplied by the World Health Organization, with the adult mosquito kit, were used to line the specimen tubes in place of the locally treated filter papers†. Six mosquitoes were exposed in each tube (7·5 cm. × 2·5 cm.) for one hour and mortality amongst the test insects was recorded after 24 hours. In some tests with *A. annularis* the exposure was extended to 24 hours after which mortality was recorded without further holding period. Results of susceptibility tests are recorded in Table I.

The data were analysed by the probit method (Finney, 1952). The results showed that LC_{50} for DDT with *A. culicifacies* was more than 2·0 per cent and

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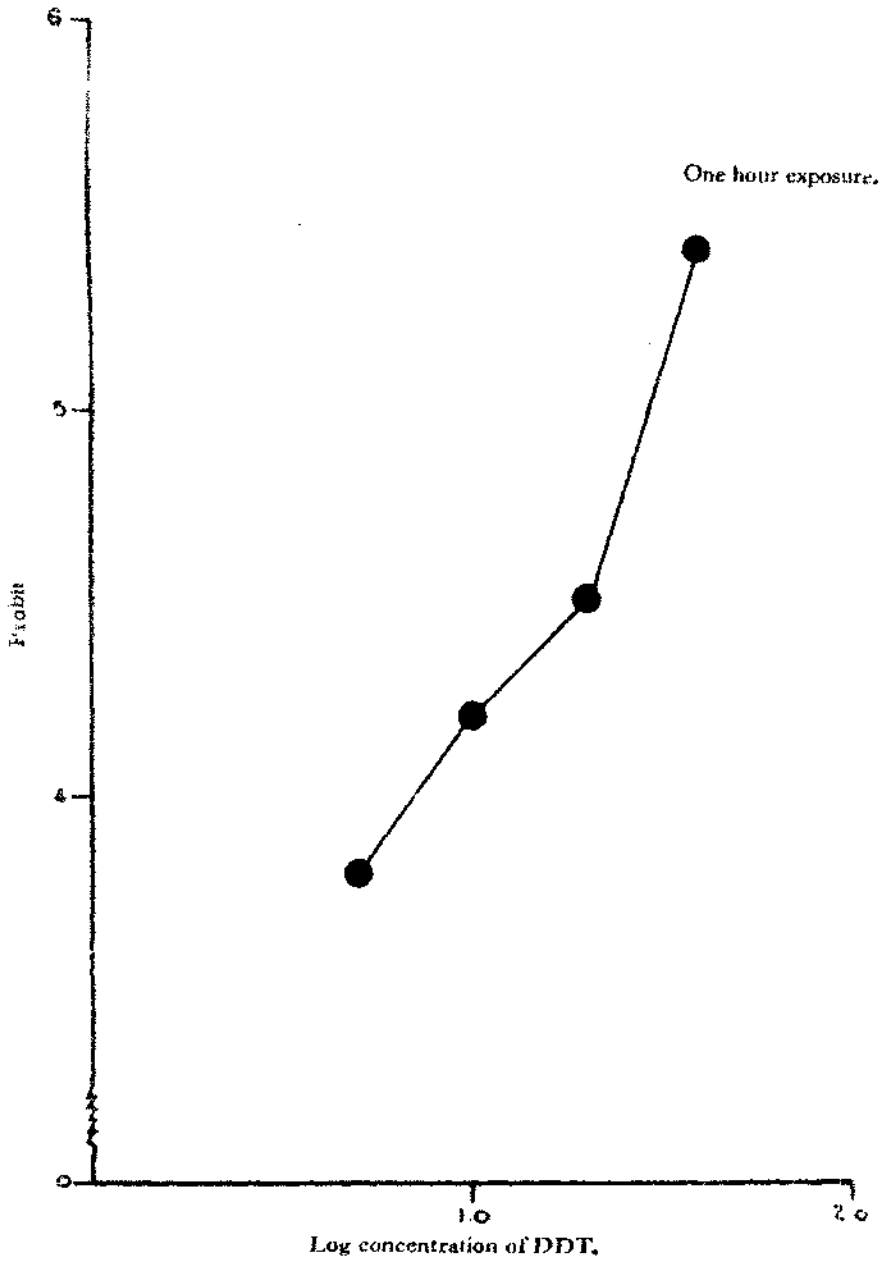
† The W.H.O. adult kit as such could not be used as the number of mosquitoes collected was small.

GRAPH I.

Relationship of probit of mortality and log concentration of DDT in respect of A. culicifacies *from Arthala village (Meerut District, U.P.)*



with *A. annularis* more than 4·0 per cent with one hour exposure. With the latter species, the LC_{50} with 24 hours exposure was observed to be 2·0 per cent. A comparison of these values, with the base-line LC_{50} values observed by Sharma *et al.* (1957), indicated physiological resistance in both the species tested. The non-linear probit/log concentration curve for *A. culicifacies* (Graph 1) supported the findings.

TABLE I.

Details of the susceptibility tests with A. culicifacies *and* A. annularis *females collected from village Arthala (Meerut Ditrict, Uttar Pradesh).*

Species.	Exposure period.	Concentration of DDT (per cent): 4·0		2·0		1·0		0·5		0·25		Control.		LC_{50} per cent; DDT
		n	p	n	p	n	p	n	p	n	p	n	c	
A. culicifacies	1 hour.	100	65·6	70	32·4	40	22·0	28	11·7	..	..	57	7·0	>2·0
A. annularis	1 hour.	24	37·5	..	...	..	..	..	..	..	..	10	10·0	>4·0
	24 hours.	65	73·8	42	50·0	14	28·6	..	..	..	..	11	0·0	2·0

n=number exposed.

p=corrected mortality rate (per cent).

c=control mortality rate (per cent).

Note:—Mortality rates in different concentrations were found consistent in different replicate tests.

SUMMARY.

The susceptibility tests carried out with *A. culicifacies* and *A. annularis*, collected from village Arthala, to DDT showed physiological resistance in both the species tested. It also indicated an extension of the DDT-resistance in *A. culicifacies* reported from the States of Gujarat, Maharashtra and Madhya Pradesh* to this area.

ACKNOWLEDGEMENT.

Assistance rendered by Shrijut Gurdial Singh, Bhagat Ram and Gunder Singh Negi is accredited.

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* Personal communication from Shri S. Nagendra, Entomologist, Public Health Directorate, Madhya Pradesh.

Indian Journal of Malariology, **16**, *4*, *December 1962*.

ECONOMIC AND DEMOGRAPHIC CONSEQUENCES OF MALARIA CONTROL IN CEYLON.

BY

HARALD FREDERIKSEN*, M.D.

[September 20, 1962]

THE death rate in Ceylon declined from 20 to 14 in the year from 1946 to 1947. The conclusions that the introduction of insecticides had drastically reduced mortality and had induced a "population explosion" quite independently of the levels of consumption and production, led to predictions of impoverishment and famine as ultimate consequences of malaria control [United Nations, 1953 ; Population Reference Bureau (Washington), 1955, 1957, 1958 ; Hagen, 1959 ; Wilcox, 1960]. But the hypothetical consequences are refuted by the observed effects of malaria control in Ceylon (Frederiksen, 1960 ; 1961).

Insecticides were first applied on a substantial scale in 1947 and on a full scale in 1948. But mortality had already declined most precipitously in the second half of 1946 (Table I). Hospital and dispensary attendances in the epidemic zone indicate, that clinically diagnosed malaria was first reduced below previous levels in the second half of 1947 (Table II).

TABLE I.

Percentage of population protected against malaria by residual spraying of insecticides and semestral death rates (all causes), Ceylon, 1944-53.*

Year.	SEMI-ANNUAL DEATH RATES†.		Per cent of national population protected.
	First semester.	Second semester.	
1944	21·6	21·1	0
1945	22·6	21·3	3
1946	25·1	15·4	18
1947	15·2	13·2	28
1948	13·5	13·2	40
1949	13·0	12·1	36
1950	12·3	12·9	35
1951	13·1	12·7	36
1952	12·3	11·7	38
1953	10·8	11·1	36

* Reproduced from : Frederiksen, H., (1960), *Malaria control and population pressure in Ceylon*. Public Health Reports, **75**, pp. 865-868.

† At an annual rate.

Less than half of the population had been exposed to malaria and was ultimately protected by the application of insecticides. Comparison of the mortality experience in the malarious and non-malarious areas fails to establish malaria control as the sole or even major cause of the dramatic decline in mortality.

* United States Agency for International Development, American Embassy, New Delhi, India. Deputed to the National Malaria Eradication Programme, India.

Between the second half of 1945 and the second half of 1946, mortality declined 26 per cent in the malarious area and 24 per cent in the unprotected non-malarious area (Tables III, IV).

TABLE II.

Dispensary and hospital attendances clinically diagnosed as malaria in the epidemic zone of Ceylon, 1943-1948 †.*

Year.	SEMI-ANNUAL ATTACK RATE PER 1000 POPULATION‡.	
	First semester.	Second semester.
1943	452	274
1944	206	216
1945	422	980
1946	844	326
1947	230	154
1948	130	88

* Source of basic data: Rajendram, S. and Jayewickreme, S.H., (1951). *Malaria in Ceylon*, Indian Journal of Malariology, **5**, pp. 1-124.

† Upper catchment of the Deduru Oya, which arises in the districts of Kandy and Kurunegala, and the Maha Oya, which arises in the districts of Kandy and Kegalla.

‡ At an annual rate.

TABLE III.

Deaths (all causes) in the malarious and non-malarious areas of Ceylon during the second semesters, 1944-48 and 1953.*

Second semester.	Malarious area†.	Non-malarious area‡.	Ceylon.
1944	26,346	39,842	66,188
1945	29,932	39,466	69,398
1946	22,186	30,058	52,244
1947	18,389	28,310	46,699
1948	17,126	29,175	46,301
1953	17,511	27,667	45,178

* Reproduced from: Frederiksen, H. (1960), *Malaria control and population pressure in Ceylon*, Public Health Reports, **75**, pp. 865-868.

† Districts with spleen rates from 10 to 74 per cent (surveys of 1939 and 1941).

‡ Districts with spleen rates from 0 to 9 per cent (surveys of 1939 and 1941).

A semi-logarithmic plot of the quarterly death rates demonstrates a fairly constant rate of decline between 1938 and 1957, which was interrupted from 1943 to the middle of 1946 (Graph 1). Statistical comparison of the trends of the quarterly death rates from 1938 to 1942 and from the middle of 1946 to 1957 fails to establish a post-war acceleration of the downtrend. The post-war levels of the death rates being a projection of the earlier downtrend, the war-time rise in mortality is the significant event.

TABLE IV.

Death rates (all causes) in the malarious and non-malarious areas of Ceylon during the second semesters, 1944-48 and 1953.*

Second semester.	Malarious area.†	Non-malarious area.‡	Ceylon.
1944**	21·0	19·2	19·9
1945**	22·8	19·1	20·8
1946**	17·6	14·5	15·7
1947**	14·6	13·7	14·0
1948**	13·6	14·1	13·9
1953††	10·9	11·3	11·2

* Reproduced from : Frederiksen, H., (1960), *Malaria control and population pressure in Ceylon*, Public Health Reports, **75**, pp. 865-868.

† Districts with spleen rates from 10 to 74 per cent (surveys of 1939 and 1941).

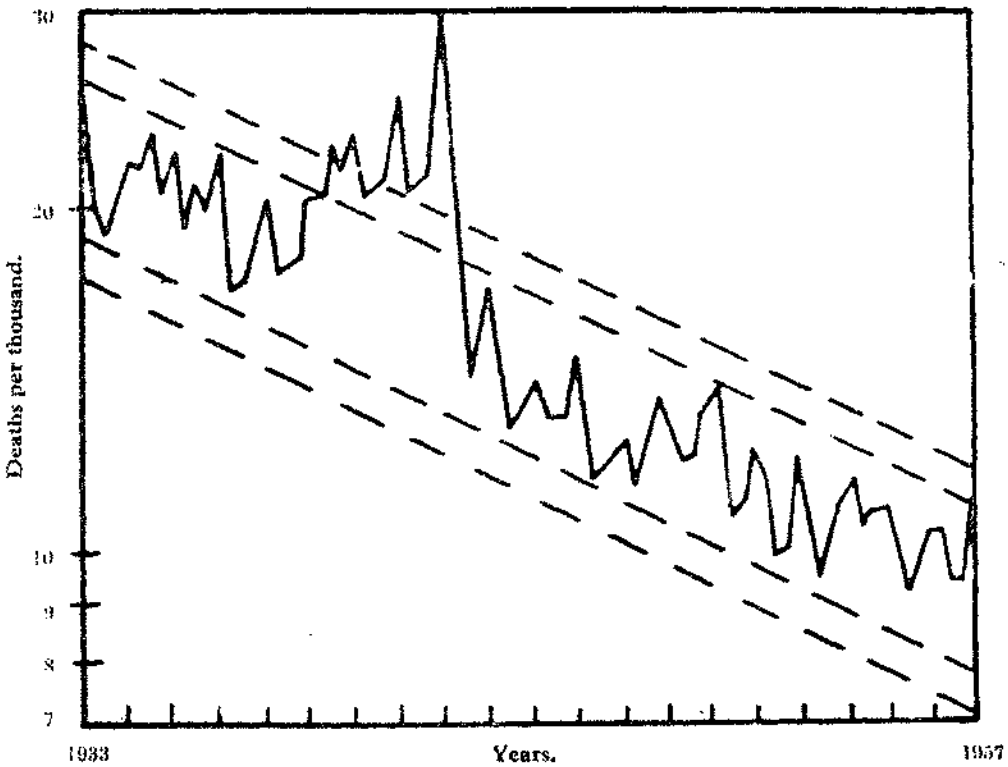
‡ Districts with spleen rates from 0 to 9 per cent (surveys of 1939 and 1941).

** Based on census population of 1946. At an annual rate.

†† Based on census population of 1953. At an annual rate.

GRAPH I.

Death rates by quarter, Ceylon, 1938-1957 (Solid line). Two and three standard deviations from the regression line for the quarterly death rates 1938-1942 (broken lines).*

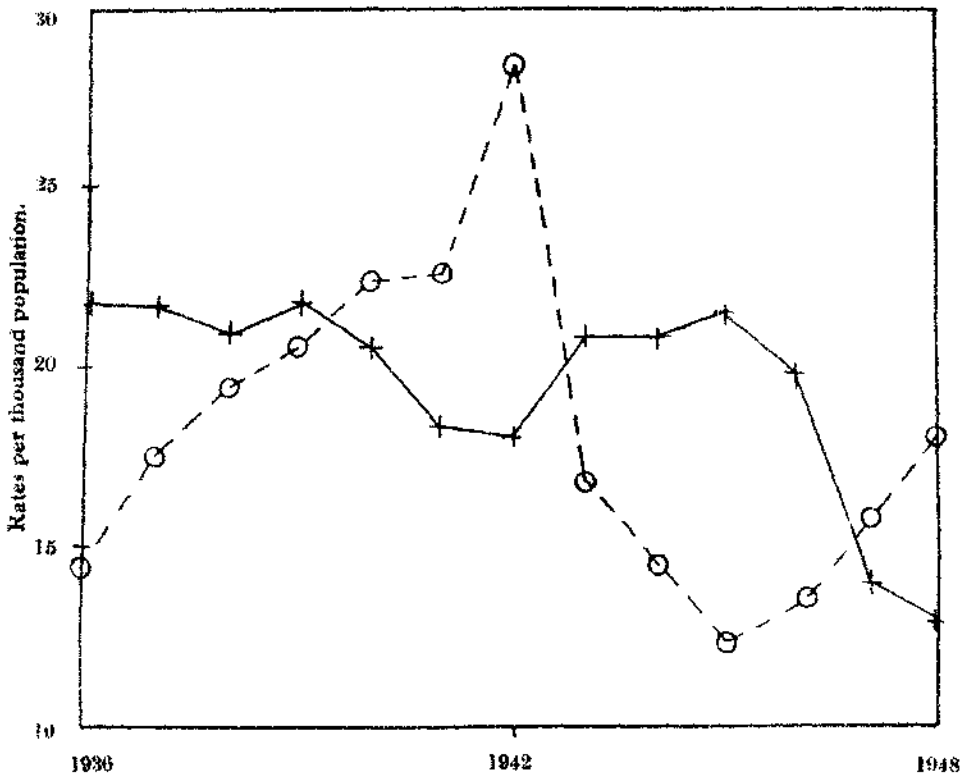


* Sources of basic data : Reports of the Registrar-General on Vital Statistics, Ceylon.

The interruption of the downtrend of mortality between 1943 and the middle of 1946 was associated with curtailment of medical services and imports of foodstuffs (Table V). Although the imports of food were sharply reduced in 1942, effects of the food deficit were first noted in 1943. Paradoxically the restriction of imports resulted in temporary improvement in nutrition in 1942. A simultaneous reduction in the imports of feed was accompanied by a sharp rise in the slaughter of livestock and a further reduction in the death rate in 1942 (Graph 2). The post-war return of the death rates to the earlier downtrend was associated with post-war recovery, notably the rise in food imports to or above pre-war levels (Tables V, VI, VII).

GRAPH 2.

Deaths (solid line) and cattle slaughtered (broken line) per thousand population, Ceylon, 1936-1948.*



* Sources of basic data : Reports of the Registrar-General on Vital Statistics, Ceylon, and Statistical Abstracts of Ceylon.

Infant mortality rates indicate that the downtrend of mortality was first resumed in urban areas, which also seems to be more readily explained by the lifting of war-time restrictions on imports than the application of insecticides which

would have favoured the rural areas (Table VIII). Rural areas, particularly the estates, were also dependent on food imports.

TABLE V.

Mortality rates, governmental health expenditures, and imports of food and other consumption goods, Ceylon, 1938-47.*

Year.	Mortality rate per 1,000†.	Per capita imports at 1938 prices‡:		Per capita governmental health expenditure at 1938 prices (Rupees)§.
		Food (Rupees).	Other consumption goods (Rupees).	
1938	21·0	17·6	7·8	2·2
1939	21·8	18·4	7·5	2·1
1940	20·6	17·2	7·7	2·0
1941	18·3	17·0	6·5	1·9
1942	18·1	13·8	4·5	1·5
1943	20·8	13·7	5·6	1·3
1944	20·8	15·8	6·5	1·5
1945	21·5	15·5	6·9	1·5
1946	19·8	16·6	6·6	1·9
1947	14·0	19·0	7·4	2·2

* Reproduced from: Frederiksen, H., (1961), *Determinants and Consequences of Mortality Trends in Ceylon.* Public Health Reports, **76**, pp. 659-663.

† Provisional corrections in the population estimates and rates for 1941 and subsequent years have been made for underenumeration of children in the 1946 and 1953 censuses.

‡ As computed by the Ministry of Finance.

§ Based on the cost of living index.

TABLE VI.

Deaths (all causes), slaughter of cattle and imports of rice and other grains and flour, Ceylon, 1938-1947.*

Year.	Deaths (all causes).	Slaughter of cattle.	Imports of rice, other grains and flour. Cwt. (thousands).
1938	122,299	112,949	11,250
1939	128,611	122,370	12,673
1940	122 738	134,065	11,764
1941	113,003	140,030	11,765
1942	112,044	175,770	8,679
1943	131,061	106,736	8,237
1944	133,985	93,918	9,090
1945	142,931	82,617	9,270
1946	135,937	92,385	10,033
1947	98,544	111,322	12,327

* Sources of basic data: Statistical Abstracts and Customs Returns of Ceylon.

TABLE VII.

Deaths and imports of rice and wheat flour, Ceylon, by semester, 1945-1947.*

Year.	Imports of rice and wheat flour, Cwt. (thousands):		Deaths:	
	First semester.	Second semester.	First semester.	Second semester.
1945	3,375	4,601	73,533	69,398
1946	4,258	5,067	83,693	52,244
1947	5,498	5,528	51,845	46,699

* Sources of basic data: Monthly Customs Returns, Ceylon, and Reports of the Registrar General on Vital Statistics of Ceylon.

TABLE VIII.

Infant mortality (per 1,000 live births), Ceylon, 1944-1948.*

Year.	Ceylon.	Urban.	Rural estates.
1944	135	156	126
1945	140	153	121
1946	141	129	131
1947	101	122	107
1948	92	105	107

* Sources of basic data: Reports of the Registrar General on Vital Statistics of Ceylon.

The greatest reduction (per cent) in mortality was experienced by the age-group 15 to 54 (Table IX). Thus greater reduction in mortality was experienced by the productive age-group than the younger age-group which, lacking immunity, is the age-group most vulnerable to malaria and therefore would have shown the greatest benefit from malaria control, if malaria in fact had been the major cause of death.

TABLE IX.

The post-war reduction in mortality by age-groups, Ceylon, 1946 to 1953.*

Year.	Number of deaths:			Proportion of deaths:			Death rates per 1000†:			Index of death rates:		
	0-14	15-54	55 and over.	0-14	15-54	55 and over.	0-14	15-54	55 and over.	0-14	15-54	55 and over.
1946	66,950	37,507	31,396	49	28	23	27·0	10·3	60·0	100	100	100
1953	44,878	17,218	26,907	50	19	30	14·0	4·0	43·0	52	39	72
Change	−22,072	−20,289	−4,489	+1	−9	+7	−13·0	−6·3	−17·0	−48	−61	−28

* Sources of basic data: The census of 1946, 1953 and the Statistical Abstracts of Ceylon.

† Based on population at the 1946 and 1953 census.

Whereas, malaria control would not have favoured the productive age-group, greater reduction in mortality was associated with improvement in nutritional status of specific age-groups. Tabulation of the average weights of representative samples of the school population by age and sex indicates that the post-war reduction in the death rates was associated with gains in weight (Table X). The greatest increase (per cent) in weight was associated with the greatest reduction (per cent) in death rate. In the groups of school-age, gain in weight and reduction in mortality increased with age.

TABLE X.

Index of average weights of the school population and death rates by age and sex, Ceylon, 1950.*

Age-group (years).	INDEX IN 1950 (1946=100):			
	Death rates.		Weights†.	
	Male.	Female.	Male.	Female.
5–9	53	52	104	105
10–14	39	38	111	111

* Sources of basic data : Statistical Abstracts of Ceylon and Administration Reports of the Director of Health Services, Ceylon.

† Based on surveys of school population in representative districts of the island.

Although not a result of malaria control, the favouring of the productive age-group by the reduction in mortality suggests consideration of health as a factor in the increased output per capita, particularly if the trend of mortality is accepted as an inverse measure of the health of the surviving population. In fact, mortality rates and the levels of production as well as consumption were inversely associated. Between 1938 and 1957, when the death rates declined to about half the pre-war level, consumption and gross national product (per capita at constant prices) almost doubled (Table XI). Health may be a determinant as well as a consequence of development.

Although malaria control had little demonstrable effect on mortality, there is ample evidence of a dramatic effect on morbidity. Whereas malaria has been recorded as only a relatively unimportant cause of death, reporting has established that, prior to control, malaria was the foremost cause of illness in Ceylon. For every death once attributed to malaria hundreds of cases were diagnosed as malaria at government hospitals and clinics (Table XII). In the course of a year the number of cases was equivalent to half the population of Ceylon. Upon the introduction of insecticides malaria has virtually disappeared. The magnitude of the problem and its disappearance is confirmed by the decline in the island-wide spleen rate (Table XII).

TABLE XI.

Mortality and economic indices of personal consumption, health and other governmental expenditures, remittances abroad, gross capital formation, and gross national product, Ceylon, 1938 and 1947-57.*

Year.	Mortality:		Per capita indices at constant prices (1938)†:					
	Rate per 1,000	Index.	Personal consumption.	Government expenditures:		Remittances abroad	Gross capital formation.	Gross national product.
				Health.	Other.			
1938	21·0	100	100	100	100	100	100	100
1947	14·0	67	146	99	113	46	110	120
1957	10·1	48	185	198	162	49	393	173

* Abstracted from: Frederiksen, H., (1961) *Determinants and Consequences of Mortality Trends in Ceylon.* Public Health Reports, **76**, pp. 659-663.

† Via cost of living index.

TABLE XII.

Malaria mortality and morbidity, Ceylon, 1938-57.*

Year.	All causes mortality rate per 1,000	Malaria mortality rate per 1,000	Malaria proportional mortality rate, per cent.	Malaria morbidity rate per 1,000.	Malaria fatality rate, per cent.	Spleen rate, per cent (March surveys).
1938	21·0	0·8	3·9	353	0·2	21·2
1939	21·8	1·7	7·8	544	0·3	18·2
1940	20·6	1·5	7·5	574	0·3	17·9
1941	18·3	1·2	6·3	535	0·2	18·4
1942	18·1	0·9	4·6	536	0·2	..
1943	20·8	1·1	5·2	349	0·3	..
1944	20·8	0·9	4·2	266	0·3	..
1945	21·5	1·3	6·0	391	0·3	..
1946	19·8	1·9	9·3	413	0·5	..
1947	14·0	0·7	4·6	211	0·3	10·3
1948	13·0	0·5	3·6	109	0·4	5·8
1949	12·4	0·3	2·6	99	0·3	2·7
1950	12·4	0·3	2·0	81	0·3	1·2
1951	12·7	0·2	1·6	58	0·4	0·8
1952	11·8	0·1	1·1	34	0·4	0·6
1953	10·7	0·1	0·8	11	0·8	0·3
1954	10·2	0·1	0·5	4	1·5	..
1955	10·8	0·0	0·3	3	1·1	..
1956	9·8	0·0	0·2	5	0·3	..
1957	10·1	0·0	0·0	3	0·0	..

* Sources of basic data: Administration Reports of the Director of Medical and Sanitary Services, Ceylon; and Visvalingam, T. (1962) *Status of the Malaria Eradication Programme, Ceylon, 1961*, mimeographed document, Colombo.

The area, until recently most malarious, was least populated. Largely as a result of malaria, three-fifths of the population had concentrated in the fifth of the island that was only little or rarely affected by malaria (Table XIII, Map 1). The control of malaria now permits the resettlement of the major part of the island where once the ancient civilization had centered. In fact, malaria control

has been associated with a reversal in the direction of internal migration and an immediate increase in the magnitude and rate of agricultural development in the area recently freed of malaria (Tables XIV, XV ; Graphs 3, 4).

TABLE XIII.

*Population, area, and population density of districts of Ceylon grouped by the endemicity of malaria in the districts.**

Endemicity malaria.	Spleen rates† (per cent).	POPULATION‡ :		AREA :		Population density per square mile.
		Number.	Per cent.	Square miles.	Per cent.	
Not endemic	0-9	4,142,889	62	5,113	20	810
Moderately endemic	10-24	1,207,569	18	5,271	21	229
Highly endemic	25-49	994,495	15	8,460	33	118
Hyperendemic	50-74	312,466	5	6,489	26	48

* Reproduced from : Frederiksen, H. (1960), *Malaria control and population pressure in Ceylon*, Public Health Reports, **75**, pp. 865-868.

† Average of surveys in 1939 and 1941.

‡ 1946 Census.

TABLE XIV.

Excess of immigrants over emigrants enumerated at the census of 1946 and 1953 in the districts of Ceylon grouped by the former endemicity of malaria.*

Former endemicity of malaria.	SPLEEN RATES† IN 1939 AND 1941 (PERCENT).	IMMIGRANTS MINUS EMIGRANTS ENUMERATED AT THE CENSUS OF 1946 AND 1953‡ :		
	Average.	1946.	1953.	Change.
Not endemic	6	102,186	75,516	—86,670
Moderately endemic	17	54,481	37,871	—16,610
Highly endemic	39	102,674	160,562	57,888
Hyperendemic	65	57,374	108,384	51,010

* Sources of basic data : Ceylon, Department of Census and Statistics and Reports of the Government of Ceylon submitted to the World Health Organization Malaria Conferences at Bangkok in 1953 and Taipei in 1954.

† Based on surveys of the school population in 1939 and 1941.

‡ Derived from the enumeration of the population by district of residence and district of birth at the census of 1946 and 1953.

TABLE XV.

Land allotments and acres developed in the formerly malarious area, Ceylon, 1938-1957, †.*

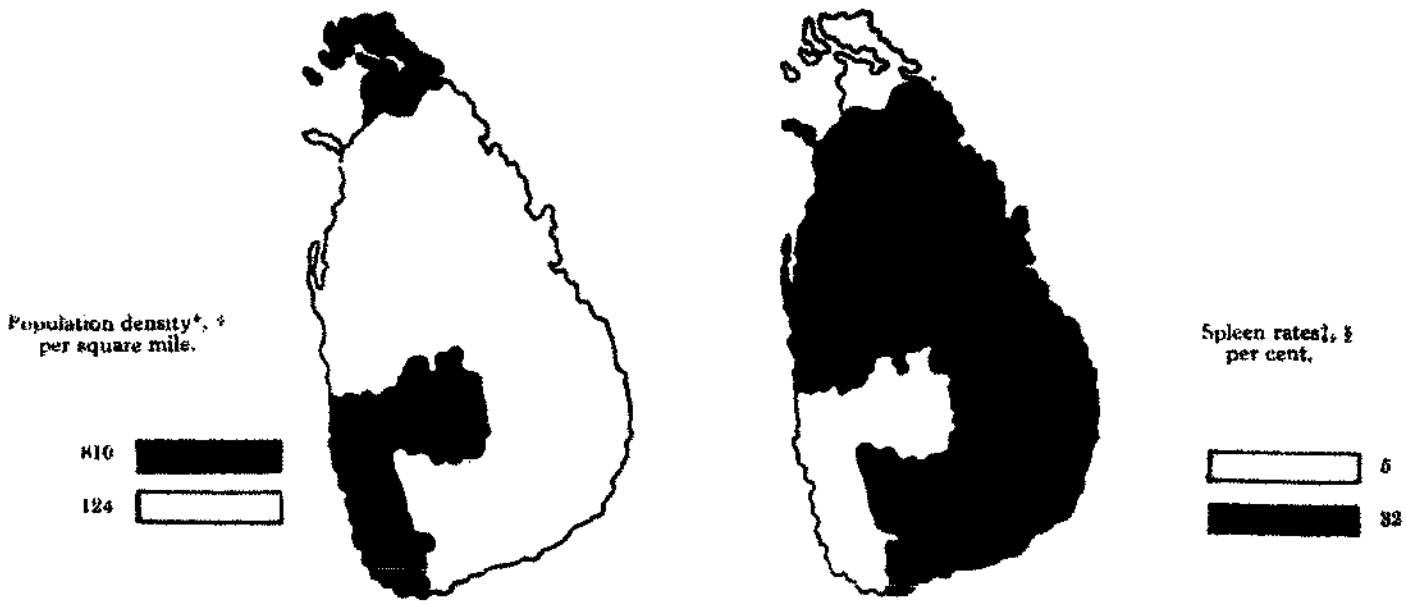
Year.	Land allotments (cumulative totals).	Acres developed (cumulative totals).
1938	1,073	6,861
1947	2,977	21,252
1957	26,329	146,855

* Source of basic data : Land Development Department, Ceylon.

† Colonization schemes only.

MAP 1.

The reciprocal distribution of population and malaria in Ceylon prior to malaria control.



* Source of basic data : Department of Census and Statistics, Census of Ceylon, 1946.

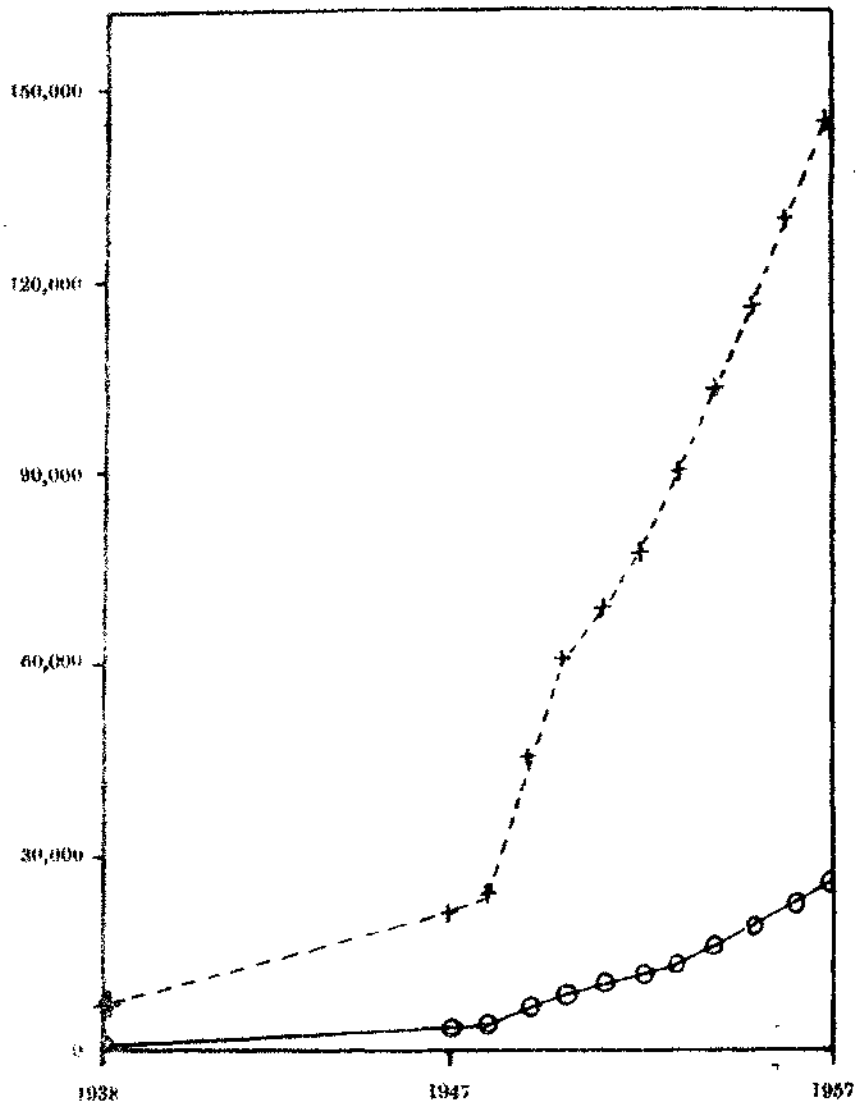
† Average density of population in districts grouped by the endemicity of malaria in the districts.

‡ Source of basic data : Reports of the Government of Ceylon submitted to the World Health Organization Malaria Conference at Bangkok, 1953 and Taipei, 1954.

§ Spleen surveys 1939 and 1941 ; average spleen rates grouping districts with rates of less than 10 and 10 or more per cent weighted by the population of the districts.

GRAPH 3.

Development of the formerly malarious area: Land allotments (solid line) and acres developed (broken line), Ceylon, 1938-1957 *, †, ‡.



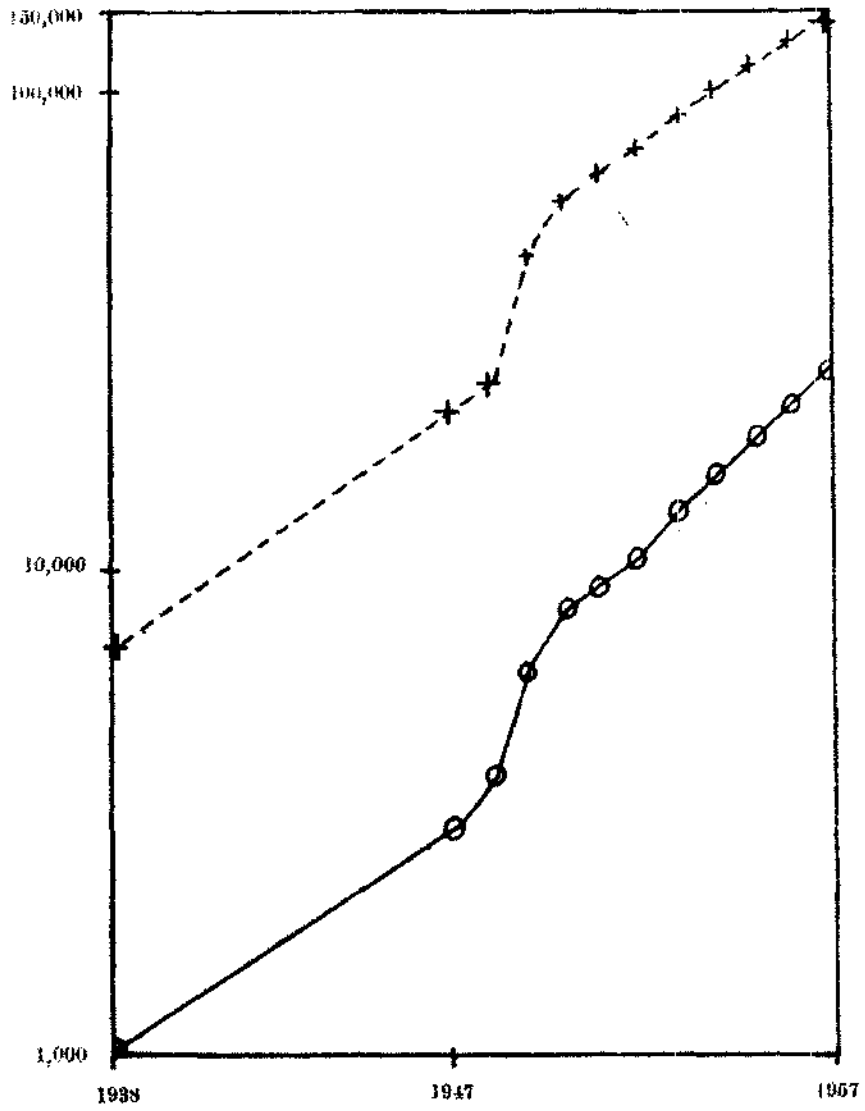
* Source of basic data: Land Development Department, Ceylon.

† Cumulative totals.

‡ Colonization schemes only.

GRAPH 4.

Rate of development of the formerly malarious area : Land allotments (solid line) and acres developed (broken line), Ceylon, 1938-1957 *, †, ‡.



* Source of basic data : Land Development Department, Ceylon.
† Cumulative totals.
‡ Colonization schemes only.

Acreage under cultivation increased from 927,078 acres in 1946 to 1,411,700 acres in 1960 and rice production increased from 246,000 tons in 1946 to 630,000 tons in 1961 as a result of malaria control (Visvalingam, 1961).

Thus, the positive contribution of malaria control to the development of Ceylon is indicated by the net effect on economic and demographic factors in development. Malaria having been the major cause of disease but not of death, malaria control has increased the quality rather than the quantity of human resources. Moreover, malaria control has removed, what had been an insurmountable barrier to the development of the greater part of Ceylon. No longer crowded into a corner of the island by malaria, the people of Ceylon will not suffer impoverishment or famine as a consequence of malaria control. The net effect of malaria control is a reduction in population pressure.

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OFFICIAL HISTORY OF THE INDIAN ARMED FORCES IN THE SECOND WORLD WAR 1939-45*.

MEDICAL SERVICES :

PREVENTIVE MEDICINE (NUTRITION, MALARIA CONTROL AND PREVENTION OF DISEASES).

PART II. MALARIA CONTROL

By

Brigadier M. K. AFRIDI, C.B.E., M.D.

CHAPTER VIII*.

ANTI-MALARIA CAMPAIGN IN 1942

PRIOR to 1941, the Indian Army was only partially mobilised, its unit raising programme being just sufficient for its allotted role of (*i*) providing forces for active service in the Middle East ; (*ii*) supplying garrison troops for Malaya and Burma ; (*iii*) protecting the north-western border of India ; and (*iv*) maintaining internal security in the country.

After the entry of Japan into the war and the invasion of Malaya and Burma the situation changed completely, as these events foreshadowed a direct threat to India on its north-eastern border. The entire resources of the country were then mobilised, and the unit raising programme was enlarged and accelerated. The fall of Rangoon accentuated the urgency of the situation still further as land communication had to be established immediately with the Army-in-Burma for supplies and for its withdrawal into India.

The choice lay between two possible land routes. One, a metalled road from Dimapur railway station to the Manipur Hill State in Assam, beyond which it continued as a mountain trail to Tamu, from where a fair-weather road ran through the Kabaw Valley to Kalewa on the river Chindwin. The second was a jungle track which began at Ledo in the north-eastern corner of Assam and after traversing successive mountain ranges and heavily wooded valleys, ended at Mogaung, a railway station in North Burma near Myitkyina.

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—EDITOR

A survey party reconnoitred these two routes and submitted its report in February 1942, which contained *inter alia* the following proposals :—

(i) the road from Dimapur (later designated as Manipur Road) to Kalewa be developed immediately for securing contact with Burma Army and the civilian refugees withdrawing from central Burma ;

(ii) Ledo be converted into a reception camp for troops and civilian refugees withdrawing from north-eastern Burma along the jungle track ; and

(iii) in view of the presence of malaria in a virulent form throughout this region, an effective organisation be provided for comprehensive control measures against the disease.

These proposals were accepted and work was started on 1 March 1942 on clearing sites for a railhead base at Manipur Road, a reception camp at Ledo and an advance base at Imphal. Simultaneously, a large labour force was mobilised for widening the existing stretch of metalled road and for extending it beyond Palel in Manipur State.

Towards the middle of April, the civilian refugees from Burma reached the border and continued to cross into India for the next two months. They were evacuated by road to Manipur Road and thence by a single line metre gauge railway to various parts of India. Partly to relieve the traffic pressure on the railway and partly for other reasons, most of the Burma Army was kept back in Imphal region for some time after its arrival there early in May.

In the meantime, the Line of Communication and the base camps were gradually taking shape. By mid-June, however, the monsoon came down in full force causing multiple land slides which blocked the road from Manipur Road to Imphal at many places. Fortunately, most of the civilian refugees had by that time been evacuated and supplies to the forward military formations could be managed by motor transport relays across the road gaps. In July, the rains washed away an important railway bridge, adding further to the transportation difficulties.

In September, the rains slackened appreciably and direct road communication to Imphal was re-established, but the railway traffic remained dislocated for another two months.

In October and November, the transportation system was particularly burdened by the large evacuation of sick to India and the flow of reinforcements back to Assam. This state of affairs continued till the end of the malaria season in December when a precipitous fall in sickness markedly relieved the situation.

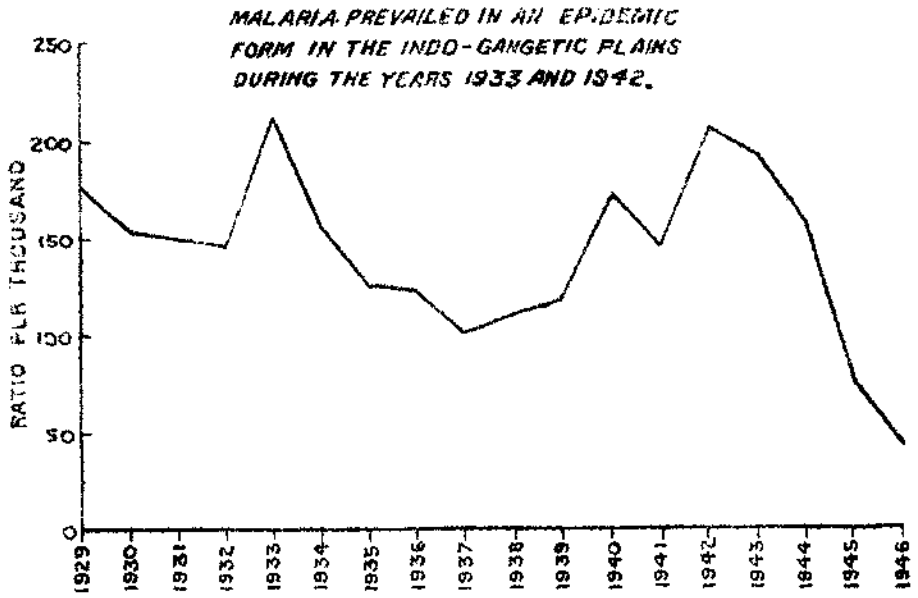
While developments on the Assam-Burma border proceeded along the lines indicated above, troops in East-Bengal remained static and were moved forward to Chittagong and Cox's Bazar early in 1943 in preparation for a drive into the Arakan. Conditions on this sector did not change materially during the year under review and are, therefore, described in Chapter IX.

ANTI-MALARIA ORGANISATION IN INDIA.

Prior to the outbreak of the war in September 1939, the Army-in-India was accommodated in permanent cantonments. Except during periodic operational activities on the North-West Frontier, malaria control measures were thus restricted to certain well-defined stations. These measures, though nominally under a senior medical officer, the *ex officio* cantonment health officer, were in actual practice conducted by anti-malaria medical officers, most of whom had received previous training at the Malaria Institute of India. The requisite funds for malaria control were provided from the annual allotments placed at the disposal of each station commander separately for recurrent and non-recurrent expenditure.

Despite certain inherent defects in this organisation, which will be referred to presently, it nevertheless produced satisfactory results in that the malaria rates in the army remained at a satisfactory level in most of the years except in epidemic years, *vide* Table I.

MALARIA INCIDENCE IN INDIAN ARMY



At the outbreak of war, however, many of the trained anti-malaria officers were, by virtue of their seniority in service, selected for appointments in field medical units in higher ranks. The newly appointed officers, who took over, besides being ill-trained and inexperienced, were equally liable to frequent transfers to the field medical units. The standard of malaria control in cantonments, as a consequence, deteriorated, resulting in an ominous rise in malaria rates in 1941.

TABLE I.

Incidence (rate per 1,000) of malaria—Viceroy's Commissioned Officers and Indian Other Ranks—India Command.

1929 to 1946.

Year	Malaria rate	Year	Malaria rate	Year	Malaria rate
1929	175·3	1935	125·9	1941	144·6
1930	153·4	1936	123·1	1942*	206·0
1931	149·4	1937	97·9	1943	192·5
1932	145·1	1938	109·6	1944	159·5
1933*	212·2	1939	118·3	1945	76·1
1934	156·5	1940	173·2	1946	43·7

*Malaria prevailed in an epidemic form in the Indo-Gangetic plains during these years.

Lieutenant-Colonel (now Major-General, Sir) Gordon Covell, Director, Malaria Institute of India, was thereupon called in by the General Headquarters (India) to review the anti-malaria organisation in the army and to prepare a suitable scheme in the light of the changed situation. In his report, Sir Gordon Covell emphasised the following defects in the existing organisation :—

(*i*) Owing to frequent transfers of anti-malaria officers, there had been a serious lack of continuity in the supervision of anti-malaria measures, more particularly since the outbreak of war. To eliminate such transfers, he recommended the formation of a cadre of civilian malaria medical officers and assistants.

(*ii*) The allotment of funds for anti-malaria measures needed immediate revision as the prevailing scales were insufficient for securing stores and equipment in the quantities required.

(*iii*) Separate provision was necessary for motor transport and for an adequate checking staff in each station in the absence of which anti-malaria measures had failed to attain the requisite level of efficiency.

(*iv*) As a rule, the civilian authorities had neither the funds nor the organisation to carry out malaria control measures in towns and villages adjacent to the cantonments. He advocated that such control be taken up by the army as a military commitment.

(*v*) For the technical direction and co-ordination of malaria control in cantonments, he recommended the appointment of an adviser in malariology at the General Headquarters (India).

Sir Gordon Covell's recommendations were accepted *in toto* in December 1941, and Lieutenant-Colonel (afterwards Brigadier) M.K. Afridi, IMS, who had held the post of malariologist in Eritrea and subsequently in Iraq, became adviser in malariology, General Headquarters (India), Delhi, on 12 January, 1942.

Tasks needing immediate attention at the time were, first, to implement the revised scheme for malaria control in cantonments and, second, to accelerate the mobilisation of anti-malaria units for malaria control in operational areas. Activities in these two directions will now be traced separately.

MALARIA CONTROL IN CANTONMENTS

Organisation.—In February 1942, the salient features of the revised anti-malaria organisation in cantonments were summarised in a circular letter, and command headquarters were asked to classify the existing military stations as large, medium and small, depending upon the size of the garrison and the degree of the malariousness of each. A scale of supervisory malaria staff, equipment and labour was laid down for each of the three categories of stations. The issue of anti-malaria stores, such as oil, Paris green and pyrethrum insecticide, was authorised on an 'as required' scale while expenditure on the remaining items was provided out of a lump sum sanctioned for each command on a generous scale.

For supervisory staff, civilian medical and science graduates were recruited in batches and given intensive courses of instruction of four weeks' duration at the Malaria Institute of India. Those declared successful at the end of each course were then allotted to commands, which posted them to the different stations. To cope with the above training programme, the Malaria Institute of India organised continuous courses except during the hot months of May and June. Simultaneously, the institute trained a large number of Indian Hospital Corps (IHC) personnel, in elementary entomological technique, who were then posted to the various stations as insect collectors in the checking organisation.

Arrangements were also made to bring all classified stations up to the revised scale of anti-malaria equipment with supplies procured direct from the manufacturers and the medical store depots. As the stocks of equipment then available in India were low, local manufacture of certain urgent items was taken up under the joint direction of the Malaria Institute of India and the General Headquarters medical staff. This enterprise, the details of which appear later, was not an unqualified success, but it certainly helped to tide over the immediate and difficult situation.

Anti-Malaria Staff Officers.—By April 1942, it became clear that with the progressive expansion of cantonments and malaria control programmes, adequate technical direction from the General Headquarters was getting increasingly difficult, especially as the anti-malaria commitments in operational areas had at the same time enlarged considerably. In May 1942, therefore, it was decided to decentralise supervision and to create the post of a Deputy Assistant Director of Hygiene (anti-malaria) [DADH (AM)] in each command headquarters. The officers for these appointments were selected from amongst trained medical officers with previous experience of malaria control in the army or those who had acquired the necessary knowledge during medical practice in tea plantations or industrial firms

in malarious areas. Apart from implementing the new malaria scheme in cantonments, these officers were required to undertake rapid malaria surveys for selecting sites for new camps.

Towards the end of the year, however, even the above scale of malaria staff officers proved insufficient and had to be augmented to deal with the expanded anti-malaria work in cantonments, training camps and operational areas. A new set-up was accordingly sanctioned in January 1943 and is discussed in Chapter IX.

Malaria Problems.—The problem of malaria in each cantonment varied with its location. Most of the major military stations were, however, situated in the Indo-Gangetic plains, Central and Peninsular India, where *Anopheles culicifacies* is the main carrier. This species breeds in rain-water collections, in pools formed by the flooding of the rivers and in seepages and water collections arising from irrigation canals. Malaria in these localities prevails during and immediately after the rainy months.

In the Indo-Gangetic plains this species is especially liable to give rise to periodic fulminant regional epidemics at intervals of seven or eight years. Since the last epidemic had occurred in 1933 (*vide* Table I), it was felt that the immunological factor was favourable for its recurrence if rainfall in 1942 was abnormally heavy which actually turned out to be the case.

Other problems arose with the expansion of the existing cantonments to accommodate additional recruiting and training establishments which entailed a corresponding increase in the controlled areas. As a consequence, anti-malaria drains had to be extended beyond the previous limits to protect the new constructions ; and villages, which had hitherto been at a safe distance, had to be included in the anti-malaria control scheme.

By August 1942, however, accommodation even in the expanded cantonments proved insufficient, necessitating new camps and installations which had to be laid out in different parts of India. In siting such camps the local anti-malaria staff officers were invariably consulted, and they made every effort to press for the selection of the least malarious areas. They were not, however, always successful, as a compromise had to be effected between the anti-malaria view-point and the engineering and military training interests. Nevertheless, such consultations gave valuable advance information about the location of the camps. It was possible, therefore, to arrange an adequate anti-malaria organisation before the camp was occupied and to prepare detailed anti-malaria drainage schemes, which could be included as a part of the camp constructional project.

Anti-larval and anti-adult mosquito measures in these camps were entrusted to trained malaria inspectors, who were recruited as a separate cadre and who worked under the supervision of the nearest anti-malaria medical officer or anti-malaria assistant.

Methods of Malaria Control.—The measures of control employed in the cantonments were on the usual lines, with chief emphasis on anti-larval measures

through oiling, application of Paris green and minor drainage. These measures were applied to all water collections, both inside the cantonment limits and in the area half a mile around. As an additional measure of safety, barracks and hutments were sprayed with pyrethrum insecticide solution on alternate days in the beginning and once a day in the peak period of malaria transmission. Villages falling within half a mile of the cantonment limits were included in this insecticide spraying programme.

Particular action was, however, necessary to promote a uniform policy of anti-malaria measures and a routine which would ensure a systematic supervision of these measures at each step. With this object in view, a pamphlet was compiled and issued in March 1942, which contained detailed instructions on the division of cantonments into supervisory circles, the proper indications for using different types of sprayers, the relative value of anti-larval and anti-adult mosquito measures in the different phases of the malaria season and a standardised method of assessing and recording results of control measures.

As a result of these efforts, anti-malaria measures in cantonments made good progress, and towards the end of the year the standard of supervision had reached a satisfactory level of efficiency. The following points, however, required to be repeatedly stressed by the anti-malaria staff officers : (*i*) the necessity for controlling the adjoining civilian areas and villages for the protection of army personnel ; (*ii*) the utmost need for continuous field training of insect collectors, supervisors, mates and labourers by the anti-malaria medical officers and assistants ; (*iii*) the importance of employing the latest types of sprayers and maintaining them in a proper state of repair.

A notable feature of the year under review was that most of the cantonments in the Indo-Gangetic plains experienced a regional epidemic of malaria. This, as already stated, had been foreseen, and the malaria officers were forewarned to institute intensive daily spraying of barracks, hutments, villages and adjacent civil areas with pyrethrum insecticide as soon as malaria rates displayed an upward trend. As a result of this timely action, the malaria rates in 1942 remained within reasonable bounds, comparing favourably with the figures for the previous epidemic year, 1933. These results might have been even more striking but for the fact that the recruits were sent on leave in unusually large batches after the initial training course when they contracted the disease in their villages.

MALARIA CONTROL IN OPERATIONAL AREAS

Organisation.—During March 1942, when the Line of Communication from Manipur Road to Imphal was being developed, malaria control in the operational areas was directed by the hygiene staff officers. Every unit was made responsible for anti-malaria measures within its own camp areas, while breeding places in the surrounding areas were treated by the anti-malaria units, or, in their absence, by the field hygiene section.

In April 1942, the civilian authorities decided to erect temporary camps for refugees from Burma and appointed Dr. D.K. Vishwanathan, an experienced malariologist, to organise anti-malaria measures. Since the military installations and the civilian camps were situated along the same route and were frequently in close proximity to each other, the direction of the military malaria control was entrusted to the same officer so as to secure a complete integration of anti-malaria effort. Dr. Vishwanathan was accordingly posted as Deputy Assistant Director of Hygiene (anti-malaria) in Headquarters Sub-Area in addition to his civilian appointment.

By the end of June 1942, however, when the civilian camps ceased to function, Dr. Vishwanathan was replaced by a military officer. In making this appointment, the complication was that since the operational areas in Assam were, at the time, under the administrative control of the Eastern Command, the Deputy Assistant Director of Hygiene, Sub-Area, would normally have had to work under that command. In the existing circumstances, however, it was essential for the General Headquarters (India) to exercise direct control over all operational areas. For technical direction, therefore, the Deputy Assistant Director of Hygiene (anti-malaria) of Sub-area and Deputy Assistant Director of Hygiene (anti-malaria) of Eastern Command were both placed under the General Headquarters (India), and the sphere of activity of the latter was restricted to East Bengal and Arakan.

Another operational area demanding attention was Ceylon, where a large body of troops had been stationed at focal points scattered all over the island. Here again a Deputy Assistant Director of Hygiene (anti-malaria) was appointed in May 1942 for the supervision and direction of anti-malaria measures in Ceylon and the Maldive Archipelago.

Establishment of Indian Anti-Malaria Unit.—The Indian anti-malaria unit had to undergo considerable evolution before it could achieve its position as the basic unit for the control of malaria in the operational areas. Thus, the first seven units raised in 1940 and 1941 were purely investigatory in character. They had a small establishment, sufficient only to undertake malaria surveys and to guide the field hygiene sections in carrying out actual control measures in the field. Of the seven units, two were raised in time for the campaign in the Sudan, two were sent to Malaya, two to Iraq and one to Burma. The first two units raised were staffed by the personnel supplied by the Malaria Institute of India.

From experience in the Sudan Campaign it was clear that the field hygiene section was unable to shoulder the responsibility for malaria control measures without considerable increase in its establishment. It was further observed that although the anti-malaria units proved extremely useful, their utility was unnecessarily restricted and could, with advantage, be augmented if they took charge of the malaria control measures as well. A decision had, therefore, to be taken on increasing the establishment of either the field hygiene section or the anti-malaria unit. The point in favour of the latter was that if responsibility both for investigation and control was assigned to one unit it would lead to speedier action and greater efficiency than if it was delegated to two separate units.

The above position was accepted by the General Headquarters (India), and the establishment of the Indian anti-malaria unit was accordingly revised in November 1941. The units raised thereafter were then subjected to close observation under actual field conditions throughout the year. As a result, certain minor additions* were made, and in April 1943 the unit achieved its final shape as a compact and self-sufficient field unit with a personnel strength of seventy-four. It had a headquarters section for carrying out malaria surveys and four sections for instituting actual control measures through local labour employed on an 'as required' scale. Each unit had, however, on its establishment 25 enrolled labourers for essential anti-malaria work in areas where labour was not immediately available. The quota of motor transport for the unit was sufficient for routine duties, but for extensive anti-malaria-schemes it received, as a rule readily, additional allotment from the local vehicle pool.

The technical equipment in the headquarters section contained all the items necessary for a malaria field laboratory for entomological and epidemiological surveys, whereas the control sections were fully equipped to undertake both anti-larval and adulticide measures, and were provided with knapsack sprayers for oiling, blowers for applying Paris green and hand-operated as well as power-driven atomisers for dispersing insecticides.

Raising Programme of Indian Anti-Malaria Unit.—In January 1942, only six anti-malaria units were available in the country, and even these had been mobilised expressly for service in Iraq and Iran. On representation, however, two of these units were allowed to be diverted to Assam, the first arriving at Manipur Road on 1 March 1942, and the second at Ledo by the end of that month.

In the meantime, the raising programme of the units was re-examined in the light of the changed operational situation. As a result, not only was a considerable increase sanctioned in the number of units to be raised but it was also decided to take all possible steps to accelerate their mobilisation. With the latter end in view, a raising centre was established at Delhi, where the malaria officers under training at the Malaria Institute of India could take over the units immediately after the course, and where the units could be equipped readily from the local medical store depot. The centre in Delhi had, however, the drawback that it lacked facilities for training in general military duties. To counteract this disability it was arranged that, after arrival in the operational areas, the key personnel of all newly raised anti-malaria units were attached to the nearest functioning anti-malaria unit for a short course of instruction in routine military administration. An advantage incidental to this arrangement was that they could, at the same time, observe in actual operation the methods of malaria control which had been evolved locally to meet the particular malaria problem of the region concerned.

In the implementation of the revised raising programme, another problem was to secure the necessary allocation of manpower which was becoming increasingly short owing to the simultaneous mobilisation of a large number of field units.

* *See* Apppendix XII.

In recognition of the importance of malaria in the Eastern Theatre, however, the anti-malaria unit was given a relatively high priority and was placed next to the field ambulance for manpower allotment.

Location of Indian Anti-Malaria Units.—Apart from the two units, posted at Manipur Road and Ledo in March 1942, a total of eleven anti-malaria units was mobilised and posted in the operational areas during 1942. These units were allotted on a regional basis, and only in rare cases were they allowed to move out with a formation. Allotments were necessarily based on the urgency of requirements, but most of the units were concentrated in Eastern and Southern India. The following statement shows the location of units at the end of 1942 :—

Location.	Number of units.
Eastern India	
Assam	6
East Bengal	2
Ranchi	1
Southern India	2
Ceylon	2
Total	13

Malaria Problems.—For a proper appreciation of the nature of malaria problems in the operational areas it would be convenient, first, to describe the varying epidemiological factors in different regions, and, second, to trace the influence of tactical conditions on the incidence of malaria. By presenting the subject in this manner, it is hoped that the resultant overall picture will be clearer than if an attempt was made to discuss separately the varying malaria conditions in different localities.

Epidemiology of Malaria in different Regions.—With the notable exception of certain limited non-malarious sectors, the area traversed by Line of Communication in Assam was almost uniformly hyperendemic. In most of the localities, spleen rates determined in February 1942, before the entry of troops into the area, ranged between 60 and 90 per cent. These hyperendemic conditions were, however, confined to foot-hill terrain where innumerable seepages, springs and the small, slow, tortuous and grassy streams exposed to sunlight provide the selective breeding places for the main regional carrier, *Anopheles minimus*. On the other hand, stagnant water in borrowpits, tanks, swamps and ricefields is not attractive to this anopheline, unless such water has an admixture of permanent seepages. As a consequence, wide alluvial rice-growing valleys in this region were practically free from malaria. In the mountainous sections, localities at an altitude above 3,000 feet were similarly non-malarious, as the hydrographic conditions there are unfavourable to the breeding of *A. minimus*.

The remarkable feature of malaria in this region was the abruptness with which an intensely malarious area changed over to non-malarious, the two types occurring side by side in places less than half a mile apart.

In most of the operational areas in Assam the atmospheric temperature and humidity favourable for the transmission of malaria prevailed at least nine months in the year. The malaria curve usually rose in March, moving up gradually to a peak in June when the monsoon brought about a small yet definite drop in July. Towards the end of August, the curve moved up again to a second peak in November or the first half of December. From December to February, a certain degree of malaria transmission continued, but fresh cases of malaria were usually so few that, for all practical purposes, these months could be regarded as non-malarious.

In most of the localities in Assam the malaria curve was thus bimodal with peaks in the pre and post-monsoon periods. The height of these peaks varied with the amount of rainfall in relation to the breeding places of *A. minimus*. For instance, excessive precipitation in March and April favoured an increased flow of seepages and was, therefore, associated with a high pre-monsoon malaria rate, whereas a weak monsoon in July and August had not the required flushing effect on streams and springs, and in such years the post-monsoon malaria season was consequently abnormally severe.

In the heavily wooded valleys in Assam, however, an additional carrier, *Anopheles leucosphyrus*, prevailed. Because of the similarity between the terrain around Manipur Road and that of Digboi environs where it had previously been incriminated as a vector, this species was treated with suspicion from the very beginning of the campaign. A special search for it had accordingly been instituted in Manipur Road and Kabaw Valley from 1942 onwards. In the former place, Captain G. Sambasivan collected this species in camps situated in the comparatively uncleared western zone in 1943, but Captain Fenn failed to capture any specimens in the Kabaw Valley from January to March 1944. In September 1944, on the other hand, the position in the same valley was reversed, as 1 British Malaria Field Laboratory recorded its prevalence there in large numbers and with a high rate of malaria infections. Subsequent observations showed that the species underwent a rapid decrease in density in October and November, forming an insignificant proportion of the routine catch by December. This explained the failure of Captain Fenn to capture this species in the early months of the year. Based on the above observations, the seasonal incidence of *A. leucosphyrus* was thus inferred to be strictly confined to the rainy months, when its favourite breeding places appeared in abundance, namely, the small rain-water pools inside the forest, formed in natural depression or in man-made excavations such as slit trenches, bomb craters, borrow-pits, etc. In such valleys, the transmission of malaria was almost continuous over the greater part of the year. In the pre-monsoon months, *A. minimus* acted as the carrier, in the monsoon *A. leucosphyrus* took over, while in the post-monsoon *A. minimus* reappeared and carried on transmission till low atmospheric temperature either stopped or slowed down the sporogony cycle.

In East Bengal, the western districts, such as Jessore, were highly endemic or hyperendemic, the spleen rates in the villages ranging between 40 and 60 per cent. The main carrier in this area is *Anopheles philippinensis* whose favourite

breeding places are village ponds and stagnant water in loops of dead or dying rivers. In eastern districts, such as Dacca and Comilla, which are liable to extensive annual flooding and have a constantly high level of subsoil water, conditions are not congenial to this anopheline and the malaria risk there was, therefore, negligible. In the deltaic region of Bengal, such as the district of Khulna and the estuary of Hoogly, the vector species is the brackish water breeder, *Anopheles sundaicus*, which, under favourable conditions, gives rise to hyperendemic or epidemic malaria.

Throughout East Bengal, however, the malaria season was relatively short, being restricted to the period from mid-July to mid-November or mid-December.

In Southern India, malaria is a problem in the foot-hills where *Anopheles fluviatilis* is the carrier, its breeding places being springs, seepages, small streams and irrigation channels, and in certain regions, ricefields. In many of the areas the seasonal incidence of malaria is from March to June, but in some a second rise occurs in the post-monsoon period from September to December.

In Ceylon, the epidemiology of malaria in different zones is conditioned by the amount of rainfall. Thus the south-western quadrant, commonly known as the 'wet zone', receives both the south-west and the north-east monsoons, and is practically non-malarious. The northern half of the island, on the other hand, gets its rainfall only in the north-east monsoon, and is relatively dry. It has an elaborate ancient system of irrigation based on tanks set at different levels and interconnected by lengthy channels wherein the carrier species, *A. culicifacies*, finds ideal conditions for breeding. The disease in this zone prevails in a hyperendemic form, its seasonal incidence being from November to June with a peak in January and February.

Midway between the 'wet' and the 'dry' zones is the 'intermediate' zone which normally receives rainfall during both the monsoons. In years when the south-west monsoon fails, however, the rivers run low, leaving pools in their bed wherein *A. culicifacies* breeds in enormous numbers. This zone is, therefore, subject to periodic fulminant epidemics of malaria, lasting from October to March with a secondary rise during April and May.

In the Maldive Archipelago *Anopheles tessellatus* was the only species of anophelines found, its larvae occurring in shallow domestic wells. It was recorded infected once in March and once in June, but, judging from the malaria curve, transmission probably extends over the whole year.

Influence of Tactical Conditions.—A severe outbreak of malaria in the early phases is a common feature of most of the military campaigns in malarious areas. As a rule, these outbreaks can be traced to the fact that at the start the commanders are so deeply concerned about the military aspects of the campaign that they fail to appreciate the danger of malaria. At first sight, the epidemic of malaria in Assam during 1942 might appear to have had a similar origin, but on closer examination the available evidence does not altogether support this view. For, as already

stated, even at the planning stage, the paramount importance of malaria on this front had been fully recognised and steps taken to develop an adequate anti-malaria organisation in the field as rapidly as possible. Nor was the need for strict anti-malaria discipline neglected, as repeated circulars and instructions on the subject were issued at the very start of the campaign.

In so far as the outbreak of malaria in Assam is concerned, a more likely explanation is partly that the rank and file, despite all efforts, did not carry out the personal precautionary measures as rigidly as they should have; but more especially that, with the anti-malaria measures then in vogue, effective control was impracticable in the tactical and epidemiological circumstances prevailing in the year under review.

There were many factors which seriously hampered such control on the Line of Communication in Assam. In the first place, the construction of base installations was started when malaria transmission had already begun. As a consequence, it was difficult to work out and establish a comprehensive malaria control scheme. Secondly, owing to continual expansion, the boundaries of the encampment areas remained ill-defined throughout the transmission season. Thirdly, in many of the base installations, units were accommodated in small clearances surrounded by patches of uncleared forest which could not be adequately explored and drained, especially in the face of unusually heavy rainfall in May and June. Lastly, fresh troops continued to move into the area throughout these months and acquired malarial infections during the prolonged road and rail journeys. Many of them became malarial casualties soon after arrival in the operational areas and had to be evacuated back for want of sufficient local hospital accommodation. Their replacements, in turn, suffered the same fate, thus establishing a vicious cycle.

A majority of the combat troops in the forward areas, however, escaped infections in transit as they happened to travel through the Line of Communication in March and April when the transmission season was still in the initial low phase. In subsequent months, however, they were exposed to local infections when they had to operate in highly malarious valleys at the height of the malaria season. Moreover, the formations were widely dispersed over an extensive sector wherein individual units were frequently shifted from place to place, sending forward patrols which remained detached from parent bodies for days together. Under such conditions, it was difficult not only to employ active anti-mosquito measures but also to supervise adequately the personal precautionary measures amongst troops, particularly those on patrols and in small detachments.

Troops in East Bengal, had a relatively static role, and the incidence of malaria amongst them was, therefore, conditioned by the endemicity of local malaria and the adequacy, or otherwise, of malaria control measures. Thus, units stationed in the non-malarious eastern districts almost completely escaped malarial infection, whereas those occupying the malarious western districts suffered to a varying extent. For instance, in one of the brigades (4th Indian Infantry Brigade)

served by an anti-malaria unit the maximum malaria rate per 1,000 men per month was 86, whereas in a neighbouring brigade (3rd Indian Infantry Brigade) with inadequate anti-malaria cover the corresponding figure was 800.

In Ceylon and Southern India, malaria was a problem only where troops were on training exercises in malarious areas when the situation was practically analogous to that of the forward contact troops already described.

Methods of Malaria Control.—In the circumstances outlined above, it is obvious that the scope for active anti-mosquito measures in Assam was extremely limited. Both in the Line of Communication and the forward areas chief emphasis was, therefore, laid on measures of personal protection through wearing long trousers and sleeves rolled down after dusk, applying anti-mosquito repellent cream to face, hands and other exposed parts of the body, and sleeping inside a mosquito-net. The efficiency, with which these measures were carried out, was an index of the state of discipline in each unit. In areas, where no protection or even partial protection could be provided through active anti-mosquito measures, troops were placed on suppressive treatment consisting of one tablet (0·1 g.) of mepacrine daily for six days in a week. It might be argued that, with the high malaria rates prevailing along the Line of Communication such treatment should have been extended to the remaining troops also, but a decision on this point had to be based on the stock position of mepacrine which was extremely low, needing careful husbanding.

In theory, these measures, if conscientiously carried out, should have afforded considerable protection from malaria, but in actual fact the results did not come up to expectations due to certain inherent difficulties which are discussed in Chapter IX. At the end of the transmission season in November, therefore, all troops were placed on 'blanket treatment' with a view to cutting short their malarial attacks and preventing relapses during the non-malarious months. This treatment consisted of (*i*) three tablets (0·3 g.) of mepacrine daily for five days, (*ii*) no drug for the next two days, and (*iii*) 0·03 g. of pamaquin for British troops or 0·02 g. of pamaquin for Indian troops daily for three days.

Wherever possible, however, anti-larval measures were enforced. In Assam, attention was focussed on the preferential breeding places of *A. minimus*, such as streams, springs and seepages, which were canalised and subjected to weekly treatment with malariol. In camps on open sites such larval control was on a fairly extensive scale, covering both the camps and the area half a mile around. But in encampments in thick forests, anti-larval measures had to be restricted to the clearances, since breeding places in the neighbouring jungle were difficult, and at times impossible, to treat.

In the prevailing circumstances, however, it was relatively easier to enforce comprehensive and systematic daily spraying of tents, huts and local habitations with pyrethrum insecticide than anti-larval measures. During 1942, therefore, malaria control through spray-killing of adult mosquitoes was utilised as the main method of attack throughout the Line of Communication and as much of the forward

areas as possible. Most of the units provided their own spraying gangs, but in some, such as hospitals and rest camps, whose strength was insufficient to spare personnel for this purpose, anti-malaria units had to make the necessary provision.

By employing these measures, local malaria was to some extent mitigated, but the problem of preventing infection during rail and road journeys still remained. An attempt was, however, made to meet this difficult situation by placing anti-malaria units at the important railway junctions of Lumding and Badarpur. But it is doubtful if anti-mosquito measures and spraying of railway trains confined to these stations helped matters, considering that the greater part of the routes still remained uncontrolled.

In Bengal, owing to the static character of the camps, conditions were particularly favourable for employing active anti-mosquito measures in the malarious sectors. Anti-larval control was concentrated on the breeding places of the main regional carrier, *A. philippinensis*, such as dead rivers, canals and village ponds which were cleared of vegetation and regularly oiled. Paris green was applied only to ponds earmarked for drinking purposes and to breeding places from which vegetation could not be removed readily. Daily spray-killing of adult mosquitoes with pyrethrum insecticide was practised in the unit areas as well as in the local villages within half a mile of the periphery of the camps. But, as already stated, these measures proved successful only when carried out under the efficient supervision of an anti-malaria unit.

In Ceylon and Southern India, the static camps were brought under fairly satisfactory control by adopting the same policy as in Bengal, except that the anti-larval measures were confined to the carrier species of the region concerned. During training exercises, however, these measures were impracticable and the commanders were accordingly instructed to select sites for night halts at least half a mile away from the local human habitations, thereby eliminating the chances of infection in troops. At first, the implications of these instructions were not fully appreciated, as an odd hut in the jungle was not recognised as a source of danger. In time, however, this principle came to be observed in detail with satisfactory results.

TREATMENT OF MALARIA

Prior to the outbreak of war, the Army-in-India followed a standard course for the treatment of malaria, the details of which are tabulated below :—

Days.	Drug.	Indian troops.	British troops.
1 to 2	Quinine	10 grains to be taken three times a day.	10 grains to be taken three times a day.
3 to 7	Mepacrine (atebrin)	0·1 gramme to be taken three times a day after meals.	0·1 gramme to be taken three times a day after meals.
8 to 9	No drug	..	..
10 to 14	Pamaquin (plasmoquin)	0·01 gramme twice a day after meals.	0·02 gramme morning, 0·01 gramme evening after meals.

For cerebral, hyperpyrexial, choleraic, dysenteric, algid or haemorrhagic cases, quinine dihydrochloride 6 grains in 10 c.c. water intravenously or mepacrine methane-sulphonate 0·3 gramme in 5 c.c. of water intramuscularly were prescribed.

In peace-time this regimen had been observed to resolve readily the clinical attacks from all the three species of malaria parasites and had proved particularly successful in reducing the relapse rates in *Plasmodium vivax* infections to an extremely low level. Nor was its efficacy doubted under operational conditions in the first three years of the war.

Towards the end of 1942, however, it was reported that malaria cases in Assam were not responding satisfactorily to the standard treatment and that many of them were observed to relapse while taking the course or immediately after it. The view expressed in these reports was that the strains of parasites in Assam were apparently refractory to mepacrine and pamaquin, therapeutic action of which was suspected to be inferior to that of the corresponding German products. In contrast, quinine was alleged to have proved remarkably efficacious in Assam, especially if given intramuscularly. These reports were viewed with serious concern, for, if confirmed, they posed not only a pressing therapeutic problem but raised larger issues concerning the supply of anti-malarials. As it was, the situation regarding these drugs was none too easy, as after the loss of Java, the Allies were faced with a serious shortage of quinine and had to rely chiefly on synthetic substitutes. If the latter had proved to be less effective in Assam than elsewhere, it would have been necessary to allocate to this front considerable quantities of quinine from out of all available stocks.

On receipt of these reports in October 1942, an immediate investigation was undertaken at Manipur Road which fortunately showed that the evidence, on which the standard treatment had been condemned, was vague and indefinite. In the first place, there was no certainty that the so-called resistant cases had actually received and swallowed the drugs as prescribed. Indeed in the overcrowded state of the hospitals, the standard of supervision over the administration of drugs was obviously below that necessary for assessing the value of a regimen. Secondly, many of the patients in Assam had been exposed to heavy multiple infections. The fact that the relapses amongst such patients might not have been due necessarily to the recrudescence of the original infection through failure of treatment but to co-existing infections with heterologous species or strains of malaria parasites, was not fully appreciated and ruled out. Thirdly, the proportion of such resistant cases was not worked out and, consequently, no allowance was made for the presence of hyper-susceptibles amongst troops who respond poorly to most forms of treatment.

Despite the reassuring outcome of this enquiry, it was thought desirable to settle this issue through a detailed investigation particularly, as in malaria therapy, quinine and atebrin had displayed significant difficulties in curative action against

different strains of malaria parasites. In 1943, therefore, a medical specialist assisted by an adequate team of pathologists and nursing staff was detailed to test the efficacy of standard treatment on a controlled series of microscopically diagnosed malaria patients at Gauhati in Assam. The findings of this enquiry are summarised below which, incidentally, indicate the scope of the investigation :—

(*i*) The standard treatment was efficacious against the local strains of malaria parasites in Assam.

(*ii*) The standard treatment was as efficacious as a regime in which quinine (20 grains) was given along with mepacrine and pamaquin.

(*iii*) Mepacrine and pamaquin were as effective as atebrin and plasmoquin.

(*iv*) The apparent failures in treatment were due to patients not swallowing the drug.

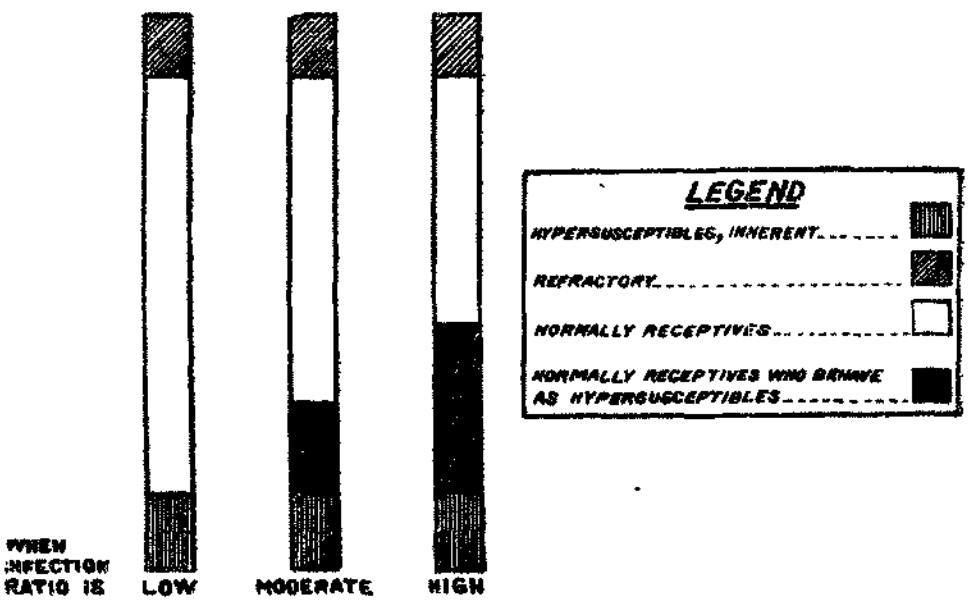
It is interesting to record that during the malaria season of 1943 the standard treatment was declared efficacious in Assam hospitals even before the findings of the above investigation were published. These improved results were probably brought about through two causes. In the first place, the hospital accommodation on this front had increased considerably which ensured a better administration of drugs to patients in 1943 than in 1942. Secondly, owing to intensive anti-malaria measures on the Line of Communication, the chances of acquiring multiple infections had been reduced considerably, so that the clinical attacks in 1943 were probably caused by a simpler type of parasitaemia than those prevailing in the preceding year.

During 1942, it was impossible to disentangle the relative importance of these two factors in Assam, as heavy malarial infections then co-existed with a shortage of hospital accommodation and lack of proper supervision over treatment. In 1943, however, a brigade of British troops was exposed to severe infections in Arakan and was evacuated direct to India for rehabilitation soon after the expedition. Despite the fact that malaria cases in this brigade received treatment under favourable hospital conditions, the standard treatment was reported to cure the clinical condition in each attack, but a significantly large proportion of cases suffered from repeated relapses of both *P. vivax* and *P. falciparum* infections. In contrast to the Arakan cases, relapses after treatment were markedly less in those patients who had acquired malarial infection in the mosquito-controlled stations in India. Admittedly, the differences in relapse rates in the two groups could have been due to differences in the parasitic strains, but it is more probable that they were caused by the varying amount of infection to which the two sets of patients had been exposed.

The above experience deserves careful note as it is akin to the conditions encountered in an epidemic of malaria in which severe and relapsing attacks of malaria even amongst adequately treated cases are so common a feature. While it is difficult to indicate with certainty the exact manner in which the infection factor enhances the severity and relapsing character of malarial attacks, it is highly probable that this phenomenon is closely related to the state of communal resistance.

For, in malaria therapy, it has been shown that in their reaction to malarial infections the non-immune individuals display wide variations, a small proportion belonging to the two extreme groups of hyper-susceptibles and refractory, while the remainder constitute the intermediary group of normally receptive. In the field, when non-immune troops were exposed to a low rate of infection the above reactionary grouping probably prevailed. Under conditions of multiple infections and re-infections with different strains and species of parasites, however, the resultant complex parasitaemia might well have disturbed the inherent resisting powers of the border-line individuals, so that despite adequate treatment a larger number of the normally receptive persons reacted as hyper-susceptibles with consequent increase in the proportion of severe and relapsing attacks. This view is illustrated diagrammatically below.

DIAGRAMMATIC SKETCH OF VARIATIONS
IN INDIVIDUAL RESISTANCE TO MALARIA INFECTION
UNDER DIFFERENT DEGREES OF INFECTION RATES



Whatever the exact explanation, it would be safe to assert that for the proper assessment of a regimen in the field, not only the strains of parasites but also the rates of infection prevailing in the area have to be constantly borne in mind. On this basis the therapeutic trials at Gauhati in 1943 are open to the objection that they were carried out on cases occurring in a mosquito-controlled section of the Line of Communication who could not, therefore, have received as heavy and repeated a dose of infection as the malaria cases in 1942. The results obtained in these trials and the conclusions reached should, therefore, have been worded as subject to this limiting factor. As an example of the opposite condition, it is

interesting to quote experiences with the organic arsenical compounds which, in combination with the standard treatment, were reported to cure many of the relapsing Arakan cases. And yet these compounds displayed no superiority when tested on local malaria cases in India. Bearing in mind the foregoing discussion, this should have been expected, as the standard treatment by itself would cure so high a proportion of the mild attacks caused by low infection rates in India that the trials would fail to show the superiority of the arsenical compounds observed in the heavily infected individuals from Arakan.

Historically speaking, however, the outstanding event in the therapeutics of malaria during 1943 was that the standard treatment regained its popularity. This course remained unaltered till 1944, when a change became necessary, as all the troops in the operational areas had by then been placed on suppressive mepacrine. It was argued that malaria cases in such troops should not be placed on quinine, as any particular advantage from the use of this drug would be counterbalanced by the loss of the mepacrine concentration in their blood in the interim. A more logical course would be to place them on mepacrine treatment straightaway, preferably in high initial doses, so that the existing concentration be raised to a therapeutically effective level as rapidly as possible. For the same reason pamaquin was deemed superfluous, as its anti-relapse action could be readily duplicated by placing such cases on suppressive mepacrine at the end of the curative course. The standard treatment was accordingly amended to a single drug regimen, shown below, which remained in force up to the end of hostilities in 1945. To avoid confusion, however, particularly in hospitals, which received cases both from the operational and non-operational areas, a parallel course was worked out for the latter troops not on suppressive mepacrine.

Days.	Drug.	Operational areas.	Non-operational areas.
1 to 3	Mepacrine ...	0·2 gramme to be taken three times a day after meals.	0·2 gramme to be taken three times a day after meals.
3 to 7	Mepacrine ...	0·1 gramme to be taken three times a day after meals.	0·1 gramme to be taken three times a day after meals.
Thereafter	Mepacrine ...	0·1 gramme once a day as suppressive treatment.	0·1 gramme daily for six weeks as anti-relapse treatment.

STORES AND EQUIPMENT

As already mentioned, the stocks of anti-malaria equipment available in India were short of requirements even at the beginning of 1942. Later, when sanction was received for the expansion of anti-malarial activities, the gap between supply and demand widened still further, and it was feared that unless immediate steps were taken it would not be possible to implement in time the revised cantonment scheme and the accelerated raising programme of the anti-malaria units.

For immediate supplies, overseas resources could not be relied upon as, owing to shipping difficulties, a minimum time lag of one year occurred between indent for and arrival of equipment. The only solution, therefore, was to secure through

local manufacture at least those items of anti-malaria equipment which were essential and in immediate demand and which could be readily manufactured. Falling in this category were the different types of sprayers used in malaria control, and efforts were accordingly concentrated on producing locally the knapsack sprayers for oiling, the hand blowers and mixers for Paris green and the hand sprayers for spray-killing of adult mosquitoes. In the last group, a special type of large hand sprayer, called Malaria Institute Special Handsprayer (MISH), designed by the Malaria Institute of India, was selected for manufacture as it gave continuous spray of fine atomised cloud covering a distance of 10 to 15 feet and was, therefore, particularly useful for large scale spraying operations. Its issue was confined mostly to anti-malaria units on an 'as required' scale to an average figure of about 200 sprayers per unit. For general issue to the troops the usual commercial type of hand sprayers were produced in bulk by the ordnance and distributed at a scale of one sprayer per officer and one sprayer per ten other ranks.

The main drawback in the locally manufactured sprayers was that, being hand made, they had all the defects incidental to this method of production. Thus, the sprayers were not mechanically identical so that each unit had to be inspected and tested separately before it was accepted by the inspectorate staff; secondly, the nuts and nozzles were not standardised and could not, therefore, be supplied as spare parts for the sprayers. These defects had, however, to be accepted and were, to a certain extent, minimised by issuing sprayers in excess of the sanctioned scale and by establishing workshops in selected anti-malaria units for repairing and refitting those with minor defects.

These sprayers were manufactured in sufficient numbers to satisfy the needs of the cantonments and the anti-malaria units raised in 1942, enabling the latter to proceed to the operational areas well equipped for instituting malaria control measures. Equipment for malaria surveys and investigations, such as microscopes and entomological outfits, was frequently short of the authorised scale, but these articles were supplied to the units in the field as and when the stocks permitted.

Owing to the extreme importance of the adulticide measures in the control of malaria on this front, the supply of hand sprayers was supplemented by importing a large number of power-driven spraying machines normally used for spray painting. The type selected was the portable petrol-driven unit No. NH 616 with a CLB cup and gun manufactured by the De Vilbiss Co., Toledo, Ohio, USA. To economise in shipping space these machines were received packed in cases $2\frac{1}{2} \times 1\frac{1}{2} \times 1\frac{1}{4}$ feet and were assembled and mounted on a trolly locally.

The scale of issue of the power-driven sprayers was determined on a regional basis, but provision was usually made to allocate at least four such sprayers to each anti-malaria unit. Incidentally a special case had to be made out for placing these sprayers on the list of articles declared essential for the prosecution of the war before their manufacture on a sufficient scale was permitted in the USA.

As regards stores, the most serious shortage was in anti-malarial drugs, for early in 1942 the total stocks of quinine and mepacrine in the army stores were about twenty tons and two million tablets respectively. Both these drugs were, therefore, placed on the list of controlled drugs, their issue to the various commands being made by the General Headquarters (India) on a specified scale every quarter. Every possible step was taken to ensure economy in expenditure and strict instructions were issued to expunge these drugs from prescriptions for non-malarial cases and to restrict supppressive treatment with mepacrine only to the troops in forward areas, where active preventive measures were impracticable.

After the loss of Java, there was little chance of replenishing the stocks of quinine as the limited yield from the cinchona plantations in India had to be set aside for the local civilian population. Immediate indents were, therefore, placed on the United Kingdom and the USA for large and progressively increasing quantities of mepacrine so that, in course of time, it practically replaced quinine as an anti-malarial.

The stocks of pyrethrum extract in India were fairly satisfactory in 1942, but even here strict rationing was enforced to prevent wastage as the produce from the East African pyrethrum plantations, which was the only source of supply left to the Allies, was insufficient to satisfy the rapidly mounting demands for this substance. In the meantime, intensive investigations were started to search for alternative insecticides amongst which lethane gave promising results. The problem of insecticide shortage was, however, solved only in 1944, when dichloro-diphenyl-trichloro-ethane (DDT) became available in large quantities and proved effective against the local vectors in India.

Even after the advent of DDT, pyrethrum was, for a time, needed in large quantities, as it was an essential ingredient of the anti-mosquito repellent cream. This cream had originally a greasy base which, however, caused such extreme discomfort through inducing excessive sweating in hot and humid climate that its formula had to be revised to a vanishing cream base. The production of the new cream, in turn, involved many manufacturing difficulties particularly in regard to the procurement of raw materials, and it took almost a year and a half before it became available in sufficient quantities to allow of unrestricted issue. Later, the synthetic liquid repellent di-methyl phthalate (DMP) was received in large quantities from the USA which, compared with the anti-mosquito cream, proved not only superior in action but was found more convenient to use, particularly in the operational areas.

No serious shortages in larvicides were encountered as, although the stocks of Paris green were low, this deficiency was effectively counter-balanced by malariol and diesel oil which were available in liberal quantities at all times.

Finally, the arrangements made for the transportation and distribution of equipment and stores are perhaps worth recording. Owing to the congested state of railway traffic in 1942, the safe and timely delivery of stores could be ensured only if

these were personally conducted. But the problem was to evolve ways and means of economising on men required for conducting such duties and to organise a regular flow of stores to widely scattered units in the field. This was effected by delivering the stores in bulk to 9 Indian Anti-Malaria Unit at Manipur Road, where a local dump was formed as a temporary measure. The above arrangement, however, proved so successful that similar dumps were established in other anti-malaria units even after the advance medical stores depots had begun to function. Later still when the manufacture and distribution of sprayers was taken over by the ordnance, the anti-malaria units continued to act as centres for the distribution of anti-malaria equipment and stores to the neighbouring units.

CHAPTER IX

ANTI-MALARIA CAMPAIGN IN 1943

OPERATIONAL SITUATION.

In 1943, operations in the cambat areas in Assam were of crucial importance to the future conduct of the war, but these did not affect the anti-malaria organisation to the same extent as activities on the Line of Communication. Here the existing bases were enlarged and established, and many new ones established, while the transportation system from Manipur Road railhead to Imphal was reorganised and provided with regular staging posts *en route*. Beyond Imphal the road to Tamu was graded and widened and two additional roads were constructed along the parallel valleys of Tiddim and Aijal into Burma, of which the former ultimately developed into a permanent Line of Communication.

The additional control work entailed by these developments was, however, set off by a certain amount of reduction in the Ledo area which, early in 1943, was taken over by the American forces to form a starting point for the Stilwell Road into Burma and China. Nonetheless, the anti-malaria unit, stationed at Ledo, continued to operate there for six months when the American authorities were able to take over control with their own anti-malaria organisation.

In Arakan, the 26th Indian Division, later reinforced by a British brigade group, advanced into the Mayu Range early in 1943. This force, however, encountered stiff Japanese resistance and had to be withdrawn before the onset of rains to the Tumbrughat line in the estuary of the Naf river.

In India, activities were concentrated mainly on organising the recruitment of personnel for the large number of new units and formations raised during the year. This, in turn, necessitated further expansion of cantonments and erection of additional camps to accommodate the continuously increasing military forces. For the training of these units a number of jungle training centres were established in suitable terrain, each of which needed extensive malaria control measures. The protection of new supply bases and hospital towns added further to the commitments of the anti-malaria organisation.

In November 1943, a major change in the overall strategic policy resulted in the creation of two independent commands, namely the South-East Asia Command and India Command. This necessitated a parallel split in the anti-malaria organisation to provide for the separate needs of the two commands.

ANTI-MALARIA ORGANISATION

Malaria Staff Appointments.—Throughout 1942, the anti-malaria staff officers had been subjected to intolerable strain owing to the continual expansion of the malaria control activities on the one hand and the organisation of anti-malaria training of the newly raised units and formations on the other. The necessity for

an increase in the cadre of anti-malaria staff appointments had thus been felt even in 1942, but the difficulty was that officers with the necessary qualifications were not then available. In 1943, however, the situation improved considerably as many of the commanding officers of anti-malaria units had in the meantime acquired sufficient practical knowledge and experience in the field to be considered for these appointments. Early in 1943, therefore, necessary action was taken whereby the anti-malaria staff appointments in most of the command headquarters were upgraded to assistant directors of malariology (ADsM) and new posts of deputy assistant directors of malariology (DADsM) were created in lower formation headquarters.

The position regarding staff appointments in October 1943 was as follows :—

Formations.	ADsM.	DADsM.
General Headquarters (India) ...	1	1
Command Headquarters ...	3	2 (one in Southern Command and one in Ceylon Army Command)
District or equivalent headquarters ...	..	9
Fourteenth Army Headquarters ...	1	..
Line of Communication Area Headquarters	..	5
Total	5	17

Anti-Malaria Units.—To meet the increase in anti-malaria commitments, both inside India and in the operational areas, provision had to be made for raising a correspondingly large number of anti-malaria units. It was realised, however, that the improvised arrangements utilised in 1942 for mobilising these units would be inadequate for implementing the new expanded programme. The raising centre was accordingly shifted from Delhi to the anti-malaria wing of the Army School of Hygiene at Babina and placed under a Deputy Assistant Director of Malariology, whose main functions were to arrange for the speedy collection of equipment and stores for the units and to carry out technical training of the personnel. As a result of these revised arrangements, a large number of well-trained anti-malaria units became available during the year for allocation to operational areas in Assam and Arakan and the jungle training centres, supply bases and recruiting and training camps in India. The disposition of these units in October 1943 was as follows :—

Location.	Number of anti-malaria units.
India	18
Operational areas	47
Ceylon Army Command	3
Total	68

With the formation of the South-East Asia Command and India Command towards the end of 1943, separate anti-malaria establishments had to be provided for both. This was effected by increasing the staff appointments and by reallocating the available units as shown below :—

Designation.	South-East Asia Command.	India Command.	Total.
ADsM	2	5	7
DADsM	6	14	20
Anti-malaria units	39	29	68

MALARIA CONTROL IN OPERATIONAL AREAS

Malaria Problems (Assam Front).—On reviewing the malaria situation at the end of the rainy season of 1942, it was obvious that the anti-malaria measures employed on the Assam front during the year, though admittedly inadequate, had succeeded in averting a complete break-down of the Line of Communication. Many incidents could be quoted which, by contrast, brought out the value of these measures, but the most striking was that of a large railway construction project at Rangapahar near Manipur Road which was started in April 1942 without anti-malaria cover and which had to be closed down six weeks later when 90 per cent of the labourers became ineffective through malaria.

Nevertheless, control measures employed in 1942 were limited in scope and, as a consequence, malaria rates, even in the protected units, reached extremely high figures and remained at that level for months so that in the course of the year most of the individuals serving in Assam had suffered repeatedly, few escaping one attack. All the units were, therefore, exposed to considerable physical and mental hardships through having to function at low strength and with personnel who were constantly going sick and were being continually replaced by inexperienced men. Control measures could thus merely claim to have succeeded in keeping the malaria rates below the levels at which the units would have disintegrated.

Another disconcerting feature of the situation was that the troops had lost confidence in the preventive value of anti-mosquito measures in Assam and were, in consequence, becoming non-co-operative. It was, therefore, of the utmost importance to make every effort to counteract this impression.

An intensive drive was accordingly undertaken early in 1943 in which the nature of the malaria problems and the methods of control applicable to Assam were explained to the troops through lectures, pamphlets and personal letters from the commanders. Despite these activities, it was recognised that the confidence of the troops in anti-malaria measures could only be restored by demonstrating a spectacular and convincing fall in malaria rates through malaria control.

The dominant consideration in such a control scheme, which was eventually sanctioned, was that its details should be based on factual data collected from

observations made in 1942. Thus the scheme could be expected to succeed only if it was sufficiently comprehensive to afford adequate protection to troops, against both local infection and that contracted in transit, as it was impossible to be certain how much of malaria in 1942 had been caused by one or the other factor. Secondly, it was essential to conform to a schedule as the successful outcome of the scheme depended upon whether or not it was completed before the onset of malaria season, i.e., before the end of March 1943. Finally, the scheme could be implemented only in a sector, such as Assam, where the Line of Communication was sufficiently stabilised to allow of anti-mosquito measures on a comprehensive scale.

As regards the forward areas in Assam, the frequent movements and constant patrolling activities of the operational units created problems of malaria in 1943 which did not materially differ from those encountered in the preceding year. Even, there, however, an accurate assessment of the locally acquired malaria could not be made as the figures did not exclude malaria cases amongst reinforcements who frequently contracted infection during transit but developed acute attacks after their arrival in the forward areas. It was, therefore, reasonably certain that the elimination of the transit infections through mosquito control on the Line of Communication would not only lower the malaria rates in the forward troops but would, at the same time, serve to bring out the amount of malaria directly attributable to the specific conditions in the forward areas.

Malaria Problems (Arakan Front).—In Arakan, the military operations which began in February 1943 created a malarial situation which was analogous to that encountered in Assam in 1942, in that the advance started just before the beginning of the malaria season and left in its wake an ill-defined Line of Communication which could not be protected by organised and timely anti mosquito measures. It was not till June 1943, when the troops retreated to the pre-arranged line in the Naf estuary, that the Line of Communication was stabilised and a comprehensive control scheme could be enforced.

Apart from this tactical factor, anti-mosquito measures in Arakan had to be suited to the prevailing epidemiological features of malaria in the region. In this regard, the foot-hill sector of Arakan need not be described in detail, for, as in Assam, malaria in such areas was carried by *A. minimus* and *A. leucosphyrus* whose breeding habits and seasonal prevalence have already been reviewed in Chapter VIII. The coastal plain of Arakan, however, presented a distinctive problem as the main carrier there was the brackish-water breeder *A. sundaicus*.

The chief requirement of this species was the degree of salinity of water in the breeding place which had to be within a specified range of tolerance. So long as this essential condition was fulfilled *A. sundaicus* displayed no particular preferences, its larvae occurring equally in all types of water collections whether large or small. For this reason, the most favourable situations were the junctions of fresh-water streams with the saline tidal creeks where the mixture of the two types of

water produced the requisite amount of salinity in the neighbouring pools, lagoons, ricefields and miscellaneous collections of water.

Nevertheless, such potential breeding places were invariably innocuous if they were exposed to the flushing action of the daily tide and it was only, where embankments around ricefields or habitations interfered with this action that the man-made breeding grounds appeared. In contrast, fresh-water pools just outside the range of the daily tide but exposed to periodic neap tides or hurricane floods constituted the natural and permanent habitats for the species.

The above breeding peculiarities conditioned the density of *A. sundaicus* which varied markedly from place to place and even in the same locality from season to season, as the existence of optimum salinity in the breeding places depended upon a precarious balance between the amount of rainfall and the fresh-water contents of the hill streams on the one hand and the occurrence of unusual tidal waves on the other. As a consequence, the amount of malaria produced by the species was equally unpredictable. In normal years the disease was restricted to localities having an abundance of permanent, natural and man-made breeding grounds, but in favourable meteorological circumstances it flared up into an epidemic sweeping over a large section of the coastal tract.

Although there is strong evidence that the adults of this species possess an unusually long range of flight, the inference that the active dispersal of *A. sundaicus* from permanent foci occasioned the epidemics of malaria along the coastal plain is open to doubt. For an equally plausible explanation for these outbreaks would be that in such localities, the species normally prevailed in small and possibly undetectable numbers, but in epidemic years, when breeding conditions became unusually favourable, it multiplied rapidly into an enormous population. This would also explain the extreme seasonal and annual variations in the severity of malaria observed along the Arakan coast where, in a given year, the disease broke out either in the spring or in the autumn or during both or in neither of the two seasons.

Policy of Malaria Control in the Assam Line of Communication.—As already explained, in planning an effective anti-mosquito scheme for the Assam Line of Communication the overriding consideration was not merely to institute controls around each base in an isolated and disjointed manner but to plan a continuous chain of protected installations for the troops in transit. Since the major portion of the Line of Communication between Gauhati and Palel was intensively malarious, malaria control, over the entire countryside, would have been an impossible task. It was necessary, therefore, to confine anti-mosquito activities to a succession of 'harbours'.

The delimitation of each 'harbour' was left to the discretion of the local commander who settled the boundary line so as to include the existing installations and sufficient space for expansion, care being taken to congregate scattered units so as to reduce the size of the controlled area to the minimum. Furthermore, in the

selection of these 'harbours', both for siting permanent installations and transit camps, preference was naturally given to those localities which were inherently free from malaria such as the banks of the river Brahmaputra near Gauhati, the large rice-growing plain of Imphal and the mountainous regions around Kohima and to the south of Palel.

Troops in transit could, according to this overall plan, escape infection by proceeding from one malaria-controlled or malaria-free 'harbour' to the next, thereby avoiding the intervening uncontrolled and highly malarious sections.

Methods of Malaria Control in the Assam Line of Communication.—In Chapter VIII, attention has been drawn to the fact that during 1942, owing to jungle growth, the larvicidal parties were unable to treat all the mosquito breeding places up to the requisite distance of one-half mile around camps. This was a serious drawback which greatly lowered the value of anti-larval measures carried out during that year. In 1943, therefore, efforts were concentrated on remedying this defect by making all such breeding places readily accessible through extensive drainage and clearing operations.

Although these drainage projects, which are described in subsequent paragraphs, were the dominant feature of the anti-malaria scheme in the year under review, improvements in, and extension of, other methods of malaria control were by no means ignored. Thus the scope of systematic spray-killing of adult mosquitoes with pyrethrum insecticide was greatly expanded by liberalising the issue of MISH (Malaria Institute Special Handsprayers) and power-driven sprayers to anti-malaria units on an 'as required' scale.

Elaborate steps were also taken to eliminate infection amongst rail passengers by reorganising malaria control of the entire railway system in Assam. Each anti-malaria unit was made responsible for a definite section and all railway stations therein were surveyed in detail. Those found malarious were allotted anti-malaria gangs who carried out malaria control measures in the station precincts and the neighbouring villages. They also undertook the spraying of night trains which, as a consequence, were treated successively at most of the main railway centres such as Gauhati, Lumding, Badarpur and Manipur Road. As an additional precaution, spraying squads accompanied troop-trains and sprayed all compartments whenever the trains halted during the night in between the railway stations.

Road convoys were similarly protected by establishing malaria-controlled staging posts. Maps showing such malaria-controlled and malaria-free areas in the Line of Communication were freely circulated and the information contained therein was supplemented by road signs showing the limits of malaria-protected or malaria-free zones.

Personal malaria precautions were rigidly and systematically enforced in the form of a daily anti-malaria drill which was originally developed by the 23rd Indian Division but was later adopted by all operational units. With the help of

this drill, frequent night checks, special orders, pamphlets and posters, the troops displayed signs of tangible improvement in malaria discipline during 1943. Even the established military practices had to give way to anti-malaria needs in certain respects. Thus the reveille in military units located in the foot-hills was postponed by one hour where practicable so as to protect troops from the bites of *A. minimus* which was known to be particularly voracious in the early hours of the morning.

Drainage Projects.—During 1943, drainage projects in many localities were installed by the anti-malaria units but the most notable was that carried out by the Military Engineering Service in Manipur Road, which, apart from being the largest scheme, served a particularly useful purpose as a virtual training centre. Here the commanding officers of anti-malaria units and others received short practical demonstration of the drainage methods which enabled them to institute similar works in their own areas through labour and transport supplied by the local commanders. The result was that, in the short non-malarious interim of three months, it was possible to establish malaria-controlled 'harbours' in most of the main concentration centres such as Gauhati, Badarpur, Lumding, Kanglatongbi, Palel, Chittagong, Cox's Bazar and the Tiddim Road.

Since the procedure followed in the execution of these works was the same in different localities, it would suffice to illustrate the drainage methods employed by giving a detailed account of the scheme in Manipur Road.

The military base at Manipur Road occupied a narrow strip of land between two rivers, the Dhansiri and the Diphu (*vide* map). The northern section of the base was comparatively open consisting of rolling hills and flat swampy valleys, but towards the south, it was covered by a dense continuous forest of high trees broken only by restricted clearings around individual camps. To the north the units were, therefore, scattered in the open countryside over a wide area, but to the south they were sited in the immediate vicinity of the road to Imphal which traversed the base along its entire length.

Mosquito breeding places in the base were many and varied in character, but the types needing particular attention were those which formed the favourite habitat for *A. minimus*, namely, the streams and the seepage swamps. The drainage operations, though mostly concentrated on these breeding places, were, however, not exclusively restricted to them, as the parallel aim was to relieve the water-logged soil and to provide a rapid run off for water, wherein the other vector species, *A. leucosphyrus*, was apt to breed. Nonetheless large tanks, borrow-pits and large rivers and their overflow pools were left untouched, as they did not constitute the favourite breeding grounds of both the carriers.

In the programme of work, the perennial streams naturally received first priority, and their canalisation was accordingly completed early in March 1943. Later, operations were extended to those streams and seepage patches in which water appeared only after the pre-monsoon showers and work thereon was completed towards the end of April.

A set routine was evolved for dealing with these streams. First, the upper reaches of the streams were defined by clearing the undergrowth for 40 feet on either side of the channel. Since the seepage contour of the streams and catchment areas was apt to move up after the rains, this was provided for by clearing the uppermost reaches for 30 to 40 feet above the point where the dry weather flow began.

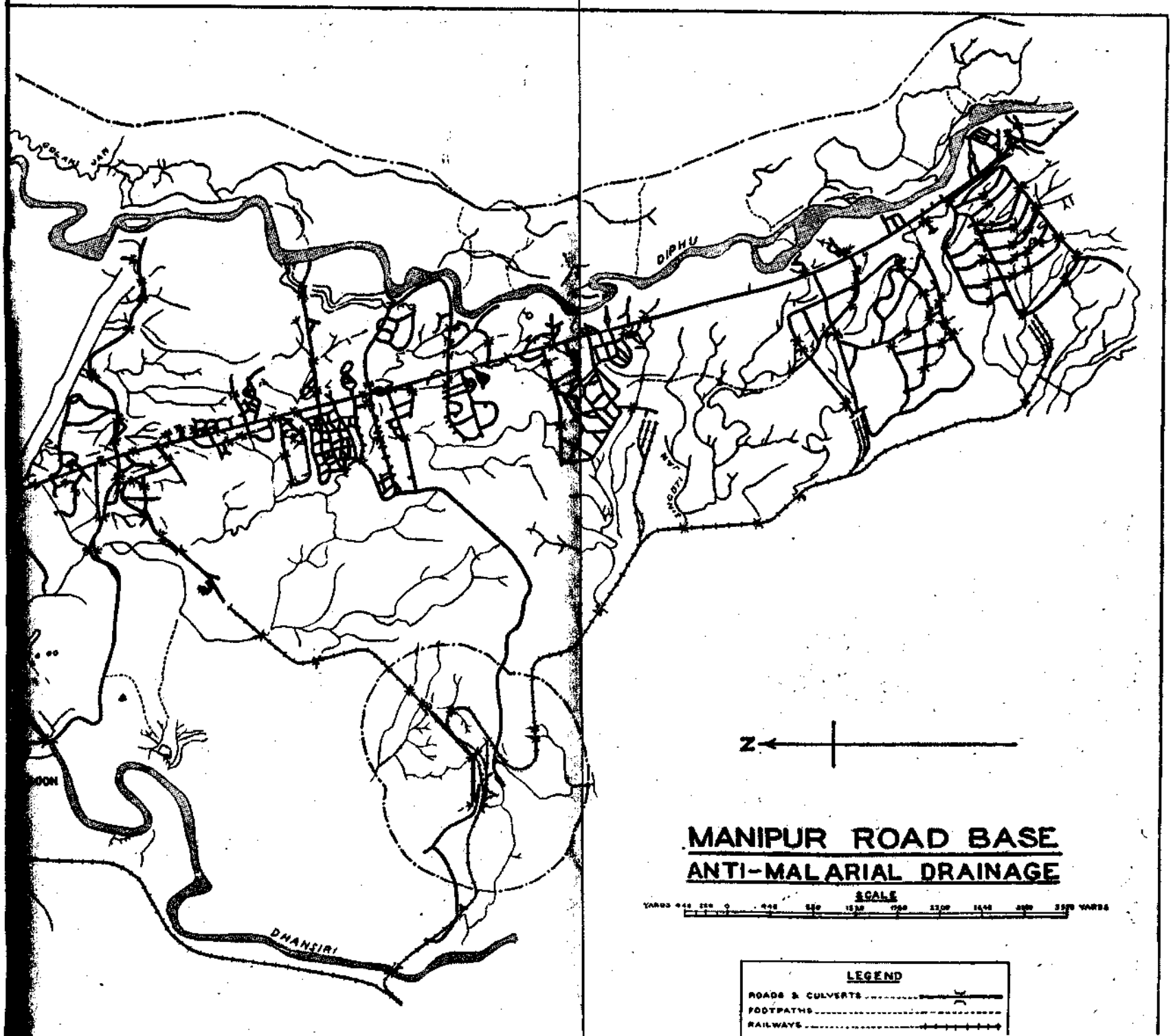
The main stretch of the stream was next taken up and canalised by straightening its course, clearing its bed and providing it with properly graded banks. These operations were progressively extended to the outfalls which were deepened and cleared of silt to let the water drain out freely. After most of the accumulated water had been drained away, the seepage patches that still remained were located and intercepted by a parallel or a herring-bone system of drains or by racket-shaped oval drains.

Finally, as work on each stream was completed, it was handed over to the anti-malaria units for larvicidal treatment and for general maintenance, adequate provision for which at the earliest possible moment constituted an essential feature of the scheme. For this purpose, two anti-malaria units, one section Royal Engineers and 1,000 labourers were kept permanently employed on Manipur Road.

The question as to whether the jungle growth should be removed from places other than around the streams and seepages evoked considerable discussion, as it was feared that after indiscriminate clearings, many seepage pools which were innocuous while shaded would be exposed to sunlight and would then form dangerous breeding places for *A. minimus*. The final decision taken was to permit only the removal of undergrowth and small trees, around the camps, with unit labour, up to a distance of 50 yards. This compromise was an unqualified success as it removed the steamy dank atmosphere and improved the general amenities of the camps by promoting the rapid drying of tents and ground without, at the same time, adding on undue number of new breeding places.

The original drainage scheme in Manipur Road envisaged operations over about ten square miles which involved canalisation and jungle clearance of about a hundred miles of water-run. In this phase of the scheme 4,000 men and 20 lorries were employed for a period of three months, the total number of man-days and lorry-days expended being 360,000 and 2,500 respectively. These figures did not, however, include unit labour engaged on clearance work around camps which was estimated at an additional 500,000 man-days. In the course of the year, however, the base area was extended on all sides and drainage measures had to cover nearly 30 square miles involving the construction of a net-work of 600 miles of drains.

As already stated, this project was executed by the Military Engineering Service through the local Commander Royal Engineer, who had at his disposal two assistant engineers, five sub-divisional officers and fifteen overseers. Although the


DIPHU
DHANSIRI
N
MANIPUR ROAD BASE
ANTI-MALARIAL DRAINAGE
SCALE
LEGEND
ROADS & CULVERTS
FOOTPATHS
RAILWAYS

engineers had no previous experience of this type of work, they had no difficulty in applying appropriate measures once the basic principles of anti-malaria drainage had been explained to them on the ground. Shortage of trained supervisors, was however, a more serious problem, particularly as there was no time to teach them how to canalise streams and to select proper sites for interception drains. This difficulty was overcome by starting drainage works over small areas under qualified overseers to whom a number of trainee supervisors were attached for practical instruction. After one or two weeks, these supervisors were placed in independent charge of fresh areas and the work was thus progressively expanded in scope till it absorbed the full quota of 4,000 labourers. This procedure ensured that every detail of the drainage operations was executed under intelligent supervision without which the scheme might well have had the opposite effect, i.e., aggrevating the malariogenic conditions.

The two assistant engineers, Lieutenant Masters and Mr. Lynn, were later placed in charge of the Royal Engineer Sections (anti-malaria), which were subsequently formed for maintaining the anti-malaria drainage projects in Manipur Road and for initiating fresh schemes in Assam and Arakan. It is tragic to record that Mr. Lynn, through whose energy and effort the drainage scheme in Manipur Road was completed on schedule and who planned many new projects for Palel and forward areas, contracted a severe and malignant form of malaria which led to his death early in 1944.

Policy and Methods of Malaria Control in the Arakan Line of Communication.—During 1943 a planned malaria control scheme, analogous to that instituted in Assam, could not be enforced in Arakan, where, in the first half of the year at least, the Line of Communication was too unstable and undefined for such action. Nevertheless, drainage operations and extensive anti-larval measures were carried out around Chittagong and Cox's Bazar, but troops operating beyond the latter had to rely only on suppressive treatment, pyrethrum spraying and personal prophylactic measures.

Drainage measures employed in the foot-hills in and around Chittagong and Cox's Bazar were similar to those enforced in Assam, but in the coastal section, the policy adopted was to keep out salt water in the creeks by constructing embankments fitted with automatic sluices which let out fresh-water only at low tide. In other localities, it was more economical to remove all embankments and other obstructions to allow a freely flowing daily tide to exercise its beneficial flushing effect.

MALARIA CONTROL IN FORWARD TROOPS

Anti-mosquito measures such as drainage and systematic spraying of insecticides could not, for obvious reasons, be enforced in areas where forward troops were operating. Throughout 1943 the prevention of malaria in such troops was, therefore, attempted through suppressive mepacrine and measures of personal precautions enforced in the form of an organised anti-malaria drill.

Personal Protection Measures.—Measures of personal protection were kept under constant study and in the course of the year many improvements were introduced. Thus, in order to ensure that the troops would, at all times, wear proper protective clothing, they were issued a cotton battle-dress consisting of a full-sleeve blouse and long trousers. For the same reason, the wearing of shorts and half sleeve bush-shirts was forbidden in the combat areas even in the day-time. The issue of light mosquito-proof bivouac tents was another useful measure, as it afforded adequate protection from rain which had frequently prevented the operational troops from setting up their mosquito-nets in the open. Similarly the issue of improved vanishing variety of anti-mosquito repellent cream was initially restricted to forward troops before the stock position had improved sufficiently to permit its general issue to the rear areas.

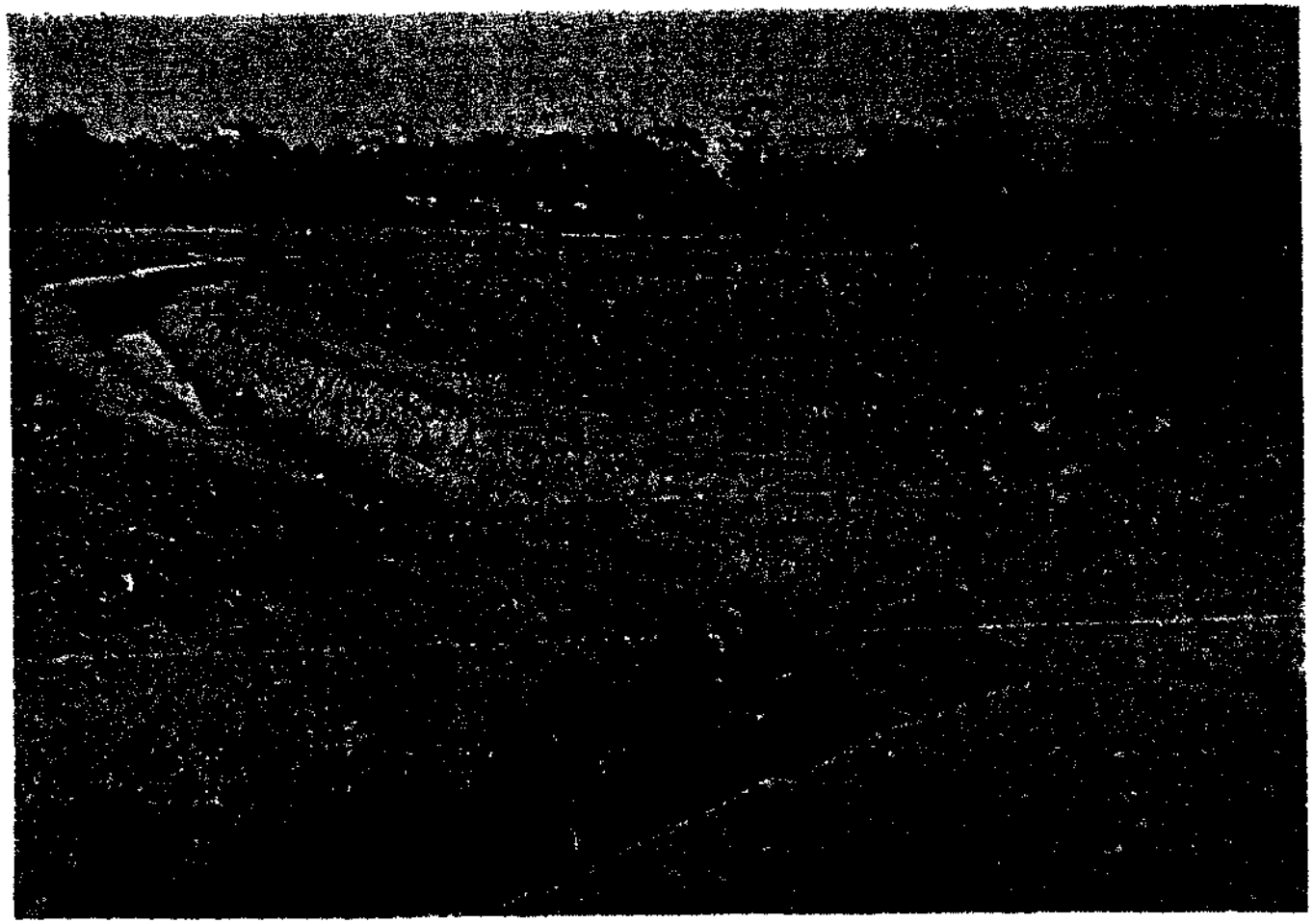
A disturbing feature of the situation, however, was that although these efforts had succeeded in raising the standard of malaria discipline to a satisfactory level, particularly amongst divisional troops, the malaria rates did not show a corresponding decrease. It was not surprising, therefore, that the troops were apt to question the protective value of such discipline and were inclined to look upon it as an eyewash and treat it accordingly.

On investigation, however, it transpired that while anti-malaria precautions in the forward areas had been rigidly and conscientiously enforced down to sub-unit headquarters, the patrolling parties did not always observe them. The latter thus acquired infections during the short period of their detachment, and since such patrols were drawn from different platoons or companies in rotation, it was not long before the entire unit or formation suffered from a general outbreak.

It was necessary, therefore, to review the routine of jungle patrols in detail, when it was found that, during contact with the Japanese, surprise attacks were so frequent and common that the patrols were disinclined to utilise those protective measures, such as veils and nets, which reduced visibility or hampered movements. The use of an effective mosquito repellent was, therefore, the only feasible alternative, but the repellent action of even the improved vanishing variety of anti-mosquito cream lasted only a limited period of time, which necessitated repeated applications during the night. This was frequently impracticable partly because of the impossibility of exercising adequate supervision over dispersed troops and partly because such troops were mostly engaged on laying ambushes when the slightest movement would be fraught with danger.

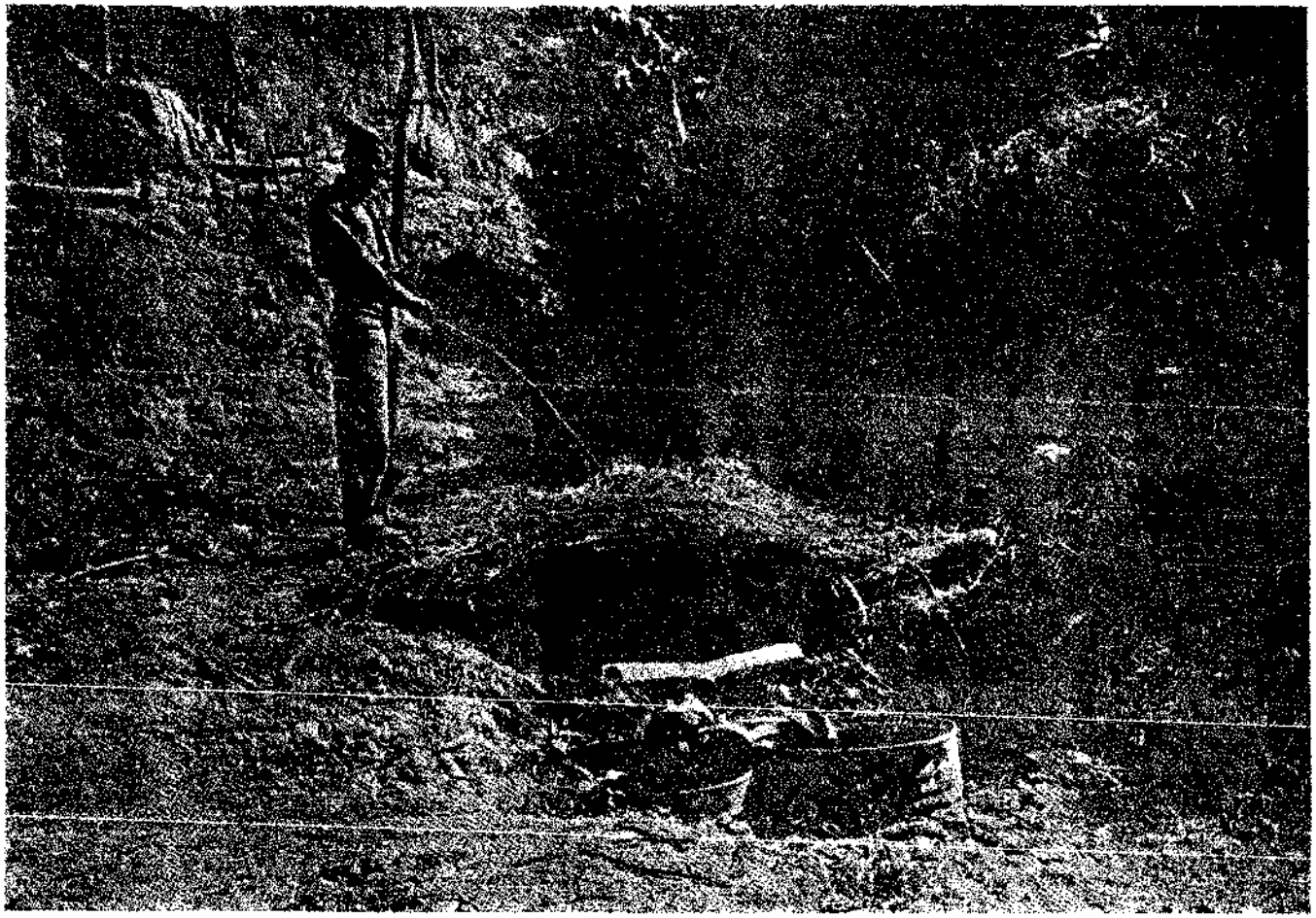
These observations, amply confirmed in Assam and Arakan, thus explained the apparent paradox of the co-existence of a high rate of malaria in forward troops with seemingly good malaria discipline. The main problem, therefore, was to devise effective and practicable protective measures for small detachments and patrolling parties, intensive investigation on which was taken up in 1944 which is described in Chapter X.

PLATE I



A typical anti-malaria interception drain.

PLATE II



Shading of a stream in a deep ravine

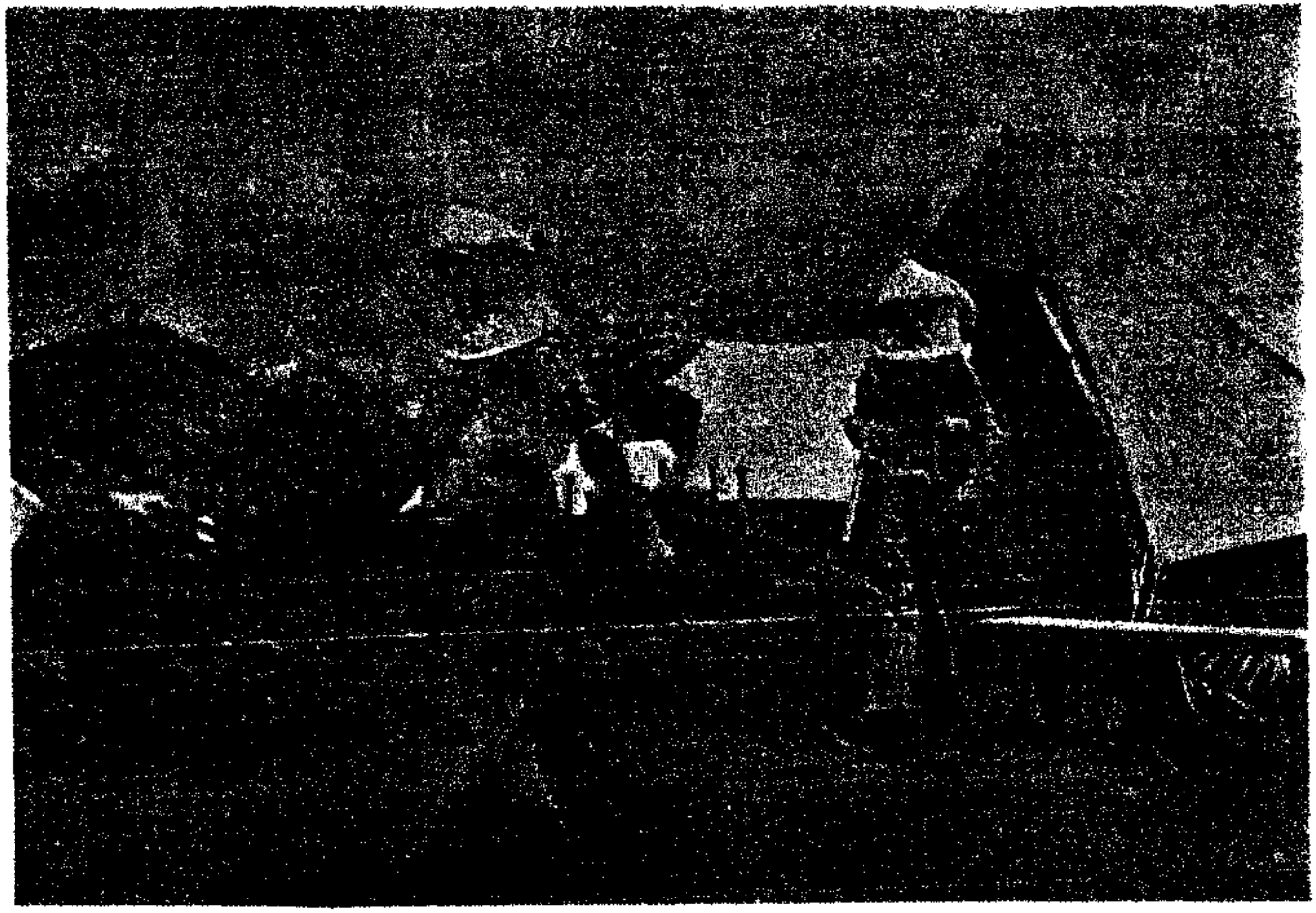
PLATE III

Oiling of a drain.

PLATE IV.

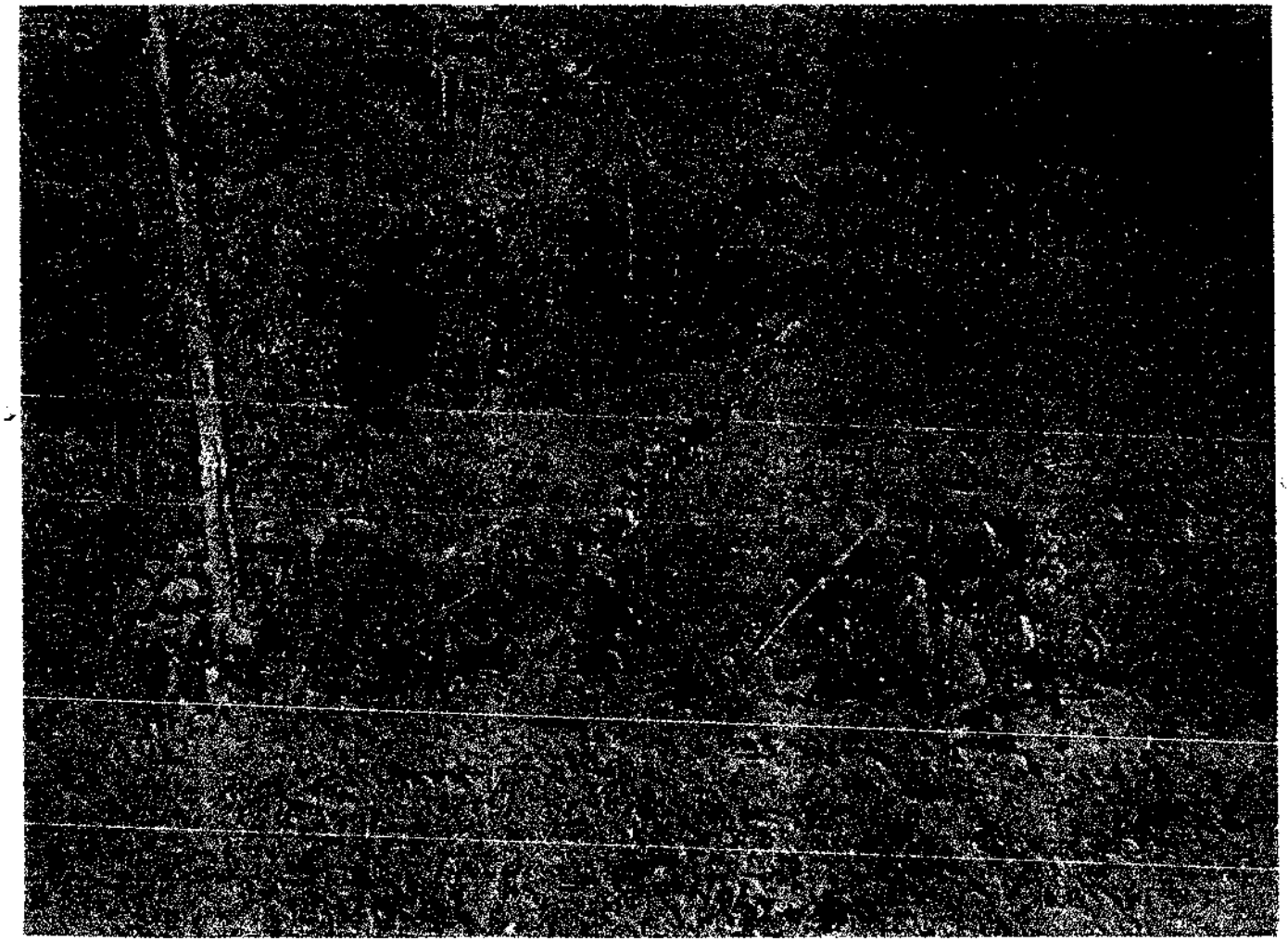
Cleaning and oiling of a drain.

PLATE V.



Protective clothing, veils and gloves.

PLATE VI.



Type of terrain over which operations took place.

Suppressive Treatment.—While prevention of malaria infections was the accepted ideal, it was realised that in the prevailing circumstances suppression of clinical attacks with mepacrine was the only alternative for keeping the forward troops on their feet. From practical experience, however, it was found early in 1943 that the prescribed break in mepacrine administration for one day in the week could not be regulated in the field and the course was, therefore, altered to a daily dose of one tablet (0·1 gramme) of mepacrine.

It was also recognised that, without stringent supervision, troops could not be relied upon to take suppressive treatment regularly. Strict instructions were, therefore, issued emphasising that mepacrine tablets must be given in the presence and under the direct supervision of an officer. To promote a suitable routine in the unit for administering the drug and to ensure a high mepacrine concentration in the blood of individuals before exposure to infection, it was also laid down that suppressive treatment should be started seven days before the entry of troops into malarious areas or the commencement of malaria season. Finally, provision was made that on the withdrawal of troops to a non-malarious locality or on the termination of the malaria season, the suppressive course was to be continued for a minimum period of 14 days before the troops were placed on a course of 'blanket treatment' described in Chapter VIII.

The efficacy of the above regimen was closely studied, both in Arakan and Assam, throughout 1943. The general experience was that while units under suppressive treatment had low malaria rates in the first four to six weeks of exposure to infection, the incidence of disease thereafter rose sharply to a very high figure. These observations posed another problem, namely, whether the delayed rise in malaria rates was due to irregularity in mepacrine or that the drug in the prescribed doses was not completely effective against repeated and massive malarial infections prevalent in Assam and Arakan. This problem was also subjected to intensive investigations in 1944 as described in Chapter X.

Malaria Forward Treatment Unit (MFTU).—The scope of the anti-malaria measures in 1943 varied on the two fronts which, in turn, produced different effects. In Assam, partly because of control of local malaria in the Line of Communication and partly because of an increase in the hospital accommodation, the great majority of malaria cases evacuated from the forward units was absorbed in the base hospitals. Many of the such cases were, therefore, saved from lengthy evacuation back to India and were able to return to their units within a reasonably short time.

The advantage secured was that owing to the low number of reinforcements required, the units could preserve their homogeneous character which had a considerable beneficial effect on their morale and fighting efficiency.

In Arakan, on the other hand, the incidence of malaria was high both along the Line of Communication and in the forward troops as had been the case in

Assam during 1942. As a consequence, the local hospitals were soon filled to capacity and the bulk of malaria cases had to be evacuated to India. In the course of time, the hospital accommodation in the neighbouring commands was used up and such patients were, therefore, evacuated farther and farther back till the evacuation traffic embraced distant stations, such as Peshawar and Poona. It was months before these men could be returned to their units and, in the interim, replacements had to be sent forward continually which had an adverse effect on the composition and morale of the units.

To meet this situation which, at the time, was considered likely to recur whenever the army was to make a forward move during the malaria transmission season, a new type of unit was devised intended solely for the treatment for malaria cases in the operational areas and was called the MFTU (Malaria Forward Treatment Unit). This unit had a holding capacity of 600 patients and was equipped to diagnose and treat malaria cases both Indian and British. It was designed to fill a role parallel to that of a casualty clearing station and was to have sufficient mobility to move forward with a division. Treatment of malaria cases in these units was to be shortened to a seven-day course, at the end of which the individuals were to receive daily suppressive mepacrine and returned to their units through a neighbouring divisional reinforcement camp after being re-clothed, re-equipped and re-armed.

It must be emphasised that the anti-malaria organisation was concerned only with assisting, and collaborating with, those who were engaged on forming and developing the Malaria Forward Treatment Units. The deployment of these units, however, remained at all times exclusively under that section of the Medical Directorate which dealt with the evacuation of casualties.

MALARIA CONTROL IN INDIA COMMAND

Since India Command, as such, was formed only towards the end of 1943, historically speaking, the descriptive heading of this sub-section should have been malaria control in cantonments. In actual fact, however, the anti-malaria activities in India had expanded so markedly that early in 1943 the cantonment scheme had lost its former importance particularly as it could develop automatically along the lines laid down in 1942.

Improvements in this scheme, however, became necessary in two directions. In the first place, the civilian malaria medical officers were permitted to join the newly formed IAMC (Indian Army Medical Corps) for service within Indian limits. This change in policy, apart from satisfying the repeated representations of these officers for an increase in pay scales, gave them a military rank which helped them considerably in the performance of anti-malaria duties in the cantonments. The need for, and advantages of, continuity in anti-malaria measures had by then been fully realised by most of the local commanders, but to prevent unnecessary transfers all such orders had to have the previous approval of the

General Headquarters (India). Secondly, the policy governing the allotment of funds for permanent anti-malaria engineering works was revised, the decision taken being to concentrate on completing such works in a few selected stations each year rather than to disperse funds in small sums over a large number of cantonments.

Amongst the fresh activities in India, undertaken by the anti-malaria organisation during 1943, the following were the most important :—

(*i*) Anti-malaria training of newly raised units and formations.

(*ii*) Malaria control in jungle warfare training areas.

(*iii*) Malaria research organisation.

Training in Anti-Malaria Measures.—In accordance with a special India Army Order, all ranks were to receive training in personal protection and other anti-malaria measures; unit commanders were to render a quarterly report certifying that such instructions had been given, and that weekly parades and demonstrations had been held embodying the anti-malaria drill, and that all ranks were in possession of protective clothing, anti-mosquito cream and mosquito-nets in a proper state of repairs. Other topical orders contained a provision that all units would, in addition to the hygiene establishment, form an anti-malaria squad for treating mosquito breeding places within their camps or lines and a spraying gang for spraying barracks, huts or tents with insecticides.

The training of these unit anti-malaria squads and spraying gangs was left to the regimental medical officers. But the difficulty was that most of the regimental medical officers were themselves not adequately conversant with these measures particularly as in many of the units the duties of such officers were performed by civil medical practitioners. Arrangements had, therefore, to be made to hold a series of training courses for the unit medical and non-medical officers in different stations and brigade groups.

In these courses, which were conducted by the anti-malaria staff officers, the general policy of military malaria control was explained to the students and the methods of training unit anti-malaria squads and unit spraying gangs demonstrated to them. The following points were, however, particularly stressed as they had been observed not to have received the desired degree of attention :—

(*i*) The devastating effect of high malaria rates on the operational role of the army.

(*ii*) The indifference of commanders to enforce detailed supervision over malaria precautionary measures and suppressive treatment.

(*iii*) Failure of commanders to visualise the exact manner in which different groups contracted malaria and to take appropriate and timely action under varying operational conditions, such as during train journey, on the line of march and on patrol duties.

(*iv*) The paramount importance of utilising malaria-controlled or malaria-free 'harbours' for halts along the Line of Communication and of avoiding local habitations in siting temporary night 'harbours' in forward areas.

(*v*) The utmost importance of selecting suitable sites for permanent camps and locating them in areas which were either naturally non-malarious or could be relatively easily controlled. The fact that the malariogenic factors varied in different parts of the country was especially stressed.

Malaria Control in Jungle Warfare Training Areas.—During 1943, intensive training of troops in jungle warfare was taken up in preparation for the forthcoming large-scale operations envisaged on the Assam-Burma border. A number of training areas were accordingly selected in different parts of India, each spread over hundreds of square miles of difficult and intensely malarious jungle terrain. The control of these vast areas by an anti-larval method presented an enormous task, impossible to execute within the time and with the resources available. Troops engaged on such training in 1943 had, therefore, to rely on personal protection measures while some of the units were placed on suppressive mepacrine.

Despite these precautions, however, the malaria rates in these troops were extremely high, ranging between 25 to 50 per cent of strength after an average period of training lasting six weeks. Towards the end of the year, therefore, the entire problem was reviewed and the following policy adopted :—

(*i*) The training in jungle warfare should, wherever possible, be restricted to the non-malarious months.

(*ii*) The sites for new jungle training centres should be selected in close collaboration with the malariologists and located, to the maximum extent possible, in the northern half of India where, with the exception of Bengal and Assam, the malaria season was short.

(*iii*) A comprehensive malaria control scheme should be planned and enforced in those training centres which could not be closed or moved.

The Jungle Warfare Training School, Managade, Mysore, was one of the centres which could not be closed or moved. Control measures in it are described below to illustrate the problems that had to be faced in such centres. This school was located in a hyperendemic foot-hill terrain where *A. fluviatilis* transmitted malaria during eight to nine months of the year.

The anti-malaria measures employed were designed to afford protection to students during rail journey on joining and leaving the school, inside the school area and during training of jungle patrols. To avoid infection during the rail journey, it was required that students should travel through the malarious sectors only in the day-time. For those who could not avoid travelling at night, protection was to be given by allocating spraying gangs to the malarious stations to spray the station buildings, the neighbouring villages and the night trains. Around the school area, an anti-malaria unit enforced the usual anti-mosquito measures such

as interception of seepages, canalisation of streams, oiling of breeding places and systematic spray-killing of adult mosquitoes with pyrethrum in school building, hutments and neighbouring villages. For the prevention of malaria in the jungle patrols the procedure adopted was to notify the proposed route to the commanding officer of the anti-malaria unit who, thereupon, arranged for the spraying of all villages and huts *en route*, with pyrethrum insecticide, a week before the patrol was scheduled to start. Furthermore, the school training staff and the commanding officer of the anti-malaria unit collaborated in selecting suitable areas separately for day and night patrols and allocated the least malarious ones for night patrolling. All students were required to practise anti-malaria drill throughout their stay in the school and were to avoid villages and small hamlets in the selection of temporary night 'harbours' during jungle patrols. These measures were included as essential parts of the training course.

Malaria Research Organisation.—Throughout 1942 and 1943 the dominant preoccupation had been to develop a malaria control organisation as rapidly as possible, and all efforts were accordingly directed to that end. Such problems of research, as emerged from routine control in the field, were referred to the Malaria Institute of India for laboratory tests and small field trials. Among such investigations may be mentioned the development of a suitable repellent cream, the preparation and testing of watery emulsion of pyrethrum insecticide, assessing the protective value of mosquito-netting of varying weaves and meshes and that of the different patterns of mosquito-nets, veils and gloves.

Towards the end of 1943, however, it was apparent that the researches carried out in the Malaria Institute of India, though extremely valuable, suffered from the serious handicap that the civilian staff of the institute was unable to carry out field investigations under actual military conditions. There was, therefore, an urgent need for creating a separate army malaria research organisation.

At first it was hoped that it might be possible to utilise anti-malaria units for this purpose, but on detailed consideration this proposal was dropped as the investigatory and control sections of these units were so closely integrated that the former could not be detached for prolonged enquiries. It was, therefore, decided to organise new units with establishment and equipment designed exclusively for research work.

Just then the War Office allotted 1 British Malaria Field Laboratory and 2 British Entomological Field Unit to India Command and to the South-East Asia Command respectively. Accordingly the former unit was, on arrival, detailed to Arakan to undertake field research on some of the outstanding problems, detailed plans for which had previously been worked out in the General Headquarters (India).

These arrangements relieved the situation to some extent, but not entirely, as provision had still to be made for initiating a number of important and urgent investigations within India, such as the formulation of malaria control schemes for

the jungle training centres, the evaluation of the newly discovered insecticide DDT under varying local conditions and the development of an effective mosquito repellent. In 1944, therefore, a malaria research organisation, consisting of an Assistant Director of Malariology (Research) at the General Headquarters (India), one Indian central malaria laboratory and two Indian malaria research units, was created. The investigations undertaken by this organisation were, in certain fields, complementary to the research programme of 1 British Malaria Field Laboratory, but the former had the additional advantage that it could cover a wider and more continuous ground than was possible in the forward areas, where a change in the tactical situation frequently cut short crucial enquiries at critical moments.

CHAPTER X

ANTI-MALARIA CAMPAIGN IN 1944

OPERATIONAL SITUATION

Towards the end of 1943, military preparations for offensive action had been completed by both sides, but the Japanese were the first to strike when, early in 1944, they made a determined attack on the Arakan front in considerable force. After some initial successes the attack was, however, effectively contained and, within a few weeks, fighting on this front resumed its previous character of local skirmishes and patrol activity. Despite this failure, the Japanese persisted in their plans and, in March 1944, launched what was obviously a preconceived and complementary offensive action on the Assam front. This attack increased in tempo during the subsequent two months, culminating in the withdrawal of the Indian forces from the Kabaw and Tiddim valleys to Imphal Vale. The scene of battle next shifted to Kohima and the stretch of the Line of Communication south of it. This resulted in the isolation of the Imphal garrison which was thereafter supplied by air lift till July, when it was finally relieved. Japanese attempts to advance northwards on to Manipur Road, however, evoked bitter and prolonged resistance which effectively stopped the drive in that direction. By this time, the Japanese forces had exhausted themselves and a timely counter-attack delivered in July and August caught them completely off their guard. This opened the way for a steady advance which cleared the Line of Communication to Imphal and beyond and, by the end of the year, enabled the Indian troops to be firmly established on the river Chindwin. In the Arakan, a similar co-ordinated offensive ended in the recapture of Maungdaw and the Mayu Range.

MALARIA PROBLEMS

The operational activities in Assam during the first half of the year simplified the work of the anti-malaria organisation, as malaria control commitments decreased considerably after the withdrawal of troops from the long and malarious areas along the Tiddim and Kabaw valleys. Anti-mosquito protection of units and formations engaged in operations on Tiddim Road and those guarding other malarious foot-hill re-entrants around Imphal was, admittedly, a fresh responsibility, but this was a light task compared to the previous effort. Introduction of these measures was further facilitated by the changed character of the operations, as troops were then concentrated in open country on a narrow and readily accessible front, instead of being dispersed over difficult jungle terrain at the end of long and malarious Line of Communication.

Further north, the situation was equally satisfactory from the malaria viewpoint, as the battle of Kohima was fought over a non-malarious mountain-side while the supply line from Gauhati to the front via Manipur Road was effectively protected by the comprehensive malaria control scheme described in Chapter IX.

In the second half of the year, the situation was just the reverse, as the counter-offensive, which began in July, was continued throughout the malaria season, along the intensely malarious valleys of Tiddim, Kabaw and Kale. The anti-malaria organisation was then faced with problems similar to those in Assam during 1942 and in Arakan during 1943, but on a much wider scale. A large force, dispersed over a large area, was then advancing steadily, leaving behind progressively lengthening and ill-defined Line of Communication on which mobile formations were strung out in continually shifting 'harbours' and camps. In such circumstances, a malaria disaster would have been inevitable had it not been for the fact that the methods of control had also, in the meantime, progressed sufficiently to cope with the situation.

In Arakan, after the initial battle which lasted only a short time in the non-malarious season, the malaria control activities soon settled down to normal. There, a particularly favourable feature of the situation was that even the main battle did not disturb the Line of Communication to any great extent, so that it was possible to attend to the deficiencies in anti-mosquito measures around malaria-controlled 'harbours' before the malaria season began.

In the course of observations made during 1943, however, it was noticed that malaria in Arakan had two obscure and somewhat abnormal epidemiological features. In the first place, the seasonal incidence of the disease in the foot-hill sector began earlier in Arakan than in similar terrain in Assam. Secondly, in some areas, a high degree of malaria prevailed even though the local breeding places were apparently unsuitable both for *A. sundaicus* and *A. minimus*. A detailed malaria survey was thus urgently needed in Arakan, and 1 British Malaria Field Laboratory was accordingly instructed to undertake the necessary enquiry in collaboration with the local anti-malaria units. These investigations, which were initiated early in 1944, led to the discovery of two additional carrier species in Arakan, namely *Anopheles jeyporiensis* var. *candidiensis* and *A. philippinensis*.

The former species, the regional prevalence of which was mostly confined to the small hillock areas between the coastal plain and the foot-hills, was encountered in numbers only in night-catches as it rested almost exclusively outdoors in the day-time. This explained why this species had been missed in previous surveys which had relied on day-catches alone.

Although the seasonal incidence of *A. jeyporiensis* was short, being strictly limited to March and April, it nevertheless played a disproportionately important role due to its regional and temporal association with *A. minimus*. For, by commencing transmission early in the year, it raised the gametocyte rates in the local population which enabled the late-comer *A. minimus* to start intensive transmission earlier and to carry heavy infections over a longer period than in regions where the latter species alone prevailed. The disease was, therefore, abnormally severe in these localities, a phenomenon also observed in other places where the seasonal

incidence of one vector species overlapped that of another, such as *A. minimus* and *A. leucosphyrus* in Kabaw Valley.

The control of *A. jeyporiensis* did not, however, present as difficult a problem as might be imagined, as its breeding habitats were practically the same as those of *A. minimus*. Drainage measures taken against the latter species, therefore, automatically eliminated *A. jeyporiensis*. As a consequence, despite the fact that this species was a potential danger over the greater part of the Arakan coast, it gave rise to little malaria during 1944, in the well drained and controlled 'harbours' extending from Chittagong to Bawli Bazar; the only outbreak traceable to it occurring in the locality where it was incriminated as a carrier.

There is no denying the fact, however, that in the military operations of 1943, when effective drainage measures could not be instituted on an adequate scale, it must have been directly responsible for the enormous amount of malaria which prevailed in forward troops in April that year.

As regards *A. philippinensis*, the malaria carrying role of this species in Bengal had been fully recognised, but there was no reliable record of its being a vector also in Arakan. Fortunately, the terrain suitable for its prevalence was limited in extent, being restricted to a small section of the Line of Communication, south of Cox's Bazar.

POLICY AND METHODS OF MALARIA CONTROL

Organisation.—In the first three months of the year, no change was made in the anti-malaria organisation but, after the withdrawal from the Kabaw and Tiddim valleys, a large number of anti-malaria units became surplus on release from the evacuated sections of the Line of Communication. These spare units were accordingly detailed for permanent duty with individual divisions and corps and, to that extent, the original policy of allocating anti-malaria units on a regional basis was modified. Nor was this arrangement altered when the counter-offensive began later in the year, as such units were then needed for organising DDT spraying among forward troops.

Another organisational innovation during the year was the appointment of staff captains (anti-malaria) to the army and Line of Communication headquarters who acted as forward malaria liaison officers and carried out special tasks such as air-spraying of DDT. The post of consultant malariologist was also created in the Headquarters Allied Land Forces, South-East Asia, to which Brigadier M.K. Afridi was appointed on 8 August 1944.

Policy of Malaria Control.—In the first half of the year, the general principles of malaria control remained unchanged both in the forward areas and along the Line of Communication, but in the second half a radical change was brought about by two factors :

(i) The newly discovered insecticide DDT was brought into use on a progressively increasing scale.

(ii) Mepacrine suppressive treatment was demonstrated to confer complete freedom from clinical attacks of malaria if the drug was regularly taken.

The influence of these two factors on anti-malaria policy and on the methods of control under varying operational conditions was so profound that it is necessary to present these two developments chronologically and in some detail.

Preliminary Trials of DDT.—Supplies of DDT for experimental purposes became available towards the end of 1943, when field and laboratory investigations were started with the main object of testing its effectiveness against the Indian species of anophelines in the dosages employed elsewhere, evolving suitable technique for its application under varying operational conditions and developing appropriate spraying equipment. These experiments were undertaken simultaneously by the Malaria Institute of India, 1 British Malaria Field Laboratory, 2 British Entomological Field Unit and Colonel Scharff of the Medical Advisory Division, Supreme Allied Command, South-East Asia.

As a result, considerable data became available by July 1944 which proved that DDT was fully effective against a variety of Indian anophelines. When sprayed on walls, its lethal action persisted for many weeks, a suitable working dose to obtain such a residual effect being one quart of 5 per cent solution of DDT to each 1,000 square feet of wall surface (about 200 mg. per square foot). Air-spraying of DDT was equally lethal to insect life, at the usual rate of three imperial quarts of 5 per cent DDT to an acre, but its effectiveness lasted over a shorter period of 10 to 14 days.

Residual Spraying of DDT.—From these preliminary results, it was concluded that residual spraying of wall surfaces with DDT would, on the whole, be the most desirable regime for general use, particularly in localities where anti-larval measures were in force, as such sprayings would effectively fill certain unavoidable gaps in larval control.

The anti-malaria action of residual spraying, however, depended upon the systematic extermination of mosquitoes resting on the treated walls which prevented them from living sufficiently long to carry the infection to the sporozoite stage. The underlying principle was thus the same as that of pyrethrum spraying but, because of its residual action, DDT applications were only required at long intervals instead of the daily sprayings necessary with pyrethrum.

While fully recognising this advantage, it was necessary at the same time to bear in mind the limitations of residual spraying. For, with this method, spraying operation had to be started at the beginning and continued to the end of the malaria season, if mosquitoes were to be prevented from becoming infective. But such optimum conditions prevailed only in stationary bases, permanent camps or cantonments, where timely and regulated sprayings could be arranged. On the other hand, during the counter-offensive in 1944, when temporary camps had to be laid out in localities where transmission was already in full swing, residual spraying of

the camps could not be relied upon to afford complete protection, as the infective mosquitoes existing in the locality could, on entry, bite and transmit infection before they were killed by the delayed action of DDT on tents or walls.

In these circumstances the alternatives were either to exterminate the entire infective mosquito population by extending spraying operations to all the resting places in the neighbourhood before the camp was occupied, or to create an effective barrier against their infiltration into the camp. The former method was largely impracticable in jungle warfare as habitations and abandoned Japanese fox-holes, which harboured these mosquitoes, were frequently scattered and inaccessible and there was hardly ever sufficient time for exploring and spraying them properly.

It was, therefore, decided to explore the second alternative, for this method, if successful, could be profitably employed not only in temporary camps but also around 'harbours' of isolated troops detachments in whom malaria prevention had hitherto presented a particularly vexing problem. 1 British Malaria Field Laboratory was accordingly detailed to evolve a suitable technique for laying a barrier with DDT, analogous to that tried in America for the protection of small groups of men from the bites of Aedes.

Trials on Barrier Spray of DDT.—The initial trials were carried out in villages near Jessore, in the paddy and jute growing part of East Bengal, where *A. philippinensis* is the principal malaria carrier. These villages were particularly suitable for the trial as the scattered small hamlets therein were located at some distance from each other and could, therefore, be used separately for differential test sprayings.

In one of these hamlets, a barrier was laid by spraying the inside and outside surfaces of walls and ceilings of all the huts and sheds and every feature in the terrain up to 50 yards around it (ground surface, tree trunks and shrubs) with 5 per cent DDT solution in kerosene, at the rate of five gallons to an acre.

Records of day and night collections of mosquitoes made before and after spraying showed that the barrier had produced a 90 per cent reduction in the mosquito population inside the protected zone during the period of observation of three nights, whereas in an untreated hamlet outside the 50-yard belt, no significant change in mosquito density was observed. A visual confirmation of the effectiveness of the barrier was obtained with fire-flies, enormous numbers of which could be seen flying outside the sprayed area but none remained on the wing inside the protected zone, where hundreds lay on the ground in a moribund state. Very small numbers, however, usually managed to penetrate the barrier on the third night.

That this reduction in mosquito population was not caused by the repellent action of kerosene was borne out by observations on another hamlet in which identical treatment with kerosene containing Di-Methyl Phthalate (eight ounces in five gallons) produced no reduction in mosquito density.

Finally, an attempt was made to compare the relative value of residual and barrier spraying and, for this purpose, only the interior surfaces of buildings in yet another hamlet were sprayed at a dosage of about one quart of 5 per cent solution of DDT in kerosene, per 1,000 square feet. The results showed that in this hamlet, the day-catch decreased by 91 per cent, but, unlike the results obtained with barrier spray, the night-catch continued at 40 to 50 per cent of the mosquito population observed before spraying.

Policy Regarding Application of DDT.—The results of these trials were communicated to the Assistant Director of Malariology, Fourteenth Army, who applied the barrier method of spraying to camps in Imphal and in the neighbouring valleys with complete success. In the course of these spraying operations, he took the opportunity to elaborate the technique into a more or less standardised form.

By October 1944, sufficient knowledge about DDT had thus accumulated, on which a well-defined policy concerning its use under varying operational conditions could be based. The main features of this policy were as follows :—

(i) In permanent camps under anti-larval control, DDT was applied as a residual spray to the interior surfaces of buildings, huts and tents once a month at an approximate dosage of one quart of 5 per cent DDT in third grade kerosene to each thousand square feet of surface.

(ii) In temporary camps, where anti-larval measures were impracticable or contra-indicated, DDT was applied as 'area' or 'barrier' spray once a week, or once a fortnight, depending on entomological reaction and availability of labour and stores. In this method, DDT was to be sprayed on :

(*a*) the inside and outside walls of tents, huts and buildings ;

(*b*) ground surface and vegetation inside the camp and to a distance of 50 yards all round it.

Walls, and vegetation received a heavier dosage of DDT than bare ground, but the overall dosage of DDT did not exceed five gallons to one acre.

(iii) Where effective control by barrier spray could not be established in time, DDT was sprayed from aircraft at an approximate dose of three imperial quarts to one acre.

Organisation of Ground Spraying with DDT.—It was soon realised that, unlike the anti-mosquito measures previously employed, DDT ground spraying, if properly exploited, had immense potentialities in that such spraying could be easily and rapidly organised under diverse conditions. Anti-malaria protection through insecticides could, therefore, be extended to operational units much further forward than was hitherto possible. Indeed, the decision to allocate anti-malaria units to individual divisions during the counter-offensive was based on the realisation of this fact.

To ensure a widespread and successful use of DDT under varying operational conditions, however, it was necessary, in the first place, to arrange for adequate instruction in spraying techniques throughout the command. With this end in view, field demonstrations of residual and barrier spraying were set up in different areas which were attended by anti-malaria and hygiene staff officers and commanding officers of anti-malaria units. The last named officers were, in turn, instructed to undertake the training of the spraying squads of units and formations in their respective areas. The procedure laid down was that the first round of spraying in each camp would be carried out by the anti-malaria unit itself, but thereafter the operations would be entrusted to camp spraying squads who would work under the general supervision of the anti-malaria unit.

Secondly, it was necessary to provide for an adequate supply of spraying equipment which, it was anticipated, would be required on a very large scale. Efforts were accordingly made to test out in the field a variety of improvised sprayers constructed from salvaged material. It soon became evident, however, that such improvisations could not possibly satisfy the rapidly mounting demands and that reliance would have to be placed on modifying the existing patterns of anti-malaria sprayers or bringing into use stirrup pumps which were available in large numbers and which could serve the desired purpose if fitted with suitable nozzles.

These trials also brought out the fact that for rear areas the knapsack sprayer was the best all-round machine, both for residual and barrier sprayings, provided the necessary precaution was taken to supplement the stocks of the locally manufactured knapsack sprayers, which were frequently liable to break down, with stirrup pumps or with a quota of sturdy foreign pattern knapsack sprayers.

For unit squads in forward areas, the MISH (Malaria Institute Special Handsprayer), with its nozzle hole locally enlarged to twice the original size, proved an adequate and feasible proposition, although even here stirrup pumps were found more appropriate for spraying relatively static camps, such as headquarters of battalion or company.

Air Spraying of DDT (Preliminary Experiments).—For air spray, the policy adopted was again to utilise only the existing spraying equipment and such aircraft as would be readily available for immediate use. The trial and operational spray flights undertaken in 1944 were, therefore, limited to Hurribombers, fitted with two 250 lb. Smoke Curtain Installations (SCI).

The preliminary experimental flights, carried out at Palam near Delhi and at Cuttack and Chilka Lake in Orissa, showed that this aircraft, flown at a height of 100 feet and at an average speed of 180 miles per hour, gave in each spraying run an effective swathe 200 yards wide and 1,000 yards long, when spraying solution was released simultaneously from both the SCIs. The average dosage cover obtained over this swathe was about three imperial quarts of DDT solution to one acre of ground surface.

Based on these findings, a selected site near Tamu, 2,000 yards by 3,000 yards (nearly two square miles), was next subjected to air spraying in October 1944, partly to protect the troops located there and partly to assess the entomological results after an air spray over a heavily forested terrain. Detailed observations showed that in one catching station the mosquito population, which comprised mostly *A. minimus*, *A. jeyporiensis*, *A. philippinensis*, *A. hyrcanus* and *Culex species*, was reduced to nil up to the twenty-fifth day after spraying, but in three other stations reduction in density to nil or very low numbers lasted only five days, although even in these a general recovery of mosquitoes was not apparent till the tenth to twelfth day after spraying. In the course of dissections, one infected *A. minimus* was encountered in the collections made from a catching station near the edge of the sprayed area, 21 days after spraying.

The conclusion drawn from this experiment was that, at the dosage and with the technique outlined above, the protection afforded by air spray could not, with safety, be relied upon for longer than 14 days and that the operation would then have to be repeated unless adequate ground spraying had, in the meantime, been started.

Organisation of Air Spraying of DDT.—Next, efforts were made to evolve a routine procedure for air spraying. For this, a suitable opportunity presented itself when spraying operations were undertaken between 30 November and 7 December over ten square miles of divisional concentration areas in Kabaw Valley, entailing 180 sorties of Hurribombers.

The successive steps in organising this spraying operation are outlined below as they formed the basis for the procedure which was subsequently followed with only such modifications as were necessitated by the employment of a different type of aircraft.

Firstly, the target area, selected by the divisional commander, was submitted for approval to corps and army headquarters. Secondly, after the necessary approval had been obtained, the dimensions of the selected area, in square miles, were intimated to Group Headquarters, RAF, which allotted a squadron of Hurribombers to carry out the requisite number of sorties, calculated on the basis of approximately 200 yards by 1,000 yards per sortie. Thirdly, the Assistant Director of Malariology, Fourteenth Army, established a direct liaison with the squadron commander to arrange for the actual spraying operations. He placed an adequate supply of DDT solution at the disposal of the squadron and prepared detailed particulars of the target for each day, on a one-inch map. For the latter purpose, the selected area was divided into squares of 1,000 yards by 1,000 yards, each of which formed a convenient spraying unit for Hurribombers sorties. Finally at the time of spraying, two smoke generators were let off on the ground, representing the central axis of the selected square on the map which enabled the six aircraft flying abreast to make an accurate run over the target. Where smoke

generators could not be arranged, spraying was equally satisfactorily carried out on a pin-point map reference.

SUPPRESSIVE MEPACRINE

As already stated in Chapter IX, the general experience in 1943 was that in units under suppressive mepacrine the malaria rates remained low for a period of four to six weeks after exposure to malaria infection, but thereafter the incidence of the disease increased sharply to a very high level.

To determine the underlying cause for this apparent break-through, a simple field test was evolved by I British Malaria Field Laboratory for estimating mepacrine concentration in the urine. The technique, which was standardised after extensive trials, was to compare the fluorescence of a chloroform extract of alkalinised urine with that of standard solutions containing known quantities of mepacrine, in a locally constructed fluoroscope in which the ultra-violet radiation was obtained by passing sun-rays through a slit fitted with Wood's glass. Preliminary field tests with this method showed that, while individual results of a single examination were subject to fallacies, mean values obtained from a group of men were consistent and reliable. Thus it was found that :

(*i*) the majority of fever cases displayed a mean concentration level which was lower than that of the healthy men in the same unit ;

(*ii*) ambulent parasitaemic cases without overt symptoms of malaria were in that part of the unit which had the lowest mepacrine concentration ; and

(*iii*) no nil reading occurred in the urine of men known to be taking mepacrine.

The inference drawn from these tests was that the estimation of mepacrine in the urine might furnish reliable evidence on the state of mepacrine discipline in a unit. To confirm this, an investigation was initiated in April 1944, on three groups of men belonging to a unit in Arakan. The first group received the daily tablet of mepacrine under the personal supervision of the investigatory staff ; in the second group the unit officers exercised the necessary supervision, while the third group comprised men on detached duty in whom daily mepacrine could not always be supervised by an officer.

Mepacrine concentrations in the urine were recorded separately for the men in three groups. Individual results, however, varied over so wide a range that for adequate analysis it was necessary to categorise them as follows :—

Category 1 from 0·0 to less than 0·5 mg. per cent.
Category 2 from 0·5 to less than 1·0 mg. per cent.
Category 3 from 1·0 to less than 2·0 mg. per cent.
Category 4 from 2·0 to less than 4·0 mg. per cent.
Category 5 from 4·0 to less than 8·0 mg. per cent.

From a study of the data, it was apparent that the mean level of mepacrine concentration in the urine, in the three groups, varied according to the degree of supervision over the administration of the drug. The better the supervision, the higher the mean level and lower the proportion of men in categories 1 and 2, i.e. below 1·0 mg. per cent. Indeed, these results were so remarkably consistent in this, as well as in the preceding trials, that the occurrence of a large percentage of categories 1 and 2 in a unit could be taken to indicate either that suppressive mepacrine had just been started or that the supervision was bad.

In planning this investigation, the original intention was to correlate the varying degree of supervision in the three groups not only with the mean mepacrine value in the urine but also with the respective malaria morbidity rates. Towards the end of June 1944, however, it was clear that because of the low incidence of malaria in the unit as a whole (seven cases in three months) enquiry into this aspect would have to be repeated in the post-monsoon months. In the meantime, however, the results of investigations in Australia were received, which proved conclusively that break-throughs in suppressive mepacrine were caused solely by the irregular taking of the drug. It was no longer necessary, therefore, to await additional confirmatory evidence, particularly as the Arakan investigation, though incomplete in respect of malaria morbidity, warranted the same general conclusion.

Thereafter, all efforts were concentrated on devising ways and means for improving the unit mepacrine discipline. The first step taken in this direction was to circulate a directive which incorporated the Australian experimental findings and their achievements in the field. This was followed by personal interviews with the formation commanders in which the consultant malariologist and the anti-malaria staff officers outlined the full implications of the Australian investigations. The point particularly stressed was that, since high malaria rates in a unit under suppressive mepacrine were definitely due to slackness in unit supervisory arrangements, disciplinary action against the defaulting unit commanders alone could bring about the desired improvement. As a result of these interviews, the formation commanders addressed personal letters containing the above viewpoint to the unit commanders, stressing the paramount need for tightening up unit mepacrine discipline.

As a second step, medical officers, conversant with the technique of quantitative estimation of mepacrine in the urine, were formed into mobile teams, which examined men in different units and sent their results direct to the superior commanders. By this means, it was possible to measure the degree of supervision which the unit commanders exercised over mepacrine consumption and to falsify claims of good discipline in units showing high malaria rates. The fact that such a check could be made, greatly assisted the disciplinary drive and had a beneficial influence far beyond the range of units actually tested.

Thirdly, the method of distribution of mepacrine to units was examined and modified where necessary. Owing to the insufficiency of reserve stocks, the RIASC (Royal Indian Army Service Corps) was reluctant to take over the distribution of mepacrine as a part of daily food rations over the entire command but, for units supplied by air-lift, they accepted bulk supplies of mepacrine at army level and undertook its distribution accordingly. On the Line of Communication and in forward areas, where ground transport was in use, distribution continued to be effected through medical channels, although in many divisions, the battalions and companies were supplied through the RIASC.

It would perhaps be pertinent to record that the amount of mepacrine actually consumed averaged about 1·3 tablets per man per day, but demands were calculated on 1·5 tablets, as provision had to be made against local losses of bulk supplies by hostile action or in air-drop, temporary increase in dosages to units showing high malaria rates, advance calls for supplies to leave parties and detachments and replenishment of forward reserve stocks to meet unforeseen demands.

Fourthly, owing to inadequate overall stocks of mepacrine, suppressive treatment could only be enforced selectively amongst troops operating south of Palel and Cox's Bazar, where anti-mosquito measures were not up to the desired standard. As a consequence, a considerable to and fro movement of troops was inevitable between sectors where suppressive treatment was in force and those where it was not. This gave rise to a multitude of problems that had to be defined and covered by specific instructions which considerably modified those issued in 1943. For example, it was stipulated that units should remain on suppressive treatment for as long as they were located in the suppressive zone, even during the non-malarious months. Troops returning to non-suppressive areas for any reason for a period of less than six months, similarly, should continue to take daily suppressive mepacrine, but those whose tour of duty covered a longer period should remain on suppressive mepacrine for only 28 days after leaving the suppressive zone.

All movement orders of individuals, parties and units contained an instructional clause laying down the particulars of suppressive treatment applicable to them, which ensured adequate-supervision during the period of transit and entitled the individuals or parties to carry the necessary stock of mepacrine tablets and replenish it from the nearest army medical establishment. Fresh troops, earmarked for duty in operational areas, were placed on suppressive mepacrine two weeks (not one week as in 1943) before entry into areas where suppressive treatment was in force. They were given an initial loading dose of two tablets a day for the first seven days to raise their mepacrine concentration rapidly to the desired level before exposure to infection. This regimen was selected, because it ensured a higher mean mepacrine level in the urine than that obtained either with a course of two tablets of mepacrine for three days or when four tablets of mepacrine were given for three days.

A more rapid rise in mepacrine level could, however, be secured with a course of three tablets for seven days, but this was considered too drastic for routine purposes. It was, however, recommended for patrols and detachments likely to remain away from strict supervision for short periods, since with this dosage a satisfactorily high mepacrine level was maintained for one week in an experimental group, even though they received no mepacrine after the termination of the seven-day course.

MALARIA CONTROL IN FORWARD TROOPS

From the foregoing paragraphs it will be evident that, in the second half of 1944, the introduction of DDT profoundly influenced the outlook on malaria control in the forward troops. For, even in a rapidly moving advance, DDT spraying gangs could readily be organised on battalion or company basis under the general supervision of the divisional anti-malaria units and, where necessary, these protective measures could be supplemented by DDT air spraying. Although it would be rash to claim that malaria infections were thereby entirely eliminated, it is perhaps safe to assert that these activities reduced the chances of infection considerably.

Another revolutionary concept, which completely altered the previous notion of malaria control in the forward troops, was the fact that absolute freedom from overt attacks of malaria could be guaranteed with mepacrine suppressive treatment, provided the drug was regularly taken.

Despite these improvements in the methods of control, it was felt that the problem of protecting small detachments and patrols still remained. These men were, as a rule, outside the orbit of DDT sprayings either on the ground or from the air, and the incidence of malaria amongst them was still dependent upon the effectiveness of supervision over mepacrine administration which, for reasons given in Chapter IX, was extremely difficult to ensure. In the last analysis, therefore, malaria rates in such troops and by extension in the combat units as a whole, depended upon the attitude of the individual towards malaria prevention. While fully appreciating the fact that this attitude could be markedly influenced by military training and junior leadership, actual experience showed that in a newly raised force, engaged on an arduous and nerve-shattering jungle campaign, this influence was apt to lose its effectiveness on men who had been through a spell of active service.

Having regard to the realities of the situation, it was, therefore, considered necessary to evolve a measure of protection which would be more attractive to patrols and comparatively less dependent on individual initiative than suppressive mepacrine.

Investigation of Repellents.—Amongst the possible measures an effective repellent was obviously the most suitable choice, as it had the added advantage of

protecting the user from painful insect bites. The common failing of all the available skin repellents, including DMP (Di-Methyl Phthalate), however, was that they had to be repeatedly applied during the night, which militated against their effectual use in forward areas. A method had, therefore, to be devised which could ensure that the effectiveness of these repellents lasted over at least one night.

With this end in view, trials were made on nets of varying meshes impregnated with DMP (Di-Methyl Phthalate). Initial experiments were conducted on the routine camouflage nets for headgear, having a mesh of 1·9 cm. and a strand thickness of 2 mm., but the results obtained were disappointing as they did not protect for longer than four hours.

Attention was next directed to fish-net commonly used in Bengal which has a mesh of 1·1 cm. ($\frac{3}{8}$ inch) and a strand thickness of 0·5 mm. Tests with these nets proved more successful as, after impregnation at the rate of 0·5 c.c. of DMP (Di-Methyl Phthalate) per gramme of net, they gave complete protection for two nights, under conditions of biting rates as high as 33 anopheline bites and 108 culicine bites in 15 minutes.

It was further observed that the protective action of such repellent nets was lost if they were covered by untreated clothing or if they were hung up as mosquito-nets. The impregnated fish-nets were, therefore, effective only if they were worn as the outermost garment in close contact with the body.

Field Trial with Repellent Fish-nets.—Since the fish-net garments did not interfere either with vision or handling of weapons, they were definitely superior to the current patterns of veils and gauntlets made from mosquito-netting and *khaki* drill, respectively.

It was still necessary, however, to assess their anti-malaria protective value, their durability in the field and whether or not the reaction of the troops was favourable to their use. A field trial was accordingly carried out, from September to December 1944, on 259 men in a training division located in a malarious forest near Saharanpur. These men remained under continuous observation over a complete malaria season and their training involved living conditions similar to those prevailing in the forward areas.

Each man in the experimental group was issued with a calico bag which contained one set of fish-net garments comprising a veil, a pair of oversleeves and a pair of stockings. The set was previously impregnated with DMP by the local anti-malaria unit at the rate of 0·5 c.c. of DMP to each gramme of net, using approximately 3 ozs. of the repellent per set. Reimpregnation was done daily by the men themselves at a formal parade by rolling each piece between the palms previously smeared with four to five drops of DMP. On an average, one 2-oz. bottle of DMP was sufficient for eight to ten days of reimpregnation.

The men wore the net garments from sunset to sunrise and took no other precaution such as skin repellents and mosquito-nets. An equal number of men

in the same company served as a comparison group who did not use repellent nets but used skin repellents and mosquito-nets.

The results showed that the impregnated fish-nets had considerable anti-malaria value and stood up well to rough wear and tear, only 20 per cent being somewhat damaged at the end of the trial, which lasted three months. The men liked the fish-nets and preferred them to ordinary mosquito-nets.

Supplies of Repellent Fish-nets.—On completion of the above field trial, sets of one veil, one pair of stockings and one pair of oversleeves in calico bags became an authorised issue of forward units at the scale of 10 per cent of strength. The manufacture of netting, however, took longer than expected, and it was only in 1945 that an initial supply became available and was issued to troops as described in Chapter XI.

ACTIVITIES OF THE INDIA COMMAND

Policy of Malaria Control.—Developments in methods of malaria control in India so paralleled those in the South-East Asia Command that it would be unnecessary to describe them in detail. As already stated, initial experimental work on DDT was carried out within India Command and action based on these findings was, therefore, taken simultaneously in both the commands. The only difference was that in India, DDT was utilised exclusively for its residual action, as barrier or air spray was unnecessary in the case of permanent military installations. The policy of malaria control in India thus remained unchanged, except that pyrethrum was replaced by DDT for insecticidal sprayings.

Suppressive mepacrine, despite the fresh evidence of its effectiveness, was also not brought into general use in India, as malaria rates amongst the permanent stationary garrison troops could be, and had been, kept at a satisfactorily low level by effective anti-mosquito measures. None-the-less, in certain jungle training centres, where adequate preventive measures were difficult to enforce, suppressive treatment was introduced for the protection of both staff and students. Similarly, in the case of troops intended for, or returning from, the South-East Asia Command, arrangements had to be made all over India to ensure that they received their suppressive mepacrine in accordance with the accepted routine.

Malaria Advisory Committee.—During 1944, the primary functional role of the India Command was to act as a mobilisation and training centre and a supply base for the South-East Asia Command. As such, the closest possible collaboration was essential for developing a closely integrated anti-malaria policy in the two commands. To facilitate this, a Malaria Advisory Committee was formed with the Deputy Director of Hygiene and Pathology, GHQ (India) as Chairman and, amongst others, the consultant malariologists and Assistant Directors of Malariology of both commands as members. Experts from the Malaria Institute of India and other local research organisations were also co-opted for different sessions.

Although nominally advisory, the decisions of this committee were supported by such expert professional and administrative opinion that, except in very rare instances, they readily received the necessary approval. The committee thus played an extremely important role in expediting action on a variety of anti-malaria problems, such as policy on suppressive mepacrine and treatment of malaria, integration of field and laboratory researches on insecticides and mosquito repellents, review of stocks, demands and allocation of anti-malaria stores and equipment to the two commands and revision of anti-malaria establishments and malaria control methods in the light of the prevailing situation and practical experience in the field.

CHAPTER XI

ANTI-MALARIA CAMPAIGN IN 1945.

OPERATIONAL SITUATION

In January 1945, the Fourteenth Army crossed the river Chindwin, overran the Shwebo plain and established a firm line on the western bank of the river Irrawaddy. After a short pause, the latter river was also negotiated at several points, the main objective being Mandalay on the opposite bank, which was captured on 14 March.

Then ensued one of the bitterest and hardest battles of the campaign in the Mandalay-Meiktila region, which ended in a decisive defeat of the Japanese. This opened the way for a twin drive along the Sittang and Irrawaddy valleys, and while the columns were still fighting around Pegu and Prome on their way to Rangoon, a sea-borne force attacked and captured that city on 5 May.

In Arakan, the islands of Akyab and Ramree were captured in the first week of January. The latter island was, thereafter, used as a forward base for landing operations in the adjoining mainland which led to the occupation of the entire coastal strip by April.

Towards the end of May, all organised resistance in Burma proper had ceased, and such Japanese troops, as still remained in the Arakan hills and the Pegu Yomas, were effectively dealt with whenever they attempted to fight their way out. More sustained efforts were, however, necessary to dislodge them from the Martaban coastal region and the Shan Hill tracts where operations lasted from mid-May to the end of July.

Meanwhile, most of the combat units and formations had been withdrawn from Burma and regrouped in India to participate in the combined operations for landing in Malaya. Just when the plans for this action were nearing completion, Japan surrendered unconditionally on 15 August. The entire scheme was then recast to make provision for mounting multiple expeditions simultaneously to Indonesia, Malaya, Siam, Indo-China, Borneo, Hong Kong and other Chinese ports.

The army, thereafter, adapted itself to its new functions comprising the repatriation of the Allied POW (Prisoners of War) and internees, the maintenance of internal security in the occupied territories and the collection and repatriation of the surrendered Japanese personnel. In most of the territories, these tasks were successfully completed by the end of the year and, thenceforth, the army was progressively reduced to a routine garrison force.

PROBLEMS OF MALARIA CONTROL BEFORE JAPANESE SURRENDER

The problems of malaria control before and after the Japanese surrender varied so markedly both in character and scope that, for convenience of description, the two phases have been treated separately.

In the pre-surrender phase, the nature of the malaria problems was conditioned by the effects of the offensive resulting in the occupation of Burma. Thus, on the Assam front, this operation reacted favourably on the forward troops inasmuch as fighting in the highly malarious foot-hills around the river Chindwin occurred before the malaria season started there, while subsequent engagements took place in the relatively non-malarious dry central plain of Burma. The final drive from Mandalay to Rangoon likewise entailed little or no malaria risk, as the amount of malaria normally prevalent in the Sittang and the Irrawaddy valleys was negligible.

After the capture of Rangoon, however, these favourable circumstances no longer prevailed as the forward troops were then exposed to extreme infections in the intensely malarious Shan Hills where operations coincided with maximum malaria transmission.

The effect of the advance on the Line of Communication in Assam, on the other hand, was mostly adverse from the very beginning, for control measures had to be enforced along a highly malarious supply route which lengthened progressively. Nor did the opening of Rangoon port ameliorate these conditions, as the inland road and rail communications had been so completely disrupted by operational bombing that troops located in the northern parts of Burma had to be supplied overland from Assam throughout the year.

Nevertheless, there were two redeeming features in the situation on the Line of Communication which lightened the task of the anti-malaria organisation. In the first place, the malaria season in the Kabaw Valley and the rest of the Line of Communication began after mid-March, allowing the organisation sufficient time to develop an adequate malaria control scheme. Secondly, owing to the progressive diminution in the volume of traffic from April onwards, the military installations on the overland communications needing control measures were substantially reduced in number.

The forward troops in Arakan were not as fortunate as those in Assam, as the operational action on this sector took place over areas which were uniformly malarious. In some localities, such as the Ramree Island, there was evidence of active transmission even in January, but on the mainland the malaria season began two months later. The troops were then exposed to extreme infections in the highly malarious foot-hills along the coastal tract.

In contrast, the Arakan Line of Communication was favourably affected by the advance, as, with the capture of Akyab and Ramree, a supply route by sea replaced the malarious overland routes before the onset of the malaria season. As a consequence, many malaria-controlled 'harbours' were abandoned and anti-malaria measures were thereafter restricted to the main concentration centres at Chittagong, Cox's Bazar, Akyab and Ramree. Later when the Japanese were finally dislodged from the Arakan coast, the holding force in this sector was still further reduced resulting in a corresponding decrease of the malaria control commitments.

To summarise, the effect of the offensive operations in 1945 was that on the Assam-Burma front, the forward troops were exposed to little or no malaria risk, but the problems of control multiplied on the Line of Communication whereas in Arakan the situation was just the reverse in that the forward troops had to face severe malaria infections, while on the Line of Communication malaria control measures were needed on a rapidly decreasing scale.

Policy and Methods of Malaria Control.—During the phase under review, the overall policy of malaria control was along the lines laid down towards the end of 1944, the main emphasis of preventive effort being on the proper use of personal protection measures, suppressive mepacrine and DDT spraying. Anti-larval measures were relegated to a secondary status and were continued only in localities where extensive drainage works existed. In the application of the control measures, however, many practical improvements were effected which are particularly worthy of note because they helped considerably in solving the difficult control problems that arose in the post-surrender phase.

Suppressive Mepacrine.—By the end of February 1945, the stocks of mepacrine had increased sufficiently to allow of the extension of suppressive treatment to all troops operating east of the line Gauhati (inclusive), Shillong (exclusive) and Chittagong (inclusive). As a result of this decision, almost the entire stretch of the Line of Communication in Assam and Arakan was brought within the suppressive zone which greatly simplified the procedural routine of suppressive treatment amongst troops moving to and from the forward areas.

The intensive drive to improve the standard of mepacrine discipline in units through the personal interest of the formation commanders and the surprise visits of the urine testing teams was continued throughout the year, which led to a rapid and progressive fall in malaria rates in the command, even after the onset of active malaria transmission. In some units, however, sharp malarial outbreaks occurred which, on investigation, were invariably traced to carelessness in supervision over mepacrine administration.

An important and common feature of these outbreaks was that the high malaria rates in the affected units continued for two to three weeks after they were placed on an increased dose of three tablets of mepacrine daily for five days. Since these units were nearly always in the front line, it was impossible to carry out adequate serial urine examinations to determine whether the persistence of high rates was due to insufficiency of mepacrine dose or to negligence in taking the drug.

On the basis of experience gained in this campaign, it is felt that, in a large mixed force, a uniformly perfect supervision over suppressive treatment is unlikely in all the units. The occurrence of isolated malaria outbreaks in future campaigns conducted in malarious regions cannot, therefore, be ruled out and should be provided against by evolving in peace-time a regime of treatment which would effectively and speedily terminate such outbreaks and would thereby avert an

unnecessary loss of considerable manpower. In the formulation of such a regime, account will have to be taken of the fact that, even at the start of an outbreak, a high proportion of the seemingly healthy individuals in the affected units will have parasitaemia bordering on the pyrogenic threshold, the suppression of which may have to be conceived not in terms of chemo-prophylaxis but of chemotherapeutics. The course laid down in this campaign was based on these considerations, but in the existing circumstances it was necessarily evolved on empirical lines without the aid of a controlled experimental check.

DDT Ground Spraying.—In the first half of 1945, DDT ground spraying was in general use throughout the command, but a special study of barrier spraying was undertaken in the Kabaw Valley and around a section of the river Chindwin, while its feasibility under mobile operational conditions was investigated in the 5th Indian Division. These latter experiences are described below in detail.

Use of DDT in Kabaw Valley.—During the phase under review, activities in the Kabaw Valley were centred chiefly on the construction of an all-weather road which had to be completed before the onset of the main rainy season. For this purpose, a large contingent of labour and Line of Communication troops was first spread out along the valley for initial levelling and earthwork. As the road approached the final stages of construction, the routine followed was to concentrate most of this labour force on one section and then shift it to the next, leaving road maintenance gangs every six or seven miles for minor repairs.

From the malaria control point of view, the fact that the labour camps did not remain in any one section of the road for more than two to three weeks on an average was the decisive feature of the project, for, under such conditions, it was impossible to install anti-larval measures. Nor was it safe to depend on residual spraying because of the insuperable difficulties in the effective treatment of neighbouring villages. Reliance had, therefore, to be placed solely on barrier spraying, and to ensure its timely application. The commanding officer of anti-malaria unit concerned was attached to the Deputy Chief of the Royal Engineers in charge of the project so that he could obtain advance information about the opening and closing of camps and arrange his spraying programme accordingly.

The above arrangements were a complete success and the results obtained with the barrier spraying exceeded all expectations. For although this method had been found effective in rendering camps around Imphal free from insects in 1944, a significant difference was that in the Kabaw Valley these sprayings produced the same effects without any anti-larval measures and, secondly, that their effectiveness was demonstrated in a notoriously malarious area at the height of the malaria season and in the presence of frequent and heavy pre-monsoon showers. This experience, therefore, proved extremely valuable, as it showed that DDT barrier spraying alone could thereafter be safely relied upon to give adequate protection from malaria which greatly facilitated control in the post-surrender phase when anti-larval measures were out of the question with the available resources.

To appreciate the intensity of malaria in the valley and the measure of success achieved in controlling the pre-monsoon, wave of malaria in 1945, figures for malaria and undiagnosed fever per 1,000 men per day for the corresponding months in 1943 and 1945 are presented in Table I.

TABLE I.

Incidence (rate per 1,000 men per day) of malaria and undiagnosed fever in the Kabaw Valley during the months of March to June of 1943 and 1945.

Year.	Rate per 1,000 men per day:				Total malaria casualties (rate per 100 of strength) for the period.
	March.	April.	May.	June.	
1943	1·25	4·40	5·00	4·03	44·7
1945	0·11	0·17	0·21	0·20	2·1

In 1944, this valley was occupied by the Japanese. Data for 1943 was compiled from the weekly situation reports of the 23rd Indian Division which operated in the upper half of the Kabaw Valley that year. Through a misunderstanding, this division did not begin suppressive mepacrine treatment till the first week of May 1943. Consequently, the reduction in malaria rates in 1945 must be attributed to the beneficial effects of chemoprophylaxis as well as of DDT spraying rather than to those of the latter alone.

Trial of DDT in the 5th Indian Division.—Despite the success of barrier spraying in the Kabaw Valley, it was still necessary to determine whether this method could be employed by units under active operational conditions. With this end in view, it was put to test in the 5th Indian Division during its advance from Meiktila to Pegu when the stocks of MISH (Malaria Institute Special Hand-sprayer) in all divisional units were brought up to scale, and men from the different sub-units especially trained in the correct method of spraying. The trial showed that not only was the method feasible in a mobile action but also it was exceedingly popular as no less than 40 per cent of the DDT solution issued to the division was sprayed by the battalion personnel. Indeed, the impression gained during the test was that, given adequate transport for carrying sprayers and DDT solution, there was an unlimited scope for applying barrier spraying under such conditions.

Use of DDT in the riverain section of the Line of Communication.—The routine evolved for the protection of personnel manning the rivercraft, used for carrying stores, is briefly recorded not because it denoted a change in the basic principle of barrier spraying, but because it illustrates the manner in which the method had to be adapted to the somewhat unusual conditions.

The chief source of danger, in the riverain part of the Line of Communication, was the section between Kalewa and Monywa, where villages on the river bank had spleen rates ranging between 24 and 80 per cent. Detachments of an anti-malaria unit were accordingly stationed at three main military centres in this section. Each detachment was made responsible for a specified sector and was allotted a

single screw tugboat to spray once a fortnight the villages and the Inland Water Transport camps on both banks of the river. As an additional precaution, the freight-carrying boats were repeatedly sprayed with DDT and were forbidden to tie up for the night in the vicinity of a village except in an extreme emergency.

Air Spraying of DDT.—Early in 1945, trials were made on the effectiveness of titanium tetrachloride smoke containing 5 per cent DDT delivered from a Hurribomber which showed that although such smoke was lethal both to adult mosquitoes and larvae, the serious drawback was that it was liable to drift and miss the target. Vertical spray of DDT solution in kerosene, therefore, continued to be the method of choice for air spraying during the year. To meet the excessive demands anticipated in 1945, however, large types of aircraft, Liberator VI, were converted for air spraying and were stationed at Digri airfield near Calcutta. The prototype, which became available early in March, was fitted with two bomb-bay tanks, each with a capacity of 330 gallons, but the remaining two machines, that joined the flight later, had four such tanks. Actual spraying was effected through two emission tubes coming off a central collector tank holding 68 gallons of solution to which all the bomb-bay tanks were connected.

With one emission tube working, the discharge rate from the collector tank was 2·83 gallons per second while its filling rate was one gallon per second. Taking the filling and discharge rates into account, the maximum spraying period for each run was about 30 seconds covering approximately 2,400 yards at the aircraft speed of 170 miles per hour. The effective swathe being 200 yards, one run thus covered approximately 99 acres. The total area per sortie was about 2¼ square miles at a dosage of about three quarters of 5 per cent DDT solution per acre.

The main advantage of these aircraft was that it was possible to organise and execute large scale spraying operations more rapidly than with the Hurribombers. Thus, the spraying of the major section of Rangoon was completed on the third day of its occupation and by the end of June 1945, the Liberator flight had carried out spraying over no less than 68 square miles in the operational areas.

Organisation of Air Spraying with Liberators.—The formation commanders submitted their demands for air spraying in square miles one month in advance, giving a rough indication of the area selected, the order of priority and the reasons for such demands. For instance, in Mandalay air spraying was requested to deal with fly nuisance ; in Rangoon it was directed against flies as well as dangue carrier mosquitoes ; while in Akyab and Ramree it supplemented inadequate anti-malaria ground spraying.

These demands were scrutinised at the General Headquarters and those approved were listed and communicated to the Air Command who arranged a suitable flying schedule with the commanding officer, DDT flight. The requirements of DDT solutions were met by an anti-malaria unit posted in Digri, the commanding officer of which also acted as a medical liaison officer settling the daily targets. For the latter purpose, map references alone sufficed, as sprayin

operations with the Liberators could be carried out without the help of elaborate ground arrangements evolved for Hurribomber, as described in Chapter X.

In the final allocation, each formation was allotted a given number of square miles of air spraying within which limit it could modify the previous order of priority. As a further precaution, an adequate number of square miles was kept in reserve so as to provide timely air spraying over new areas occupied on short notice.

It may also be mentioned that the above procedure proved equally satisfactory for air spraying in combined operations where it was difficult to specify definite targets. When, however, these needs were expressed in terms of square miles, the Air Command could work out the required number of sorties, the ground forces could settle ultimate priorities within the limits of their allocations, while the anti-malaria organisation could calculate the necessary amount of DDT solution*.

Limitations of Air Spraying with Liberators.—In the course of the year, certain limitations were observed in the working of the Liberator flight. In the first place, each mission of this flight entailed the crossing of the Bay of Bengal, where bad flying conditions frequently prevailed. Second, even after the bad weather over the Bay had been negotiated, the distances were too great to be sure of the exact state of weather conditions over the target on arrival. Thus, despite the usual precaution to specify alternate targets, no less than eight out of twenty-eight missions of the flight proved abortive in May and June 1945.

The remedy, of course, lay in shifting the flight to within an easy range of all the spraying areas so that the time of arrival at a target could be adjusted to coincide with favourable weather forecasts. With Liberators, however, such a move was not immediately feasible, as these aircraft required elaborate landing and maintenance facilities which were not available in Burma till the second half of August.

Evaluation of Air Spraying.—In the evaluation of air spraying, the difficulty was that these operations were usually undertaken over areas where the anti-malaria organisation could not provide competent observers for making an accurate entomological assessment. Nor could the variation in malaria rates be used for appraisal, as it was impossible to assess the influence of the varying standards of suppressive mepacrine discipline in different units. Recourse was, therefore, had to the method of assessing the value of air spraying by tracing its effects on the incidence of dengue. For this purpose, the most suitable formation was the 26th Indian Division, as it had landed near Rangoon on 4 May and had remained in the city for two days before and for one week after air spraying. On enquiry, it was found that this division had suffered an initial outcrop of dengue cases, which caused a sharp rise in the fever rates in the second week after its entry into Rangoon (*vide* Table II) but that the disease had thereafter stopped abruptly, coincidental with

* For further details *see* "Malaria Control in Combined Operation" p. 458.

the after-effects of air spraying. Nor did dengue or an undue rise in fever rates occur in units that entered the city later, even though the seasonal prevalence of this disease lasts till July. It is realised that the latter units were also protected by ground spraying of DDT, but until such measures were fully established, air spraying must have given a considerable amount of protection.

TABLE II.

Incidence (rate per 1,000 men per day) of malaria and undiagnosed fever—26th Indian Division—21 April 1945 to 9 June 1945.

Week ending.	Rate per 1,000 men per day.	Remarks.
21 April 1945	0·16	
28 April 1945	0·18	
5 May 1945	0·12	Entry into Rangoon.
12 May 1945	0·18	
19 May 1945	0·33	Dengue outbreak.
26 May 1945	0·14	
2 June 1945	0·08	
9 June 1945	0·12	

Anti-larval measures.—In the first quarter of 1945, anti-larval measures were continued in most of the malaria-controlled 'harbours' along the Assam Line of Communication as, despite the forward move of the army units, such 'harbours' were still fully occupied by a large number of civilian and enrolled labour. Later, however, the garrison strength of these bases decreased, and it was then possible to reduce the area under larval control by vacating the peripheral camps.

In Arakan, a similar policy was followed, anti-larval measures being restricted to the progressively contracting perimeters of the military areas in Chittagong and Cox's Bazar.

In the new stretch of the Line of Communication created by the forward move of troops, no anti-larval measures were undertaken either on the Assam or Arakan front, the only anti-mosquito measures employed being DDT barrier spraying. The only exception to this rule was Tamu, where hand operated sluices were used to control breeding in the streams and where drains originally constructed in 1944 were, as far as possible, reconditioned. The sluicing mechanism was designed by Major Pettman, Commanding Officer, Royal Engineer's Section (Anti-Malaria), who made an intensive and excellent study of the engineering aspects of this method of larval control. Even in Tamu, however, anti-larval measures were by no means comprehensive, as in many areas, containing uncleared minefields, mosquito breeding places could not be oiled or drained.

Repellent Fish-nets.—As mentioned in Chapter X, a limited supply of repellent fish-nets became available early in March 1945 when approximately 4,000 sets were issued to different units in the Fourteenth Army for trial during the offensive from Meiktila to Rangoon.

Reports received from the units, formation headquarters and the Operational Research Group, were generally agreed that these nets afforded satisfactory protection from mosquito bites even though the repellent lotion was re-applied every fourth day instead of daily as it should have been. They were declared to be more comfortable, gave a greater sense of security, and a better sense of readiness to use weapons than the mosquito-nets, and were particularly favourably commented upon by the armoured columns, signallers and troops travelling light.

The only valid objection raised against them was that the troops had to use the same clothes for days, on occasions when, for lack of transport, spare clothes were not carried. A recommendation was accordingly made that long-sleeve jerseys and leggings made from the netting be issued as additional items, so that the troops could sleep in such garments when necessary.

PROBLEMS OF MALARIA CONTROL AFTER JAPANESE SURRENDER.

As already stated in the new plan formulated after the surrender, the army had to mount a number of expeditions simultaneously to Malaya, Siam, Indo-China, Java, Borneo, Andamans and Japan. In the order of battle of each of these forces one or two anti-malaria units were included, while in the subsequent build-up, many more units were moved under very high priority. Large quantities of anti-malaria stores and spraying equipment were shipped so that, by about D-Day +27, approximately 30 tons of Di-methyl Phthalate, 113 tons of DDT, 130 knapsack sprayers, 7,000 stirrup pumps, 9,300 MISH (Malaria Institute Special Hand-sprayers) and 33 power-driven sprayers reached these areas over and above the equipment issued as initial unit supply.

The policy laid down for malaria control was that all areas, unless naturally free from malaria, were to be treated as intensely malarious, even though some of the localities were known to have been effectively controlled before the war. This policy was vindicated by subsequent events when it was found that the Japanese forces had neglected to maintain the elaborate control projects in Singapore, Kuala Lumpur, Penang, Port Swettenham, Hong Kong and Batavia.

The malaria problems, which these forces had to face, can be divided into those encountered on first landing and those arising during occupation. Since the former problems are likely to be somewhat of wider application and interest, they are described in detail under 'Combined Operations'. The problems that arose during occupation, on the other hand, varied with the local conditions, but in most of the localities the following were the dominant issues :—

(*i*) The control of urban areas.

(*ii*) The control of operational formations.

(*iii*) The adjustment of anti-malaria organisation and policy of malaria control to changed conditions.

Control of Urban Areas.—In the control of urban areas the difficulty was that the military installations were mostly located in the congested sections of the town where there were no open spaces to permit the application of the routine barrier

spray. Residual spraying was found equally impracticable as it involved the spraying of the neighbouring houses up to a depth of at least 200 yards which in most countries could only be carried out with the help of a strong military guard. Moreover, these military centres were so numerous and so scattered that even the restricted residual spraying around each added up to an enormous task.

It was necessary, therefore, to evolve a modified method of barrier spraying which, on trial, proved a complete success. In this method, the floor, the ceiling and the inside and outside surfaces of walls of the military centres were first sprayed. Next, the street surfaces of the two blocks of buildings adjoining the centre were subjected to spraying, employing, where necessary, power-driven sprayers capable of throwing jets of spray for treating high walls. Bombed buildings in the neighbourhood received special attention, the plinth area and both surfaces of the standing walls, if any, being thoroughly sprayed. Finally, the barrier was completed by spraying the traffic islands, the parking places, the pedestrian side-walks and shrubbery along the road up to a depth of 50 yards. Road surfaces in constant use by the vehicular traffic were, however, excluded from spraying operations.

Spraying was carried out at the usual rate of one gallon of 5 per cent solution of DDT per acre. With this treatment the anophelines remained absent for long periods, but the more resistant culicines were liable to reappear early. The latter were, therefore, used as an index for timing the next round of spraying, the standard evolved after trials being a total catch of five culicine mosquitoes per night. This might, at first sight, seem an unnecessarily stringent standard but the fact was that the troops, having lived in an atmosphere free from insects, were in any case, prone to undertake spraying immediately on sighting a single mosquito. The adoption of the above standard, therefore, entailed spraying at longer intervals than those actually observed by most of the units.

Control of Operational Formations.—Since the operational formations lived under conditions similar to those encountered throughout the campaign, the application of the routine barrier spraying in their case presented no difficulty. The unit spraying squads working under the supervision of the anti-malaria unit personnel were employed on this task, and in the effort to concentrate attention on a single method of control, no anti-larval measures were permitted. This was necessary, as experience showed that the numbers and categories of men available for anti-malaria work being insufficient wherever DDT spraying and anti-larval measures were combined contrary to instructions, the resultant mosquito control was invariably less complete than that achieved with DDT alone.

Air Spraying.—Both in urban and operational areas, air spraying was employed on a fairly extensive scale. For localities in Burma, Siam and Indo-China, the DDT Liberator flight based on Pegu (Burma) was utilised, whereas for air spraying in Malaya and Java, Dakotas stationed at Singapore and Batavia were employed. In some localities, such as Batavia, air spraying was carried out soon after occupation, but in most other areas, such applications supplemented ground spraying.

Anti-Malaria Organisation.—Towards the end of the year when the army had completed most of its operational tasks and was gradually settling down to the static role of a garrison, it was considered necessary to plan the readjustment of the existing anti-malaria organisation to changed conditions. The following scheme was accordingly proposed :—

(i) In permanent garrison stations, an organisation on the lines of the Indian cantonment scheme was to be developed. In countries, such as Malaya, where trained personnel were available, the change-over from control through anti-malaria units could be readily effected, but in others the necessary staff would have to be recruited and trained.

(ii) Reduction in the number of anti-malaria units was to correspond to the development of the control schemes in permanent garrison stations on the one hand, and to the decrease in the army strength on the other. It was, however, advised that a sufficient number of units be retained to meet the requirements of the operational mobile columns. The same considerations were to apply to retrenchment in the anti-malaria staff appointments, keeping in view the special need for the supervision of malaria control in outlying garrison stations such as Borneo, Hong Kong and other Chinese ports.

(iii) Anti-larval measures in large towns were to be developed by the civilian authorities, the military participation in the scheme being restricted to the control of breeding places in and around the army camps. Once anti-larval control had attained an adequate standard of efficiency, DDT barrier spraying was to be replaced by residual spraying.

(iv) In static troops, the prevention of malaria by anti-mosquito measures was to replace the suppression of clinical attacks by mepacrine. The cessation of this treatment, however, was to be staggered in different units so as to avoid the simultaneous occurrence of a large number of malaria cases.

MALARIA CONTROL IN COMBINED OPERATIONS

The combined operations described below relate to the landing of XXXIV Indian Corps on the coast of Malaya at Penang, Morib and Port Dickson. Plans for this action had been developed after numerous tactical exercises prior to the Japanese surrender, but actual landing was undertaken after the surrender. None the less, as a precaution against possible Japanese resistance, the operation was mounted in accordance with the original plan except that the covering fire was to be used only if Japanese opposition was encountered. Since the latter contingency did not arise, the operations provided an exceptional opportunity for a detailed study of the anti-malaria plan.

The sequence of operational events from 9 September (D-Day) onwards was as follows : Landing began at Morib where HQ 25th Indian Division, divisional troops and 74th Indian Infantry Brigade disembarked on D-Day and D-Day+1. The remaining two brigades (51st and 53rd Indian Infantry Brigades) of this division landed there on D-Day+2 and moved inland by D-Day+4, but the 74th Indian Infantry Brigade remained in the beach-head area up to D-Day+13.

In the meantime, HQ XXXIV Indian Corps disembarked at Penang on D-Day+1 and completed the landing on D-Day+3, while the second division (23rd Indian Division) of the corps landed at Port Dickson on D-Day+3 and moved off to Seramban, Malacca and Tampin areas by D-Day+4.

Anti-Malaria Plan.—As already mentioned, the anti-malaria scheme, as a part of the overall plan, had been evolved over many months, but it assumed its final shape only in July 1945. Measures of malaria control which could be utilized in this operation and for which full provision was made were suppressive mepacrine, personal protection measures and DDT spraying. Suppressive mepacrine was to be started two weeks before landing, at a dose of two tablets daily in the first week and one tablet daily thereafter. For training in personal protection measures, anti-malaria drill had been practised by all troops in camps and during field exercises.

As regards DDT, the possibility of air spraying was first explored. Since all the air strips within the flight range of the proposed beach-heads were in the Japanese hands, the use of land based fighters was out of the question while those on aircraft carriers, being fully earmarked for operational tasks, were also not available for DDT spraying. Nor were the Liberators considered suitable for the purpose as they were extremely vulnerable to hostile fire. In these circumstances, air spraying was deemed impracticable till a landing ground had been captured.

Bearing in mind the capacity of the air strips existing in Malaya, the Air Command considered that Dakotas would be the most suitable aircraft and arrangements were accordingly made to convert three of these machines for air spraying. It was estimated that in the initial phases air spraying would be needed on 30 square miles, for which the anti-malaria organisation was to provide 15,000 gallons of 5 per cent DDT solution. Exact targets were left undefined, but it was envisaged that spraying would be needed, *inter alia*, over Klang, Port Swettenham, Port Dickson, Seramban and Kuala Lumpur.

In the absence of air spraying, it was necessary to plan the ground spraying of DDT so that it could be started immediately after landing. This provision was essential as on the intensely malarious coast of Malaya, any delay in spraying would have been disastrous to units with lax mepacrine discipline. Moreover, immediate mosquito control was necessary to prevent dangue infection, as no chemoprophylactic agent could be employed against this disease.

In considering the problem of organising ground spraying, it was felt that the most promising approach would be to utilise unit spraying squads to the fullest possible extent, particularly as trials in the 5th Indian Division had demonstrated their usefulness under similar conditions. Accordingly, HQ XXXIV Indian Corps, which had then taken over the organisation of DDT spraying, issued a directive on 6 August 1945 to all commanders stressing the utmost need for giving an intensive training to unit squads. All units were provided with MISH (Malaria Institute Special Handsprayers) at the scale of 5 per cent of strength and with suitable cans to carry DDT solution in haversacks.

The anti-malaria units were to concentrate mainly on spraying the beach-head installations and the concentration area behind the advancing troops but, if circumstances permitted, they were required to assist the forward units in the organisation of spraying. For this purpose, one anti-malaria unit was to land at Morib on D-Day and a second unit on D-Day+1. At Port Dickson and Penang, one anti-malaria unit was similarly to accompany the 23rd Indian Division and HQ XXXIV Indian Corps respectively on the first day of landing. Three units were to follow on D-Day + 23 and two units on unspecified dates later.

It was recognised that a decisive phase in the plan would be to make an adequate provision for supplies of DDT which, in consequence, received an XX priority in early convoy lifts. DDT was to be carried as pre-mixed solution in the initial phase while the bulk powder and solvents were to follow in later convoys, the total requirements of ground spraying being estimated at 200 lbs. of DDT per 1,000 men per month. Combat troops were to carry their own pre-mixed solution by loading into each unit vehicle a two-gallon tin of DDT solution and two to three MISH (Malaria Institute Special Handsprayers). For replenishment, they were to indent on the beach medical unit until the anti-malaria units could take over this function. The former was accordingly allotted 200 gallons of pre-mixed solution and 200 lbs. of DDT powder. All ranks of anti-malaria units were to carry on their person at the time of landing a two-gallon tin of DDT solution and two to three MISH (Malaria Institute Special Handsprayers) guns apart from the quota carried in the unit vehicles.

In this manner, the total supplies of DDT in phase D-Day to D-Day+3, shown in Table III, were partly carried by the different units and partly arranged as bulk supply. In this table are also included particulars of lifts in convoys up to D-Day+44 after which monthly maintenance supplies were estimated at five tons for barrier spraying and five tons for air spraying and general sanitation purposes.

TABLE III.

Schedule of DDT and solvents for XXXIV Indian Corps.

Period.	Premixed DDT solution (Gallons).	DDT powder (lbs.).	Kerosene (gallons).	Malariol (gallons).
D-Day	2,000	3,000	..	1,000
D-Day+3 ...	8,900	1,500	10,500	4,000
D-Day+8 ...	2,000	4,000	41,000	8,000
Total up to D-Day+8	12,900	8,500	51,500	13,000
D-Day+23 ...		4,000	28,000	8,000
D-Day+31 ...		4,000	34,000	8,000
D-Day+44 ...			34,000	4,000

Execution of Anti-Malaria Plan.—Instructions regarding the use of suppressive mepacrine prior to landing were duly implemented while, after landing, measures of personal protection were rigidly observed. All units carried out DDT spraying efficiently and immediately after landing as planned, and there were no complaints of shortage of DDT or of sprayers.

One of the anti-malaria units landed at Morib, complete with all its stores and equipment, but the second unit failed to implement the standing instructions concerning the transportation of stores with the result that it remained ineffective till D-Day+8. Although the spraying requirements of this sector were successfully met by the first unit, this experience emphasised the inevitable lack of reserve stores in the initial stages of combined operations and the paramount need for each unit to be self-sufficient on landing.

At Port Dickson, the landing occurred without a mishap, and the Deputy Assistant Director of Malariology (Major A.P. Ray) found that all unit camps from front to rear had been effectively sprayed before D-Day+8 when he visited that sector.

Supplies arrived on schedule up to D-Day+3 but were delayed thereafter, stores included in D-Day+8 convoy arriving on D-Day+14 and D-Day+27. None the less, there was no actual shortage of DDT, as, owing to the postponement of air spraying, 2000 lbs. earmarked for it were diverted to ground spraying.

Sufficient kerosene and malariol arrived by D-Day+3 and the antimalaria units began mixing the same day, using heated DDT to obtain rapid solution. Stocks of DDT solution were then stored at Morib and Port Swettenham from which the forward units received their supplies through field ambulances from D-Day+4 onwards. The source of supply prior to that date was the beach medical unit as planned.

Results.—While the achievements of XXXIV Indian Corps in this operation were impressive, it is difficult to say how far the unit spraying programme would have been implemented had the Japanese been able to resist. Even if the favourable circumstances of unopposed landing be discounted, due credit must be given to the fact that the scale of and plans for the handling of stores and equipment from D-Day to D-Day+8 and the tasks assigned to the anti-malaria units had been conceived on realistic lines.

Had full facilities been available, air spray could have been carried out from D-Day+1. The Dakota earmarked for this campaign 'Operation Zipper' was received on D-Day+11, and air spray was commenced from D-Day+12. Although, in view of this delay, spraying could not be carried out as originally planned, it had been possible to arrange spraying operations in Kuala Lumpur, Taiping and Klang.

The lack of timely air spraying was a definite handicap and although it was compensated for by adequate ground spraying in Malaya, it would be unwise to expect that conditions would be equally favourable in landing operations elsewhere. Provision for air spraying in all combined operations conducted in a malarious country must, therefore, be regarded as an inescapable necessity particularly if other mosquito-borne diseases, such as dengue, co-exist in the region of operaions. Even if vertical spraying be impracticable, efforts should be made to incorporate DDT in titanium tetrachloride or other forms of smoke screens planned for tactical purposes. Recommendations to this effect were made, but it is not known whether they were actually carried out in the Malayan operation or not.

As regards the evaluation of the anti-malaria plan in terms of malaria incidence, the figures for the 23rd Indian Division are of limited value as this formation was moved to Java on 1 October. During the weeks ending 15 and 22 September, however, the malaria and undiagnosed fever rates per 1,000 men per day in this division were 0·17 and 0·22 respectively.

Detailed figures can be cited in respect of the 25th Indian Division which are presented in Table IV.

TABLE IV.

Incidence (rate per 1,000 men per day) of malaria and undiagnosed fever—25th Indian Division—15 September 1945 to 20 October 1945.

Week ending.	Divisional troops.	51st Indian infantry brigade.	53rd Indian infantry brigade.	74th Indian infantry brigade.
15 September 1945	0·13	0·11	1·57*	0·24
22 September 1945	0·12	0·37	0·10	0·09
29 September 1945	0·11	0·28	0·15	0·24
6 October 1945	0·09	Separate figures for individual brigades for October are not available.		
13 October 1945	0·20			
20 October 1945	0·15			

* 82 per cent of these cases were later diagnosed as 'upper respiratory tract infection.'

Since the rates in this division compare favourably with the average for the whole command (*vide* Chapter XII) during the same period, it is reasonable to conclude that the control measures employed at the time of landing had succeeded in preventing excessive incidence of malaria and dengue.

MALARIA AS A WEAPON OF WAR

Before closing this section, which brings to an end the narrative of the anti-malaria campaign in the South-East Asia Command, it would perhaps be appropriate to discuss the question of malaria as a weapon of war in the light of actual events.

At the outset, however, it should be clearly understood that the scope of this discussion has been restricted to, first, how far malaria influenced the conduct of war and second, whether malaria was used as a weapon of war, and that the debatable and speculative topic of how malaria could be used as a weapon of war has been purposely omitted.

The events which pointedly demonstrated the influence of malaria on the conduct of war occurred mostly in 1942-43, even though the operational activities in those years were restricted to patrolling and actions with limited objectives, such as the Mayu Range expedition in Arakan. It was then all too common an experience that the formations literally melted away with malarial casualties, particularly if engagements took place in the middle of the malaria season. Thus, the figures for malaria in the 23rd Indian Division quoted in Table I are, by no means, an extreme example of the enormous losses through malaria in patrolling activities.

In the Mayu Range expedition, conditions were even worse. The 9th British Infantry Brigade, which took part in this expedition, had, over a period of eight weeks from 9 March to 7 May 1943, 2,228 cases of sickness against 495 battle casualties out of a total strength of 3,500. In other words, 81·8 per cent of all admissions to hospitals were caused by sickness. A further break down of these sickness figures showed that out of 2,228 admissions, 1,886 were for clinical malaria, i.e. 84·6 per cent of the total sick, while dysentery, the other great military scourge, accounted for only 120 cases or 5·4 per cent.

During the period of eight weeks, therefore, this brigade group suffered a loss of 53·9 per cent of its strength from clinical malaria, despite the fact that it had been on suppressive mepacrine. To appreciate the influence of these rates on the fighting efficiency of the brigade, it should be mentioned that, at the height of the malaria prevalence, this disease was carrying off approximately one battalion strength each week and that it incapacitated 3½ times as many men as were wounded in action, even though the brigade was engaged in two major and a large number of minor actions against the Japanese.

There is no record of how the Japanese troops fared during the same period, but it is almost certain that they must have suffered equally severe losses through malaria. Subsequently, in so far as the South-East Asia Command was concerned, the conditions changed progressively for two reasons. In the first place, comprehensive malaria control measures were organised on the Line of Communication which brought about a marked reduction in malaria cases both among the stationary troops and those in transit. Secondly, through intensive research, malaria in the forward troops was reduced to relatively negligible proportions with suppressive mepacrine and DDT. As a result of these developments, the South-East Asia Command gained complete freedom of operational action even at the height of the malaria season, so that the overall malaria rates did not exceed 3 to 4 per cent of strength per year from the end of 1944*.

In contrast, the Japanese neglected to make a parallel effort and failed to develop either a comprehensive anti-malaria organisation or a uniform malaria control policy. Indeed, from the available documentary records it is clear that even to the end of the war the problem of malaria had received scant attention. As a consequence, the gap between the standards of malaria control on the two sides widened progressively with time so that by 1944, the Japanese forces were still incapable of enduring prolonged operational ventures in the malarious terrain during the malarial months. Thus the fate of the battle around Imphal in 1944 was effectively sealed when, after the onset of malaria season, the Japanese supply line broke down completely through sickness. No one, who has witnessed the heaped up bodies of the sick and dying Japanese soldiers in Tamu and Kabaw Valley in August 1944, can have much doubt on that score.

Similarly, in the offensive of 1945, when the Japanese troops were driven into the highly malarious Pegu Yomas and Shan Hills, malaria must have played an important role in paralysing their efforts to regroup for renewed resistance against

* *See* Chapter XII.

the Burma Army. This was testified by the Japanese medical officers and was borne out by the examination of the Prisoners of War at the time of their capture, a few examples of which are given below to illustrate the conditions prevailing there :—

In the Shan Hills :

Out of 89 prisoners, 18 had fever. In 81 prisoners (91 per cent.), malaria parasites were found in their blood at the time of capture. Amongst the eight negatives, seven gave a history of recent attacks of malaria and only one professed to have escaped the disease.

In Pegu Yomas :

Out of 200 men, the daily average number of men sick with malaria was 10 in April ; it rose to 30 men in mid-June and caused a total of 10 deaths.

From May to July, 16 out of 18 men got malaria with 3 deaths despite the fact that they were placed on two tablets of quinine daily.

From May to July, 50 out of 80 men on daily suppressive mepacrine got malaria with 12 deaths.

Between May and July, everyone in a group of 100 men got malaria with 6 deaths despite taking one tablet of quinine daily.

Turning to the second question, namely, whether malaria was used as a weapon of war, there is no denying the fact that malaria was reckoned to be an important factor in tactical planning. Thus, early in 1943, the safeguarding of troops from malarial infections had a considerable influence on selecting the final line of withdrawal from Mayu Range which, incidentally, committed the Japanese to a highly malarious area. Again in January 1943, the Commander, 23rd Indian Division, conformed his schedule of withdrawal from the Kabaw Valley into the non-malarious Imphal Vale to the seasonal incidence of malaria in the former valley, although unfortunately this schedule was later upset through unavoidable circumstances with a disastrous outbreak of malaria indicated in Table I. Finally, the malaria factor was given due weight in the planning of offensive actions along the Kabaw Valley in 1944 and the Sittang and the Irrawaddy Valleys in 1945.

On the other hand, it is equally clear from a study of other operational actions that it was not always possible to adjust the programme to the exigencies of malaria control. Thus, the attack on the Mayu Range in 1943, the offensive action in the Kabaw Valley in 1944 and the operations in the Shan Hills in 1945 had all to be undertaken at the height of the malaria season, involving extreme malaria risk for the troops.

From the foregoing discussion, it is apparent that while in the South-East Asia campaign there is no evidence of malaria having been consciously employed as a weapon of war, the outcome of many of the battles was profoundly influenced by the enormous drain on manpower through malaria. In planning, full consideration

was given to malaria, but it influenced final decision only if it happened to coincide with the general tactical considerations. In other words, the contribution which the antimalaria organisation made towards the success of the South-East Asia campaign was not so much in influencing tactical moves as in providing a high level of anti-malaria protection which rendered such moves feasible. It would be difficult to conceive how an anti-malaria organisation could ever be expected to play any different role.

CHAPTER XII.

RESULTS OF ANTI-MALARIA CAMPAIGN IN 1942 TO 1945.

For a proper appraisal of the anti-malaria campaign it would be convenient to recapitulate the salient features of the anti-malaria activities from 1942 to 1945 before discussing their effects on malaria rates. For, by minimising cross references to previous chapters, this furnishes a coherent summary in which it should be easy to follow the influence of the different methods of control on malaria rates and to assess their effectiveness under different circumstances.

ANTI-MALARIA ACTIVITIES

In chapters VIII to XI, anti-malaria activities in the South-East Asia campaign have been described year by year but, viewed in retrospect, the progress in anti-malaria measures can be traced through three well defined consecutive phases. These were :—

(*i*) The initial phase when the main emphasis was on spraying with pyrethrum insecticide.

(*ii*) The second phase when anti-larval control played the dominant role.

(*iii*) The third phase when DDT spraying and the rigid application of suppressive mepacrine overshadowed all other methods of control.

The different phases, however, coincided with the malaria seasons rather than with the calendar years as, for several reasons, changes in control methods were usually made in the non-malarious winter months. In the first place, the actual experience of malaria control in the preceding seasons could then be passed in review to determine the need and nature of such changes. Secondly, the anti-malaria routine was not disturbed in the middle of the malaria season. Thirdly, the widely scattered units had then sufficient time to complete the necessary preparations for the next malaria season. The last consideration was particularly important in far-flung commands, such as India and South-East Asia, where allowance had to be made for the inevitable time lag between the introduction and implementation of a change in control methods.

Initial Phase.—During the malaria season of 1942, which constituted the initial phase, the operational conditions were such that only improvised anti-malaria measures were feasible and even these could be employed on a limited scale only. In the operational areas, therefore, malaria control yielded disappointing results. Although these measures could be credited with saving the Line of Communication from a complete breakdown, the disease nevertheless accounted for a high rate of casualties throughout 1942.

In the cantonments, on the other hand, anti-malaria measures were undertaken on a more comprehensive scale and produced immediate and demonstrable effects

in that the sickness rate was maintained at a reasonably low level despite the severe regional malaria epidemic that prevailed in Northern India in 1942 (*Vide* Chapter VIII).

The most outstanding developments in this phase were, however, in the organisational and technical fields, progress in which had far reaching effects on the conduct of the anti-malaria campaign in subsequent phases. Thus it was in the malaria season of 1942 that the anti-malaria organisation attained concrete shape and that the establishment and functions of the anti-malaria units were finalised. The basic policy then developed (and adhered to in later years) was to entrust the execution of control measures to anti-malaria units, each of which operated in its own allocated sector of the Line of Communication, while the direction, supervision and co-ordination of these measures were to be effected through anti-malaria staff officers placed at different command levels. In this manner, a closely integrated system of decentralised control was evolved which was readily adjustable to local conditions and yet possessed a basic structural uniformity that greatly facilitated anti-malaria planning. The only minor change introduced later was that anti-malaria units were allocated to individual divisions for DDT spraying operation in the forward areas. Even this was not a fundamental change but a logical extension of the organisation to facilitate the application of a new method of control.

Technically, it was recognised in 1942 that an effective control plan had to provide anti-malarial protection step by step from the rear to the forward positions in the following order :

(*i*) cantonments and training areas in India ;
(*ii*) road and rail communications ;
(*iii*) installations on the Line of Communication ; and
(*iv*) fighting troops and formations.

In the control scheme, ultimately developed, anti-malaria measures were accordingly designed to meet the specific requirements of the different situations listed above, each of which posed distinctive problems needing appropriate action but all of which had to be dealt with simultaneously to achieve the desired objective. With improvement in resources and knowledge, much progress in anti-malaria technique was registered in subsequent years, but the above basic control policy developed in the initial phase remained unchanged to the end of the war.

Second Phase.—In the malaria seasons of 1943 and 1944, which comprised this phase, the main difficulties arose not so much from the limitation of effort or resources as from the inherent shortcomings of the anti-malaria measures then in vogue. Indeed, in no other phase were the anti-malaria activities so vigorously pursued, entailing the installation of gigantic drainage projects, widespread larvicidal operations and systematic daily spraying with pyrethrum insecticide in all the main troop-concentration areas. Nor was the degree of success achieved altogether negligible considering that it was in this phase that the malaria rates declined sufficiently to constitute a convincing demonstration of the value of malaria control measures.

None the less, the incidence of the disease, though reduced to manageable proportions, still remained much too high, particularly amongst forward troops to whom protection through anti-larval measures could not be extended. Even on the Line of Communication, which provided the most favourable conditions for the successful application of these measures, the results achieved were, in retrospect, not commensurate with the amount of effort and energy expended.

Despite these shortcomings of anti-larval control an important collateral gain in this phase was that, with the reduction of malaria on the Line of Communication, the specific problems of malaria control in forward troops became clearly defined, and it was, therefore, possible to extend appropriate investigations in this direction. A number of crucial enquiries on suppressive mepacrine, DDT and repellent nets were accordingly undertaken, which paved the way for the success achieved in the succeeding phase when uniform control of malaria was effected in all parts of the operational zone.

Third phase.—This phase actually began at the end of the malaria season of 1944, as DDT sprayings and a drive for rigid suppressive mepacrine discipline were initiated in October of that year. From the practical point of view, however, the phase can be regarded as covering only the malaria season of 1945, for it was then that the potentialities of these measures were fully identified and received proper emphasis.

A striking feature of the activities in this phase was that the anti-larval measures, which had hitherto formed the backbone of the entire malaria control scheme, were almost completely displaced by DDT sprayings and were restricted to cantonments and to those operational bases where extensive drainage measures existed. This swing-over could be effected all the more readily, because control through DDT did not require prolonged training of personnel and the slow building up of the ponderous organisation essential for anti-larval measures.

INCIDENCE OF MALARIA.

The statistics of the formations that succeeded each other in the operational areas during 1942 are so inextricably mixed that it is impossible to present adequate collective figures for the entire force. Records of malaria in the Manipur Road Base during the last four months of 1942 are, however, available and are fairly representative of the then existing conditions though not completely so, as control measures were better organised in this base than elsewhere. These figures are given in Table I.

The steep decline in malaria rates during October and November 1942 was occasioned largely by the fact that the returns did not include relapse cases. Owing to multiple infections and superinfections, repeated attacks of malaria were, however, so common in Manipur Road in 1942 that the distinction between fresh infections and relapse could only have been made on an arbitrary basis. As a result, many of the malarial attacks, wrongly labelled as relapses, were excluded from the returns. Moreover, because of the extreme congestion in hospitals and

the evacuation system in 1942, a large number of ambulatory malaria cases were treated in the unit lines, many of which did not appear in the records. The figures for malaria in Table I, high as they are, do not, therefore, reflect the factual position which was perhaps more accurately portrayed by the general impression that placed the monthly malaria casualty rate in the base at between 40 and 50 per cent of the garrison from June to December 1942.

TABLE I.

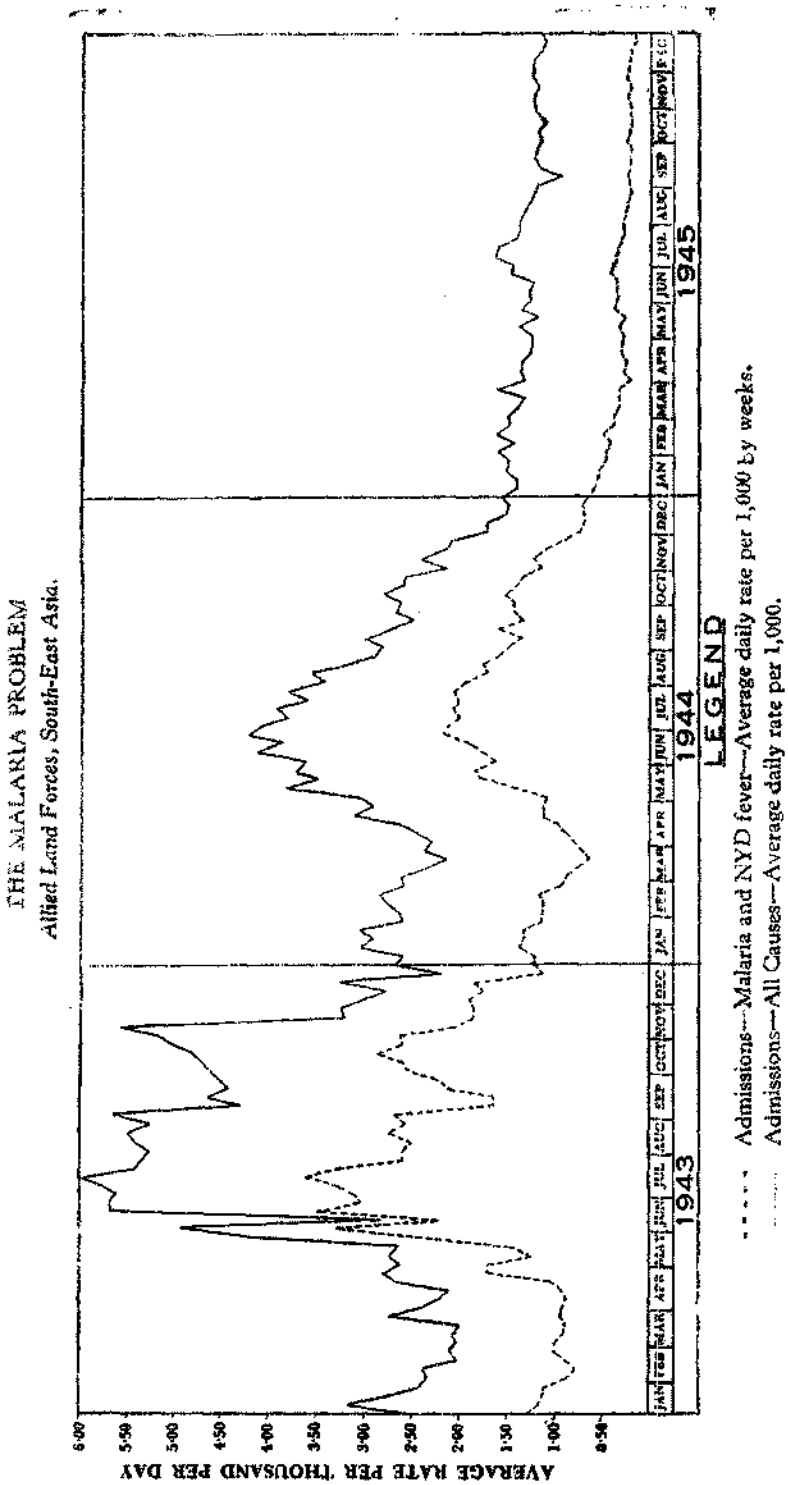
Incidence (rate per 1,000 men per day) in Manipur Road Base, September 1942 to December 1943.

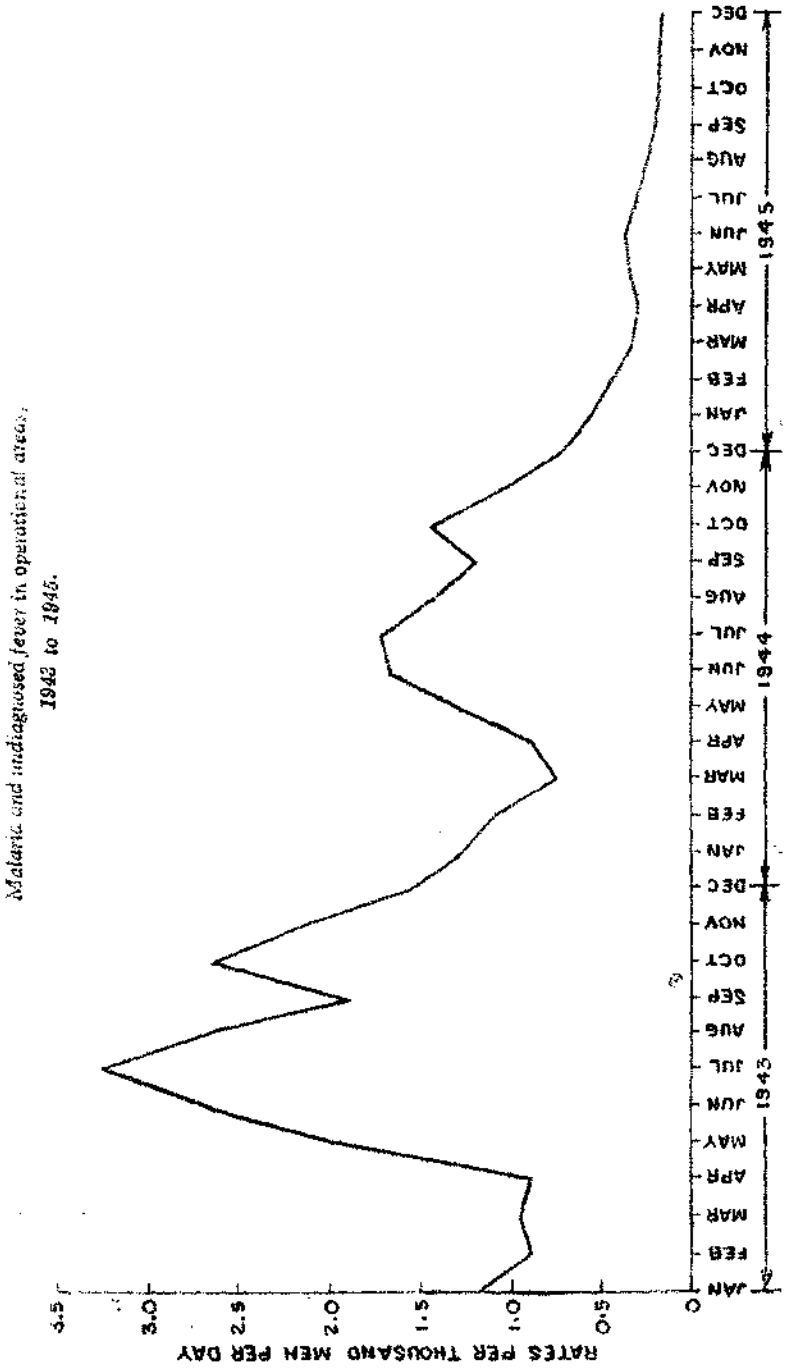
Months.	Rate per 1,000 men per day :	
	1942.	1943.
January	..	4·0
February	..	3·0
March	..	1·5
April	..	0·9
May	..	1·1
June	..	2·4
July	..	2·8
August	..	2·6
September	14·4	2·4
October	9·9	5·3
November	6·1	4·0
December	6·0	2·3

From 1943 onwards, the situation in the operational areas became sufficiently stable to permit a systematic compilation of malaria returns. These are presented in Table II, the figures for 1943 having been culled from the Eastern Army records while those for 1944 and 1945 are extracted from the weekly situation reports of the Fourteenth Army and the Allied Land Forces, South-East Asia, respectively.

It should be pointed out that in Table II the collective head malaria includes both fresh infections and relapses, for, as already stated, an accurate distinction between these two categories of cases was not feasible under the malarial conditions in Assam and Arakan. In the absence of hard and fast rules such a classification, if permitted, would have been coloured by the varying personal opinions of the local physicians and data from the different localities would not have been comparable. For the same reason, microscopic diagnosis was not insisted upon as an essential condition, for, even if this resulted in some of the non-malarial fever cases being returned as malaria, this was preferable to the microscopically negative malaria cases appearing under a camouflaged diagnosis.

A study of Table II clearly brings out the progressive improvement that occurred in the malaria situation during the three years. It should, however, be borne in mind that these figures pertain to a large population scattered over a wide area, and that they do not, therefore, always reflect the high malaria rates in localised epidemics. All the same, the collective results convincingly demonstrate the efficacy of anti-larval control, for even at the height of the transmission season,


THE MALARIA PROBLEM
Allied Land Forces, South-East Asia.
AVERAGE RATE PER THOUSAND PER DAY
6·00
5·50
5·00
4·50
4·00
3·50
3·00
2·50
2·00
1·50
1·00
0·50
JAN FEB MAR APR MAY JUN JUL AUG SEP OCT NOV DEC
1943
JAN FEB MAR APR MAY JUN JUL AUG SEP OCT NOV DEC
1944
JAN FEB MAR APR MAY JUN JUL AUG SEP OCT NOV DEC
1945
LEGEND
Admissions—Malaria and NYD fever—Average daily rate per 1,000 by weeks.
Admissions—All Causes—Average daily rate per 1,000.



Malaria and indiagnosed fever in operational areas, 1942 to 1945.

the rates in 1943 remained between 2 and 3 per 1,000 men per day, whereas in the succeeding year, when these measures were more fully organised, not only did the malaria curve run along at a much lower level but the maximum rate in the year was also brought down to 1·71 which was practically half of the corresponding figure in 1943.

TABLE II.

Incidence (rate per 1000 men per day) of malaria and undiagnosed fever in operational areas—1943 to 1945.

Months.	Rate per 1,000 men per day :		
	1943	1944	1945
January	1·18	1·30	0·57
February	0·90	1·10	0·46
March	0·95	0·78	0·32
April	0·90	0·90	0·30
May	1·92	1·30	0·34
June	2·63	1·65	0·37
July	3·26	1·71	0·31
August	2·68	1·43	0·23
September ...	1·90	1·18	0·20
October	2·68	1·42	0·19
November	2·16	1·03	0·19
December	1·59	0·73	0·17

The value of anti-larval measures is further demonstrated by their effect on malaria rates in the Manipur Road Base (*vide* Table I) where malaria displayed a spectacular fall in the pre-monsoon transmission season of 1943, and even the maximum incidence rates in October and November were much lower than the incomplete figures for 1942. These rates would have been lower still, had it not been for the severe outbreaks of malaria in units that had to be located just outside the range of complete control, because of the continued expansion of the base during the year.

The effect of malaria control on infections in transit troops was more difficult to assess. Some evidence is, however, forthcoming about reinforcement parties which, on their way to Imphal, traversed the entire Assam Line of Communication in June 1943, when the pre-monsoon malaria transmission was at its height. The incidence of malaria in these parties, followed up for one month after their arrival in the non-malarious Imphal Vale, was found to be under 6 per cent. This might appear a high figure but a comparable example, illustrative of conditions in 1942, was that of the 21st Indian Auxiliary Pioneer Battalion, which arrived at Manipur Road in the second half of September 1942, when the post-monsoon transmission was at its height. On the tenth day of its arrival, i.e., prior to the expiry of the average incubation period of locally acquired infections, 770 men in this unit reported sick with malaria and fever out of a total strength of 1,300 men. In other words, the malaria sickness rate in this unit reached a height of 59 per cent in a single day. While some of these cases might have been locally infected, it would

be conceded that a great majority of them must have contracted the disease *en route*. Indeed, it was the tragic story of this unit which emphasised the paramount need for instituting a methodical system of malaria control, covering the entire Line of Communication and for ensuring the maximum possible protection to troops during road and rail journeys.

Turning to the figures for 1945 in Table II, it will be observed that not only was the maximum incidence rate reduced to about one-fifth of the corresponding figure for 1944, but also that the malaria curve ran a straight course without displaying the expected rise in the malaria transmission period. Taking the command as a whole the anti-malaria activities in 1945 had thus succeeded in lowering the incidence of malaria to a point where it was no longer the serious problem it had been in previous years. None the less, even in this phase, a significant amount of residual sickness continued and, while a proportion of this might have been due to non-malaria fevers, the major portion was undoubtedly due to genuine malaria attacks. For actual observations showed that, despite all precautions, stray cases of malaria kept on occurring in most of the units, while in others, with defective mepacrine discipline, the disease often assumed epidemic proportions.

EVALUATION OF DIFFERENT METHODS OF MALARIA CONTROL

Because of the enormous casualty producing potentialities of malaria in the war, it is but natural that this disease was countered by every possible means of protection. In these circumstances, opportunities for conducting adequate experimental trials and for evaluating the different methods of control by orthodox scientific analyses were necessarily limited.

The impressions gained from a close study of these measures, in the course of an extensive control scheme spread over a large territory presenting a variety of epidemiological conditions, have an undeniable value. In the discussion that follows, therefore, opinions are expressed, which though not always supported by altogether adequate evidence, deserve consideration as they are mostly rooted in deeper experience that could not be fully brought to light in this short narrative. In any case, the main objective is not to attempt a final judgement on the value of these measures, but to bring out their strong and weak aspects as actually observed under active service conditions, so as to stimulate further investigations, the need for which was keenly felt during the campaign under review.

Personal Protection Measures.—These measures were common to all the three phases and were in universal use. Theoretically they should have conferred a high degree of protection if rigidly enforced and properly carried out. In practice, however, their effectiveness was observed to vary not only with the state of discipline of the units and the living conditions of the troops concerned, but also with the biting habits and rate of infectivity of the local malaria carrying species. Thus, the main feeding period of *A. minimus* being after midnight, its bites could be readily avoided by the proper use of mosquito-nets. Accordingly, in stationary units located in main camps in Assam, personal protection measures, if rigidly

enforced, were frequently successful. On the other hand, in mobile units, or in stationary units with a high proportion of men constantly on night duty, they proved almost ineffective, as the risk of infection was then greatly increased by the second factor, namely, the excessively high infective rates in *A. minimus.* In areas where the local carriers, such as *A. culicifacies, A. philippinensis* and *A. leucosphyrus,* were inclined to feed in the early evening, troops were exposed to infective bites before they went under mosquito-nets. Fortunately this adverse factor was counterbalanced by the low infectivity rates in these species, particularly the first two. As a consequence, the use of protective clothing and anti-mosquito cream was in general more efficacious against these species than against *A. minimus.*

Of all these measures, mosquito-net gave the maximum protection, provided its use was not hampered by rain, intolerable heat, night air attacks or other unfavourable conditions under which the troops had to live. Protective clothing and anti-mosquito cream, though useful, were commonly observed to have a disappointing influence on malaria rates.

The limitations of the personal protection measures against *A. minimus* were demonstrated most convincingly by the experiences of the 23rd Indian Division in Kabaw Valley in 1943. The commander of this division had taken special steps to provide all units with an adequate scale of protective clothing, mosquito-nets, anti-mosquito creams, gloves and veils, and had evolved and enforced special anti-malaria drill before proceeding to the Kabaw Valley. Despite these precautions, however, the division suffered severely from malaria, as shown in Table I, Chapter XI. The underlying causes of such outbreaks are fully discussed in Chapter IX and it only remains to add that, in situations where troops had to rely exclusively on personal protection measures, the malaria rates were excessively high, both in Assam and in Arakan.

In the closing phases of the campaign the standard of personal precautions undoubtedly improved considerably and, while it is difficult to define their exact value, it is none the less considered that much of the reduction in malaria rates usually ascribed to them was probably brought about by the other concomitant measures such as suppressive mepacrine and DDT spraying.

Spraying with Pyrethrum Insecticide.—This method received a fair trial at Manipur Road Base in 1942, but the results obtained were far from satisfactory (*vide* Table I). In combination with the extensive anti-larval measures, undertaken in the succeeding malaria season, a substantial reduction in malaria was brought about, but it is not certain how much of this was attributable to pyrethrum insecticide sprayings. From general observation the impression gained was that, under active service conditions, this measure appeared to be less effective against a highly efficient carrier like *A. minimus* than against a poor carrier such as *A. culicifacies.* This does not necessarily imply that variation in the malaria carrying capacity was the actual cause but that certain unknown differences in the resting and biting habits of the two species conditioned their infectivity rates as well as their susceptibility to pyrethrum spraying.

Anti-larval Measures.—The measure of success achieved with anti-larval control has already been discussed as well as its spectacular role in demonstrating the value of anti-malaria efforts at a time when the prevalent opinion was inclined to be sceptical.

Anti-larval control is, however, a long range programme, particularly suited to peace-time conditions when given adequate funds, its success can be assured through the medium of an effective organisation. In war, when there is a serious shortage of equipment and trained staff and when the primary need is to bring numerous scattered camps under rapid control, often in the middle of the malaria season, the inherent weakness of these measures became only too apparent. For it takes time to build up the ponderous anti-larval organisation and to bring it up to a pitch of efficiency, which ensures the requisite incessant and expert supervision of each aspect of the complicated larvicidal routine.

The newly raised anti-malaria units, composed largely of hastily trained personnel, recognised these features of anti-larval control after actual experience during one malaria season, and it was only in 1944 that the measures received the necessary detailed attention. This improvement, however, occurred largely in static camps, for, as already stated, anti-larval measures were not feasible in forward areas, which marked another serious limitation of the usefulness of this method of control under active service conditions.

Use of DDT.—Compared with anti-larval measures, the use of DDT produced a rapid and complete control of malaria with considerably less organisational effort. In these respects, the contrast between the two methods was so marked that the decision to give up anti-larval measures was taken not without a sigh of relief.

On the Assam and Burma front, however, DDT was employed in combination with suppressive mepacrine and it was impossible to assess the effect of each of these measures separately. For a direct proof of the effectiveness of DDT, the malaria rates in Ceylon Command are, therefore, more suitable for study, as suppressive mepacrine was not in force there.

It should, however, be pointed out that the two areas were not strictly comparable, for, although the living conditions of the troops were nearly the same in the two sectors, the carrier species were different.

Nevertheless, the steep decline in malaria rates shown in Table III, that occurred after the initiation of DDT sprayings in February 1945, both in the Trincomalee and Kandy areas of Ceylon, provides convincing proof of the effectiveness of this measure, particularly as the steep fall in the rates compares favourably with that observed in Assam, where DDT spraying was combined with suppressive mepacrine.

The low rates in the Kandy area are all the more remarkable considering that this zone experienced a severe epidemic in 1945, owing to deficient rainfall. This epidemic did not effect the sickness rates in the army, but the figures, given in Table IV, for civilian patients in Kurunegala hospital, adequately reflect its severity.

TABLE III.

Incidence (rate per 1,000 men per day) of malaria and undiagnosed fever in Ceylon—November 1944 to October 1945.

Months.	Rate per 1,000 men per day: Trincomalee area.	Kandy area.
November 1944	1·6	0·6
December 1944	2·1	1·0
January 1945	2·2	1·2
February 1945	2·0	0·8
March 1945	0·4	0·3
April 1945	0·3	0·4
May 1945	0·5	0·3
June 1945	0·4	0·4
July 1945	0·2	0·3
August 1945	0·3	0·3
September 1945	0·1	0·2
October 1945	0·1	0·1

TABLE IV.

Average weekly outdoor attendances for malaria at Kurunegala hospital—September 1943 to October 1945.

Months.	Average weekly number: 1943	1944	1945
January	..	328	796
February	..	241	523
March	..	211	413
April	..	161	384
May	..	149	497
June	..	165	566
July	..	150	1,038
August	..	153	1,173
September	262	136	723
October	194	244	490
November	210	521	..
December	306	905	..

The effectiveness of DDT spraying was also strikingly demonstrated in the 3rd (Special) Indian Pioneer Battalion which was employed in the Trincomalee headworks, in the hyperendemic zone of Ceylon, from August 1943 onwards. The personnel of this unit, about 1,500 strong, were not changed throughout this period as the men had been recruited on special terms which did not permit them to proceed elsewhere on leave or on transfer. Prior to February 1945, their camp had been controlled by anti-larval measures and daily spraying with pyrethrum insecticide, but towards the end of that month DDT spraying was introduced, the effects of which are apparent from the figures presented in Table V.

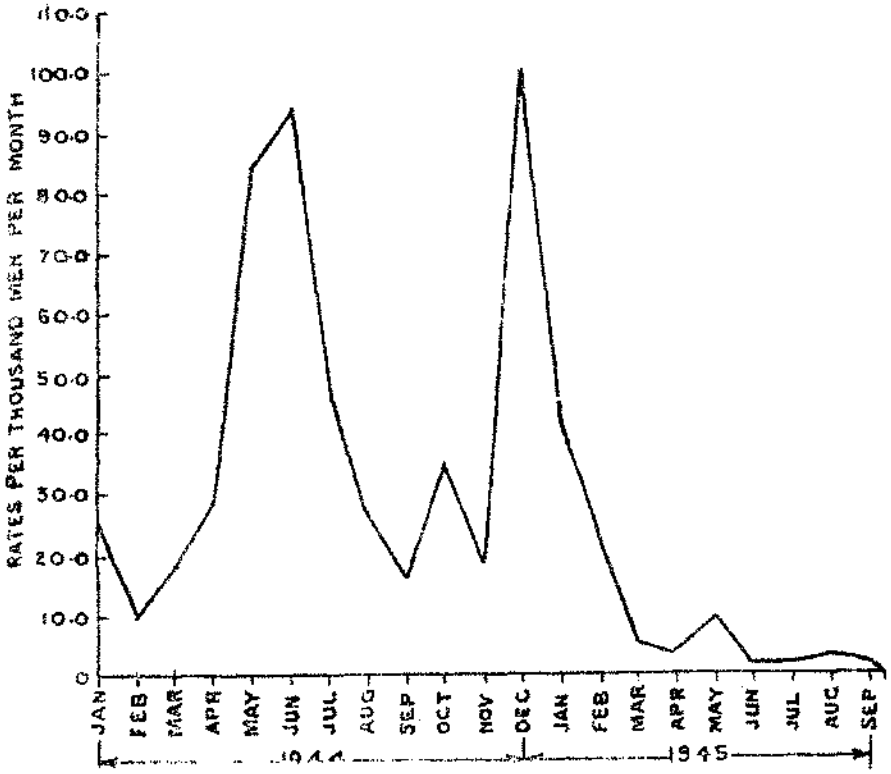
Judging by the evidence from Ceylon, there can be little doubt that DDT spraying must have played an equally important role in the prevention of malarial infections in Assam and Arakan. But, in the absence of more direct evidence, the

achievements in the latter sectors must necessarily be ascribed to the combined effect of mepacrine suppressive treatment and DDT spraying.

TABLE V.

Monthly incidence (rate per 1,000) of malaria and undiagnosed fever—3rd(Special) Indian Pioneer Battalion—January 1944 to September 1945.

Months.	Rate per 1,000	
	1944	1945
January	24·7	42·7
February	10·0	22·0
March	18·7	5·3
April	28·7	3·3
May	84·7	9·3
June	95·3	1·3
July	47·3	1·3
August	26·7	2·7
September	15·3	2·0
October	34·7	0·0
November	18·7	..
December	100·7	..



Malaria and Undiagnosed fever in the 3rd Special Indian Pioneer Battalion.

SUPPRESSIVE MEPACRINE.

Knowledge about suppressive mepacrine grew with experience. In 1942, the view in common with that held elsewhere was to look upon it as an emergency measure suitable for prevention of overt attacks of malaria for restricted periods. It was, therefore, advocated only for operational troops and others to whom protection through anti-mosquito measures could not be extended. Experience in 1943 seemed to confirm this thesis because suppressive mepacrine was usually observed to be effective only for a limited period of four to six weeks. In 1944, however, the Australian investigations and, to a lesser extent, the local enquiry in Arakan, finally established the complete suppressive value of mepacrine for long periods, if regularly taken.

During 1943 and 1944, another factor which seriously restricted the scope of this measure was the continued shortage of mepacrine, the available and 'due in' stocks of which were at any given time, sufficient only for a small number of troops. The zone of suppressive treatment had, therefore, to be adjusted to the stock position so as to avoid periodic stoppages through lack of supplies which would have had serious demoralising effect on the troops. It was only in March 1945 that the supply situation improved sufficiently to permit the extension of this measure to all operational troops, including those stationed on the Line of Communication.

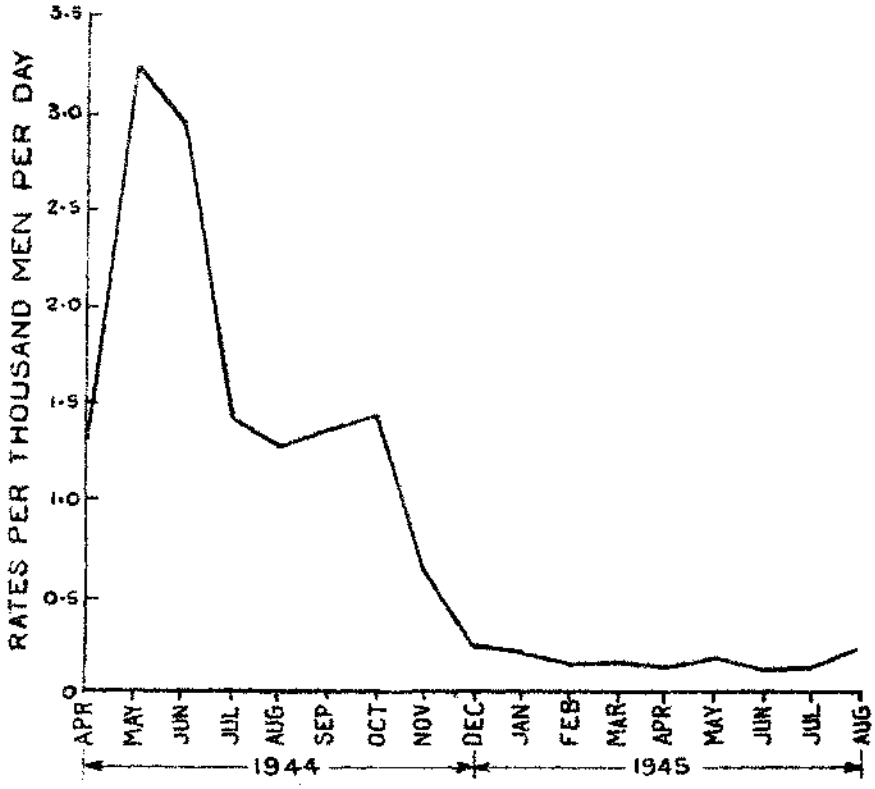
Suppressive mepacrine produced definite results only after the institution of the drive for strict supervision of its administration but, as already stated, this action happened to coincide with the advent of DDT spraying. To assess the actual value of this measure in the field, therefore, it is necessary to pass in review some pertinent examples of its effectiveness, as well as of its apparent failures.

Of the formation in which suppressive mepacrine displayed definite beneficial effect, despite exposure to heavy malarial infections, the most striking records were those of XV Indian Corps and 2nd British Division. Figures, over a protracted period are, however, available only for XV Indian Corps, and are shown in Table VI.

TABLE VI.

Incidence (rate 1,000 men per day) of malaria and undiagnosed fever —XV Indian Corps—April 1944 to August 1945.

Months.	Rate per 1,000 men per day.	
	1944	1945
January	..	0·22
February	..	0·16
March	..	0·16
April	1·29	0·15
May	3·24	0·20
June	2·96	0·15
July	1·42	0·15
August	1·26	0·24
September	1·37	..
October	1·44	..
November	0·67	..
December	0·25	..



Malaria and Undiagnosed fever in XV Indian Corps.

From a study of Table VI it will be seen that, after October 1944 when rigid supervision was enforced throughout the corps, the rates fell precipitously and remained at a low level during the period of high transmission of malaria from April to June 1945.

In the case of the 2nd British Division the monthly rates are not available, but Colonel J.M. Milne, Assistant Director of Medical Services, has traced the effect of cessation of suppressive mepacrine on the malaria rates in the division which gives equally valuable information. His observations were made on the divisional troops and two brigade groups, with a total strength of about 11,000 men, in which mepacrine was stopped in July 1945 after their withdrawal from the operational area to a practically malaria free cantonment in India.

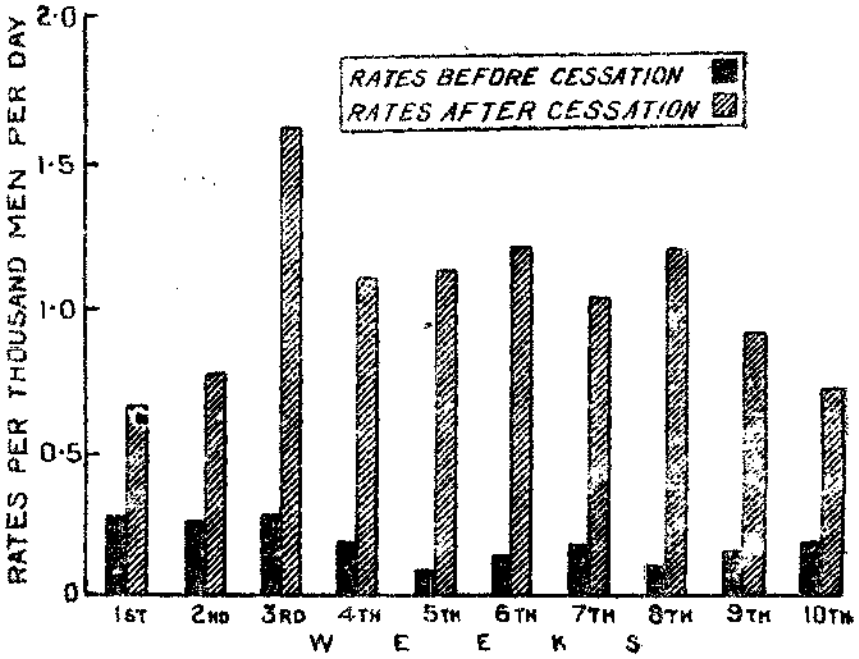
The cessation of mepacrine was staggered on different dates but for compiling results, the time of stoppage was treated as a dead-line and the incidence rates in the division were worked out from it for ten weeks before and for ten weeks after cessation, irrespective of the actual date of stoppage in individual units. The figures thus compiled are given in Table VII.

TABLE VII.

Incidence (rate per 1,000 men per day) of malaria and undiagnosed fever in the 2nd British Division before and after cessation of mepacrine suppressive treatment.

Weeks.	Rates before cessation.	Rates after cessation.
1	0·27	0·66
2	0·25	0·77
3	0·27	1·60
4	0·18	1·10
5	0·08	1·12
6	0·13	1·20
7	0·18	1·03
8	0·10	1·20
9	0·14	0·90
10	0·18	0·72

Malaria and undiagnosed fever in the 2nd British Division before and after cessation of mepacrines suppressive treatment.



The small rise in malaria rates noticeable in the three weeks prior to the stopping of mepacrine was due to a misunderstanding of the staggered system, as the units which had not received orders to stop the drug, relaxed their discipline when they saw other units stopping it. The rise that occurred after stopping, on the other hand, furnished factual proof of the value of suppressive mepacrine. From Table VII it will, however, be seen that, unlike the suppressive period, the

incidence curve in the post-suppressive phase fluctuated over a wide range, rising sharply immediately after the cessation of mepacrine to a peak in the third week and then declining slowly. It is only possible, therefore, to compare the two periods by averaging the rates during the ten weeks before and the ten weeks after the stopping of mepacrine which work out at 0·18 and 1·02 per 1,000 men per day respectively. These figures are of profound importance as they reveal that suppressive mepacrine was holding in check about six times the amount of malaria that was breaking through the suppressive phase. It will further be noticed that, even at the end of observation period of ten weeks, the rates did not come down to the range obtaining in the suppressive period. Indeed, a longer observation on a smaller number of men of this division showed that the rates touched 0·2 per 1,000 men per day only in the sixteenth week after the cessation of mepacrine. The amount of malaria which was suppressed was, therefore, perhaps even greater than that calculated above.

The improvement in the malaria situation, exemplified by XV Indian Corps and 2nd British Division, was not, however, common to all the formations in the command, as, despite the intensive drive for rigid discipline in October 1944 a number of units suffered severely from malaria, the most notable outbreaks being those recorded in the 36th British Division and 19th Indian Division, described below.

The outbreak in the 36th British Division occurred while it was engaged in severe fighting in the intensely malarious jungle terrain around Myitkyina. The figures for malaria and undiagnosed fever, given in Table VIII, show that the epidemic attained its maximum severity in November, when the rates assumed alarming proportions. The entire division was then placed on three tablets of mepacrine daily for five days but, despite this, the malaria rates did not fall till January 1945. Even that fall cannot with certainty be ascribed to the high mepacrine dose as the transmission of malaria also normally slackens in that month.

TABLE VIII.

Incidence (rate per 1,000 men per day) of malaria and undiagnosed fever —36th British Division—October 1944 to March 1945.

Week ending.	Rate per 1,000 men per day.
14 October 1944	1·90
28 October 1944	2·95
11 November 1944	3·50
25 November 1944	5·10
16 December 1944	2·20
30 December 1944	1·10
13 January 1945	0·46
27 January 1945	0·74
10 February 1945	0·80
24 February 1945	1·00
10 March 1945	0·98
24 March 1945	0·56

The course of the outbreak in the 19th Indian Division was almost identical. This division had been suspected of lax mepacrine discipline, which was confirmed by urine examinations in one of the brigades in April 1945. Shortly thereafter, however, it went into action in the highly malarious Shan Hills when the malaria transmission season was at its height, and suffered a severe epidemic reflected by the figures given in Table IX.

TABLE IX.

Incidence (rate per 1,000 men per day) of malaria and undiagnosed fever —19th Indian Division—April 1945 to August 1945.

Week ending.	62nd Indian Infantry Brigade.	64th Indian Infantry Brigade.	98th Indian Infantry Brigade.	Total.
14 April 1945 ...	0·66	1·03	1·25	0·52
28 April 1945 ...	1·40	0·63	1·65	0·68
12 May 1945 ...	1·43	0·39	0·79	0·59
26 May 1945 ...	8·54	1·18	2·74	1·94
16 June 1945 ...	1·67	0·88	1·98	1·04
30 June 1945 ...	2·80	0·18	1·75	1·07
14 July 1945 ...	1·02	0·17	0·46	0·43
28 July 1945 ...	0·46	0·06	0·29	0·26
11 August 1945 ...	0·16	0·05	0·35	0·18
25 August 1945 ...	0·29	0·00	0·11	0·19

A study of Table IX brings out two interesting features. In the first place, the rise in malaria rates in different brigades occurred during different weeks, coincident with the dates of their entry into the foot-hills. Secondly, even severe malaria in individual brigades was not always adequately reflected in the total figures for the division. Indeed, it was only towards the end of May that the rates rose sufficiently to indicate a general outbreak. The entire division was then placed on three tablets of mepacrine daily from 1 to 8 June, which did not, however, secure a substantial reduction in malaria up to the end of June.

From the evidence so far surveyed, it is permissible to conclude that suppressive mepacrine had proved effective wherever the drug had been administered under adequate supervision and that its apparent failure was invariably due to laxity in mepacrine discipline. The extent to which this measure succeeded in suppressing clinical attacks is well illustrated by the rise in malaria rates in the 2nd British Division when mepacrine was stopped. There can, therefore, be no doubt that the steep fall in malaria rates in the South-East Asia Command after October 1944 could, to a considerable extent, be justifiably ascribed to suppressive treatment. At the same time, it would be unwise to ignore the share of DDT spraying in this reduction, particularly considering the results obtained with the latter in Ceylon. The probability, therefore, is that the two factors were complementary; DDT proving more beneficial on the Line of Communication and in headquarters camps, whereas the effectiveness of suppressive mepacrine was more marked in the forward troops.

The evaluation of suppressive mepacrine would be incomplete without referring to certain difficulties that were encountered in its practical application under operational conditions. It was observed that in the hyperendemic areas of Assam and Arakan the margin of safety for troops under suppressive mepacrine was exceedingly small, as overt attacks of malaria usually supervened if the drug was missed for a single day. Under operational conditions, when proper supervision of the daily dose could not always be ensured, particularly in detachments, patrols and composite units, it was, therefore, felt that suppressive treatment would have been much more effective had a drug with more lasting effects than mepacrine been in use. An ideal drug which would probably satisfy many of the exacting requirements of active service would be one which need only be taken once a week but which would remain almost fully effective for another week.

ANTI-MALARIA ORGANISATION.

The anti-malaria establishment in the India Command and South-East Asia Command has evoked adverse criticism chiefly on the score that, since malaria control is an integral part of hygiene, it should not have been necessary to create a separate anti-malaria organisation. This criticism, however, applies only to the developments that occurred in the operational areas, as the cantonment scheme was concerned primarily with the reorganisation of the anti-malaria services that already existed.

To put the case for the India Command in its proper perspective, it is necessary to review the situation at the time when the Assam front was established early in 1942. This event produced a multitude of administrative medical problems but, from the anti-malaria point of view, the dominant consideration was to evolve and to put into the field an adequate organisation as speedily as possible, as the area of operations was not only intensely malarious, but the malaria transmission season had already started.

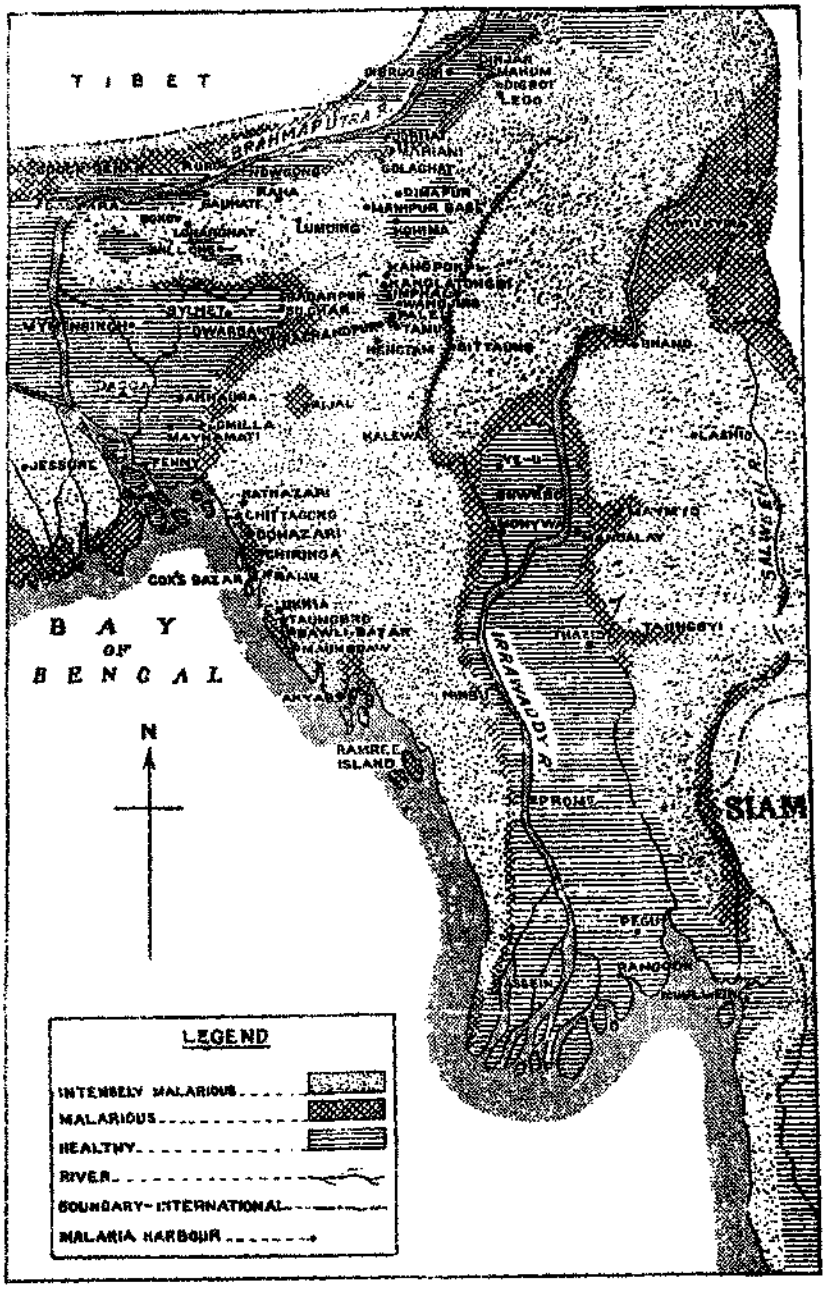
The situation was further complicated by the fact that there was an equal and simultaneous need for general sanitary services, which required the rapid expansion of the hygiene establishment as well. These dual operations had, therefore, to be organised side by side and, although they had many common features, they also possessed certain important divergent characteristics that had to be taken into consideration. Thus, the anti-larval measures, which were then the mainstay of anti-malaria operations, required detailed specialised knowledge which the hygiene officers had to acquire but which could just as easily be imparted to an average medical officer, through a relatively short course of training. It was unnecessary, therefore, to divert the qualified hygiene officers to this task, particularly as they were urgently needed to man the general hygiene services. Secondly, as already explained in Chapter VIII, the field hygiene section as such could not carry out anti-malaria measures without a considerable increase in its establishment and, for reasons given there, it was preferable to expand the anti-malaria units for this

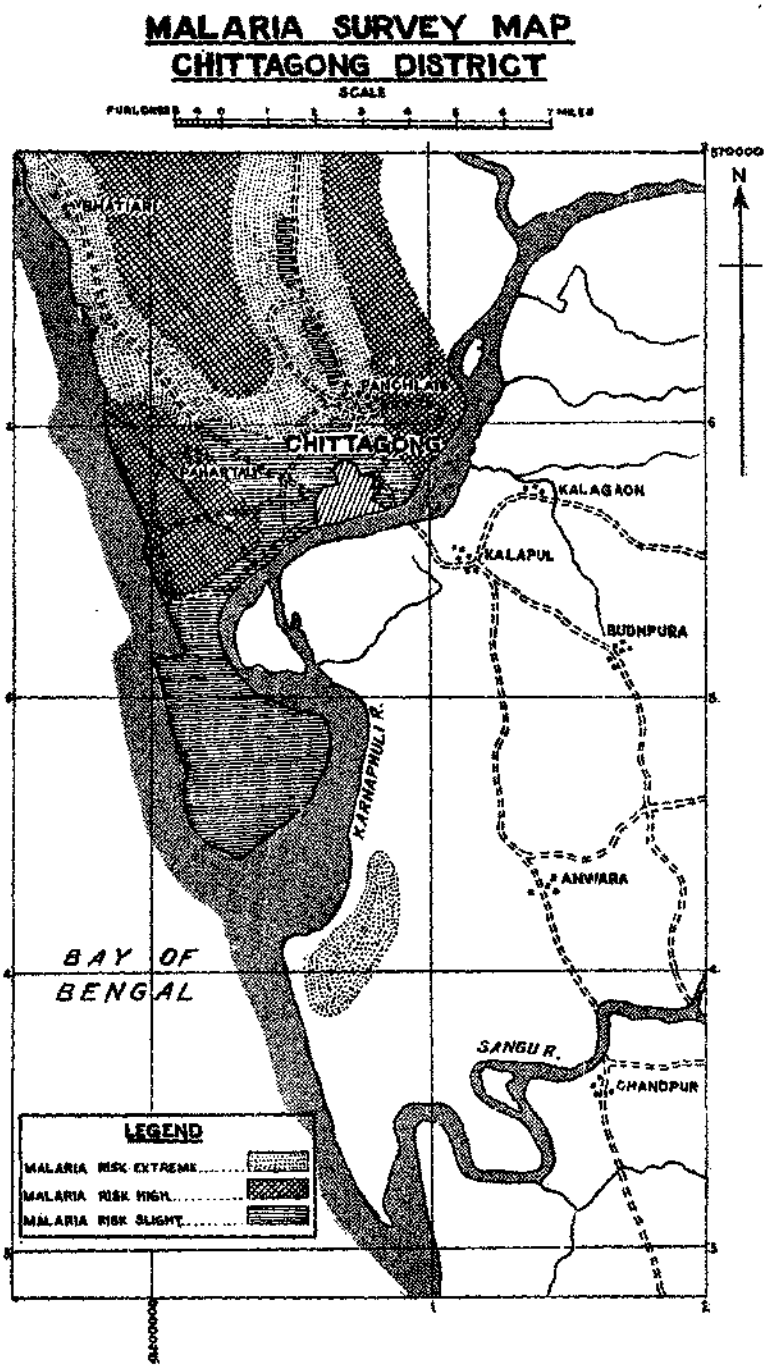
purpose. Thirdly, the rapid increase in the scope of anti-malaria as well as hygiene operations necessitated an immediate decentralisation of control but the magnitude of the task was such that the hygiene staff officers could not undertake the dual responsibility without loss of efficiency in one sphere or the other. The creation of anti-malaria staff officers was, therefore, the only possible solution to the problem.

It will thus be seen that the anti-malaria organisation in the India Command and South-East Asia Command was moulded by the pressure of organisational and technical circumstances before which all theoretical considerations had to give way. Organisationally, the dominant need was to ensure the speediest possible development and this could best be ensured by planning the twin organisations of hygiene and anti-malaria on parallel lines, rather than by expanding the former to cover the functions of both. The semi-independence of anti-malaria field work was, however, offset by bringing the anti-malaria staff officers at the General Headquarters (India) within the hygiene department of the Medical Directorate, thus securing a close integration of anti-malaria and hygiene policies. At no time, therefore, was a complete cleavage between the two sets of activities allowed to occur, as is implied by the critics of the anti-malaria organisation in India.

Technically, the pattern of the organisation was largely influenced by the exacting requirements of anti-larval measures which then dominated all other methods of malaria control. In 1945, when DDT spraying superseded larvicidal operations, there was an obvious need for the recasting of the establishment of anti-malaria units as well as of hygiene and anti-malaria staff appointments but the war ended before the necessary adjustments could be effected and tested in the field.

MALARIA SURVEY MAP
BURMA—ASSAM

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CHITTAGONG DISTRICT
SCALE
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BHATIARI
PANCHLAIS
CHITTAGONG
KAHARTALI
KALAGAON
KALAPUL
BUDHPURA
KARNAPHULI R.
ANWARA
BAY OF
BENGAL
SANGU R.
CHANDPUR
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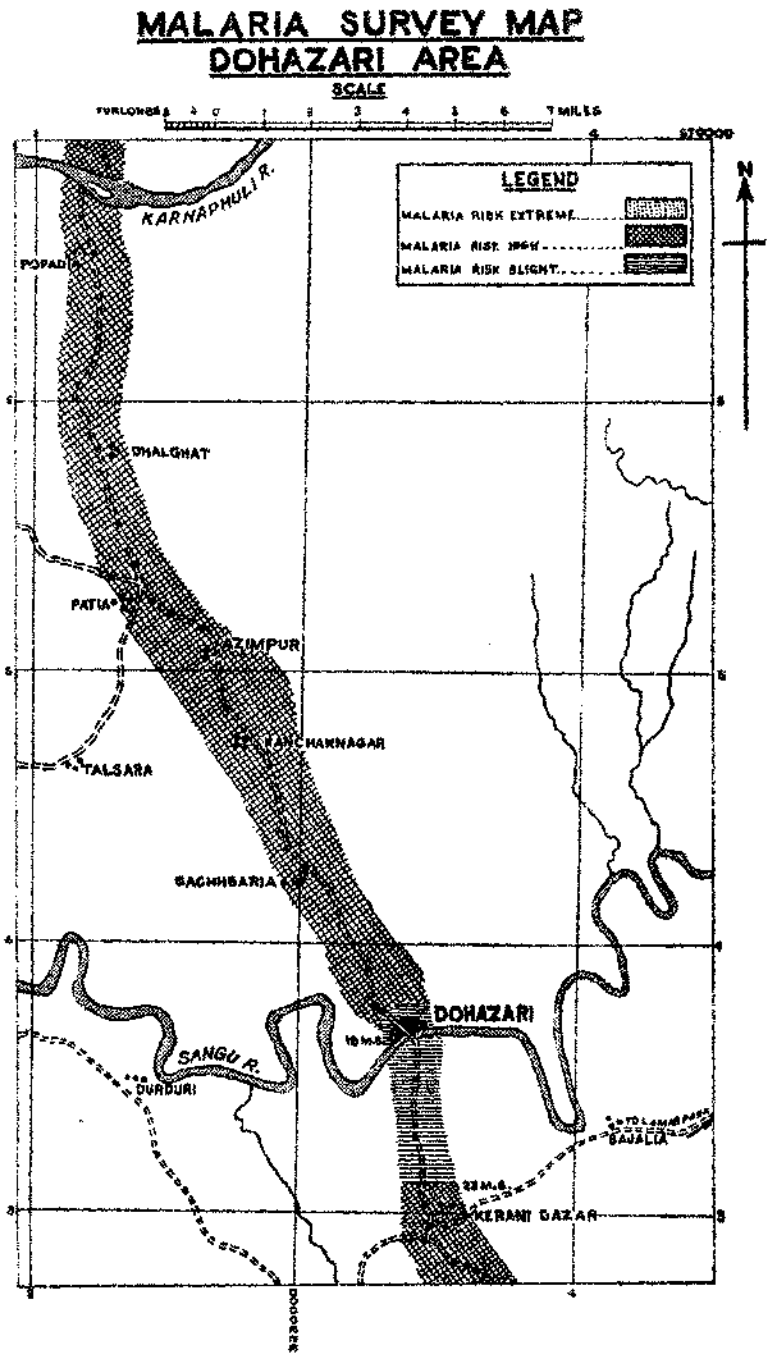
MALARIA RISK HIGH
MALARIA RISK SLIGHT


MALARIA SURVEY MAP
DOHAZARI AREA
SCALE
LEGEND
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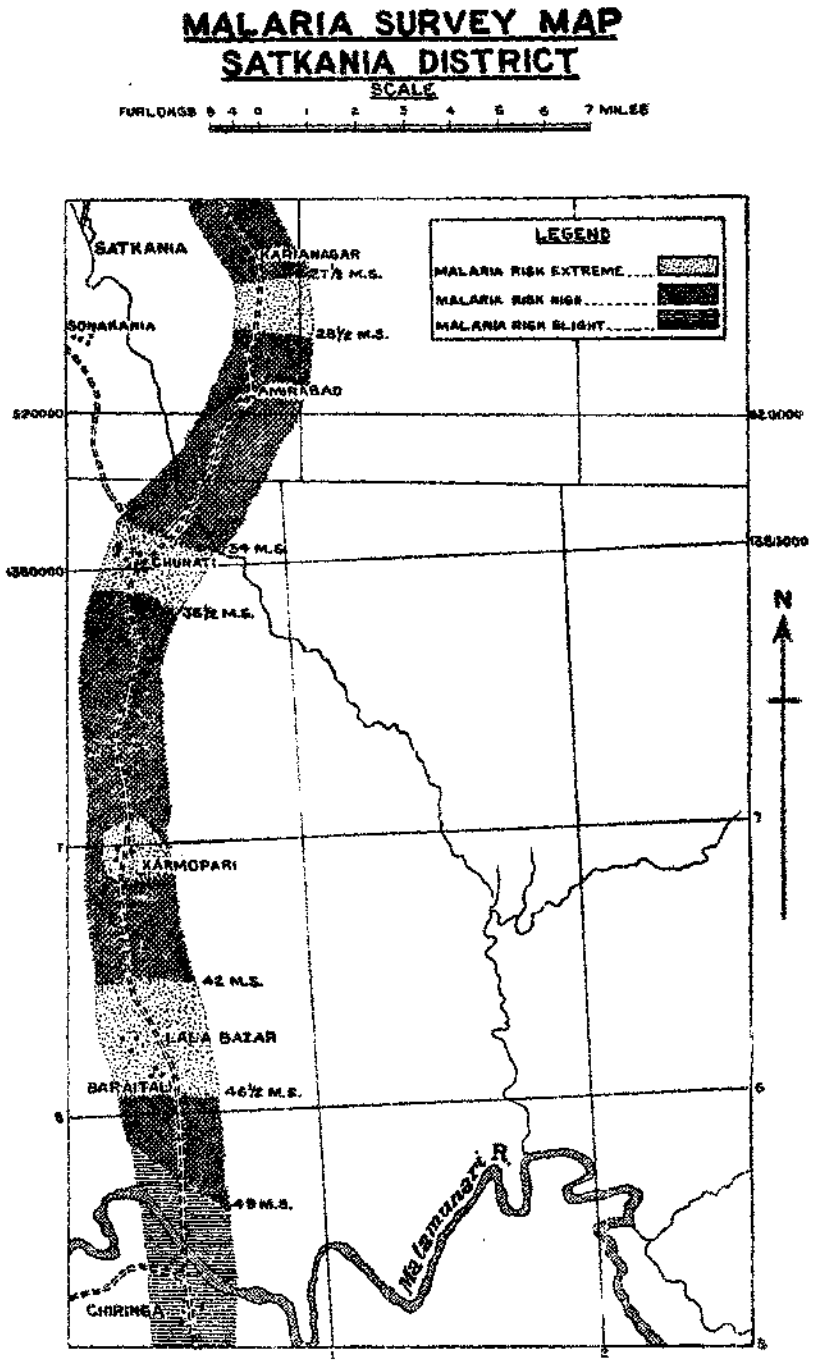
DOHAZARI
SANGU R.
DURDURI
BAJALIA
KERANI BAZAR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MALARIA RISK SLIGHT
SATKANIA
KARIANAGAR
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SONAKANIA
28½ M.S.
RAMRABAD
520000
34 M.S.
CHUNATI
35½ M.S.
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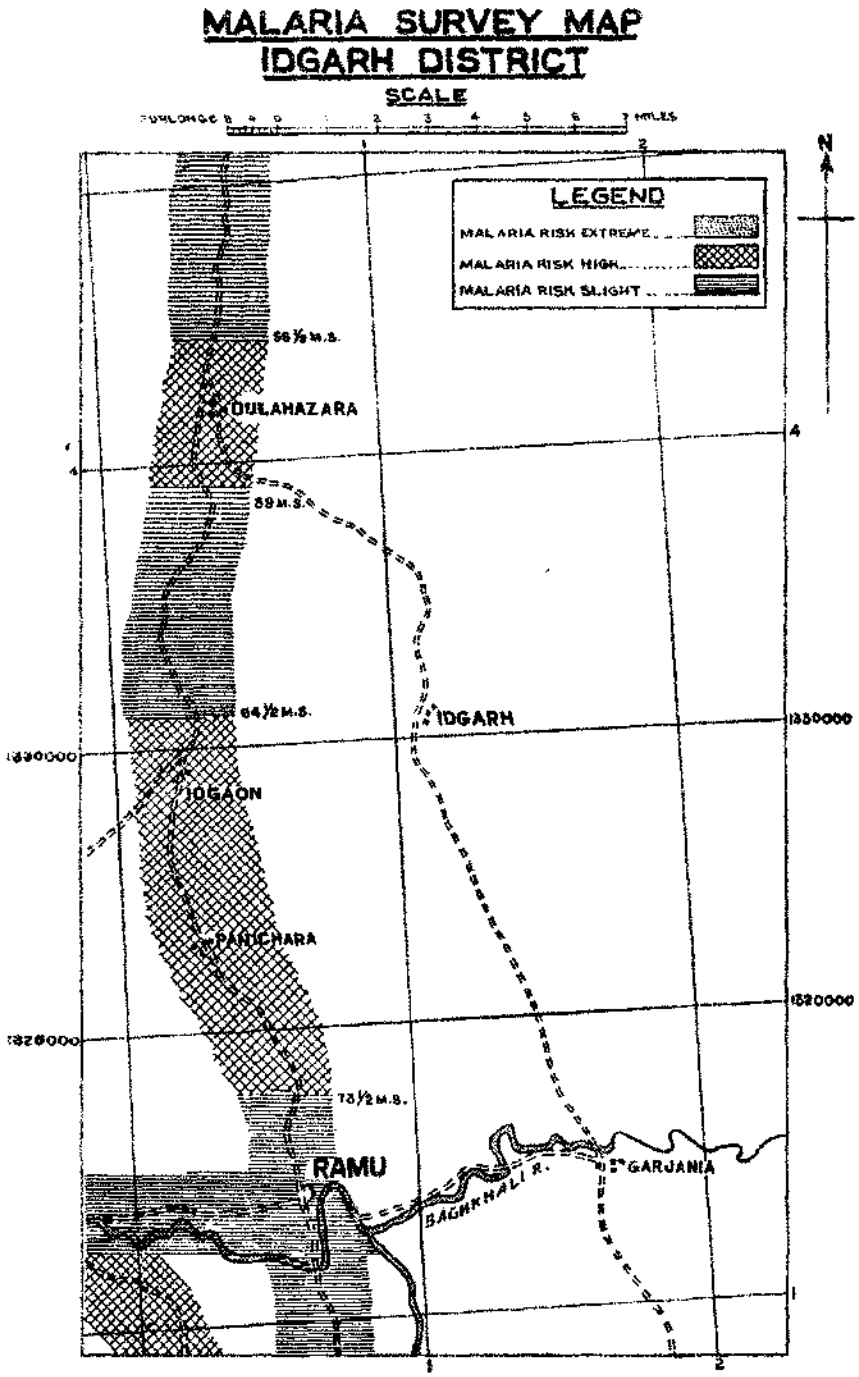
CHIRINGA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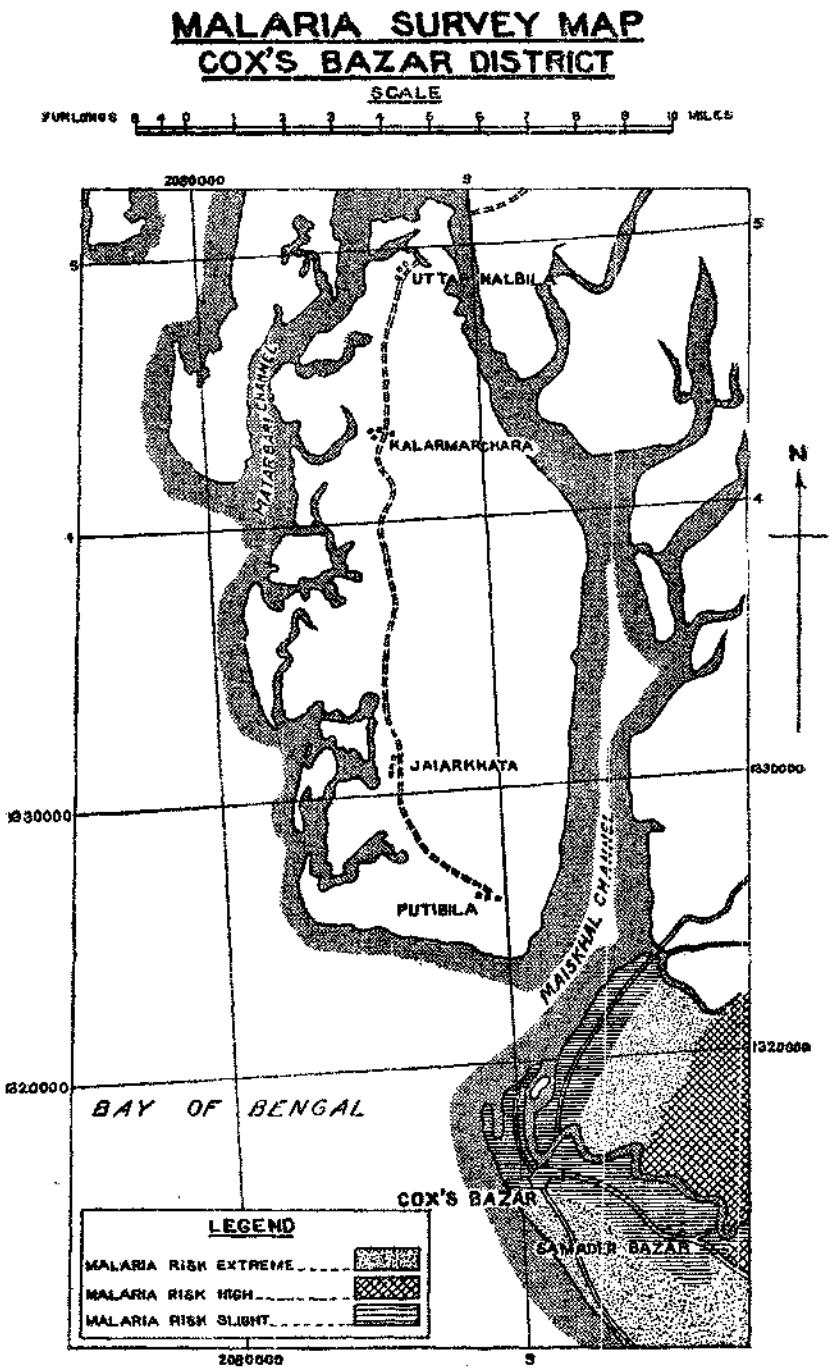
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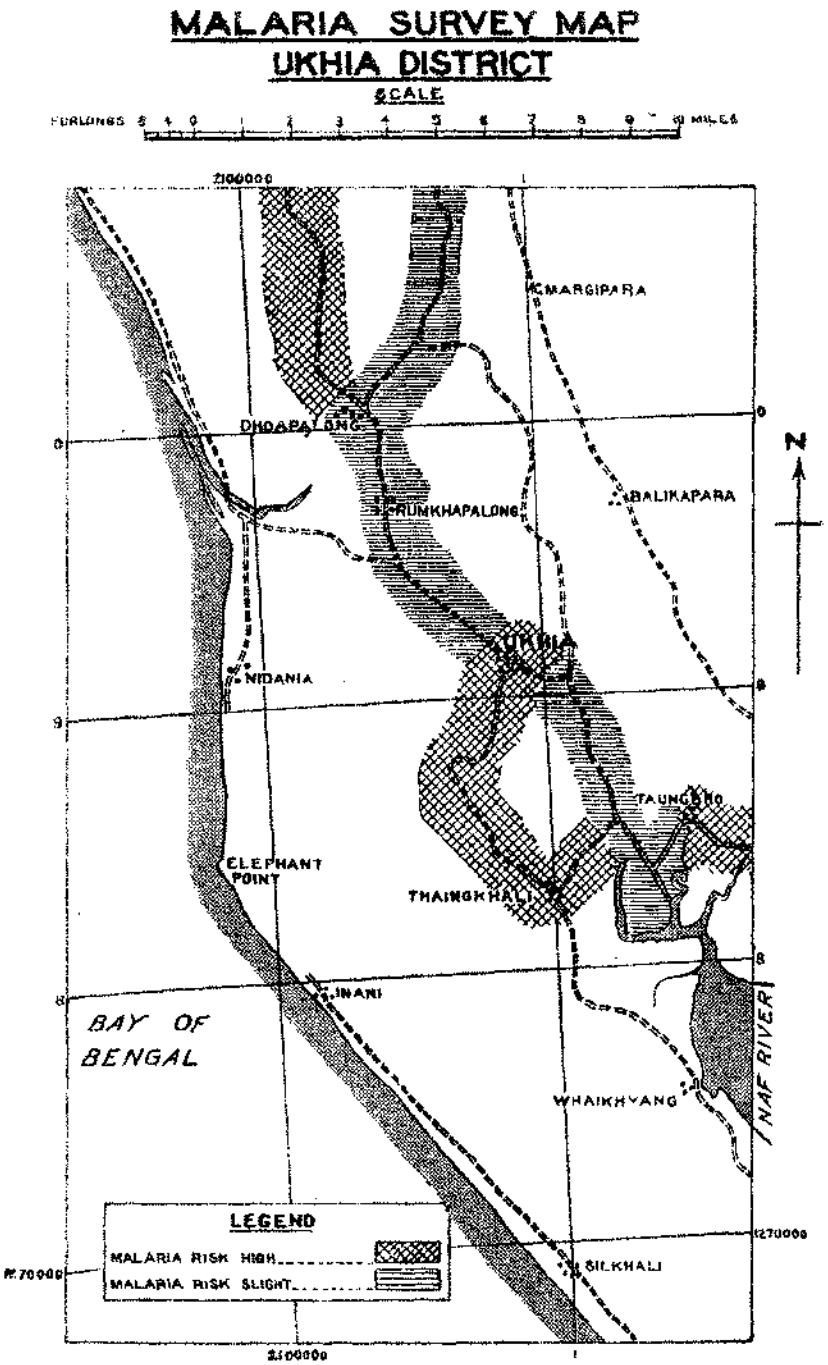
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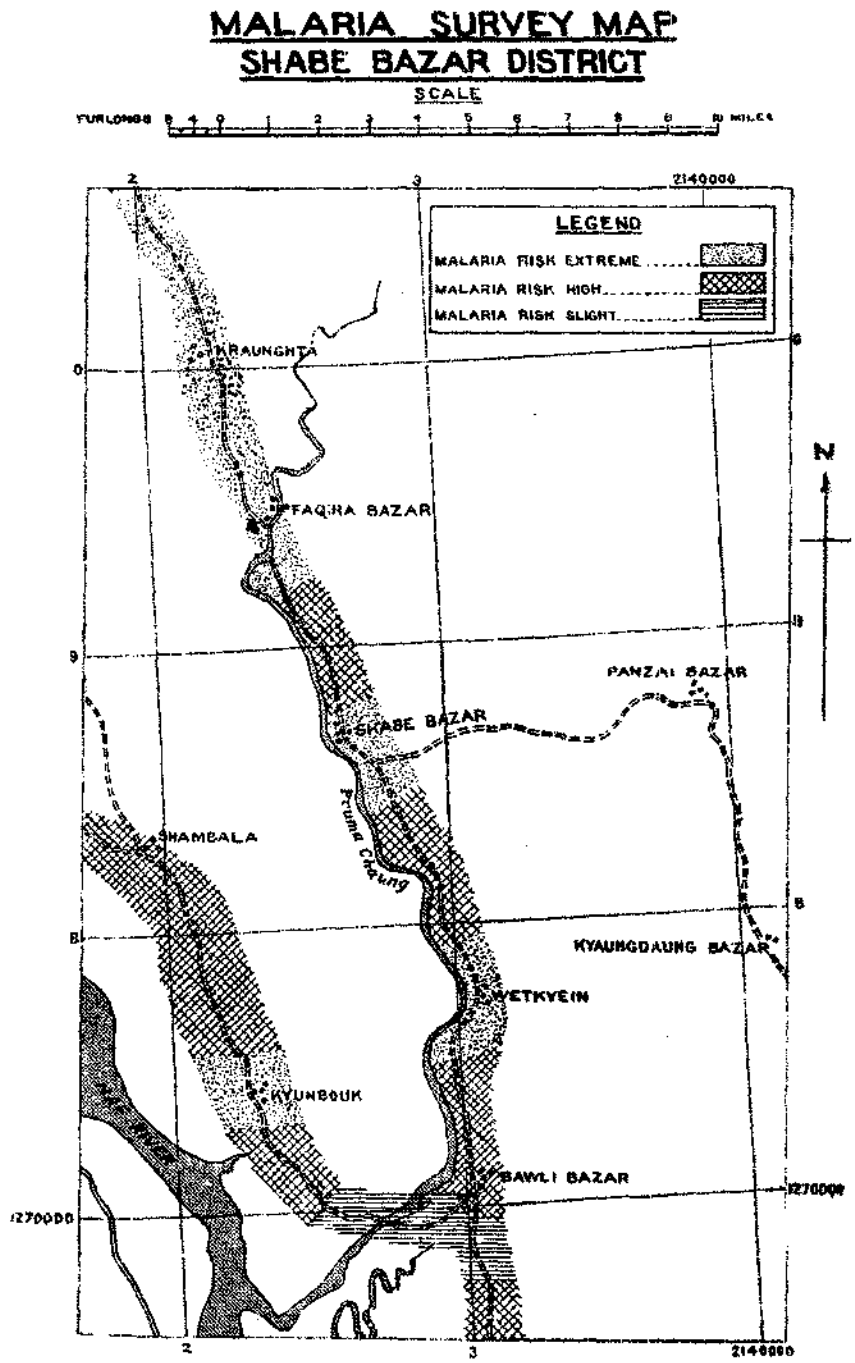
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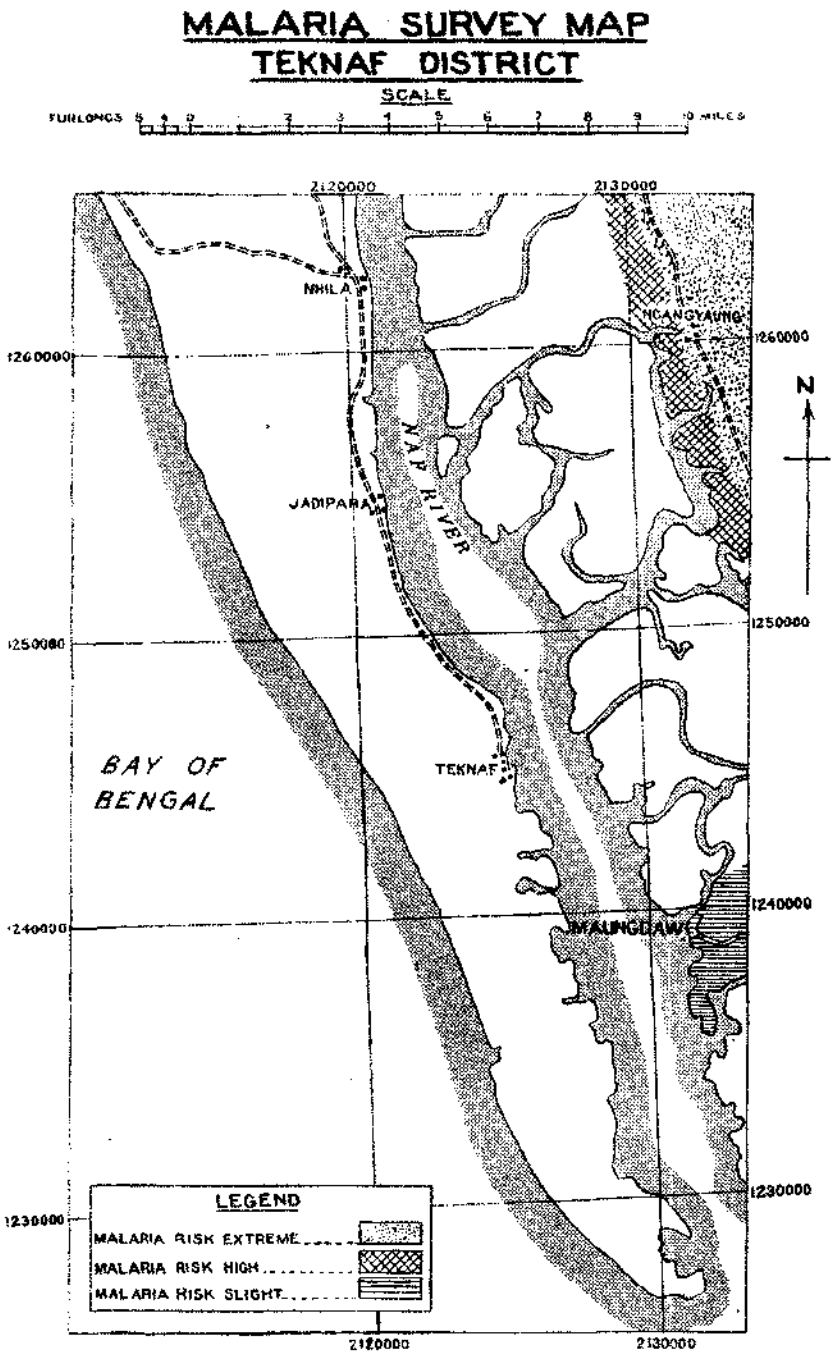
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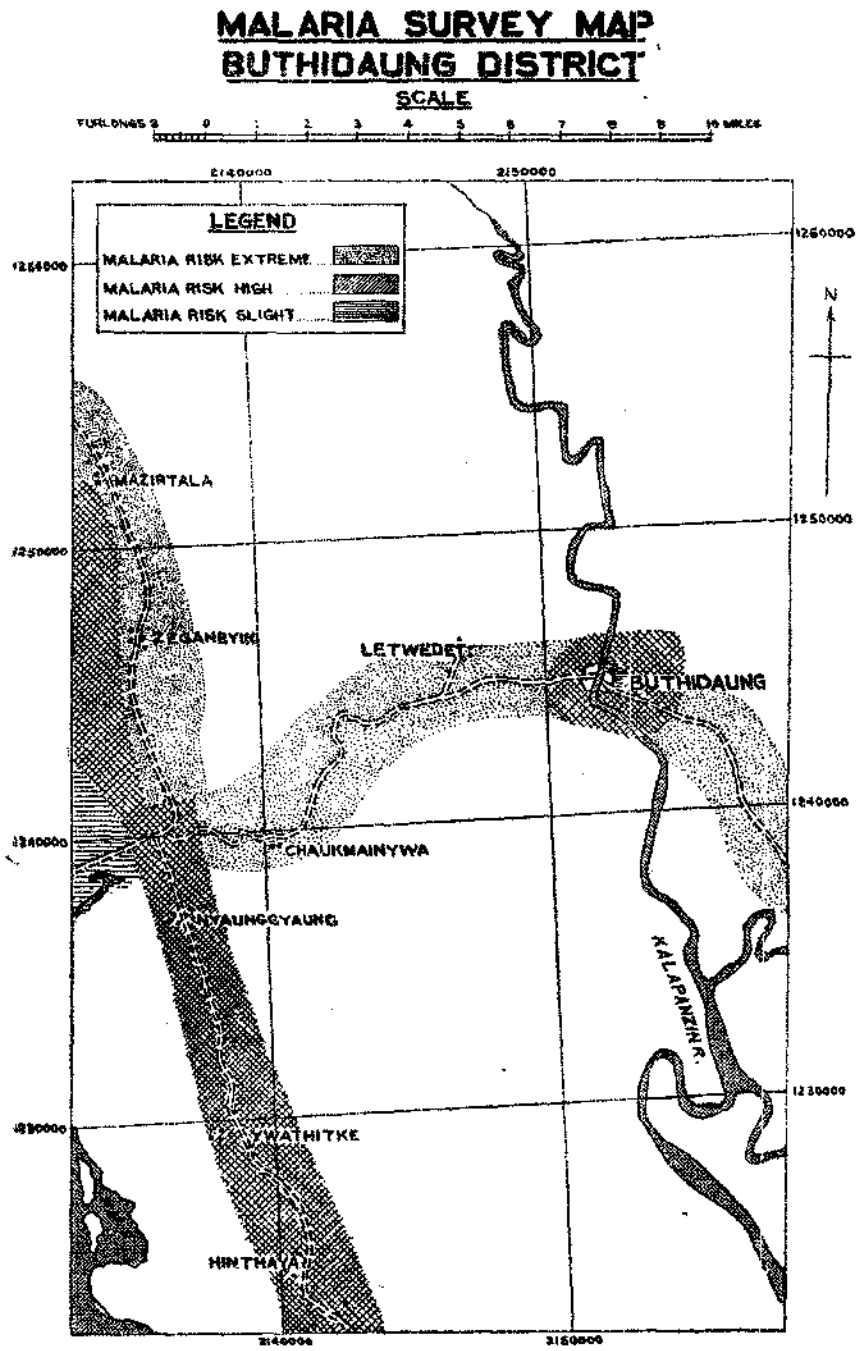
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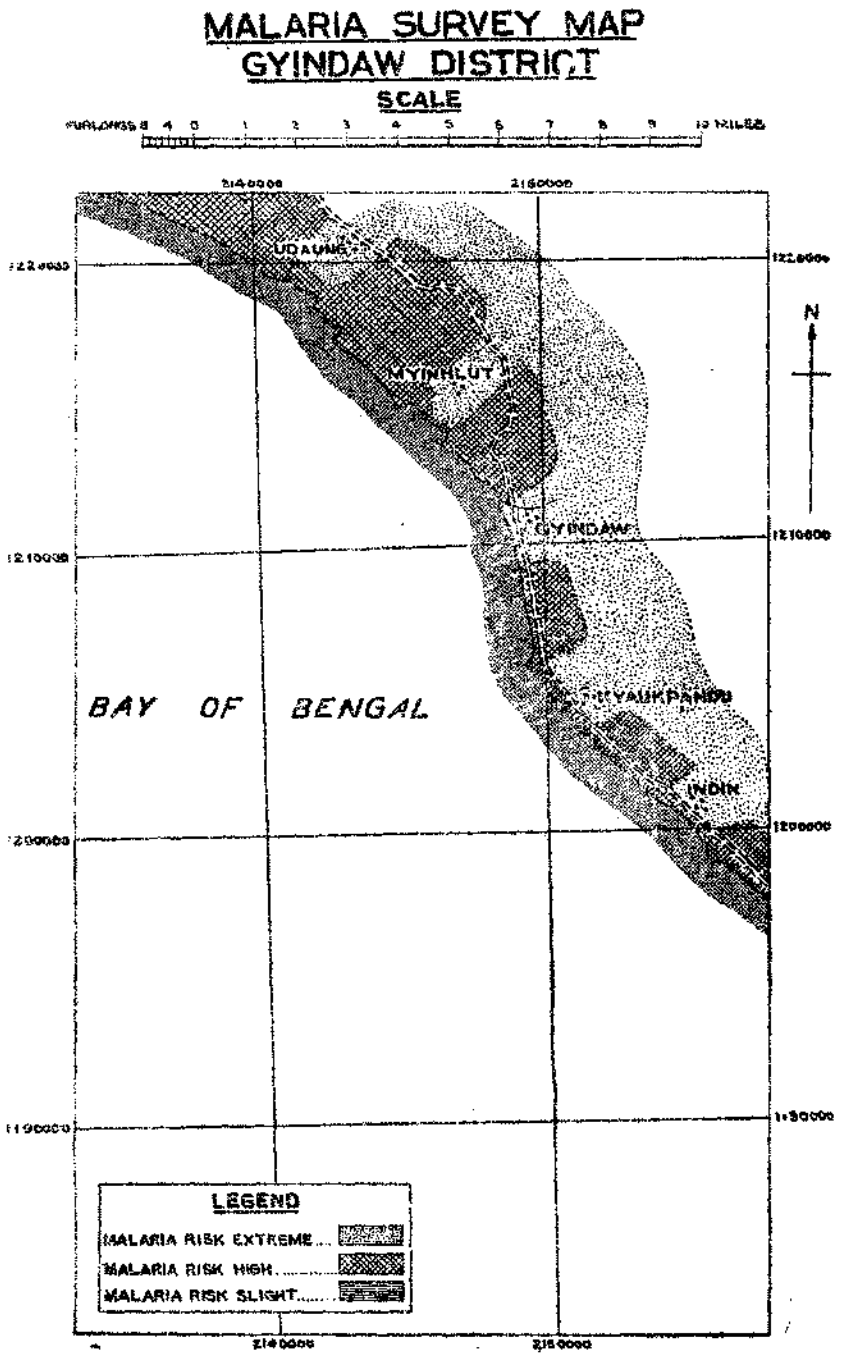
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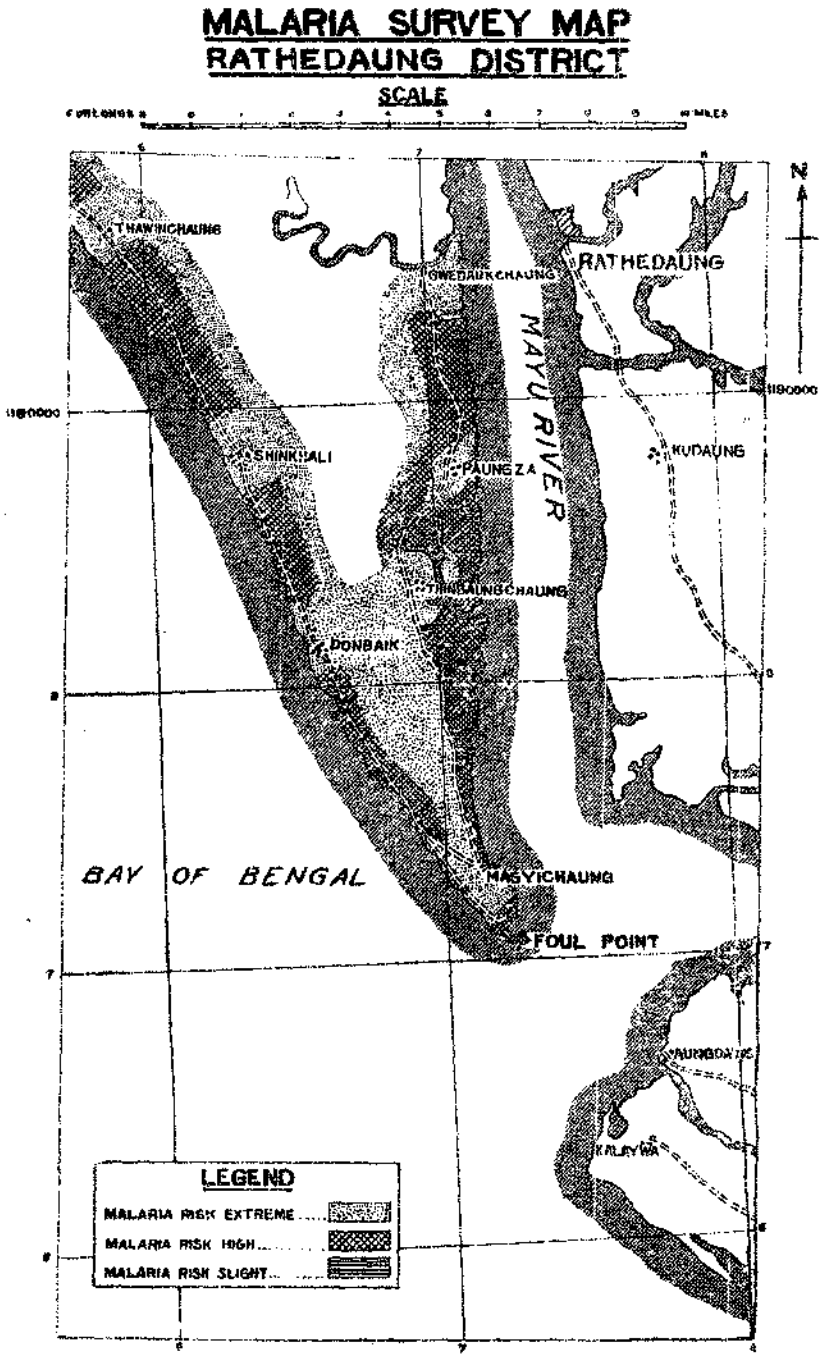
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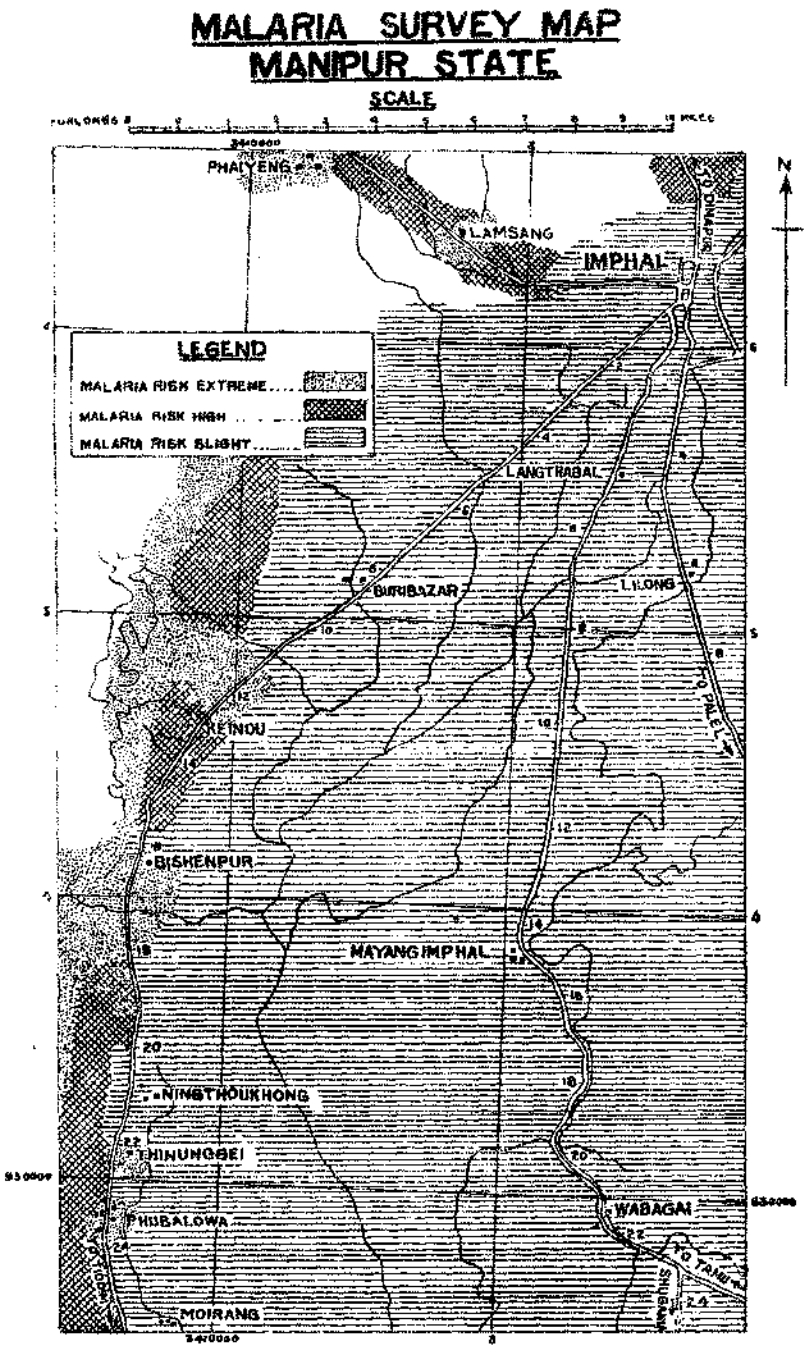
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SCALE
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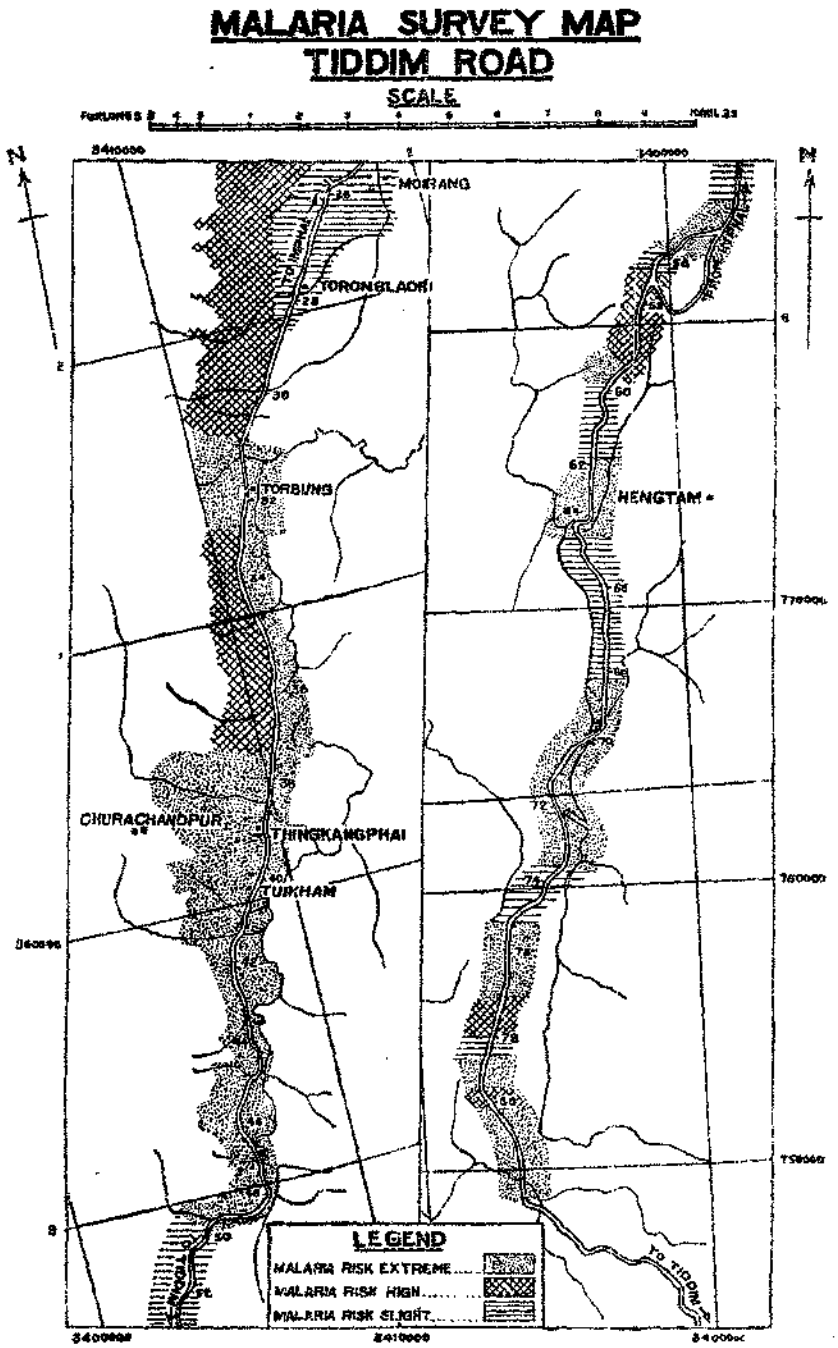
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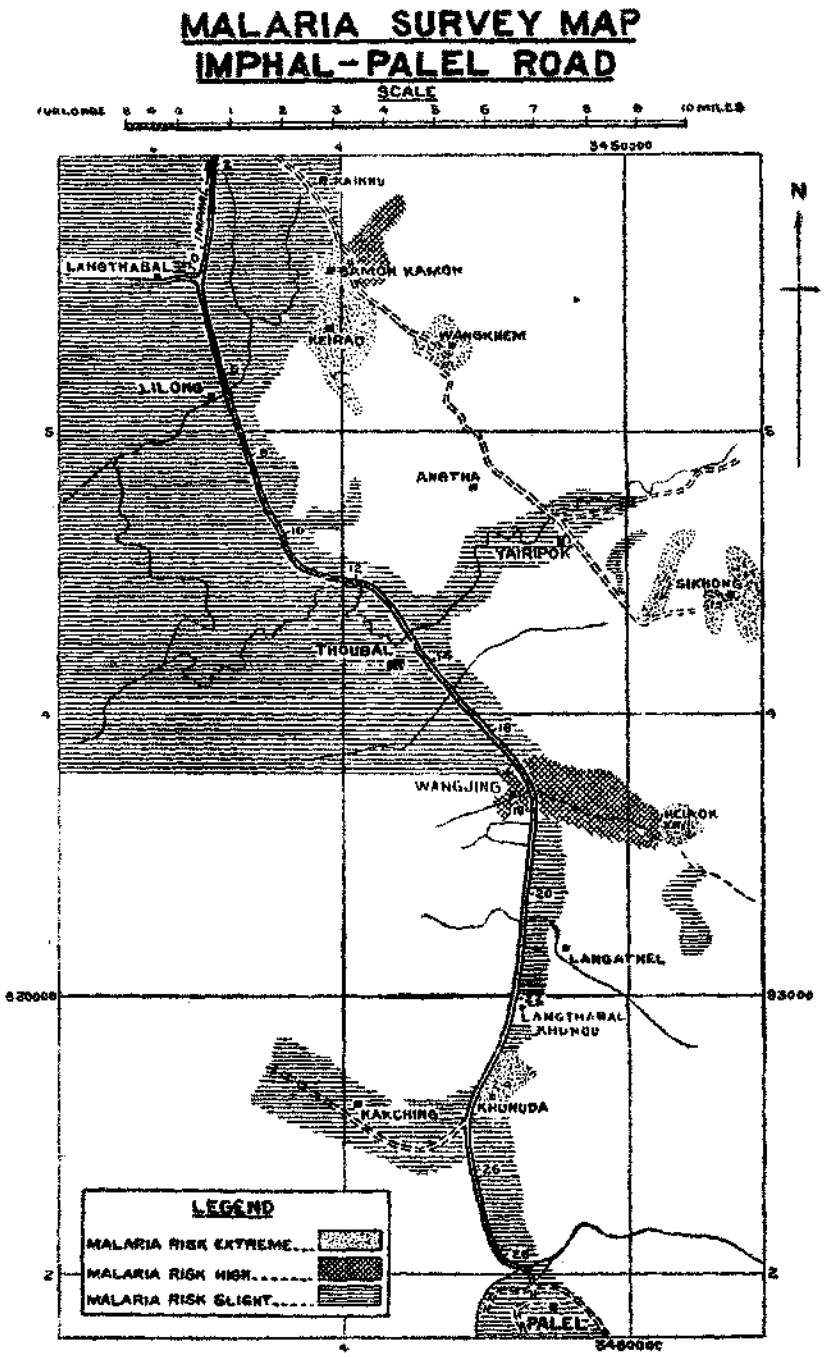
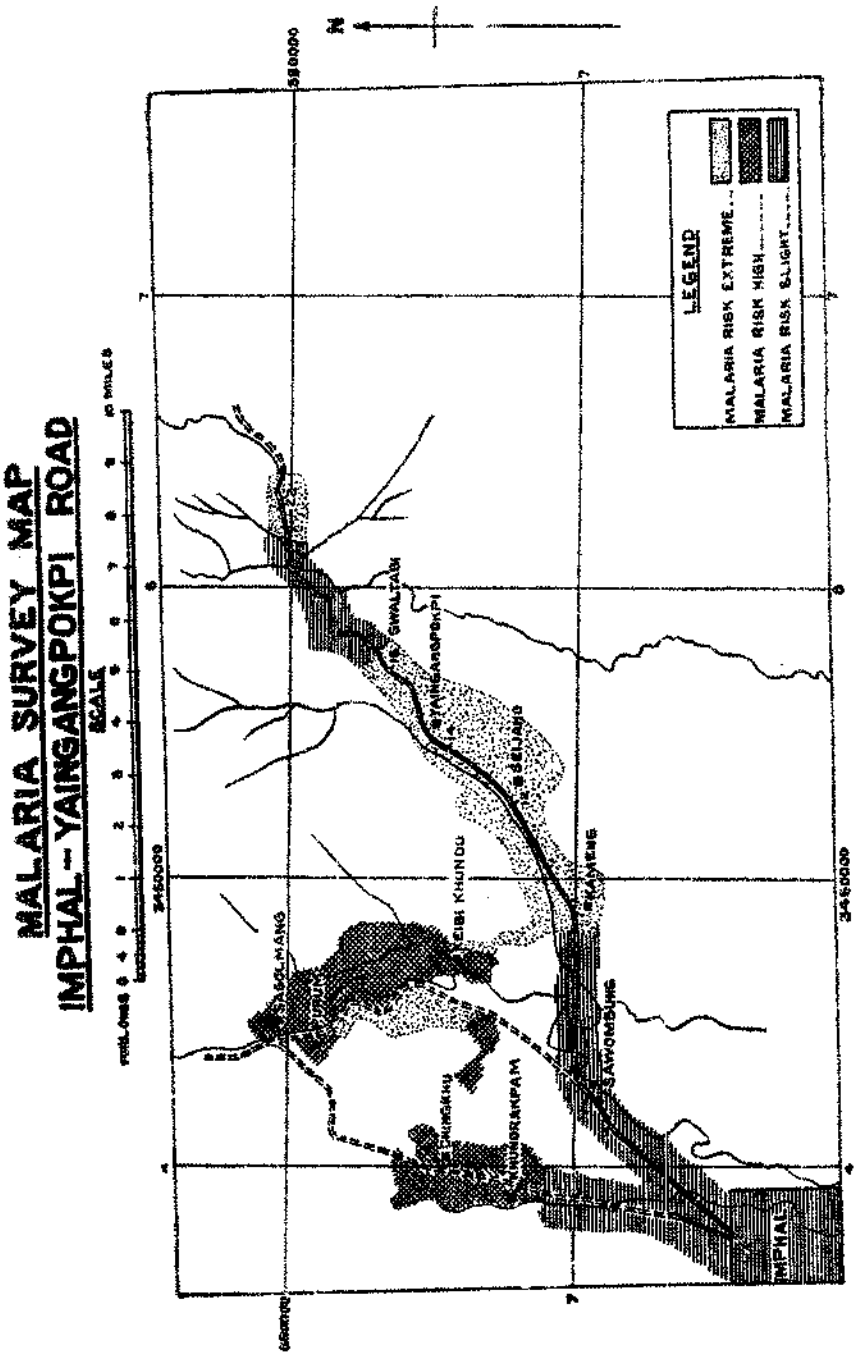
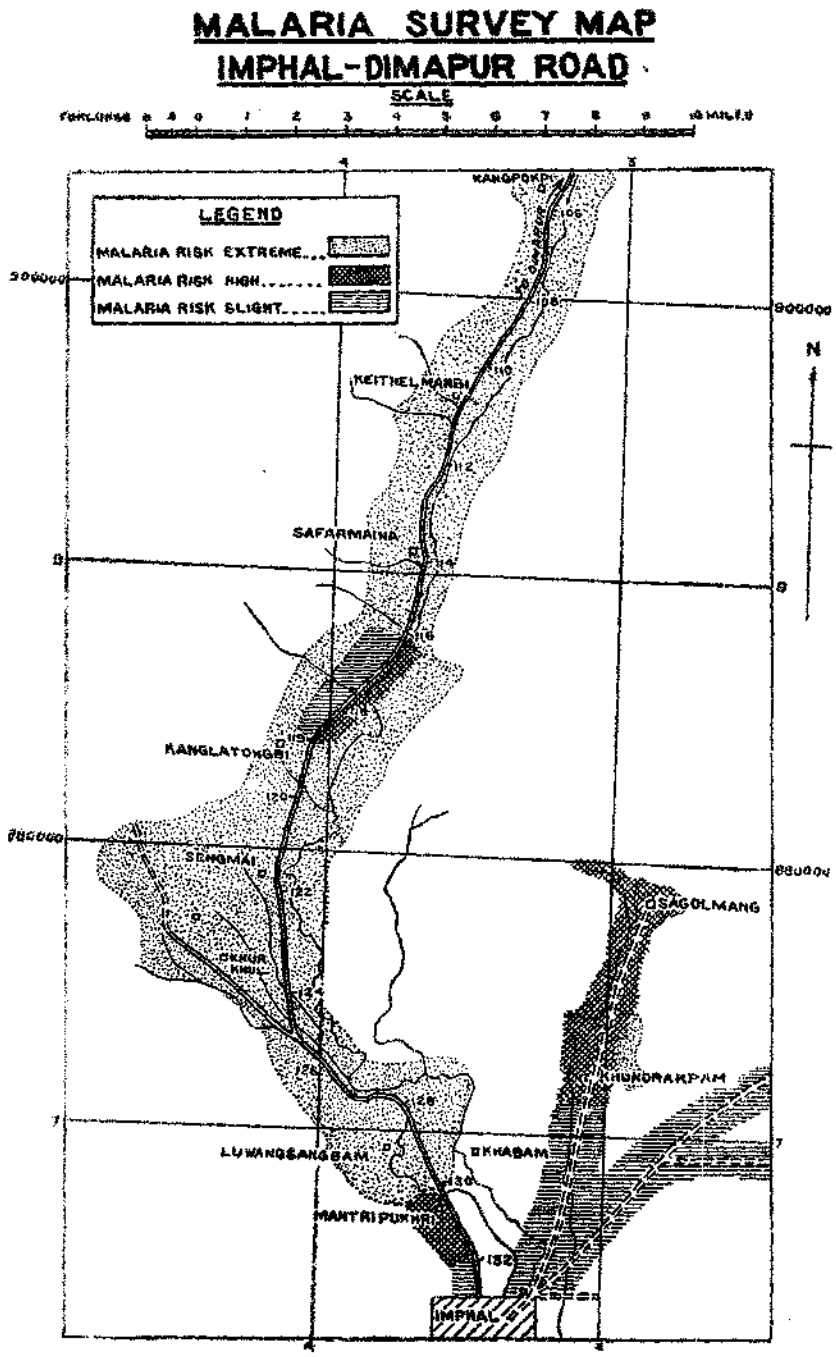
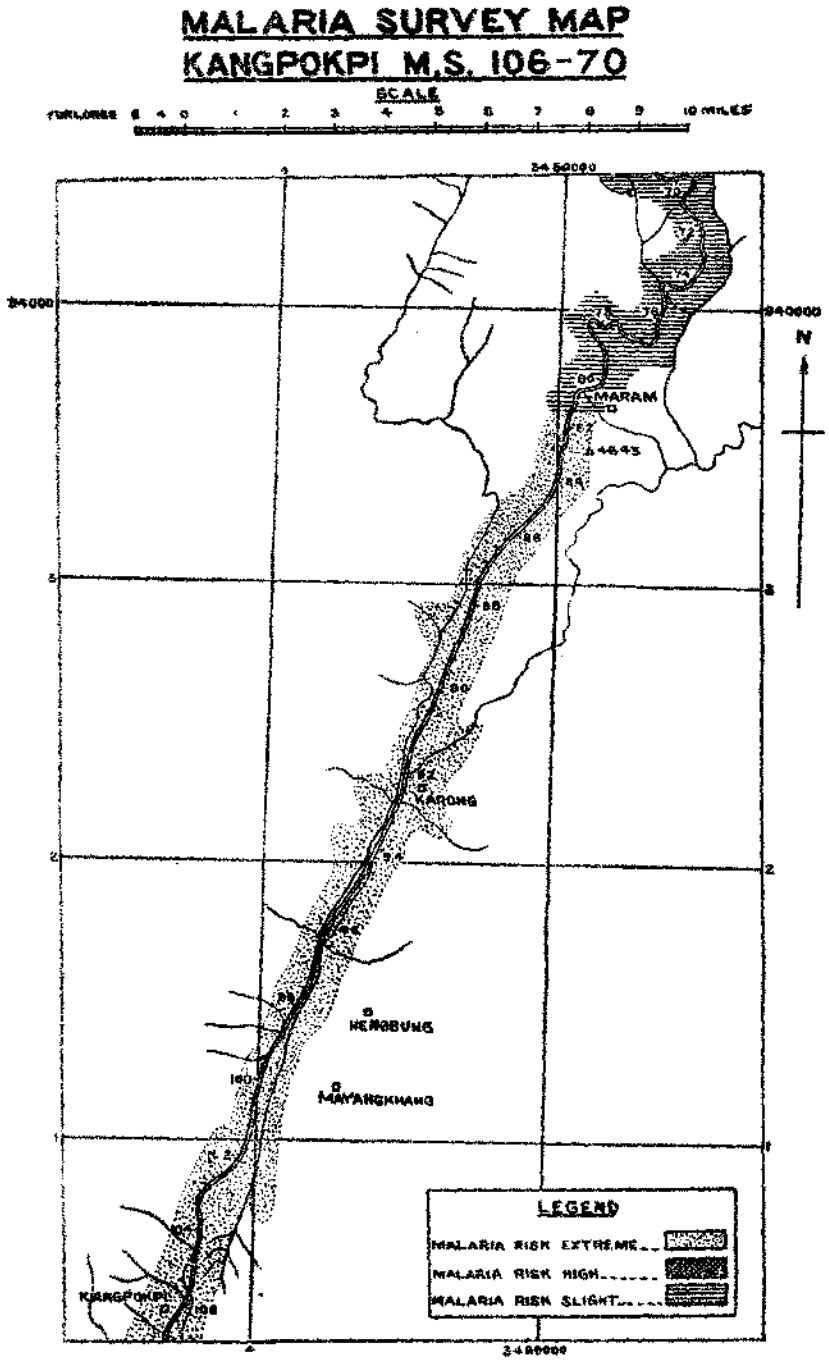
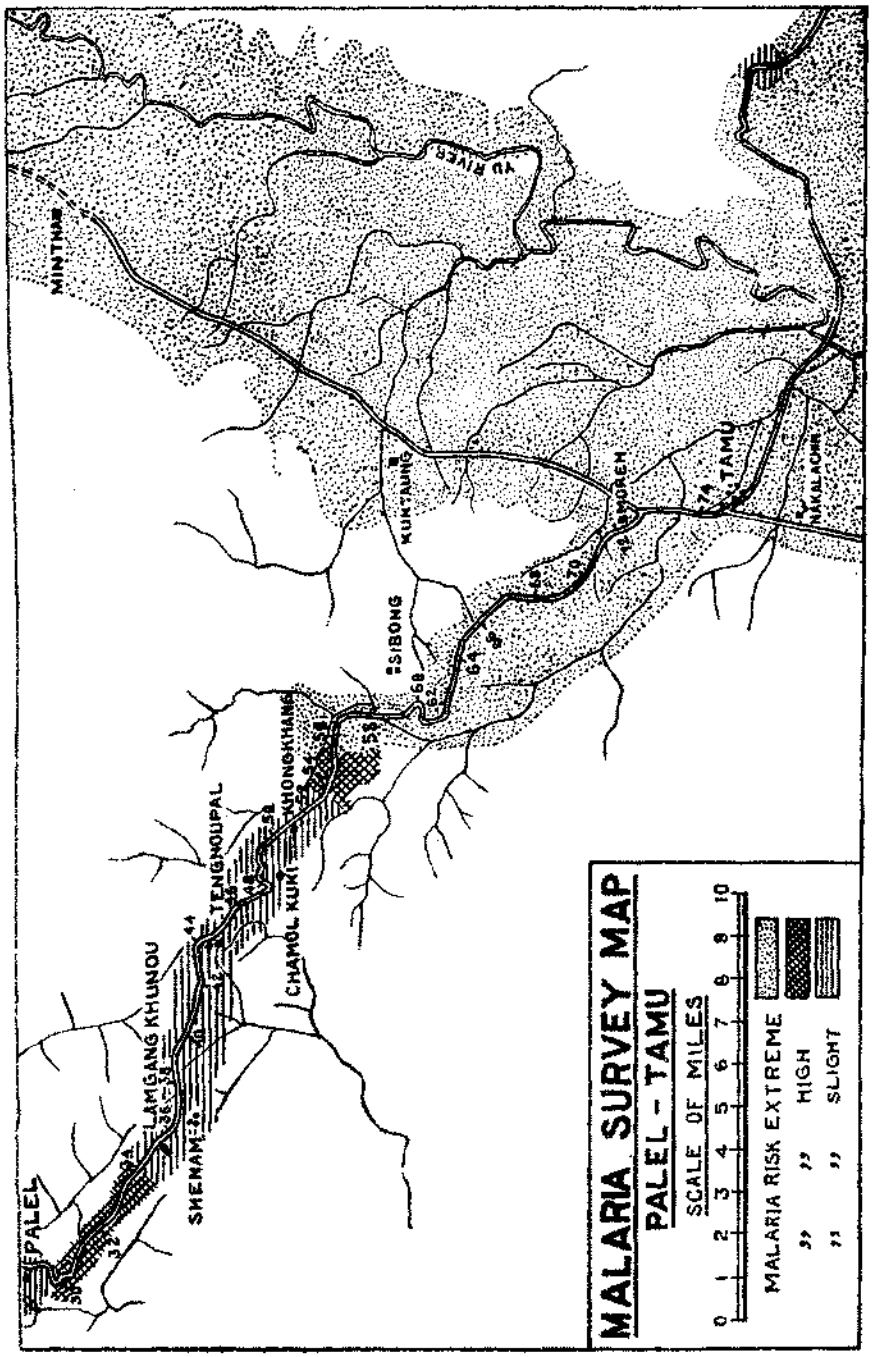
TUKHAM
LEGEND
MALARIA RISK EXTREME
MALARIA RISK HIGH
MALARIA RISK SLIGHT
TO TIDDIM


MALARIA SURVEY MAP
IMPHAL-PALEL ROAD
SCALE
N
LANGTHABAL
KEIRAO
WANGKHEM
JILONG
ANGTHA
YAIRIPOK
SIKHONG
THOUBAL
WANGJING
LANGATHEL
LANGTHABAL KHUNOU
KAKCHING
KHUNUDA
PALEL
LEGEND
MALARIA RISK EXTREME
MALARIA RISK HIGH
MALARIA RISK SLIGHT


MALARIA SURVEY MAP
IMPHAL — YAINGANGPOKPI ROAD
SCALE
LEGEND
MALARIA RISK EXTREME
MALARIA RISK HIGH
MALARIA RISK SLIGHT
N
IMPHAL
SAWOMBUNG
KANGLATONBI
KHUNDU
GWALTABI
YAINGANGPOKPI
SAGOLMANG
KHUMBRAKPAM
KHUMAN
2460000
580000


MALARIA SURVEY MAP
IMPHAL-DIMAPUR ROAD
SCALE
LEGEND
MALARIA RISK EXTREME
MALARIA RISK HIGH
MALARIA RISK SLIGHT
N
KANGPOKPI
KEITHELMANBI
SAFARMAINA
KANGLATONGBI
SENGMAI
SAGOLMANG
KHUNDRAKPAM
LUWANGSANGBAM
KHABAM
MANTRIPUKHRI
IMPHAL


MALARIA SURVEY MAP
KANGPOKPI M.S. 106-70
SCALE
N
MARAM
YARONG
HERGBUNG
MAYANGKHANG
KANGPOKPI
LEGEND
MALARIA RISK EXTREME
MALARIA RISK HIGH
MALARIA RISK SLIGHT


MALARIA SURVEY MAP
PALEL - TAMU
SCALE OF MILES
0 1 2 3 4 5 6 7 8 9 10
MALARIA RISK EXTREME
" " HIGH
" " SLIGHT
PALEL
LAMGANG KHUNOU
SHENAM
TENGNOUPAL
CHAMOL KUKI
KHONGKHAM
SIBONG
KUKTAUNG
YU RIVER
MINTHAM
TAMU
MAKALONG

CHAPTER XIII.

ACTIVITIES OF THE MALARIA INSTITUTE OF INDIA DURING WORLD WAR II.

BY

LIEUTENANT-COLONEL JASWANT SINGH, M.B., Ch.B. (Edin.), D.T.M. & H. (London), D.P.H. (England), I.M.S.

India has during the past 50 years or so attained an imposing record of achievements in the field of malaria research. The classical investigations of late Sir Ronald Ross, an officer of the I.M.S., who at the close of the last century demonstrated for the first time that mosquitoes transmitted malaria, evoked an unprecedented interest in this subject, and a great mass of details has since been worked out in connection with the transmission of the disease and its control. It was not till the year 1927 that the central organisation* "Malaria Survey of India" was established to carry out planned investigations for the prevention of malaria in India and to provide a medium for a concerted study of the subject. The name of the organisation was changed to Malaria Institute of India in 1938. This organisation was financed entirely by the Indian Research Fund Association from its inception till 1940 when the Public Health Section was taken over by the Government of India. Its research activities continued to be financed by the Indian Research Fund Association till 1946, when the Government of India assumed responsibility for that section also.

In 1942, advantage was taken of the generous offer of the Rockefeller Foundation to donate the equipment of its malaria research unit at Coonoor to form a much needed Southern India Branch of this institute. Owing to shortage of personnel during World War II, the activities of the Southern India Branch were greatly restricted. Expansion of the staff sanctioned in the post-war period will, it is expected, enable its activities to be considerably extenced. Among its functions will be the provision of advice and guidance to the numerous tea and coffee plantations and other industrial concerns located in South India.

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* The idea of a central organisation owes its conception to Colonel Sir S. Rickard Christophers, CIE, FRS, IMS (Retd.), whilst its subsequent development is attributed a great deal to the able and energetic direction of Lieutenant-Colonel J.A. Sinton, VC, OBE, IMS (Retd.) and Major-General Sir Gordon Covell, CIE, IMS (Retd.).

The Expert Committee on Malaria of the Interim Commission, World Health Organisation (1948) has recommended the establishment of malaria control organisations like the Malaria Institute of India in other countries which do not possess such an organisation, and have commended the excellent results which have been achieved by this institute. Dr. M.C. Balfour, Regional Director in the Far East, international Health Division of the Rockefeller Foundation, while discussing the research and teaching in tropical medicine in the Far East before the Fourth International Congress on Tropical Medicine and Malaria (1948), referred to the malaria Institute of India as the driving force in studies in malariology and in promotion of malaria control in the Far East and paid tribute to its notable history in malaria research, teaching and practice.

The functions of the institute were wide and varied and fell under three broad categories, advisory, training and research. Thus, it advised the Central Government on all aspects of the malaria problem. Advice was also given to the provincial governments as and when required. In the field of training, regular courses in malariology were conducted for medical officers, engineers and malaria inspectors. Instructional manuals on malarial surveys, entomology, anti-mosquito measures, etc., were published for the use of students and the general public*. Field and laboratory researches on parasitology, mosquito bionomics, laboratory techniques and methods of malaria control, including the testing of insecticides and anti-malarial drugs, and epidemiological investigations constituted an important feature of the institute's activities. Attention was also devoted to the study of allied diseases like filariasis, kala-azar, yellow fever, etc. The results of such researches were published in the *Journal of Malaria Institute of India* (later called *Indian Journal of Malariology*) which was edited by the director of the institute. The institute was also responsible for the effective promotion of liaison between the central and the provincial malaria organisations and with malariologists working in other countries. The functions and activities of the institute continued to be the same in the post-war period.

WAR ACTIVITIES

During World War I, malaria work in India came to a temporary standstill as most of the officers engaged on that work were recalled to military duty. During World War II, on the other hand, the Malaria Institute of India did not only survive the shock of the fateful years of the war but played an active and important role in the war effort as described in the succeeding paragraphs. The success on the Burma and the other Eastern fronts was largely due to the remarkable results in malaria control achieved by the anti-malaria organisations of the Allied armies.

Major M.K. Afridi, Assistant Director at the institute, as already stated before, was recalled to military duty in August 1940. He served as malariologist to the forces in Eritrea and Iraq, and subsequently held the post of Assistant Director of Malariology, General Headquarters (India) and later the more responsible post of Consultant Malariologist, Allied Land Forces, South-East Asia, with the rank of brigadier. Major Jaswant Singh, Research Officer, succeeded him. All the six military assistant surgeons, attached to the institute, were also recalled to military duty.

All activities of the institute during the war were carried out with the pre-war staff which remained depleted, as stated elsewhere, until August 1944, when the Government of India sanctioned certain additions to the staff for the period of war. These included a new post of deputy director, one additional assistant director, two additional malaria assistants and some clerks and technical assistants. The concentration of activities of the institute at Delhi due to the war, however, necessitated the move of the headquarters from Kasauli to Delhi in the beginning of 1943.

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* *See* Appendix XIII.

From the outbreak of the war, the director of the institute, Lieutenant-Colonel (now Major-General Sir) Gordon Covell, discharged the functions of consultant malariologist, General Headquarters (India) and the R.A.F. The appointment of consultant malariologist was, however, officially sanctioned in April 1944.

The appointment of the director as consultant malariologist to the General Headquarters (India) entailed on him constant and heavy touring, involving long absence from the headquarters. The personal inspections, however, stimulated the malaria control work carried out on the various war fronts, while the contacts established in the foreign countries had a considerable effect in co-ordinating the activities of malaria workers of these countries and India. The first tour to the U.S.A. was undertaken in 1943 with the special object of securing a favourable quota of pyrethrum. As a result of this tour, the position of allocation of pyrethrum to India improved considerably.

The impressions gained by the tour in Australia and New Guinea were utilised as the subject matter of an extensive lecture tour to the military stations and camps of the India Command and the South-East Asia Command. These lectures had a considerable effect in improving the standard of anti-malaria discipline in the various formations.

Before the last war, the Army-in-India did not possess a regular organisation for the control of malaria, either in cantonments or in the field. The first two anti-malaria units to be formed and mobilised were staffed mostly by the personnel of the institute and were placed at the disposal of the armed forces for service overseas in the Middle East theatre*. These two units were the forerunners of about 120 such units. Practically all the officers attached to these units, as well as the anti-malaria staff officers and the malaria officers of the cantonments in India, underwent intensive courses in malariology at this institute.

Training.—Normally, a six-week course in malariology was held each year for medical officers deputed by the Public Health Departments of the provinces, Indian States, Defence Services, Railway Board, Local Bodies, etc., but, owing to the requirements of war, it was necessary to hold five or six intensive courses every year though the duration of each was curtailed to four weeks. Twenty-one such courses for military personnel were held during the war at which over 500 officers received training. The subjects taught included the identification and dissection of mosquitoes and their larvae, the bionomics of mosquitoes, the parasitology, pathology and epidemiology of malaria, methods of investigating and measuring malaria incidence and principles and practice of control measures†.

A number of short courses were also held for officers and various classes of subordinate personnel. The total number trained in the anti-malaria duties during the war was 1,068 including 555 officers, (426 IHC Indian Hospital Corps) personnel and sanitary inspectors and 87 engineers.

* One of these units received special commendation for its work in the field from the General Officer Commanding, Eritrea.

† For syllabus *see* Appendix XIV.

Research.—The institute carried out research in anti-malaria drugs, insecticides, mosquito repellents and spraying equipment, etc. A special technique of rapid staining of malaria parasites was developed and production of antisera was started.

ANTI-MALARIA DRUGS

Java had been holding the monopoly of cinchona and quinine supplies in the world for a considerably long time, her output supply being 97 per cent of the world's requirements. In the beginning of the year 1942 and thereafter, the war exigencies made it impossible for the Java supplies becoming available to the Allies, and the acute shortage of quinine was being felt seriously. India's pre-war production of quinine was only 70,000 lbs., and another 140,000 lbs. had to be imported from Java to make up her total consumption of 210,000 lbs. The requirements of other Far Eastern countries, and the drying up of the only source of supply, i.e., Java, made the situation critical in the countries of the Eastern theatre. Conservation of existing stocks, prohibition of the use of quinine for any other purpose than anti-malaria and limiting the course of treatment to actual abortion of the clinical symptoms were among the many measures to be explored to meet the emergency.

This also led to the stimulation of interest in the search for substitutes which, could be used with the same degree of efficiency as quinine. Synthetic anti-malarial drugs had already made their appearance, but India and other Asian countries had to depend for their supplies from the United Kingdom and the U.S.A. The other possible source was the drugs of indigenous origin. The Malaria Institute of India therefore, undertook considerable amount of research work for evaluating the efficacy of the synthetic drugs under the local conditions and investigating the anti-malarial properties of the indigenous drugs. Many preliminary trials were made on Northern India rhesus (*Macaca mulatta*) monkeys before the drugs were tried on human beings.

Of the indigenous drugs the following were investigated with somewhat discouraging results.—

Name of drug.	Results.
A-9 ..	No more effect than would be expected from the administration of cinchona in it.
A.B.N. 41 or Dita-quinine ..	No more effect than would be expected from the administration of quinine in it.
Alstonia scholaris ..	No effect.
A-Gad	No effect.
Antique quine	No effect.
Ayurvedic anti-malarial medicine	No effect.
Chirvasal	No effect.
Coffee husk	No effect.
Degan's remedy ..	No effect.
Fraxine malacophylla ..	No effect.

Name of drug.	Results.
Hindscout	No effect.
H.T. 7	No effect.
Jwaraghna	No effect.
Saptaparna	No effect.
Karanjwa seeds (Pongamia glabra)	No effect.
Lichtenstein cure ..	No effect.
Malaridol	No effect.
Sunine	No effect.
Tinospora cordifolia ..	No effect.
Vitex peduncularis ..	No effect.

Synthetic anti-malarials like mepacrine (atabrine, atebrin, acriquine, chemisochin, chinacrin, crinodora, erion, haffkinine, italchine, metoquina, metoquine, quinacrine) and M3349, M4888 later called paludrine (proguanil, chloroguanide) were given extensive trials both in the laboratory and in the field. After the initial favourable results in India and in Australia by Hamilton Fairley, large scale experiments were conducted in hospitals, rural areas and in well organised labour populations. Paludrine proved quite successful both in its prophylactic and therapeutic use (Afridi, 1947).

J.S.B. STAIN

During the war, it became increasingly difficult to procure ingredients for the preparation of various modifications of Romanowsky's stains, such as Giemsa, Leishman and Wright's, at reasonable cost. Work was, therefore, undertaken at the institute to prepare a stain from ingredients which were readily available and could be substituted for the costlier imported dyes and their solvents. Methyl alcohol (acetone free) and glycerine were being used as solvents, but in rapid methods of staining not only the costly ingredients were substituted by less costly and more readily available materials but these were eliminated as detailed below. A technique of rapid staining of malaria parasites by water soluble stain (J.S.B. Stain) was thus developed by Jaswant Singh and Bhattacharji (1944).

The stain consists of two solutions, I and II. The first is a solution of methylene azure prepared under acid oxidation and subsequently dissolved in a weak solution of a strong base, e.g., caustic potash. The ingredients of solution I are given below :—

Medicinal methylene blue	0·5 gramme
Potassium dichromate ($K_2 Cr_2 O_7$)	0·5 gramme
Sulphuric acid ($H_2 SO_4$) 1 per cent by volume ...	3 c.c.
Potassium hydroxide (KOH), 1 per cent ...	10 c.c.
Water	500 c.c.

The methylene blue is dissolved in 500 c.c. of water by constantly shaking in a narrow-mouth flask. It is acidified with the dilute sulphuric acid and then the chrome salt is added. A heavy deposit of flocculated amorphous purple-coloured precipitate is then formed. The solution is thoroughly shaken and heated in a water-bath at boiling point. The original liquid and the precipitate begins to

change colour, first becoming greenish and on further heating for about three hours at the above temperature the colour changes to blue. This change may be apparent after two hours of boiling, but it is advisable to continue boiling for another hour. At the end of this period the flask is allowed to cool, and the precipitate appears as steel-blue needle-like branched crystals. With concentrated sulphuric acid the precipitate gives a deep green colour thus indicating that it belongs to the thionine series like methylene blue. With ether it gives an orange-red colour showing that it is an azure. 10 c.c. of 1 per cent KOH solution is then added drop by drop, while shaking the flask continuously, when the greater portion of the precipitate dissolves. This liquid is then filtered several times to ensure complete solution of the dye remaining on the filter paper. The filtrate is blue with a violet iridescence and is a mixture of the azures with only a trace of methylene blue.

The presence of methylene azure and traces of unoxidised methylene blue can be demonstrated by adding two drops of the filtrate to 2 c.c. of water and 5 c.c. of ether. On shaking vigorously for a few seconds, ether absorbs the azures and turns faint orange or eosin red, while the watery layer below shows a faint blue colour due to the presence of some unaltered methylene blue. If instead of ether, chloroform is added, this will settle at the bottom of the test tube, giving an intense violet colour which indicates the presence of azure. The watery portion at the top remains colourless. If the oxidation of methylene blue is incomplete the watery portion will stain a definitely blue colour. In actual practice a trace of unaltered methylene blue does not interfere with the staining.

Solution II is readily prepared by dissolving 1 gramme of water soluble eosin in 500 c.c. of tap water. A freshly-prepared eosin solution may not yield as satisfactory a solution as one which has turned deep red after some use.

Solutions I and II should be kept in wide-mouth-stoppered jars, 1½ inches diameter by 3½ inches height, and set aside for 48 hours to mature. Both the solutions keep well for several months, but in solution I a thin golden yellow scum is likely to form on its surface due to slight precipitation of the dye. This does not, however, interfere with staining, and the staining power of the solutions does not deteriorate with age*.

ANTISERA

Antisera, which was previously being imported from the Instituto Sieroterapico Milanese, Milano, through the Rockefeller Foundation, International Health

* For method of staining with JSB stain *see* Appendix XV.

The stain was tried by the Chief of Laboratory Service, 20 General Hospital, APO 689, CBI Laboratory and the Director, Army Medical Centre, Army Service Forces, War Department, USA, Washington, and its usefulness was recognised. Microscopical specimens of *P. vivax* and *T. brucei* stained with JSB stain were exhibited at a laboratory meeting of the Royal Society of Tropical Medicine and Hygiene held at the Royal Army Medical College, Millbank, London, on 16 March 1945. Sharp differential staining and general utility of this stain for demonstrating parasites in thick and thin films were also mentioned.

Slightly modified technique is at present being tried out in the National Institute of Health, Bethesda, as well as in the London School of Tropical Medicine and Hygiene, London. The reader is referred to some of the important references [*i.e.*, Boyd (1949) ; Manwell (1945) ; Manwell and Philip Feigelson (1948) ; Russell, West and Manwell (1946)] regarding the later developments.

Division, Instituto di Sanita Publica, 299 Viale Regina Marghereta, Rome, Italy, for use in precipitin tests for the determination of the source of blood meals of mosquitoes, was not available during the war. The production of high titre antisera was, therefore, given a high priority particularly in view of an enquiry from the Medical Research Liaison Committee for India in London.

Human and bovine antisera, which are commonly used in this country, were prepared to meet the immediate needs of India. Precipitin tests are now being carried out in large numbers on behalf of the World Health Organisation teams in the South-East Asia region.

INSECTICIDES

In the years preceding the outbreak of the war, the spray-killing of adult mosquitoes with sprays containing pyrethrum was hailed as the greatest advance in the methods of malaria control. As a result of observations carried out in the field since 1936 in India and elsewhere, the efficacy of this method had been established as an outstanding weapon against rural, urban, industrial and incidental malaria.

In the early war years, this method proved effective in securing immediate control of malaria under field conditions and constituted probably the greatest help which could be given in military campaigns in malarious countries.

Japan, with its annual output of 12,000 tons of pyrethrum cineraria folium, was the biggest pre-war supplier of pyrethrum flowers, but at the commencement of hostilities, this source was no longer available to the Allies, as Japan probably started to conserve stocks of these flowers for their own consumption long before it entered the war in 1942. With the extension of military operations to the Middle East and the Far East, demands for anti-mosquito sprays by the Services of all nations gradually increased, and the position soon became acute as the stocks previously built up in the Allied countries were being seriously depleted. Kenya, which was the second biggest supplier producing nearly 6,000 tons of flowers annually, had to be mainly depended upon, the other sources, i.e., Brazil and Belgian Congo playing only a minor role. Unfortunately, in 1943, the pyrethrum crops in Kenya were adversely affected by the failure of the rains.

It had already been demonstrated that high quality pyrethrum flowers could be grown in India in the Punjab hills, North-West Frontier Province and Kashmir. With the increasing demand for pyrethrum by the Allied countries, extension of pyrethrum cultivation was given a very high priority. Cultivations in the Palni hills, Nilgiris, Mayurbhanj, Khasi hills and Kashmir were tried, and ultimately extensive plantations on a commercial scale were established in Kashmir Valley and the Nilgiris, which yielded increasing supplies of pyrethrum in the following years. In the meantime, as the stocks of pyrethrum flowers had greatly been depleted, it was decided by the Allied High Command that the allocation of pyrethrum sprays should be restricted for the use of troops in operational areas only and should not be issued for permanent and semi-permanent camps where screening and other anti-malarial measures could provide reasonably adequate protection to the troops. This restriction chiefly affected India as it had the largest number of

permanent and semi-permanent camps in highly malarious areas. It was at this critical juncture that the Malaria Institute of India was called upon to undertake extensive laboratory and field trials on insecticides and various kinds of mosquito repellents and other methods of mosquito control. Intensive investigations were started by the institute on methods of achieving effective results in malaria control by using concentrates containing reduced quantity of pyrethrins in the presence of activators and synergists and alternative insecticides, particularly those available from indigenous sources. An account of the researches carried out in this respect by the institute is given below :—

Comparative biological tests on the mosquitocidal value of a number of proprietary insecticidal preparations, such as pyrocide 20, pyefly, pyrin, pyrefume 20 and 100, deskito, pymulso, lethane 384 etc., and pyrethrum flowers from Kenya, Coonoor and Kashmir were undertaken first in an experimental chamber of 50 cubic feet capacity and later in a room of 1,500 cubic feet capacity. Experiments undertaken in the large room, which was fitted with an exhaust fan, gave more satisfactory results than those carried out in the small chamber. A spray prepared by soaking 1 lb. of pyrethrum flowers in one gallon of kerosene oil was found to be as efficient as that prepared by diluting pyrocide 20 with kerosene. In fact fresh sprays prepared by soaking powdered flowers from Kashmir for 72 hours in kerosene gave better results than those obtained with imported extracts.

It was usual to spray oil solutions of various insecticides for the control of mosquitoes. As, however, kerosene was not only in short supply but more expensive, a series of experiments were conducted by this institute, both in the laboratory and in the field, to find out some other suitable and more economical vehicle for the preparation of sprays, containing pyrethrum and other insecticides. In search for a suitable emulsifier for the preparation of an emulsion of pyrethrum extract in water, in the presence of which the pyrethrins would not become hydrolysed, over 100 biological experiments were carried out. Of the 25 different emulsifiers tested, one was found to satisfy the necessary conditions. As a result, pyrethrum emulsion in water could be used in place of kerosene oil solutions, and this emulsion did not lose its insecticidal properties for long periods. About 250 further insecticidal tests were carried out in the search for synergists and activators of pyrethrins. Combinations of pyrethrins, lethane 384, lethane 384 special, I.N. 930, sesame oil and diesel oil were tried in order to effect economy in the use of pyrethrum in mosquitocidal sprays.

On account of the large increase in air traffic from the West to India through the African Continent, the fear of introduction of yellow fever* into India loomed

* In addition to its main function of dealing with the problems connected with malaria, the institute was called upon during the war to advise on measures directed against the possible introduction of yellow fever in India. Since 1941, the officers of the institute have been undertaking a number of special tours in Egypt, the Sudan, Eritrea and Kenya Colony to study and report on the anti-Aedes measures adopted in these countries in connection with seaports, ships, airfields and aircraft. A number of visits were also made to Bombay, Calcutta and Karachi with a similar purpose in view. The experience gained during these tours together with the data, obtained from investigations carried out at the institute in regard to insecticides, aerosol bombs and other spraying equipment, have formed the basis of the quarantine regulations issued from time to time by the Government of India for preventing the introduction of yellow fever into this country.

large. A number of experiments were carried out in aircraft at Karachi with view to find most suitable insecticides for the destruction of mosquitoes in the a craft coming from yellow fever areas. As the air companies and the R.A.F object to the use of kerosene solutions in the aircraft, various types of aqueous prepa tions, such as deskito, pymulso, pyrethrum aqueous emulsion, etc., were tried. phantomyst atomiser, producing a very fine spray, was not considered satisfacto as the particles produced were much too fine. A number of tests were carried o with aerosol bombs, containing pyrethrins dissolved in sesame oil and freo These, adequately used, gave very good results.

All insecticides tried were contact poisons and the size of the particles of t spray discharged from sprayers was found to have direct bearing on the percenta of the specimens killed. It was found that, when an insecticide was discharged the rate of about 50 c.c. per minute with a pressure of about 25 lbs., it produc the highest kill of mosquitoes as compared to any other discharge rate of the san insecticide.

Towards the end of 1943, DDT was received for the first time at this ins tute. It was to be used against mosquitoes as a solution in kerosene or as xylene-triton-aqueous emulsion. As both these solvents were in short supply India and comparatively expensive, a number of experiments were conducted find a suitable and cheap solvent. The Council of Scientific and Industri Research for the first time suggested turpentine (indigenous and of vegetab origin) as a solvent for such a formulation. As turpentine was required for oth more important uses as well, General Headquarters (India) offered toluene whic at that time was available in India in large quantities. DDT emulsion concentrat readily miscible with water were prepared with different solvents individually or combination with others, using various kinds of emulsifiers. A number of tests we carried out in the field to determine the duration of the residual effect of vario emulsions against adult mosquitoes. It was found that emulsion prepared wi medium kerosene extract as a solvent was as successful as a kerosene solution DDT and was more economical.

Comparative experiments were also carried out to determine the duration the residual effect of DDT when sprayed inside huts as a 5 per cent solution kerosene or as a 5 per cent aqueous emulsion in Bengal Duars. Both were found be equally effective, appreciably reducing the infectivity rate amongst mosquitoe Barrier spraying was also tried applying 5 per cent DDT solution and emulsic at the rate of 1·5 gallons per acre. This amount which did not completely kill th vegetation in the area was found to be insufficient for adequate control of mosqu toes in the area.

DDT was also tested as a larvicide both in the field and in the laboratory. was applied in solutions in used-engine-oil or in diesel oil. Emulsion concentrate containing DDT in different solvents received from I Anti-gas Laborator Jubbulpore, were tested for their larvicidal value. All of them, except thos

containing turpentine as solvent, appeared to sink to the bottom of the sprayed water within a few minutes of their application. These emulsions were also tried against adult mosquitoes, and the one containing turpentine and toluene as solvent of DDT yielded satisfactory results. It was, however, found that DDT solutions in kerosene or used-engine-oil sprayed on the water surface as larvicide did not have appreciable residual effect.

Effect of aerial spraying of DDT solution in kerosene was also tried in Delhi. It was found that when DDT was sprayed from a low-flying aircraft at the rate of 660 gallons of 8 per cent DDT-kerosene solution per square mile, the prevalence of mosquitoes fell to the extent of about 50 per cent, but this number went up very quickly and also that kerosene oil was found deleterious to vegetations in a number of places. Three other experiments were carried out to determine the efficacy of DDT-turpentine-toluene emulsions when sprayed over the breeding places from a low-flying aircraft. The emulsion was applied at the rate of approximately 16 c.c. per ten square yards. In all these, a 75 per cent mortality among 3rd or 4th instar larvae was observed after 24 hours, but in larvae in 1st and 2nd instar the mortality was less than 50 per cent. The dosage applied, however, killed a large number of small fish which were present in the water over which DDT was sprayed. These experiments amply showed that the spraying of DDT from aircraft, though useful as an emergency measure in warfare, is unlikely to come into general use for civilian purposes. It was excessively expensive and the pilots had to be specially trained for this type of work. Even in warfare, this method, generally speaking, is restricted to 48 hours immediately following the occupation of the enemy position to tide over the danger period before routine spraying of the interior of the buildings and their immediate surroundings by the anti-mosquito ground staff can be established. Application of DDT from the air is less accurate than from the ground owing to changes in the direction of the wind. Apart from this, the risk to crops, flowers and fruit trees from the destruction of beneficial insects, which are necessary for pollination, limits the use of this method.

REPELLENTS AND PROTECTIVE CLOTHINGS

It was felt that the army personnel on night duty needed personal protection in the form of suitable repellents for which ordinary bamber oil was not found satisfactory. Repellent vanishing cream, containing 4·2 per cent pyrethrins, was used on a large scale by the army in the Far Eastern fronts. Similar vanishing creams supplied by the various makers, including Stafford Allens' deskito cream, were tested, but not found superior to the anti-mosquito cream containing pyrethrins. Extensive trials were also conducted with Di-Methyl Phthalate fluid and Di-Methyl Phthalate cream (the latter containing 60 per cent of the former). These were found highly effective both against Aedes and Anopheles. Extracts of a number of indigenous aromatic plants were also tried, but none of them had any appreciable repellent properties. While working on comparative protection

from the bites of mosquitoes afforded by different types of clothing, it was found that mosquitoes did not bite through a cloth to which Di-Methyl Phthalate had been applied. Experiments were carried to assess deterrent action of these repellents, when applied to fine or coarse camouflage nets. The protection ratio afforded by drill to cellular cloth was found to be 95·8 to 50 per cent. Other cloth material like utility gaberdine was also tested.

SPRAYING EQUIPMENT

Experiments were conducted to find out the particular type of sprayer which would discharge the requisite size of particles of the insecticide to obtain the maximum kill. Sprayers which worked at constant air pressure were found to be superior to those in which the pressure gradually decreased. Certain types of electric and petrol-driven sprayers were selected for the spray-killing of adult mosquitoes. In aircraft the use of aerosol bomb was found to be more satisfactory.

For the atomisation of solutions and emulsions of insecticides, hand sprayers of the continuous type, popularly known as Malaria Institute Special Handsprayer, were extensively used by the army especially in the forward areas. This pattern has been modified by different workers and used for indoor residual spraying of DDT, but stirrup pumps fitted with a lance with a wheel cock at one end and a nozzle with a bore of 1/32 to 1/64 inch at the other, were found most suitable. A large number of knapsack sprayers were also tested and hand pressure knapsack sprayers fitted with nozzle of the American contaminated type were found very effective.

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APPENDIX X

ANTI-MALARIA OPERATIONS IN DELHI, JAMNAGAR AND COALFIELDS.

ANTI-MALARIA OPERATIONS IN DELHI

The first authentic reference to malarial conditions in Delhi was made in the report of the commission of 1845 which investigated the cause of unhealthiness in the tract lying along the course of the Western Jamuna Canal. Apart from this report, observations, recorded prior to 1912, dealt chiefly with conditions in Delhi Fort and Daryaganj Cantonment, which were notorious in the army as hotbeds of malaria. The malaria incidence recorded for the troops for the years 1896 to 1900 was 1,146 cases per mille. Captain Lelean in 1903 commented that the havoc, caused by malaria to troops in Delhi, was so intense that in 1904 the bulk of the garrison was removed to the ridge during the worst months of fever. Expert advice was sought and given from time to time between 1912 and 1934, and, owing to lack of funds, only a small proportion of the recommendations were carried out at various periods and consequently the results achieved were only partial. It was not till 1936 that a well planned anti-malaria operations for the Delhi urban area were seriously undertaken.

The city of Delhi is situated in a zone of Northern India which is susceptible to periodical fulminant epidemics, which tend to occur especially in years of excessive monsoon rainfall following a series of years of deficient rainfall, provided also that the level of communal immunity is low. Recent epidemics in Delhi have occurred in 1926, 1933 and 1942. The chief malaria problem in Delhi is due to the excessive canal irrigation, coupled with interference with natural drainage by railway, road and canal embankments, and annual flooding of the Bela by the rise in water level in the river Jamuna, resulting in heading back of water in the various storm-water drainage channels and the formation of prolific breeding places as the floods recede. The presence of a vast number of excavations throughout the area, in the form of railway and roadside borrow-pits, pits in the brick-fields and stone quarries, form additional hazards.

Conduct of Anti-malaria Operations in Delhi.—The anti-malaria operations in Delhi urban area can be reviewed in two phases, namely, (*i*) the period between 1936 and 1940, and (*ii*) the period during the war from 1941 to 1946.

Phase I.—In June 1936, the Government of India decided that an attempt should be made to improve the malaria control in Delhi urban area then extending over approximately 50 square miles. The policy adopted was to bring into effect such temporary measures as were considered necessary for the control of mosquito breeding as rapidly as possible and at the same time to prepare and examine projects for the requisite permanent anti-malaria engineering measures as funds became available. A list of such projects was prepared by expert engineers, and a number of these were completed at a cost of about rupees 25 lakhs.

The essential features of these anti-malaria operations were (*a*) vesting the responsibility and direction of the measures in the whole area under one authority, *i.e.*, the Director, Malaria Institute of India ; (*b*) provision of an independent checking organisation directly under his charge ; and (*c*) the introduction of an adequate and comprehensive anti-malaria legislation similar to that in the city of Bombay.

The chief method of control of malaria was the recurrent use of larvicides, levelling and minor drainage works and spraying of pyrethrum solution in selected malarious localities in Delhi (for the first time in India). The experiment proved so successful that this method of using the insecticide opened a new method of attack that was full of promising results. The end of this period (1936 to 1940) set the anti-malaria operations on a well organised basis, with solid results. Though the cost of the permanent anti-malaria works was high, it has been many times recovered in the form of amelioration of unhealthy conditions and enhanced value of landed property.

Phase II.—Routine measures indicated in the previous section were followed up during this period also. The year 1942, however, was an epidemic year in the Indo-Gangetic plains and a year of supreme test for the efficiency of the existing organisation in Delhi. The abnormal features of this year were :—

(*a*) Unusually heavy excessive rains and river flooding.

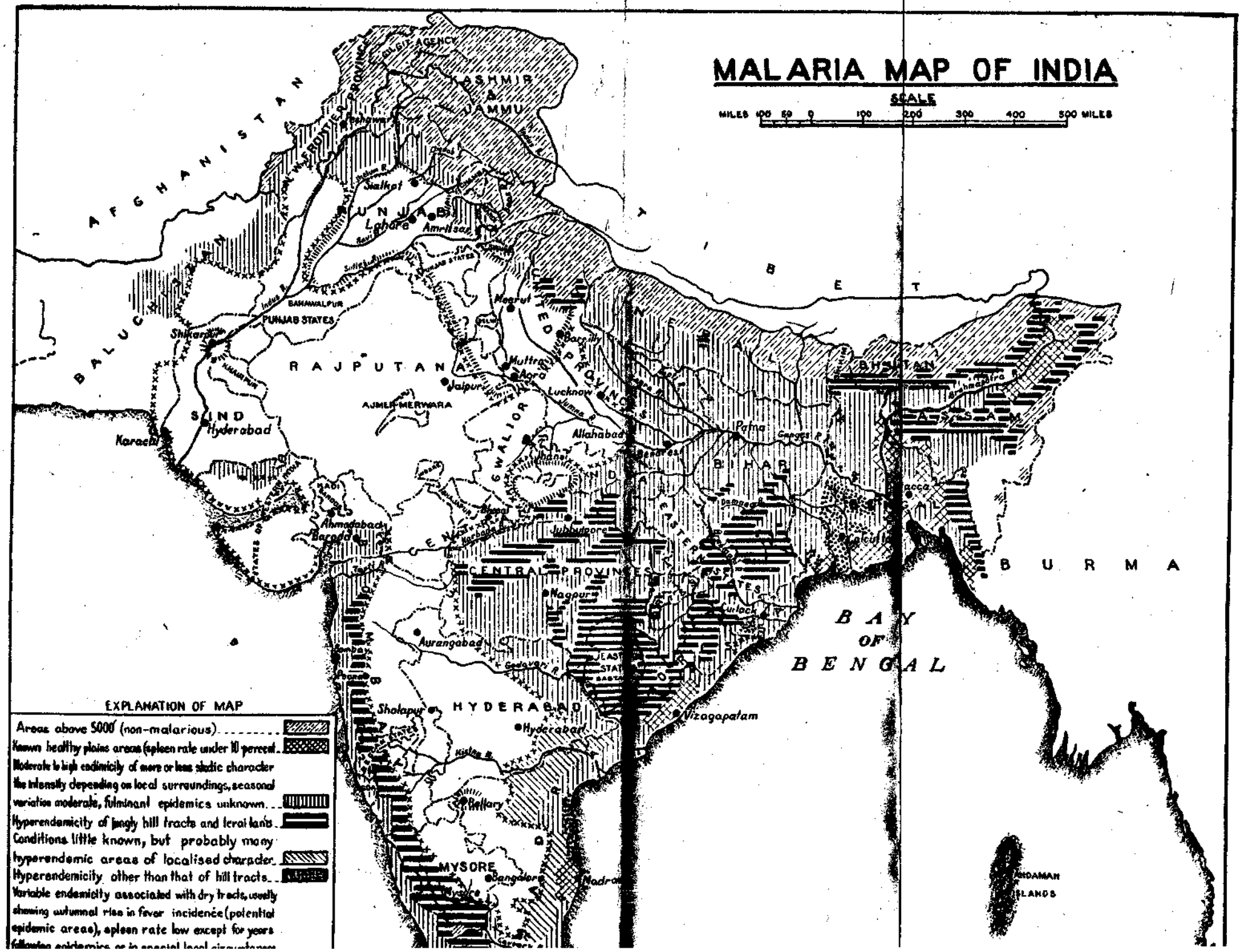
(*b*) A huge influx of population consisting of both civilians from adjoining uncontrolled areas as a result of river-floods and troops with malarial infections returning from countries in the eastern theatre of war, thus bringing about conditions typical of tropical aggregation of labour.

(*c*) Short supply of anti-malaria stores and drugs due to sudden stoppage of supplies of quinine and pyrethrum from Java and Japan respectively.

(*d*) A great increase of excavations in the shape of slit trenches and borrow-pits due to air-raid precautionary measure and erection of new buildings respectively.

That the epidemic was in full swing during the year was specially evident from the great increase in morbidity and mortality figures in the areas adjoining Delhi. Despite all the above mentioned handicaps, the incidence of malaria was kept under check in Delhi, the chief measure, employed to prevent the spread of the disease, being the use of pyrethrum for spray-killing of adult mosquitoes.

A notable feature during these years was that the anti-malaria organisations of both the defence services and the civilian public health authorities worked in the closest collaboration. In fact, the funds for the control of malaria in troops stationed in the Kingsway Camp in 1941 were placed at the disposal of the Director, Malaria Institute of India. Shortly thereafter, the army, navy and air force personnel were stationed in various camps in and around Delhi, and consequently as many as three anti-malaria units, each under the command of a medical officer


MALARIA MAP OF INDIA
SCALE
MILES 100 50 0 100 200 300 400 500 MILES
AFGHANISTAN
BALUCHISTAN
KASHMIR & JAMMU
GILGIT AGENCY
PUNJAB
PUNJAB STATES
BAHAWALPUR
RAJPUTANA
AJMER-MERWARA
SIND
Karachi
Hyderabad
Shikarpur
Jaipur
Agra
Lucknow
Allahabad
Patna
Benares
Calcutta
Lahore
Amritsar
Sialkot
Meerut
Bareilly
Muttra
GWALIOR
Indore
Bhopal
Jubbulpore
Ahmadabad
Baroda
CENTRAL PROVINCES
Nagpur
Aurangabad
HYDERABAD
Hyderabad
Sholapur
Bellary
MYSORE
Bangalore
Madras
Vizagapatam
Cuttack
BHUTAN
ASSAM
BIHAR
BURMA
BAY OF BENGAL
ANDAMAN ISLANDS
EXPLANATION OF MAP
Areas above 5000' (non-malarious)
Known healthy plains areas (spleen rate under 10 percent)
Moderate to high endemicity of more or less sporadic character the intensity depending on local surroundings, seasonal variation moderate, fulminant epidemics unknown.
Hyperendemicity of jungly hill tracts and terai lands.
Conditions little known, but probably many hyperendemic areas of localised character.
Hyperendemicity other than that of hill tracts.
Variable endemicity associated with dry tracts, usually showing autumnal rise in fever incidence (potential epidemic areas), spleen rate low except for years following epidemics, or in special local surroundings

specially trained at the institute, were detailed. Each unit had a total personnel of 75 and was fully equipped with material, appliances and transport. It was as a result of the combined efforts that the 1942 epidemic, which caused havoc in the surrounding uncontrolled areas, was effectively checked. In the post-epidemic years of 1943 and 1944, the control measures were further strengthened by the use of DDT, a powerful insecticide with prolonged toxic effect. In 1945, DDT was sprayed from air by aircraft specially fitted with necessary equipment over many square miles of the low-lying riverain sector.

The total population in Delhi urban area, including the army personnel, increased from about 4,64,000 in 1933 to 10,50,000 in 1942. Despite a tremendous increase in the population, the malaria incidence, as indicated in Table I, decreased progressively.

TABLE I.

Total cost and cost per capita on anti-malaria campaign and the malaria incidence per 1,000 population—Delhi urban area—1939 to 1946.

Year.	Total expenditure. (Rupees)	Total population.	Cost per capita. Rs. As. Ps.	Malaria incidence per 1,000 population.
1939—40	63,200	5,08,000	0—2—0	62·1
1940—41	69,900	6,43,000	0—1—9	65·4
1941—42	71,500	6,64,200	0—1—8	43·2
1942—43	74,200	10,00,000	0—1—2	92·5
1943—44	1,82,400	10,50,000	0—2—9	72·0
1944—45	1,92,300	10,50,000	0—2—11	36·0
1945—46	2,48,400	10,50,000	0—3—9	30·9
1946—47	3,00,700	10,50,000	0—4—5	26·6

ANTI-MALARIA OPERATIONS IN JAMNAGAR

There was an influx of large number of Polish refugees, and a camp for thousands of children was established at Balachadi, Jamnagar State. Within a very short period of their arrival in the camp, malaria occurred in an epidemic form in August-September 1942. An investigation unit was detailed from the institute soon after. An anti-malaria organisation was set up and its work for the following two to three years was co-ordinated from Delhi. By continuous supervision, it was ensured that the measures recommended were effectively carried out with consequential satisfactory results.

ANTI-MALARIA OPERATIONS IN COALFIELDS

Increased output of coal was recognised as an essential war effort in 1943, and it was also conceded that, for the industrial development of the country, the labour engaged in the coal mining industry must be protected from the ravages of malaria. Accordingly, in 1944, the Malaria Institute of India was asked to undertake malaria control measures in the coalfields in India.

In view of the urgency of the situation and the inevitable delay in setting up an efficient civil orgiansation, it was decided to start work in the larger coalfields in 1944 with the aid of army anti-malaria units. One unit each was detailed to Jharia, Raniganj and Pench Valley coalfields. The civil organisation under the Malaria Institute of India began to function in the coalfields of Jharia, Raniganj, Pench Valley and Margherita from 1 December 1945.

The control measures employed in the coalfields were recurrent anti-mosquito measures involving the use of insecticides for the destruction of adult mosquitoes, and of larvicides for killing their aquatic forms. Minor engineering works, such as drainage, levelling, clean-weeding of tanks, etc., were also undertaken wherever economically feasible.

On account of the constantly shifting nature of the mining industry, control measures in the coalfields had been confined to recurrent measures. Engineering works of a permanent nature like drainage had to be postponed till townships and residential areas for large groups of miners were established.

In the course of two years' work, the reduction in the incidence of malaria in some of the bigger coalfields ranged from 24 to 57 per cent. The reduction in the spleen rates was particularly significant, especially when it is taken into consideration that the control measures had been in operation only for a relatively short period. The rates of malaria morbidity also showed a similar downward trend. These results were achieved in spite of the fact that a large proportion of the cases were amongst imported labourers, particularly those recruited from Gorakhpur, Bilaspur and other malarious areas.

In the opinion of the Chief Sanitary Officer, Raniganj Mines Board of Health, "available statistics of malaria morbidity have shown a steady decline in Raniganj coalfields since 1944. This reduction is more real than apparent as no such corresponding reduction is manifest in the rural areas in the neighbourhood."

Notable work on control of malaria was carried out in a number of places in India, like Panagarh and Kangra Valley.

Panagarh (3 Reserve Base) Project.—Panagarh, one of the largest advance base area in India for the Burma Campaign, was highly malarious prior to control measures. In view of the strategic importance of this area and large troop-concentration, very extensive anti-malaria campaign had been undertaken. The programme consisted initially of engineering project, perhaps the largest for any static camp like this, and other anti-larval measures, followed thereafter by both anti-larval and anti-adult measures. The operation was such a success that almost within a year not only malaria was stamped out but mosquito nuisance was eliminated to a very large extent.

Kangra Valley.—During the survey of Kangra Valley, it was noticed that a few streams were exceptionally free from mosquito larvae, whereas at the same height and identical conditions mosquito larvae were breeding in other streams. After a very careful investigation, no cause could be found except the presence of fish which was identified as *Schizothorax progastus* and found to be a larvivorous fish. [(Raina, 1945), *Ind. Med. Gaz.*, **80**, 273]. In the same area, a large tract of marshy land was turned into grazing ground by a network of 'sub-drainage'.

APPENDIX XI

NOTES ON THE DISTRIBUTION, BREEDING PLACES, ADULT HABITS AND RELATION TO MALARIA OF THE ANOPHELINE MOSQUITOES OF INDIA AND THE FAR EAST*.

BY

MAJOR-GENERAL SIR, KT. G. COVELL, C.I.E., M.D., D.P.H., D.T.M. & H., I.M.S.

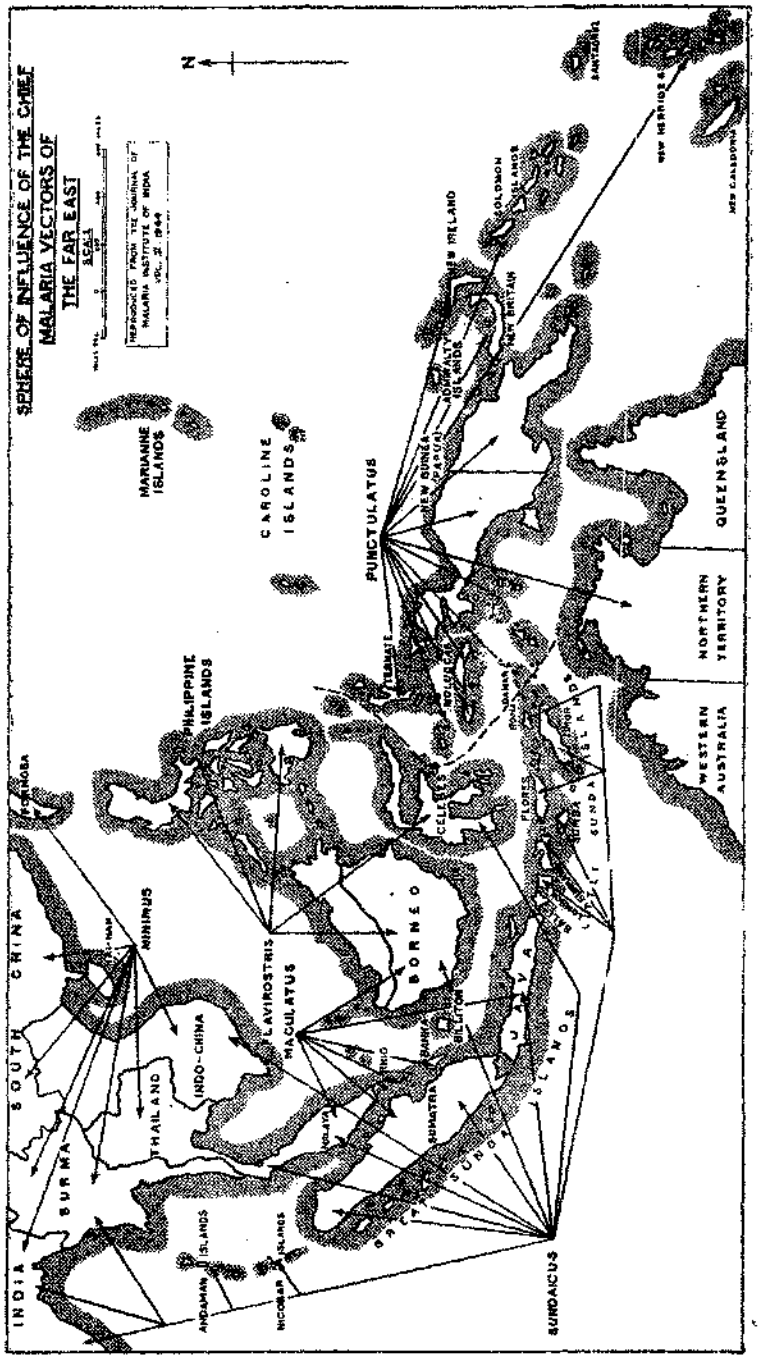
A critical review of the data recorded regarding the transmission of malaria by the different species of Anopheles, with notes on their distribution, breeding places, adult habits and relation to malaria, was published by Covell (1927b). The information contained in this memoir was brought up-to-date in a later article (Covell, 1931). During the 13 years, which have since elapsed, a large amount of additional data has accumulated. Pressure of other work has prevented Covell from producing an up-to-date edition of the original memoir, which related to all the known species and varieties of Anopheles throughout the world. The present article deals only with those species, recorded in India and the Far East, which have either been found naturally infected with malaria parasites or have been suspected to be vectors of malaria on epidemiological grounds. It is felt that the presentation of up-to-date information on these species may be useful to those who are concerned with the control of malaria under war-time conditions in this region.

The area dealt with includes India, Burma, Thailand, Indo-China, Southern China, Formosa, the Andaman and Nicobar Islands, Malaya, the East Indian Archipelago, Northern Australia and certain of the South-West Pacific Islands. The East Indian Archipelago includes the Great Sunda Islands (Sumatra, Java, Borneo, Celebes, Banka, Billiton), the Little Sunda Islands (Bali, Lombok, Sumbawa, Flores, Sumba, Timor), the Moluccas, New Guinea and the Philippine Islands. The South-West Pacific Islands referred to are New Britain, New Ireland, the Admiralty Islands, the Solomon Islands and the New Hebrides.

As a general rule the mosquitoes of the East Indies may be differentiated into Asiatic and Australian forms, the line of demarcation passing between Celebes and the Moluccas to the north and between Roma and Damar Islands to the south (vide map). This rule is not, however, absolute, for *bancrofti*, a typically Australian species, has recently been reported on the west coast of Celebes.

More than 100 species or varieties of Anopheles have been described from regions, but the majority have never been suspected to be malaria carriers, and only 41 are included in the present review. Of these, 12 are classed as vectors of major importance and a further 16 of local importance in certain areas. The remaining 13 probably play little or no part in the transmission of malaria in nature.

* COVELL, G. (1944). *J. Mal. Inst. Ind.*, 5, 399-434.


SPHERE OF INFLUENCE OF THE CHIEF
MALARIA VECTORS OF
THE FAR EAST
SCALE
REPRODUCED FROM THE JOURNAL OF
MALARIA INSTITUTE OF INDIA
VOL. 2 1944
N
INDIA
SOUTH CHINA
BURMA
THAILAND
INDO-CHINA
ANDAMAN ISLANDS
NICOBAR ISLANDS
MINIMUS
LAVIROSTRIS
MACULATUS
SUNDAICUS
PHILIPPINE ISLANDS
MARIANNE ISLANDS
CAROLINE ISLANDS
PUNCTULATUS
BORNEO
SUMATRA
JAVA
SUNDA ISLANDS
CELEBES
FLORES
SUMBA
TIMOR
NEW GUINEA
NEW IRELAND
NEW BRITAIN
SOLOMON ISLANDS
NEW HEBRIDES
NEW CALEDONIA
WESTERN AUSTRALIA
NORTHERN TERRITORY
QUEENSLAND

Among the species, listed below as vectors of major importance, there are two groups which have particularly affected the conduct of military operations during the present war : (*i*) the *fluviatilis-minimus* group which is responsible for the intense malaria prevailing throughout the foot-hill tracts of India and the northern sector of the Far East from Burma to Southern China, and (*ii*) *punctulatus* and its variety *moluccensis* which play a similar role in New Guinea and the South-West Pacific Islands wherever the disease is rife.

Important malaria carriers.	Of local importance in certain areas.	Probably of no practical importance
culicifacies	*aconitus*	*amictus*
fluviatilis	*annularis*	*annulipes*
maculatus	*bancrofti*	*baezai*
minimus	*barbirostris*	*jeyporiensis*
minimus var. *flavirostris*	*hyrcanus* var. *nigerrimus*	*karwari*
philippinensis	*hyrcanus* var. *sinensis*	*mangyanus*
punctulatus	*hyrcanus X*	*pallidus*
punctulatus var. *moluccensis*	*jeyporiensis* var. *candidiensis*	*pulcherrimus*
stephensi	*kochi*	*ramsayi*
sundaicus	*leucosphyrus*	*separatus*
superpictus	*novumbrosus*	*splendidus*
umbrosus	*pattoni*	*subpictus*
	stephensi var. *mysorensis*	*vagus*
	subpictus var. *indefinitus*	
	tessellatus	
	varuna	

The present study has served to emphasise certain gaps in the knowledge of malaria vectors of this region. In particular, further intensive research is urgently required as to the day-time resting places, time of biting, preferential feeding habits and effective flight range of the different anophelines concerned. It is known that certain species include two or more distinct biological races, which though morphologically indistinguishable, differ widely in their habits and consequently in their effectiveness as transmitters of malaria. It is most important that this aspect of the problem should be thoroughly investigated throughout the area under review.

Of the numerous publications, which have been consulted in compiling these notes, the following have proved particularly useful : *Keys to the anopheline mosquitoes of the world* (Russell, Rozeboom and Stone, 1943) ; *The geographical distribution of the malaria-carrying mosquitoes* (Kumm, 1929) ; and *Mosquito intermediary hosts of disease in Australia and New Guinea* (Taylor, 1943).

The various species have been arranged in the text in alphabetical order, as in original memoir.

ACONITUS DONITZ, 1902.

Distribution—India (Assam, Bengal, Bihar, Central Provinces, Hyderabad, Konkan, Madras Coast North, Malabar, Mysore State, Orissa, United Provinces);

Ceylon ; Andamans ; Burma ; Thailand ; Indo-China ; Malaya ; Sumatra ; Java ; Bali ; Sumba ; Borneo ; Celebes.

Breeding Places.—Irrigation channels, swamps, ponds, ricefields (especially just before harvesting the crop and in fallow-fields), pools in creeks and river-beds, roadside drains and tanks with grassy margins. Hacker (1920) classed this species among the swamp fauna, and found the larvae most often in association with those of *barbirostris*, *hyrcanus* and *annularis*.

Adult Habits.—Adults are found in houses, cowsheds and outdoor resting places. Barber (1918) stated that in Malaya they were often found in houses, readily took blood (presumably human) and were apparently capable of long flight, since they were to be found at considerable distances from their breeding places. Ave Lallemant, Soerono and Stoker (1932) showed that in the Dutch East Indies, *aconitus* could cover a distance of nearly 1,000 yards. Venhuis (1942*a*) noted that in Java the adults were often found on stream banks in far greater numbers than in houses. Gaschen and Raynal (1937) in Tonkin, where cattle were very abundant, recorded 94·2 per cent with animal blood in series of precipitin tests. Walch (1932) in the Dutch East Indies, where cattle were in normal numbers, found 12 per cent with human blood (out of 229), but where cattle were scarce the percentage with human blood was 61 (out of 137).

Relation to Malaria.—This species has been found infected in India, Indo-China, Malaya and the Dutch East Indies. In India, Das (1943) found 2 specimens infected (1 gut, 1 gut plus gland) out of 24 dissected at Chandpur, East Bengal, and concluded that it was a carrier of first importance in this area. Natural infections have also been recorded in the Jaipur Hills, one gut infection out of 107 dissected (Senior White, 1937) and in the Orissa coastal plain, 3 gut and 2 gland infections out of 951 (Senior White, Adhikari, Ramakrishna and Roy, 1943). Viswanathan, Das and Oommen (1941) recorded one gut infection out of 1,145 dissected in an area (Lakhimpur, Goalpara District) in Assam where *annularis* is considered to be the chief vector. These are apparently the only records of natural infection of *aconitus* published in India, and the species is probably of little importance as a malaria carrier in this country. In Burma, Feegrade (1924*b*) recorded a single gut infection out of 58 dissected at Akyab. In Indo-China it is looked upon as a vector of secondary importance (Toumanoff, 1936). The only occasion, on which gland infections have been recorded in that country, was during an epidemic at Ha-Tri in the Tonkin Delta, 2 specimens out of 67 dissected (Genevray, Toumanoff and Try, 1937). Very few infections have been recorded in Malaya. Stanton (1914) reported 3 out of 78 *albirostris*, without stating whether they were gut or gland infections, whilst Gater (1927) recorded 2 gut infections out of 192 dissected at Petaling. It is only in the Dutch East Indies that *aconitus* appears to be a vector of real importance. Infection rates recorded in Western Java range from 2·9 to 17·8 per cent, and here it may be almost as important a carrier as *sundaicus* especially along the coast. In Lamongan Residency in the Solo Valley an infection rate of 42

per cent is said to have been reported, but details as to numbers dissected are not available. Smalt (1937) recorded an infection rate of 7 per cent in Bali, and infections have also been reported in Sumba and in Minahassa, Celebes. In Sumatra, *aconitus* has been found infected only in Tapanoeli (West Sumatra) and Djepara (South Sumatra). Doorenbos (1931) published records of 3,032 dissections in East Sumatra, with an infection rate of only 0·2 per cent.

It appears probable that these variations in the effectiveness of *aconitus* as a malaria carrier in the Dutch East Indies are related to its zoophilic tendencies, for it appears to feed on man only in areas where cattle are scarce. If it is present continuously and in great numbers in such areas hyperendemic conditions may result.

AMICTUS EDWARDS, 1921

Distribution.—Dutch New Guinea, Australia (Queensland, Northern Territory, North-West Australia).

Breeding Places.—In North Queensland there are two distinct varieties with different breeding preferences. The type-form breeds in fresh water in association with *annulipes* and *punctulatus* var. *moluccensis* ; var. *hilli* breeds in open brackish water collections in association with *moluccensis*. In the laboratory, larvae will develop in 80 per cent sea-water.

Adult Habits.—The adults enter houses and attack man.

Relation to Malaria.—Roberts (1943, unpublished) dissected 79 specimens of var. *hilli* at a late stage of an epidemic among the civil population of Cairns, North Queensland, and found one gland infection (1·3 per cent). The chief vector in this epidemic was *punctulatus* var. *moluccensis*. *A. amictus* is considered to be potentially dangerous under locally favourable conditions.

ANNULARIS VAN DER WULP, 1884

Distribution.—India (widely distributed throughout, chiefly in the plains, but also in the hills up to 7,000 feet) ; Ceylon ; Burma ; Thailand ; Indo-China ; Southern China ; Formosa ; Malaya ; Sumatra ; Java ; Borneo ; Little Sunda Islands.

Breeding Places.—Predominantly still waters, associated with floating vegetation and filamentous algal growth : water in slow movement, though certainly not inimical, is not favoured (Senior White, Adhikari, Ramakrishna and Roy, 1943). Larvae are chiefly found in tanks, swamps, ricefields and borrow-pits. Borel (1926) in Indo-China observed them in tree-holes and cut-bamboos, whilst Walch and Soesilo (1927) recorded them in saline water in Java.

Adult Habits.—The adults are found in great numbers in cattlesheds, but are also frequently taken in houses. Senior White, Adhikari, Ramakrishna and Roy (1943) noted that in Orissa they showed an increasing tendency to enter houses in November. Their life is greatly prolonged in the autumn, thus increasing their chances of a double contact with man. They are said to be able to fly great distances. Gill (1914) noted that they were active and bit freely at temperatures as low as 54° F. They are markedly zoophilic. Ramsay, Chandra and Lamprell

(1936) recorded 1·6 per cent with human blood out of 192 subjected to the precipitin test. Fourteen gave no result and the remainder contained animal blood only.

Relation to Malaria.—This species was found naturally infected by Adie (1903) at Ferozepore, Punjab (one gland infection out of 148 dissected), but it is not considered to play any part of importance in the transmission of malaria in this part of India. It is, however, a vector of some importance in Orissa and Bengal, and is considered to be the principal malaria carrier in the Orissa coastal plain by Senior White, Adhikari, Ramakrishna and Roy (1943), who dissected 9,183 specimens and found 14 guts (0·15 per cent) and 7 glands (0·03 per cent) infected. Senior White (1943) also recorded one gut infection out of 1,030 dissected in the Hazaribagh Ranges. In Birbhum District, Bengal, Timbres (1935) dissected 49,698 *annularis*, of which 8 had sporozoites in the salivary glands (0·02 per cent). Covell and Pritam Singh (1942) recorded one gut infection out of 20,844 dissected in the vicinity of the Chilka Lake, Orissa, whilst in the some area Senior White and Adhikari (1939) dissected 1,048, with 3 gut infections.* In Assam, Viswanathan, Dass and Oommen (1941) reported 20,043 dissections of this species, of which 11 showed natural infection (7 glands, 4 guts). These infections were all encountered during 1940-41, when 7,481 specimens were dissected (infection rate 0·15 per cent). All the infected specimens were from a locality in Goalpara District where *minimus* was very rare, and it was noted that *annularis* was apparently the sole vector.

Outside India, natural infections of this species have been recorded in Burma, Southern China, Malaya and the Dutch East Indies. In Burma, infected specimens were reported in Katha, Kyaukpyu and Akyab by Feegrade (1924*a*, 1924*b*) and Lalor (1912, 1913). In Southern China, Robertson (1940) recorded 6 infections out of 380 dissected in Yunnan, in a region where *minimus* is the principal vector. This is apparently the only record of natural infection in *annularis* in that country. In Malaya the sole record of infection seems to be that of Stanton (1914), one gut out of 23 dissected, and this species is not considered of any importance as a carrier there. In the Dutch East Indies, Swellengrebel and Swellengrebel-de Graaf (1920) reported 2 gut infections out of 691 dissections and noted that in a region of severe malaria in Java this species and *sinensis* were the only two found infected, although *aconitus* and *maculatus* were also present. No further infections of *annularis* appear to have been recorded from the East Indies during the past 25 years, and it is not considered to be a vector of any practical importance in this region.

ANNULIPES WALKER, 1856

Distribution.—Australia (dry inland districts, coastal areas and in mountains up to an elevation of 5,000 feet); Tasmania; Port Moresby, New Guinea; reported, perhaps incorrectly, from New Hebrides (Russell, Rozeboom and Stone, 1943).

Breeding Places.—Shallow grassy pools, among vegetation on the edges of marshes, slow running creeks, surface wells, rock-pools and occasionally brackish

* A specimen of *annularis* with sporozoites in the salivary glands had since been encountered at Kespur, Chilka Lake by Senior White.

water (Cooling, 1924). It breeds in all clean or muddy, but not foul, ground water, casual or permanent, along the grass-grown banks of creeks or rivers, etc. (Taylor, 1943).

Adult Habits.—This species will bite at any time of the day, in secluded spots, or at night (most freely at sunset and for about two hours after). Adults enter houses quite freely (Taylor, 1943).

Relation to Malaria.—Considered to be a vector in Australia on epidemiological grounds (Breinl and Taylor, 1918), and is listed among species which are potentially dangerous under locally favourable conditions. It has been associated with small local outbreaks of benign tertian malaria on the Australian mainland in places where it has been the only anopheline species present.

BAEZAI GATER, 1933

Distribution.—Malaya, Indo-China (Farinaud, 1938).

Breeding Places.—Brackish water, in stagnant pools and swamps under shade along the coast. As compared with *sundaicus* it prefers shade, whilst the latter favours sunlit-breeding places.

Relation to Malaria.—Kingsbury (1939), referring to trap-catches in the vicinity of Kuala Lumpur, states : "Most of the mosquitoes caught were dissected but the only one infected was one *baezai* which showed apparently degenerated sporozoites in the salivary glands." This appears to be the only record of natural infection of this species, but it has been suspected to be a vector on epidemiological grounds.

BANCROFTI GILES, 1902

Distribution.—New Guinea (interior and south coast) ; Australia (Queensland, Northern Australia) ; Eastern Moluccas ; Celebes (West Coast).

Breeding Places.—Shaded pools, along the grass-grown banks of slowly-running creeks and rivers ; also in pot-holes in creeks and rivers (Taylor, 1943).

Adult Habits.—The adults rarely frequent houses (Hill, 1925). The Cairns race scarcely feeds on man at all in nature, whilst the same species from the northern territory is one of the fiercest biters and greediest feeders of all the Australasian mosquitoes. Precipitin tests of blood meals in a small series from Merauke, Dutch New Guinea, showed 62 per cent as having fed on man, but these were all caught in houses (Heydon, unpublished). It is considered that even this race is not highly anthropophilous, and that it feeds on man rather by chance contact than by deliberately seeking him.

Relation to Malaria.—This species is said to be a vector in New Guinea. de Rook recorded a natural infection rate of 4·3 per cent among specimens caught at Tana Merah in that Island (Swellengrebel and Rodenwaldt, 1932).

BARBIROSTRIS VAN DER WULP, 1884.

Distribution.—India (widely distributed, except in the extreme north-west); Ceylon; Burma; Thailand; Indo-China; Southern China; Malaya; Sumatra; Java; Borneo; Celebes; Little Sunda Islands; Philippine Islands; New Guinea.

Breeding Places.—Shady pools containing vegetation, tanks, borrowpits, rice-fields, swamps and slow-flowing streams. Hacker (1920) classed this species amongst the swamp fauna, its most frequent associate being *hyrcanus*. Covell (1927*a*) recorded the larvae in the Andamans in a salt water swamp, and in a disused well, in the latter case accompanied by those of *leucosphyrus*.

Adult Habits.—The adults will bite in the shade out of doors in the day-time. As a rule they are rarely found in houses, but Banks (1907) noted that on one occasion in the Philippines large numbers were observed in huts, during an epidemic of malaria, among clothing. According to Toumanoff (1936), this species feeds by preference on animal blood but is more anthropophilic than *sinensis*, *vagus* and *subpictus*. Venhuis (1939) believes that there is a great difference between the habits of the type-form and the variety *vana*, the former being markedly zoophilous, whilst the variety feeds on man.*

Relation to Malaria.—This species has never been found naturally infected in India, but infections have been recorded both in Malaya and in the Dutch East Indies. In Malaya, Hacker (1923) reported 5 infections out of 103 dissected near Birnam river, North Selangor. The final results (unpublished) were 7 gut and 3 gland infections out of 133. Williamson (1926) dissected 89 with one gut infection at Krian, North Perak, and Hodgkin (1934; 1935) reported 2 individual infections, one in Kinta and the other on the Selangor coast. Later, Hodgkin and Johnston (1935) incriminated *barbirostris* as the chief agent in the maintenance of endemic malaria at Batu Gajah, Perak, where there was a dense human population, large numbers of mosquitoes and relatively few domestic animals. In 1933, out of 914 dissected, 15 gut and 11 gland infections were recorded (2·8 per cent); in 1934, out of 667 dissected there were 3 gut and 4 gland infections (1·05 per cent). The only other infections in this locality were *hyrcanus* var. *nigerrimus* and *hyrcanus* var. *sinensis* (one gut infection only, in each case), whilst about 1,000 specimens of *aconitus* were dissected with negative results. *A. barbirostris* was also held responsible for outbreaks at Krian, North Perak, and Butterworth, Province Wellesley. Subsequently, Hodgkin (1938*b*) published records of 2,860 dissections over two years in a village on the Klang-Selangor Road. He pointed out that though only 15 were found infected, this would represent one infective bite every second month.

In Sumatra, Dutch East Indies, Swellengrebel and Rodenwaldt (1932) recorded 3 infections observed in 544 specimens of *barbirostris* dissected by Swellengrebel and his colleagues, and one out of 277 by Doorenbos. In 1938, a high natural infectivity rate was recorded in this species at Wonoredjo, Celebes (Venhuis, 1939).

* The name *vanus* was originally given by Walker 1860 to an anopheline from Celebes which is usually considered to be *barbirostris*, or possibly one of varieties of *hyrcanus*.

This was thought to be not the type-form, but variety *vana*, as noted above. The figures in 3 sets of dissections were :

Set number.	Number dissected.	Number infected:		Percentage.
		Glands.	Total.	
1	517	22	68	13·15
2	524	6	58	11·07
3	242	0	4	1·65
Total	1,283	28	130	10·13

These figures are far higher than any others recorded and indicate that in the Celebes, *barbirostris vana* must be a vector of very great local importance.

CULICIFACIES GILES, 1901

Distribution.—India (widely distributed throughout, extending also to South Arabia) ; Ceylon ; Burma ; Thailand ; Indo-China (Annam, Laos, Tonkin) ; China (Lahati, Yunnan). Toumanoff (1936) found this species at Do-Luong, province of Vinh, North Annam, and stated that previous to this the furthest easterly record was that of Barnes (1923) at Chiengmai, Thailand. *A. culicifacies* has never been recorded in Malaya or East Indies, nor is it present in the Andamans or Nicobars.

Breeding Places.—Usually in fresh clean water, irrigation channels, sluggish streams, often with sandy margins and little vegetation, sheets of fresh rain-water, ricefields (whilst the plants are small, and in fallow-fields), borrow-pits, shallow wells, pools in river-beds, ornamental waters and swimming pools, occasionally in brackish water (Chalam, 1926).

Adult Habits.—Rests in cowsheds and houses, bites man freely, but the anthropophilic index is usually a low one. Afridi, Jaswant Singh and Harwant Singh (1939) in Delhi recorded precipitin tests of 4,172 positive specimens in 1937, of which 75 (1·80 per cent) contained human blood, and of 854 positive specimens in 1938 in which 8 (0·93 per cent) contained human blood. These observations were carried out during an inter-epidemic period. Covell and Jaswant Singh (1943) recorded an anthropophilic index of 22·3 per cent out of 547 positive tests carried out in the same area during an epidemic year. They suggested that *culicifacies* probably feeds on human and bovine blood indifferently ; that in normal years in Delhi Province it exists under almost exclusively rural conditions, where it has greater opportunities for feeding on cattle than on man, and where the cattle byres provide attractive resting places ; whereas in epidemic years, when conditions not only for its production in large numbers but also for its longevity and general activity are exceptionally favourable, its range of infiltration into urban areas is greatly increased. Under such conditions the cattle to man ratio is considerably reduced and this may explain the higher anthropophilic index. Barber and Rice (1938) found 1·9 per cent with human blood out of 730 positive specimens caught at Poona. The human positive percentage before the rains was 3·4 ; after the rains

had set in, 0·3. At Pattukkattai, Madras Presidency, Russell, Menon and Rao (1938) recorded the results of 394 positive precipitin tests of this species, of which only one contained human blood. In the following year, however, in a limited series of 70 positive tests in the same area there were 56 (80 per cent) with human blood (Russell and Jacob, 1939). They noted that this exceptionally high rate for *culicifacies* may have been due to a relative absence of cattle. Ramsay, Chandra and Lamprell (1936) in a small series of tests in Assam found 9 with human and 10 with cattle blood out of 19 positive specimens.

The effective flight range is normally about half a mile, but may extend to one mile when climatic conditions are exceptionally favourable.

Relation to Malaria.—A. culicifacies is the most important vector of the plains of peninsular and North-Western India, and is particularly notorious as the causative agent of regional malaria epidemics of great severity and wide range. In North-Western India these epidemics are particularly associated with extensive flooding and occur in years of high rainfall. In Ceylon, on the other hand, where this species is the only vector of any importance, they occur in years of drought when the river-beds, instead of being filled with water during the monsoon, are converted into a series of pools with a sluggish stream flowing down the centre, forming ideal breeding places for the production of *culicifacies* in enormous numbers. Senior White (1940) considers that this species is not a vector of primary importance in the north Gangetic plain, in the Satpura and Chota Nagpur Ranges, the Jaipur Hill tracts and the eastern coastal plain north of Godavari.

Russell and Rao (1940) dissected 13,156 specimens at Pattukkottai, Madras Presidency, with an infection rate of 0·11 per cent. They pointed out that in spite of this low figure it was able to maintain average endemic spleen and parasite indices of over 40 and 30 per cent respectively, and questioned the validity of Senior White's conclusion that it plays no part of importance in the transmission of malaria in the Singhbhum and Jaipur Hills.

Though the infection rate in *culicifacies* is usually so low, much higher figures have been recorded during epidemics. Thus, Covell and Baily (1932) reported sporozoite and total infection rates of 11·7 and 24·5 per cent out of 649 specimens dissected during a regional epidemic in Sind, whilst at one time at the height of the epidemic a sporozoite rate of 30 per cent was recorded out of 50 specimens caught in human habitations. This is in accordance with what one would expect from the trend of the precipitin results cited above.

In Assam and Burma, *culicifacies* appears to play little part in the transmission of malaria, the sporozoite and the oocyst rate being 0·4 per cent in each case out of a total of 1,662 specimens dissected in these areas (Senior White, 1940).

Although the species extends through Burma to Thailand, Indo-China and Southern China, it is nowhere found in large numbers in these countries and is not considered to be a vector of any importance. The discovery of a single specimen with 2 oocysts in the gut at Lahati, Yunnan, has, however, led Gaschen (1934) to suspect that it may be responsible for some of the malaria in that province and in North Tonkin.

FLUVIATILIS JAMES, 1902

Distribution.—India (widely distributed throughout); Ceylon, Burma, Thailand, Indo-China [according to Russell, Rozeboom and Stone (1943) but not listed by Toumanoff (1936)], Hong Kong (Burke, 1937). Occurs also in Turkestan, Iraq and Bahrein Islands, Persian Gulf. According to Stanton (1914), this species (*listoni*) was recorded from Formosa by Miyajama and Kinoshita; Secrete (1916) alluded to it as a common species in the district of Toeucho, but Koidzumi (1923) did not include it in his list of the anophelines of Formosa, and it seems possible that the early records really related to *minimus*.

Breeding Places.—Foot-hill streams with grassy margins, springs, irrigation channels: sometimes in ricefields and at the edges of swamps, lakes and tanks. Not commonly found below 1,000 feet or above 5,000 feet altitude in Southern India: occasionally higher elsewhere (Russel, Rozeboom and Stone, 1943). Covell and Harbhagwan (1939) describe this species as a clean water breeder, normally found in channels or streams with grassy edges and a moderate current, but making use of shallow wells in the monsoon season when these breeding places are washed out by heavy rains. In the Wynaad, Southern India, it was noted that the breeding places were invariably located in the immediate vicinity of human dwellings. Adisubramaniam and Vedamanikkam (1943), confirming this observation, concluded that anti-larval measures in respect of this species could be limited to within 1,500 feet of habitations. Though it prefers sunlit-breeding places, larvae are also found in waters partially shaded.

Adult Habits.—This species comprises at least two biological races, which differ markedly as regards their preferential feeding tendencies, although no morphological characteristics have yet been demonstrated by which they can be distinguished. The Southern India race is strongly anthropophilic; out of 1,681 specimens from the Wynaad, which gave positive reactions, 97 per cent contained human blood, whilst the percentage of human positive reactions among other species caught in the same houses during the same period was less than 5 per cent (Covell and Harbhagwan, 1939). Jaswant Singh and Jacob (1944) reported 64 per cent with human blood out of 88 positive tests on specimens from North Kanara. On the other hand, out of 350 specimens from Bazpur, in the foot-hills of the United Provinces, only 1·4 per cent contained human blood. In this area the local race of *fluviatilis* feeds almost exclusively on cattle. Barber and Rice (1938) recorded an anthropophilic index of 4·6 per cent out of 62 positive tests.

The domestic habits of the Southern India race are illustrated by the observations of Measham and Chowdhury (1934) who collected only 4 specimens in cowsheds as compared with 199 caught in human dwellings.

Viswanathan and Rao (1943) observed that in North Kanara females of *fluviatilis* entered houses for feeding almost exclusively during the first quarter of the night, that most of them took their blood meal before midnight, and that feeding might commence within 1½ hours after dusk had set in. The same observers showed that a high proportion of this species rests outdoors during the

day-time, notably under stream banks (Viswanathan, Rao and Rao, 1944). Senior White (1941), using a human-baited trap-net in the Satpura Ranges, noted that this species was attempting to feed at 2 a.m. Perry (1914) observed the adults biting during the day-time in the Jaipur Hill tract.

Relation to Malaria.—The Southern India race is one of the most efficient vectors known, and very high infectivity rates have been recorded on many occasions. Covell and Harbhagwan (1939) in the Wynaad dissected 2,781 specimens, of which 562 were infected (oocyst rate 16 per cent, sporozoite rate 7·6 per cent, total infectivity rate 20·2 per cent). Out of 1,303 dissected during May, the oocyst rate was 22·8 per cent, the sporozoite rate 10·1 per cent and the total infectivity rate 26·3 per cent. Measham and Chowdhury (1934) also recorded high rates from the Annamalai Hills, where the percentage of infections in April was 12·2; and Mathew (1939) reported an oocyst rate of 23·7 per cent and a sporozoite rate of 12 per cent out of 2,602 dissections in Travancore, South India. Jaswant Singh and Jacob (1944) dissected 897 specimens in North Kanara with a sporozoite rate of 7·1 per cent (total infection rate 11 per cent). Senior White and his colleagues have shown this species to be a vector in the Singhbhum and Jaipur Hills, and in the Satpura Ranges.

The only published records of natural infection of *fluviatilis* in North India appear to be those of Robertson (1910) at Saharanpur, United Provinces, who gave no details of his dissections, and Macdonald and Majid (1931) who reported 2 specimens with gut infections out of 23 dissected at Karnal. Clyde (1931) dissected 460 at Banbassa, in the foot-hills of the United Provinces, an intensely malarious tract, with negative results and it is probable that the northern race is of little, if any, importance as a vector. *A. fluviatilis* is not considered to be a malaria carrier in any area east of peninsular India.

HYRCANUS VAR. *NIGERRIMUS* GILES, *1900*

Distribution.—India (widely distributed throughout); Ceylon; Burma; Thailand; Indo-China; Southern China; Malaya; Sumatra; Java; Borneo; Celebes; Philippine Islands.

Breeding Places.—Ricefields, swamps, tanks with much vegetation, borrow-pits, and along the edges of slow-moving streams.

Adult Habits.—Adults are rarely found in houses, more often in cattle-sheds. They will bite man out of doors in the evening and in the shade during the day. Ramsay, Chandra and Lamprell (1936) recorded an anthropophilic index of 3·8 per cent out of 453 specimens subjected to the precipitin test.

Relation to Malaria.—This species has been found naturally infected in Indo-China, Malaya and the Dutch East Indies and is considered to have some importance as a vector in these countries. No natural infections have been recorded in India or Burma. In Indo-China the only record of infection is that of Gaschen (1936) who dissected 68 specimens out of which 1 contained oocyst and sporozoites. In Malaya, Hodgkin (1933) dissected 200 specimens at Kuala Lumpur, among which were 9 gut infections (4·5 per cent) and 5 gland infections

(2·5 per cent). This species had never before been considered to be a vector in that country. In Sumatra, Soesilo *et al.* (1933) recorded 4 infected specimens out of 163 dissected, and Soesilo (1935), during an epidemic in Java, reported 3 infections out of 386 dissected during August (when no other species was found infected) and 7 out of 334 dissected in November. He noted that the first series was a mixed collection of *nigerrimus* and *sinensis*, whilst the second contained all *nigerrimus*.

HYRCANUS VAR. *SINENSIS* WIEDEMAN, 1828

Distribution.—India (Assam only) ; Burma ; Indo-China ; Southern China ; Korea ; Japan ; Formosa ; Sumatra ; Java ; Riouw ; Celebes. It is recorded by Baisas (1931) in the Philippines ; but is not listed as occurring in these islands by Russell, Rozeboom and Stone (1943).

Breeding Places.—As for *nigerrimus*.

Adult Habits.—This form appears to differ conspicuously from *nigerrimus* as regards its preferential feeding habits. Walch (1932) in house-catches recorded an anthropophilic index of 83 per cent out of 381 specimens in a locality where cattle were present in normal numbers. Where these were scarce the human positive percentage was 90 (102 tests). Toumanoff and Hu (1934) at Shanghai recorded 295 human positive and 4 mixed human and cattle results out of 300 tested. The same observers reported 95 per cent human positive in specimens caught in houses and 99 per cent with cattle blood in those caught in cowsheds, suggesting that this form feeds on cattle or man indifferently. Chang (1940) observed that *sinensis* in Yunnan voraciously sought human blood, even when cattle and pigs were numerous. On the other hand, Morishita and Katagai (1933), as the result of a series of precipitin tests carried out in Formosa, reported only 20·8 per cent of those caught in houses, 1·2 per cent of those caught in cowsheds and 2 per cent of those caught in the open with human blood and concluded that *sinensis* usually feeds on cattle, whilst Raynal and Gaschen (1935) in Indo-China found 95 per cent of specimens with cattle blood out of 1,163 tested. Ave Lallemant, Soerono and Stoker (1932), in the Dutch East Indies, showed that this species could cover a distance of at least 1,600 yards.

Relation to Malaria.—Walch and Walch-Sorgdrager (1921) incriminated *sinensis* as the main vector in an epidemic at Sunei Tuan, Sumatra, where they encountered 107 infected specimens out of 7,257 dissected (1·5 per cent). Soesilo *et al.* (1938) recorded 1 infection out of 6 specimens dissected, also in Sumatra. The form found in Indo-China is not usually considered a carrier of importance because of its generally zoophilic tendencies, but in two epidemics near Hanoi it was apparently the chief vector. Gaschen (1936) dissected 661 of which 28 were infected (14 guts, 16 glands, infection rate 4·24 per cent) in the Red River Delta, Tonkin.

Sinensis is considered to be the chief malaria carrier in the plains of China and is also thought to play a part of importance in the transmission of the disease

in Formosa. Feng (1932) reported a natural infection in Central China, and Anazawa (1931) recorded an infectivity rate of 0·8 per cent in Formosa.

HYRCANUS X

Venhuis (1940) has pointed out that one of the species of *hyrcanus* widely distributed in Sumatra, Java, Borneo and Celebes differs from both *nigerrimus* and *sinensis* in its larval and pupal stages. This has been provisionally named *hyrcanus X*, since it is possible that it may not be a single entity. Those who recognise it as a new species believe that typical *hyrcanus-nigerrimus* and *sinensis* are very rare in Java and Borneo. During a malaria epidemic at Benteng, Celebes, 54 out of 587 *hyrcanus* were found infected. Examination of 63 sets of larval and pupal pelts with corresponding adults from Benteng at the time showed that all were *hyrcanus X*; moreover, about 6 larvae and 160 adults of *hyrcanus* from the same locality were examined and no examples of either *sinensis* or *nigerrimus* were discovered. In this locality 50 infections were also recorded among 524 specimens of *barbirostris*. During an epidemic at Karangbinangoen, Java, 46 out of 466 *hyrcanus* were found infected; many hundreds of larvae and adults and about 50 sets of pelts and adults were examined, and all were *hyrcanus X*. In a restricted part of the epidemic area, 15 out of 32 *aconitus* were infected, but as this mosquito was absent elsewhere, *hyrcanus X* was judged to be the most important vector.

JEYPORIENSIS JAMES, 1902

Distribution.—India (Assam, Bengal, Bombay, Deccan, Central India, Central Provinces, Chota Nagpur, Gujarat, Hyderabad State, Konkan, Madras Presidency, Malabar, Mysore State, United Provinces); Burma; Indo-China (Toumanoff and Try, 1937); Chapa, Tonkin.

Breeding Places.—Streams, flowing water connected with rice cultivation, and along marshy edges of streams, lakes and ponds.

Adult Habits.—The adults enter houses, but are more frequently found in cattlesheds (Senior White, 1937).

Relation to Malaria.—Nursing, Rao and Sweet (1934) recorded 2 gut infections out of 674 dissected in Mysore State (0·3 per cent). Senior White (1937) in the Jaipur Hill tract dissected 318, of which 4 were infected (gut only). Ramsay, Chandra and Lamprell (1936) reported over 2,000 dissections of this species in Assam, with 1 infection only (gut). Stephens and Christophers (1902), and Perry (1914) suspected this species to be a carrier on epidemiological grounds, but no gland infection has yet been reported, and it is not generally considered to play any part of importance in the transmission of malaria.

JEYPORIENSIS VAR. *CANDIDIENSIS* KOIDZUMI, 1923

Distribution.—India (Assam, Bengal, United Provinces, Malabar); Burma; Indo-China; Southern China; Formosa.

Breeding Places.—Slow-running water, river margins, streams with grassy edges, ditches, swamps, ricefields, seepage outcrops, especially in foot-hill areas.

Jackson (1936) noted that it was found in ricefields in China after the reaping of the second crop.

Adult Habits.—This form was listed along with *minimus* as strictly entophile* by Toumanoff (1936), who stated that in Indo-China when caught in houses it always contained human blood, and occasionally did so even when captured in stables or cattlesheds. He classed it as markedly anthropophilic although animal deviation was much greater than was the case with *minimus*. Raynal and Gaschen (1935) recorded 506 precipitin tests with *candidiensis* of which 41 per cent contained animal blood as compared with 26 per cent with *minimus*. According to Jackson (1935*a*) the flight range of *candidiensis* in Hong Kong exceeds half a mile.

Relation to Malaria.—Prior to 1944, the only records of natural infection of this form in India were those of Iyengar (1934*a*, 1934*b*) who encountered 4 gut infections out of 3,833 dissected in Travancore State. Recently, however, it has been shown to be a vector in Arakan on the Bengal-Burma border. In Indo-China it is considered an efficient vector in the foot-hills, second only to *minimus* in importance. Toumanoff (1936) recorded 4 gut and 2 gland infections out of 826 dissected in Tonkin (infectivity rate 0·7 per cent), 1 gland infection out of 58 in the foot-hills of South Indo-China, and 3 gut infections out of 424 in the high plateau of Annam (0·7 per cent). Feng (1935) described it as an important secondary vector in the hilly regions of Southern China, and later recorded 2 gut infections out of 56 dissected at Kwangsi (Feng, 1936). Jackson (1936) reported an infection rate of 3·3 per cent among 554 dissected in Hong Kong ; Ling, Liu and Yao (1936) recorded 1 gland infection among 53 dissected in Yunnan ; and Robertson (1941) found 6 infections (2 gut, 1 gland, 3 gut plus gland) out of 108 dissected in the same district along the Burma Highway (infectivity rate 5·55 per cent).

KARWARI JAMES, 1903

Distribution.—India (Assam, Bengal, Bihar, Bombay, Deccan, Chota Nagpur, Konkan, Madras, Malabar, Mysore State, Orissa) ; Ceylon ; Burma ; Thailand ; Indo-China ; Southern China ; Malaya ; Borneo ; Celebes ; Philippine Islands.

Breeding Places.—Clear shaded streams, spring pools, drains, seepages and swamps. Watson (1921) characterised this species in Malaya as a pool breeder, which prefers a gentle stream of water flowing through the pool, such conditions often being found at the foot of a hill. Larvae were also found in ravine streams, but these always had grass or weeds along the margins. Senior White (1926) noted that they were to be found in water flowing over rock rather than earth with high acidity and low conductivity. Hacker (1920) classed *karwari* as a small pool breeder, associated with *maculatus*, *vagus* and *kochi*. Iyengar (1926) found it breeding in seepages and seepage pools in Bangalore.

Adult Habits.—Adults are found in houses and stables. Laurel (1934), from a limited number of precipitin tests in the Philippines, concluded that they fed on man. Ramsay, Chandra and Lamprell (1936) recorded precipitin tests of blood meals on 311 specimens, of which 16·4 per cent contained human blood.

* Species which seek their hosts inside habitations or closed shelters, cattlesheds or stables.

Relation to Malaria.—Barber (1918) found this species highly susceptible to experimental infection in Malaya, and Watson (1921) suspected it to be a vector in that country on epidemiological grounds, but there are no records of infections in nature, excepting 1 gut infection reported by Strickland (1929) out of 125 specimens dissected in Assam in July. Ramsay, Chandra and Lamprell (1936) stated that over 15,000 specimens have been dissected in Assam with negative results. It seems unlikely that this species plays any part of importance in the transmission of malaria.

KOCHI DONITZ, 1901

Distribution.—India (Assam, Bengal); Burma; Thailand; Indo-China; Southern China; Formosa; Malaya; Sumatra; Java; Little Sunda Islands; Borneo; Moluccas; Philippine Islands.

Breeding Places.—Small muddy pools during the rainy season, unplanted ricefields; shaded or exposed streams and irrigation channels; sometimes in artificial containers, cut-bamboos, etc. (Russell, Rozeboom and Stone, 1943). This species was classed as a small pool breeder by Hacker (1920) who noted that the larvae were found most frequently alone, but fairly often in association with *hyrcanus* and *vagus*. Watson (1921) in Malaya noted that it bred in dirty water, such as buffalo wallows, water in wheel tracks, and drains contaminated with sewage, whilst Lalor (1912) found the larvae in standing water between furrows on sugar-cane plantations in Burma.

Adult Habits.—According to Russell, Rozeboom and Stone (1943) this species is moderately domestic, the adults frequenting houses and feeding on both man and animals. Toumanoff (1936) describes it as markedly zoophilic, attacking animals in shelters and out of doors, but occasionally entering houses to feed on man. Morin (1930) stated that in Cochin-China *kochi* is commonly found in houses at night, but entirely absent from day-time collections. A proportion of the adults certainly rest out of doors in the forest, and Venhuis (1942*a*) records them as being found under stream banks. Walch (1932) who performed a limited number of precipitin tests with this species found that in house-catches where cattle were present in normal numbers only 4 per cent contained human blood. The figure recorded by Ramsay, Chandra and Lamprell (1936) was almost identical (3·8 per cent human positive out of 213). Ave Lallemant, Soerono and Stoker (1932), in the Dutch East Indies, showed that *kochi* could cover a distance of over 1,000 yards.

Relation to Malaria.—This species has been found naturally infected in India and Indo-China, but is not considered an important vector in either of these countries. The only record of natural infection in India is that of Ramsay (1930) who reported 2 gut infections out of 77 specimens dissected in July at Cachar, Assam; but the total number dissected during the year was 2,113 and no other infections were encountered. In Indo-China the sole record of natural infection in this species is that of Toumanoff (1936) who observed oocysts in 2 specimens out of 45 dissected. In the Dutch East Indies, however, it is considered to be a vector

of some importance, and natural infections have been recorded on a number of occasions, sometimes with a high infectivity rate. Walch and Walch-Sorgdrager (1921) recorded a sporozoite rate of 11·5 per cent (70 dissections). Bosch (1925) reported an epidemic, considered to be caused by this species, in which 10 specimens were found infected out of 268 dissected (3·7 per cent). On the whole, however, the infectivity rate even in the Dutch East Indies is low, the figure given by Doorenbos (1931) as the result of 8,920 dissections in Sumatra being 0·75 per cent. The general conclusion is that where the species is present in large numbers it may become a vector of considerable local importance provided that local conditions are favourable.

LEUCOSPHYRUS DONITZ, 1901

Distribution.—India (Assam, Bengal, Konkan, Madras, Malabar, Mysore State); Ceylon; Burma; Thailand; Indo-China; Southern China (Hainan); Andamans; Malaya; Sumatra; Java; Borneo; Philippine Islands.

Breeding places.—Cogill (1903) noted that in Western India where water was plentiful the larvae of this species were found in the open, but that as a rule they occurred in jungle streams, being particularly partial to waters containing decaying leaves. Christophers (1912) found them in the Andamans in pools beside forest streams. Covell (1927*a*), also in the Andamans, recorded them in a pool in the open along with those of *tessellatus* overhung with *Mimosa pudica*, and also in a disused well with those of *barbirostris*. Senior White (1926) in Ceylon found the larvae along with those of *maculatus* in very acid water of high purity, and noted that they had been collected from springs at the head of a ravine, with no forest of any kind within some miles. Clark and Choudhury (1941) in Assam recorded them in stagnant water in open jungle, small pools in marshy areas and elephant footprints.

Adult Habits.—Roper (1914) in Borneo recorded that the adults were to be found abundantly in coolie huts inside mosquito-nets, engorged with blood. Bais (1919) collected them in houses in the Dutch East Indies, 800 meters from the nearest breeding place. Clark and Choudhury (1941) found them exclusively in houses at Digboi, Assam, and Toumanoff (1936) made a similar observation in Indo-China. Venhuis (1942*a*) observed them resting on stream banks in East Java. Walch (1932) noted that they fed readily on man, and recorded 90 per cent with human blood out of 102 specimens collected in houses in a locality where cattle were scarce. Ramsay, Chandra and Lamprell (1936) in Assam found 75·5 per cent with human blood out of 102 tested.

Relation to Malaria.—Roper (1914) suspected *leucosphyrus* to be a malaria carrier in Borneo on epidemiological grounds. Bais (1919) dissected 235 specimens of which 4 had gut infections (1·7 per cent) and considered it to be an important vector in the Dutch East Indies. In more recent years it has been found infected in several localities in Sumatra. Doorenbos (1931) reported 504 dissections in that island with an infectivity rate of 1 per cent. Stoker (1934) recorded 7 infections out of 110 specimens caught in houses at Sarang-Tioeng, an island off the coast of

Borneo, and Goelarso (1934) found 1 out of 13 infected in a locality in East Borneo. In India until recently this species was not looked upon as a carrier of any importance on account of its rarity, but Clark and Choudhury (1941) recorded an infection rate of 3·7 per cent out of 135 dissected in the vicinity of Digboi, Assam, in 1938, and of 4·9 per cent (82 dissected) and 3·1 per cent (642 dissected) in two different localities in the same area in 1940. More recently it has been incriminated as the chief vector in the Shingbwiyang area, North Burma.

In Indo-China, Toumanoff (1936) recorded 1 gut infection out of 77 dissected and considered it probable that this species, though not generally abundant in that country, is of some importance as a vector on account of its intimate contact with man. Farinaud (1938) reported 1 infection out of 50 dissected at Poulo-Condore in the deltaic region. Lefebre (1938) suspected *leucosphyrus* to be a vector in the forest-covered plains of Laos, where it comprised 66 per cent of the total anophelines collected. In the wooded valley of the Mekong 2 specimens were found with infected guts out of 29 dissected.

MACULATUS THEOBALD, 1901

Distribution.—India (widely distributed throughout the sub-mountain tracts along the foot of the Himalayas up to 8,000 feet, Nilgiri Hills, Salem Hills, Assam Hills, Chota Nagpur, Jaipur Hills); Ceylon; Burma; Thailand; Indo-China; Southern China; Formosa; Malaya; Sumatra; Java; Banka; Riouw; Borneo; Little Sunda Islands; Philippine Islands.

Breeding Places.—Streams and river-beds, with a preference for seepages. Strickland (1916) in Malaya noted that the larvae were found in cleared hill areas and not in hill-land jungle. Watson (1921) stated that this species alone of all the species in Malaya can make its home in fast running streams, free from grass and other vegetation. Larvae are also found in ricefields in hilly districts, especially in those where a variety of rice is grown, the stalks of which bend over and lie on the ground when the heads become ripe. Selwyn-Clarke (1931) stated that in Malaya the maximum breeding periods were March and September. The first is not affected appreciably by the April rains, but the heavy rains of October and November may wash out the larvae produced in the second period.

Adult Habits.—The adults enter houses and bite man readily at night, chiefly between 9 p.m. and 2 a.m. Thereafter the majority leave the houses and rest outdoors, notably along stream banks (Venbuis, 1941). Essed (1925) described them as chiefly out-of-door mosquitoes. Morin (1930) noted that in Cochin China they were common in houses at night, but rare in the day-time. Chang (1940), however, stated that in Yunnan they are taken commonly in houses, though rarely in cattlesheds, and Wallace (1939b) stated that in Malaya many more were caught in human habitations than in cattlesheds. Precipitin tests by Ramsay, Chandra and Lamprell (1936) in Assam indicated a preference for cattle blood, only 5·4 per cent containing human blood out of 130 tested. Walch (1932), in the Dutch East Indies, recorded a human positive percentage of 97 among 38 specimens caught in houses where cattle were scarce, but of only 11 among 45, also

caught in houses, in a locality where they were present in normal numbers. In Malaya, Green (1934), in a series of 304 precipitin tests with various species of anophelines, found 93 per cent of *maculatus* with human blood; the corresponding figure for *umbrosus* was 95 per cent and for *barbirostris* and *separatus* 80 per cent. Hodgkin *et al.* (1935) stated that precipitin tests once more indicated that *maculatus* has a strong preference for human blood. Toumanoff (1936) on the other hand stated that in Indo-China *maculatus* was markedly zoophilic, attacking animals not only in shelters but also in the open, and only occasionally entering houses to feed on man. Jackson (1936) stated that precipitin tests of this species in Hong Kong indicated a strong preference for cattle, whilst Laurel (1934) in the Philippines recorded only 7 specimens with human blood out of 249 tested.

It has been suggested that the importance of *maculatus* as a malaria carrier in Malaya is due to the scarcity of cattle in that country.

The effective flight range of *maculatus* was formerly considered to be not more than half a mile, but in recent years observations in Malaya indicate that it may on occasions exceed this distance (Evans, 1938; Holmes, 1939; Strahan, 1940). Wallace (1939*a*) recovered marked specimens at a distance of nearly 1½ miles from the point of release, but infiltration as far as this from breeding places is probably exceptional.

Relation to Malaria.—This species is not considered to be a vector of any importance in India, the only natural infections recorded in this country being from Shillong, Assam. In the 10-year period 1931-41, 25 gut and 5 gland infections were encountered out of 8,952 specimens dissected (0·34 per cent). During 1940-41, when it was the only species found infected in this locality, 13 gut and 1 gland infections were met with out of 1,573 dissected (3·96 per cent) (Viswanathan, Dass and Oommen, 1941). Ramsay (1930) dissected 3,374 *maculatus* in Assam with negative results. Feegrade (1926) recorded 1 gut infection out of 13 dissected at Lashio, Burma, but it is not considered a vector of importance in that country or in Thailand, where it is rare (Anigstein, 1932).

In Indo-China, Toumanoff (1936) recorded 1 gut and 1 gland infection of this species out of 90 dissected in Tonkin from 1931-33, whilst at Vinh-Thuy, one specimen was found infected out of about 50. It is probably of little importance as a vector in this country, though Morin (1930) considered on epidemiological grounds that it played some part in transmission in Cochin China. It has been found naturally infected in Hong Kong, but Jackson (1936) concluded that it could be a vector there only in exceptional circumstances. Chang (1940) thought it unlikely to be a carrier of any note in Hunan. Robertson (1940) records 3 natural infections of this species out of 42 dissected along the Burma Highway, Yunnan, in an area where *minimus* is the chief carrier.

In Malaya and in the Dutch East Indies *maculatus* is a vector of major importance in hilly areas, although the number of records of dissections published in the former country are remarkably few. In the Dutch East Indies it has been

found infected in Sumatra, Java, Banka, Riouw, several of the Little Sunda Islands and in Borneo. Doorenbos (1931) recorded 6,980 dissections on the east coast of Sumatra with an infection rate of 2·8 per cent. Venhuis (1941), during an epidemic at Kediri, East Java, dissected 393 specimens caught in houses with 1 gland infection. Out of 136 caught along stream banks, one was found with infected gut and 2 with infected glands. Only about one-third of those caught in houses contained blood, whereas blood was present in almost all those captured on the stream banks.

Venhuis (1941) concludes that perhaps those caught in houses were chiefly young adults waiting for a blood meal, or alternatively that there may be two biologically separate races, as suggested above.

In the Philippines, Russell (1934) considered that *maculatus* was an occasional vector. Ejercito (1934) recorded 3 infections (2 gut and 1 gut plus gland) out of 42 caught in a room containing several malaria patients. Another series of 296 dissections included 1 infected specimen (gut plus gland). He agreed with Russell that this species is probably a vector in the Philippines, but not one of major importance.

MANGYANUS BANKS, 1906

Distribution.—Philippine Islands.

Breeding Places.—Shallow, slow-flowing streams with sandy or rocky beds in or close to mountainous areas ; the larvae prefer clear water and are frequently found among mats of aquatic vegetation along edges of streams or irrigation channels, particularly among exposed bamboo roots in undercut banks (Russell, Rozeboom and Stone, 1943).

Adult Habits.—Russell and Santiago (1934*a*) recovered 3 marked females of this species at distances of from 218 to 546 yards from the point of release, and one male at 982 yards.

Relation to Malaria.—It is suspected to be a vector, apparently on epidemiological grounds (Jesus, Jao and Gracia, 1938). King (1932) states that most of the experimental results attributed to *febrifer* probably relate to this species, implying that it is readily infected with malaria parasites, and that it is found in situations with which malaria is usually associated.

MINIMUS THEOBALD, 1901

Distribution.—India (Assam, Bengal, Bihar, Chota Nagpur, Hyderabad State, Konkan, Madras, Malabar, Mysore State, Orissa, United Provinces) ; Burma ; Thailand ; Indo-China ; Southern China ; Formosa ; Sumatra ; Celebes ; Moluccas.

Breeding Places.—The characteristic breeding place of this species is in clear unpolluted slowly-moving water with grassy edges. It is only in such a situation that continuous dense breeding takes place ; but the larvae are found in many other breeding places, such as seepage outcrops, borrow-pits, tanks, ricefields, irrigation channels and shallow earth wells at low or moderate altitudes. From a practical

point of view, the vast areas of stagnant water formed by marshes and ricefields which may be present in *minimus* areas are of little or no importance (Thomson, 1940*a*). According to Lefebre (1938) this species breeds at altitudes up to 4,900 feet in Laos, Indo-China. It will not breed under shade, if this is absolutely dense, but partial shade is no deterrent : in fact, under such conditions the larvae are found in the shady rather than in the sunlit portions of the breeding place. Thomson (1940*a*, 1940*b*) has shown that the reasons for it not breeding under dense shade are secondary, due to the fact that marginal vegetation does not grow under such conditions, and, therefore, the influence of water movement is exerted right up to the bank. *A. minimus* breeds in moving water not because it prefers water in movement, but because it is usually only under such conditions that the water is fresh enough and cool enough for its needs. The larvae are unable to resist a current velocity exceeding 0·29 feet per second. The pre-monsoon breeding places are permanent waters, such as rivers, streams, seepages and wells fed by springs. When the monsoon breaks the larvae are washed out from many of these situations; but new seepage outcrops appear at the junction of the hills and the low lands, and many *nullahs* previously dry now contain fresh slow-moving water with grassy margins forming ideal *minimus* breeding places. Thomson (1940*c*) has shown that the thermal death point of *minimus* larvae is 40°C. considerably lower than is the case with *hyrcanus, barbirostris, culicifacies* and *vagus*. This renders it impossible for *minimus* to breed in ricefields whilst the plants are young or in shallow collections of sunlit water during most of the rainy season in Assam.

Adult Habits.—The chief day-time resting places of *minimus* in Assam and North Bengal are inside dark houses and coolie huts. They are frequently found on the dark earth floor under the large bamboo beds and the great majority rest in the lower half of the room. By far the greatest number are to be found hanging from the underside of the bamboo beds, under piles of firewood supported on a bamboo frame-work or similar dark horizontal surfaces. During the hot, damp monsoon season, this species takes two days to digest its blood meal and develop its ovaries, ovipositing on the second night after feeding. In the cold weather this period is increased to from four to six days. There is a considerable daily turnover of the *minimus* population in houses. About 90 per cent of its blood feeding takes place after midnight, and there is very little feeding activity during the first two or three hours after sundown (Thomson, 1941).

Ramsay, Chandra and Lamprell (1936) in Assam recorded precipitin tests on 622 specimens of which 60 yielded no result. The percentage with human blood was 85·7. Toumanoff (1936) stated that in Indo-China specimens subjected to the precipitin test almost invariably contained human blood when caught in human habitations, and frequently did so when taken in stables or cattlesheds. Mesnard and Toumanoff (1933), in a series of such tests, recorded 86·5 per cent with human blood, the remainder yielding no result. Raynal and Gaschen (1935) recorded 509 precipitin tests with this species of which only 26 per cent contained animal blood. Morishita and Katagai (1933) in Formosa found 69·2 per cent of specimens caught

in houses, 20·1 per cent of those caught in the open and 6·4 per cent of those caught in cowsheds to contain human blood.

Chang (1939) states that in Hunan, Southern China, *minimus* is taken only in small numbers in houses, and appears to enter them only for a blood meal, leaving again after feeding, and thus resembling *flavirostris* in its adult habits. It seems probable that this form belongs to a different biological race from that found in Assam.

Relation to Malaria.—This is one of the most dangerous malaria carriers known, by reason of its domestic habits and its preference for human blood. It is the chief vector of the foot-hills of the United Provinces, Bengal, Assam, Burma, Indo-China, Southern China and Formosa. Iyengar (1940) in the Darjeeling foot-hills dissected 172, and recorded a sporozoite rate of 9·3 per cent and a total infectivity rate of 18·6 per cent. Viswanathan, Das and Oommen (1941) in Assam reported 5,120 dissections with a total infectivity rate of 3 per cent. Infections were encountered in every month except February. They noted that in all centres transmission occurs from March or April to the end of the year, with the peak in October. The highest percentage of infections were in March, June, August and October. Ramsay, Chandra and Lamprell (1936) noted that out of over 100,000 mosquito dissections carried out in Assam, 98·3 per cent of the infections encountered were *minimus*. Senior White (1937) in the Jaipur Hills dissected 201 and recorded 17 gut and 8 gland infections (sporozoite rate 4·7 per cent). The same author later reported 195 further dissections from the same area, with an oocyst rate of 5·1 per cent and a sporozoite rate of 4·1 per cent. (Senior White, 1938). In the Singhbhum Hills, Senior White and Das (1938) dissected 334 specimens with an oocyst rate of 6·9 per cent and a sporozoite rate of 3·9 per cent.

In Indo-China, Toumanoff (1936) noted that infections of *minimus* were scattered throughout the year, the highest figures being recorded in December, January, June and October. Toumanoff (1932) observed that the percentage of this species infected in Tonkin rose from 0·8 in undulating country with irrigation canals and 1·2 in a region of wooded hills and marshy valleys to 3·43 in wooded mountainous areas with numerous permanent streams. Treillard (1938) noted that *minimus* lived from 5 to 10 times as long as *vagus* under identical conditions in the laboratory, and surmised that this might be a cogent reason for its efficacy as a vector of malaria. Raynal and Gaschen (1935) stated that among 4,488 specimens of *minimus* dissected in Indo-China the sporozoite rate was 3·69 per cent. Morin (1929), in Cochin China, recorded 5 infections (2 gut, 3 gland) out of 63 dissected (8 per cent).

In China, Feng (1932) recorded an oocyst rate of 8·57 per cent and a sporozoite rate of 29·85 per cent during a malaria epidemic in Amoy Island. The same author reported 6 specimens with gut plus gland infections out of 37 dissected in Kwangsi, Southern China (Feng, 1936). Jackson (1936) dissected 11,447 specimens in Hong Kong in four years, and recorded an infectivity rate of 4·5 per cent.

In Formosa, Anazawa (1931) reported an infectivity rate of 2·4 per cent in this species (? numbers dissected).

Hell (1933) stated that the type-form of *minimus* occurred in Sumatra, Celebes and the Moluccas, but there appears to be no record of natural infection of this species in these islands except that of Soesilo *et al.* (1932) who incriminated it as a carrier in Minahassa, Celebes (infectivity rate 3 per cent).

MINIMUS VAR. *FLAVIROSTRIS* LUDLOW, 1914

Distribution.—Philippine Islands ; West Java ; Borneo ; Bali.

Breeding Places.—Clear water of shaded streams ; especially round roots of bamboo ; also rivers, flowing or stagnant irrigation channels, pools and wells (Russell, Rozeboom and Stone, 1943). Smalt (1937) recorded it breeding in rice-fields in Bali.

Adult Habits.—Enters houses at night to attack man, but does not rest there. In the Philippines its favourite day-time resting places are under overhanging stream banks (Russell, Rozeboom and Stone, 1943). Venhuis (1942*b*) noted that in Java also it was rare in houses though present in great numbers along stream banks. According to Russell and Santiago (1934*b*) this species can fly with the wind at least one mile from its breeding place. Laurel (1934) recorded precipitin tests on 1,433 specimens in the Philippines, of which 94 contained human blood, 920 cattle blood and 4 both human and cattle. He concluded that there was apparently no preference for either man or cattle.

Relation to Malaria.—It is the principal vector in the Philippine Islands. Russell (1933) dissected 705 of the *funestus-minimus* group and found 4 specimens of *flavirostris* infected.

According to Overbeek and Stoker (1937) it has been found naturally infected in Tjipandani, West Java (3 per cent) ; Sidoardjah (1 per cent) ; and on the island of Poelau Laoet near Borneo. Venhuis (1942*b*) in Java, during an epidemic presumed to have been caused by *aconitus*, captured 258 *flavirostris* on stream banks, of which 235 were dissected. Among these there were 3 with infected guts. In the last five months of the epidemic, 5 guts were found infected, 4 from specimens caught on stream banks and one from a specimen caught in a house. In four years (1937-40) in East Java, 511 guts and 673 thoracic fluids from specimens caught in houses and stables only were examined. None of these were infected except 1 mid-gut from a specimen caught in October 1940.

NOVUMBROSUS STRICKLAND, 1916

Distribution.—Malaya.

Breeding Places.—Jungle pools, drains and swamps. Hodgkin (1938*a*) notes that it apparently breeds only in jungle, most frequently in swampy jungle such as covers extensive tracts of the coastal districts ; in contrast to *umbrosus* which, according to this author, contrary to general belief does not apparently breed in jungle.

Adult Habits.—Resemble those of *umbrosus*

Relation to Malaria.—Hodgkin (1937) recorded 2 natural infections out of 189 specimens dissected and the same author reported 3 specimens later with oocysts and 6 with sporozoites out of 752 dissected (Hodgkin, 1938*b*). He considers that this species is a vector of importance, and that it is probably responsible for a number of the infections which have been attributed to *umbrosus* (Hodgkin, 1938*a*).

PALLIDUS THEOBALD, 1901

Distribution—India (widely distributed, except in the north-west); Ceylon; Burma; Thailand; Sumatra (Djambi) (Soesilo *et al.*, 1932); possibly in Malaya (Gater, 1933).

Breeding Places.—Ricefields, tanks with vegetation, borrow-pits in connection with rice cultivation. Kenrick (1914) noted that in the Central Provinces, as this crop gives way to jungle, so do the numbers of this species diminish, till none at all are found where there are no ricefields. Iyengar (1926) observed the larvae in ditches and stagnant pools with a species of Chara forming dense sub-aquatic bushes, on the top of which they were found along with those of *annularis, barbirostris* and *hyrcanus*.

Adult Habits.—The adults are found chiefly in cattlesheds, but also enter human habitations. Satyanarayana (1934) recovered marked specimens which had flown across the harbour at Vizagapatam, at points 800 to 900 yards from the place of release.

Relation to Malaria.—This species has been found infected in nature, but is nowhere considered an important malaria carrier. Kenrick (1914) stated in a paper read at the Third All-India Sanitary Conference that Goverdhan had encountered gut infections in 2 specimens dissected at Chanda, Central Provinces. Sur and Sur (1929) dissected 1,236 at Krishnagar, Bengal, with 3 gut infections (0·24 per cent). Bose (1931) at Birnagar, Bengal, recorded one infected gut out of 403 dissected. Timbres (1935) dissected 35,429 specimens in Birbhum District, Bengal, and recorded a sporozoite rate of 0·03 per cent. Roy and Biswas (1942) reported 6 gland infections (0·7 per cent) out of 854 dissections in Udaipur State, Central Provinces. Iyengar (1939) reported 2 gut infections out of 408 dissected in Bengal. In the East Satpura Ranges, Senior White and Adhikari (1940) dissected 3,157, of which gut infections were met with in four instances.

PATTONI CHRISTOPHERS, 1926

Distribution.—China, not south of 30° North (Russell, Rozeboom and Stone, 1943).

Preeding Places.—Small collections of water in beds of hill streams, pockets along streams with considerable algal growth, rain pools in rocks (Russell, Rozeboom and Stone, 1943). Larvae have been recorded in ricefields in Szechwan (Best, 1932).

Adult Habits.—It is considered to be an important vector in the hilly regions of Northern China (Feng, 1935), apparently on epidemiological grounds only. Hindle and Chow (1929) succeeded in infecting 9 specimens out of 11 experimentally (82 per cent). There appear to be no published records of natural infection.

PHILIPPINENSIS LUDLOW, 1902

Distribution.—India (Assam, Bengal, Bihar, Bombay, Deccan, Chota Nagpur, Konkan, Madras Coast North, Malabar, Mysore State, Orissa) ; Burma ; Thailand ; Indo-China ; Southern China (Hainan, Yunnan) ; Andamans ; Malaya ; Sumatra ; Philippine Islands.

Breeding Places.—Tanks, pools, borrow-pits and ditches with vegetation; recorded in ricefields in the Andamans by Christophers (1912) and Covell (1927*a*). Krishnan (1940) in Bengal found no larvae of this species in ricefields, the species breeding chiefly in reservoirs, borrow-pits and canals. Ramsay and Macdonald (1936) concluded that this species requires cool water and hence favours permanent pools with vegetation under light shade. Bose (1931) stated that he could attract or repel this species from village tanks by introducing or removing suitable vegetation. Iyengar (1944) stated that *philippinensis* prefers ponds exposed to sunshine, containing water with a high dissolved oxygen content, and that it breeds only in clear water, being absent from those even feebly contaminated with sullage or decaying organic matter. Larvae are found in the greatest numbers in water with a dense growth of sub-aqueous bushes (*Hydrilla verticillata*, the most favoured of all, *Ceratophyllum demersum* and certain species of Utricularia and Najas). Duckweeds and water-hyacinth (*Eichornia speciosa*) are inhibitory, due to the cutting off of sunlight and consequent changes brought about in the phytoplankton in the water. Some forms of non-filamentous green algae, which flourish best in sunlight, are favourable to the breeding of *philippinensis*, whilst certain algae of the family Cyanophyceae, notably *Microcystis aeruginosa*, and the blue green algae (e.g. Anabaena and Oscillatoria) are inimical. Euglena, which is frequently an indication of organic contamination, is also unfavourable. The same author (Iyengar, 1942) notes that, in Bengal, the depth of the subsoil water level has a marked influence on the prevalence of *philippinensis*. In villages where the level of the water table is low the density of this species is high ; conversely where the subsoil water level is high the *philippinensis* density is low. In conformity with this finding, in the deltaic region of Bengal, areas with a high subsoil water level during the monsoon are comparatively healthy, whilst those with a low subsoil water level tend to be highly malarious.

Adult Habits.—In Bengal, Krishnan (1940) notes that *philippinensis* is chiefly a domestic species, adults being found in from 34 to 90 per cent of the houses at Abujhati. Timbres (1935) also observed that it definitely prefers human habitations to cowsheds. Iyengar (1944) collected 3,940 specimens in Bengal, of which 97·2 per cent were caught in houses. Of a total of 37,622 anophelines of all species collected in houses, 10·2 per cent were *philippinensis*, whereas out of 59,624 caught

in cowsheds, only 0·2 per cent were this species. The most favoured day-time resting place was on the wall within 1½ feet of the floor in bedrooms, but specimens were also found in similar situations in kitchens and on pots and other utensils on the floor. Biting is said to take place at night chiefly from 8 to 10 p.m. and 2 to 4 a.m. In Assam the species appears to be zoophilic. Ramsay, Chandra and Lamprell (1936) recorded the results of precipitin tests on 343 specimens, 31 of which yielded negative results. The percentage with human blood was only 6·4. No adults of this species were found in the Andamans either by Christophers or Covell, and it seems probable that this species may include two or more distinct biological races.

Relation to Malaria.—This species is an important vector in deltaic Bengal. Sur (1928) recorded 3 gut and 5 gland infections out of 223 dissected (infectivity rate 3·1 per cent). Sur and Sur (1929) at Krishnagar dissected 770 with a sporozoite rate of 2 per cent, and at Birnagar dissected 458 with a sporozoite rate of 1·5 per cent. Timbres (1935) dissected 12,594 specimens with an infectivity rate of 1·04 per cent. The highest sporozoite rate was 2·88 per cent recorded in mid-August. Iyengar (1939) reported 1,918 dissections with a total infectivity rate of 7·2 per cent, and Iyengar (1940) recorded a further series of 1,830 dissections with an infectivity rate of 6·3 per cent. Niogi and Khan (1937) dissected 101 specimens in the Bengal Dooars of which 2 were infected. In Assam, however, this species does not appear to play any part of importance in transmission. Ramsay and Macdonald (1936) stated that the Ross Institute workers have dissected 12,464 specimens in that province, all of which proved negative. Viswanathan, Das and Oommen (1941), also working in Assam, recorded dissections of 4,239 specimens carried out during the period 1931-41, among which 4 were infected (2 guts, 2 glands). The infections were encountered in two years only, 1931 and 1933.

In Burma, Feegrade (1926) recorded 2 gut and 1 gland infection in this species at Bhamo (1·6 per cent), but it is not generally regarded as a vector of importance in that country.

Toumanoff (1936) dissected 592 specimens in Tonkin in 1931-33, all of which proved negative. There appears to be no record of natural infection of this species in that country. It has been suspected as a carrier in Hainan, Southern China, but there appears to be no definite evidence to support this supposition. There is no evidence to suggest that it is a vector in the Philippines.

PULCHERRIMUS THEOBALD, 1902

Distribution.—India (Baluchistan, North-West Frontier Province, Punjab, Rajputana, United Provinces, Waziristan, Sind, Delhi Province, Gujarat); Bahrein Islands (Persian Gulf); Caucasus; Iraq; Iran; Turkestan; Bokhara.

Breeding Places.—Open water containing vegetation, large swamps, stagnant pools, ricefields (in Turkestan).

Adult Habits.—The adults are frequently found in human habitations and cattlesheds. They are voracious feeders, attacking men and animals in the open both by day and night. They are strong fliers, and may be carried long distances bythe wind, especially during dust-storms. They rest in partially lighted quarters rather than in the dark. Chodukin and Sternchold (1938) recorded the results of precipitin tests on 986 anophelines of various species in Central Asia. Among these, 18 per cent of *pulcherrimus* contained human blood only, and a further 17 per cent contained both human and cattle blood.

Relation to Malaria.—This species has been found naturally infected in Central Asia. It was listed by Zaitzev (1934) as an important vector in the Caucasus. Zavadskii (1929) recorded that in the autumn of 1928 sporozoites were found in the salivary glands of one of the numerous females of this species captured in houses in a village of the Tashkent District, where an epidemic of malignant tertian malaria was in progress. Geller (1939), writing on the culicid fauna of Kazakhstan, states that *pulcherrimus* is an important vector in many districts. There appears, however, to be very little specific evidence to support this assumption.

Christophers and Shortt (1921) recorded a gut infection in this species in Basra, but did not state the numbers dissected. It is not considered to be a carrier of any importance in Iraq. In India the only record of natural infection appears to be that of Covell and Baily (1930) at Larkana, Sind, where oocysts were observed in 5 specimens out of 453 dissected. It probably plays no effective part in the transmission of malaria in this country.

PUNCTULATUS DONITZ, 1901.

PUNCTULATUS VAR. *MOLUCCENSIS* SWELLENGREBEL AND SWELLENGREBEL-DE-GRAAF, 1920

This species has been regarded as comprising two well-defined varieties but recent investigations have thrown considerable doubt on this point. Specimens of the type-form from Milne Bay, New Guinea, differ both biologically and morphologically from *moluccensis* from Cairns, and the two forms are distinguishable and behave differently in parts of coastal New Guinea. In other areas, numerous intermediate forms occur, and examination of a long series of adults and larvae from many localities has failed to reveal any constant differential characters which can be universally applied. Since the position is so obscure, and since in any case the distribution, breeding places and adult habits of the two forms are very similar, whilst both are undoubtedly dangerous malaria carriers, they are here considered together.

Distribution.—Islands east of Celebes and Timor ; most of Melanesia, west of 170° East and north of 20° South, Moluccas ; New Guinea ; New Britain ; New Hebrides ; New Ireland ; Solomon Islands ; Northern Australia.

Breeding Places.—Sunlit natural and artificial water, e.g., banks of rivers and creeks with or without vegetation, drains, trenches, swamps and hoof-prints. The type-form breeds only in fresh water, whilst *moluccensis* has a much wider range

and will breed either in fresh or in very brackish water. Follows man when he cuts down and clears away the scrub, thus admitting the sun to ground water which was previously unsuitable to it for lack of sunlight (Taylor, 1943).

Adult Habits.—Both the type-form and the variety will feed at any time of the day, provided the sky is overcast. They are small grey mosquitoes which do not attract much attention when feeding because their bite is not severe, nor do they make any noise. They enter houses and remain there if undisturbed. Observations in New Guinea on *moluccensis* showed that the great majority feed during the night. The activity of those entering human dwellings to feed was marked by a distinct periodicity, the greatest activity occurring about 9 p.m. It then declined, with minor peaks at approximately three-hourly intervals until biting virtually ceased at or immediately before dawn. It was observed that a strong wind during the earlier part of the evening might lower the peak of activity at 9 p.m. and raise it correspondingly at midnight, whilst a steady breeze or continuous rain flattened the curve of activity throughout the night.

Walch (1932), as the result of a series of precipitin tests, concluded that *punctulatus* and *moluccensis* both fed exclusively on man, but in a recent series of tests carried out in New Guinea, only 19 per cent (of var. *moluccensis*) contained human blood, most of the remainder having fed on dogs and pigs. They certainly bite man when an opportunity arises, but are apparently not highly anthropophilous.

It appears that the majority of *moluccensis* leave the house after gorging fully, and find shelter in the surrounding scrub in spots close to the ground where humidity is highest and where they are protected from the surrounding wind. They are found under fallen coconut leaves, in empty coconut husks, amongst the exposed roots of bushes and in crab holes a few inches below the surface of the ground. A minority remain in dwellings during the day and are to be found in cool dark corners, behind packing cases, under the thatch and in any other position sufficiently sheltered.

Precise data regarding their effective flight range are not available, but in the course of a study of the bionomics of *moluccensis* in New Guinea it was noted that no mosquitoes had fed on man among those caught beyond 300 yards from a village, and that the density of the species varied inversely with the distance from the village, very few being found at 600 yards distance, whilst they were captured in large numbers at 300 yards. It seems probable that the effective flight range does not exceed half a mile.

Relation to Malaria.—*Punctulatus* type and var. *moluccensis* are the dominant malaria vectors throughout the area of their distribution. Heydon (1923) dissected over 200 specimens at Rabaul, New Britain, and recorded an oocyst rate of 3·6 per cent and a sporozoite rate of 3·9 per cent, the corresponding figures among specimens taken in huts being 6·2 and 6·4 respectively. Most of these were probably *moluccensis*. Rook (1924) recorded 12 gut infections out of 448 *punctulatus* type dissected in New Guinea (2·9 per cent). The same observer dissected 82 specimens

of *moluccensis* at Tanah Merah, New Guinea, in November and 125 in December-January with an infection rate of 4·9 and 11·0 per cent respectively (Swellengrebel and Rodenwaldt, 1932). More recent unpublished records include results of dissections of *punctulatus* type at Milne Bay, New Guinea (infection rate 11 per cent, sporozoite rate 8 per cent out of 61), and of *moluccensis* at Cairns, North Queensland (infection rate 1·16 per cent, sporozoite rate 0·7 per cent out of 1,163). In the Ramu Valley, New Guinea, a sporozoite rate of 1·8 per cent (330 dissections) was recorded by Atherton and Lemerle (1944, unpublished). The total infection rate was 3·3 per cent.

RAMSAYI COVELL, 1927

Distribution.—India (Assam, Bengal, Bihar, Orissa); Ceylon, Burma; Thailand; Sumatra; Java.

Breeding Places.—Rain-water pools, tanks and swamps with heavy growth of vegetation. Sen (1941) notes that in Bengal it appears to be almost exclusively associated with *Pistia stratiotes*.

Adult Habits.—The adults rest both in houses and cowsheds.

Relation to Malaria.—It was found naturally infected in Assam by Ramsay (1930), who recorded one gland infection out of 287 specimens dissected (0·36 per cent). Later, Ramsay and Macdonald (1936) reported 2,217 dissections (including the above) with no further infections. This species probably plays no part of any importance in the transmission of malaria.

SEPARATUS LEICESTER, 1908

Distribution.—Malaya; Sumatra; Riouw Archipelago.

Breeding Places.—Pools and streams up to 5,000 feet (Russell, Rozeboom and Stone, 1943). Hacker (1920) classes this species among the swamp fauna, and notes that it prefers the open swamp just outside the jungle, in contradistinction to its close relative *umbrosus*, which breeds typically in swampy jungle. (According to Hodgkin, 1938*a*, however, *umbrosus* does not breed in jungle though *novumbrosus* does). Essed (1925) found the larvae in a shady well.

Adult Habits.—The adults rest in deep jungle, and rarely enter houses (Russell, Rozeboom and Stone, 1943). Green (1934), recording the results of precipitin tests of the blood meals of 304 mosquitoes, found 80 per cent of *separatus* with human blood, as compared with 95 per cent in *barbirostris* and *umbrosus* and 93 per cent in *maculatus*.

Relation to Malaria.—This species is not considered to be a vector of importance. Hodgkin *et al.* (1935) recorded one gland infection (0·3 per cent) in a series of dissections of specimens caught in a rubber estate in a locality intermediate between the inland hills and the coastal plain in Malaya. This appears to be the only published record of natural infection in the species.

SPLENDIDUS KOIDZUMI, 1920

Distribution.—India (widely distributed throughout); Thailand; Indo-china; Southern China; Formosa.

Breeding Places.—Small pools containing algae and other vegetation often in connection with river-beds, seepage pools, tanks and sluggish streams.

Adult Habits.—The adults are found, usually in small numbers, in houses and cattlesheds. Toumanoff (1936) stated that this species was markedly zoophilic, attacking animals not only in shelters but also in the open, but that it occasionally enters human habitations and feeds on man. Ramsay, Chandra and Lamprell (1936) recorded the results of precipitin tests on 214 specimens, all of which had fed on cattle. This was the only species out of 11 subjected to more than 100 tests each in which the anthropophilic index was nil.

Relation to Malaria.—Robertson (1910) recorded natural infections in this species at Saharanpur, India, but gave no details as to the numbers dissected or infectivity rate. Macdonald and Majid (1931) recorded 1 gut infection out of 26 dissected at Karnal, Punjab. These appear to be the only natural infections reported in India. Jackson (1935*b*) dissected 48 specimens at Hong Kong and found 2 with gut infections, but noted that the only animals in the neighbourhood were a few goats, and that conditions for contact with man were exceptionally favourable. Anazawa (1931) recorded an infectivity rate of 2·3 per cent in the species in Formosa. Although it has occasionally been found to be infected, there appears to be no specific record of a gland infection. It is improbable that this species plays any part of importance in the transmission of malaria.

STEPHENSI LISTON, 1901

Distribution.—India (widely distributed throughout); Burma; China (Yunnan) (Williams, 1941); extends westwards through Bahrein Islands (Persian Gulf), Iran and Iraq to East Arabia.

Breeding Places.—In towns larvae are found in wells, cisterns, fountains, garden tanks and tubs, water used for soaking bricks and keeping the surface of cement concrete wet during building construction, cellars into which subsoil water percolates, leakages from reservoirs, improperly graded roof-gutters, discarded tins and receptacles of all kinds, hollows in machinery and scrap iron, etc. In rural areas it is found in pools, in stream beds and at the margins of the stream itself, in seepages and marshy areas with a gentle flow of water, irrigation channels, reservoirs and springs, showing a preference for sunlit breeding places (Mulligan and Baily, 1936). In Bahrein larvae were found in drains, shallow wells and garden pits containing seepage water. The upper limit of salinity, in which larvae were found, was 2,750 parts of chlorine per 100,000 (Afridi and Majid, 1938). In Bombay, this species had been found breeding in water with high salinity, sometimes reaching or even exceeding that of sea-water (Bentley, 1911; Chalam, 1926; Bana, 1943). It can tolerate water with a high degree of organic pollution and has been found breeding in drains contaminated with sewage (Roy, 1931).

Adult Habits.—According to Russell, Rozeboom and Stone (1943) the adults are strong fliers and may travel three miles. Afridi and Majid (1938) concluded that their range of dispersal in Bahrein Islands was 1½ miles. On the other hand the extreme localisation of malaria in urban areas in India would seem to indicate that under such conditions the effective flight range does not exceed half a mile at most. Sweet and Rao (1937) stated that the type-form is hardier and lives longer than var. *mysorensis* and feeds avidly on man, whilst the latter appears to prefer animal blood. Afridi, Jaswant Singh and Harwant Singh (1939), however, in a series of 360 positive precipitin tests carried out with the type-form in Delhi, found only 1·4 per cent with human blood.

Relation to Malaria.—This species is an important vector under rural conditions in Western and North-Western India, the Persian Gulf and Iraq, and under urban conditions in certain localities in peninsular and Northern India. It is the chief carrier in Bangalore and Bombay cities. Hodgson (1914) recorded 2 gut infections out of 110 specimens dissected in Delhi, but it does not appear to be a vector of any importance in that city at the present time. It was incriminated as the cause of a severe outbreak of malaria in Lucknow in 1929 by Banerjea (1930) who recorded an infectivity rate of 9·3 per cent out of 75 dissected. This epidemic was peculiar in that it commenced in the late spring instead of in the autumn as is usual in this part of India. Two-thirds of the cases were benign tertian infections.

Natural infections of *stephensi* have been recorded in Bahrein Islands (Afridi and Majid, 1938). Kutch State (Afridi, Majid and Jaswant Singh, 1938), Khairpur State, Sind (Covell and Baily, 1934), Quetta (Mulligan and Baily, 1936), Kohat (Sinton, 1917), Bombay (Bentley, 1911 ; Covell, 1928), Mopad, Madras (King and Iyer, 1929) and Bangalore (Sweet and Rao, 1931 ; Nursing, Rao and Sweet, 1934).

STEPHENSI VAR. *MYSORENSIS* SWEET AND RAO, 1937

Distribution.—India [(Poona, Sind, Mysore State (not Mysore City or Bangalore), Vizagapatam)]. It may be much more widely distributed, since it can be differentiated from the type-form only from the character of the egg, and it is possible that many of the records given above relate to this form.

Breeding Places.—Sometimes wells, but more often irrigation channels and along the margins of streams.

Adult Habits.—This species was thought by Sweet and Rao (1937) to be less hardy than the type-form and more zoophilic in its feeding tendencies. Early attempts to maintain laboratory colonies failed, whilst the type-form breeds without difficulty in captivity. Russell and Mohan (1941) have, however, succeeded in maintaining a laboratory colony of *mysorensis* through nine generations.

Relation to Malaria.—This form has been considered of less importance as a vector than the type, but Senior White (1943) considered it to be the chief rural carrier at Vizagapatam, where a sporozoite rate of 1·75 per cent was recorded. Russell and Mohan (1939) found both forms easily infected with *P. falciparum*

and equally susceptible. It is possible that some of the records of infection given above under the type-form may really relate to *mysorensis*.

SUBPICTUS GRASSI, 1899

Distribution.—India (widely distributed throughout); Ceylon; Burma; Thailand; Indo-China; Formosa; Great and Little Sunda Islands; Philippines; Moluccas; New Guinea.

Breeding Places.—Almost any temporary or permanent collection of water, even sewage polluted pools or brackish water. Common in borrow-pits, buffalo wallows, brick-pits, roof-gutters and artificial containers. Walch and Walch-Sorgdrager (1926) from a study of the eggs consider that in the Dutch East Indies there are at least two races, one breeding in salt water, the other in fresh.

Adult Habits.—The adults are found in large numbers both in cattle-sheds and houses. They feed on man, but prefer the blood of cattle. Roy (1943) found 25 per cent with human blood in a series of 1,515 precipitin tests, but in all other records of such tests with this species the human positive percentage in India has been very low as shown below :—

Authority.	Locality.	Number of positive results.	Number with human blood.	Anthropophilic index, per cent.
Ramsay, Chandra and Lamprell (1936)	Assam	261	6	2·3
Barber and Rice (1938) ...	Poona	224	1	0·4
Senior White (1938) ...	Jaipur Hills	42	0	0·0
Russell and Jacob (1939) ...	Pattukkottai, Madras	224	7	3·1
Afridi, Jaswant Singh and Harwant Singh (1939) ...	Delhi	1,824	0	0·0
Covell and Harbhagwan (1939) ...	Wynaad, South India	41	1	2·4

In Indo-China, Raynal and Gaschen (1935) recorded 6·2 per cent with human blood out of 316 *subpictus* tested.

Relation to Malaria.—This species has been occasionally found infected in nature. It is believed to be a carrier of some importance in Celebes and Modoera, but not elsewhere. Russell and Rao (1940) recorded 13,277 dissections at Pattukkottai, Madras Presidency, out of which 2 specimens were infected (1 gut only, 1 gut plus gland). Russell, Rao and Jacob (1939) reported 2 gut infections out of 4,728 dissected at Ennore, in the same province.

The following records have been published for the Dutch East Indies :—

Authority.	Number dissected.	Infectivity rate per cent.
Swellengrebel and Swellengrebel-de-Graaf (1920) ...	4,898	0·4
Porten (1924)	1,544	0·16
Rodenwaldt and Essed (1925)	3,875	0·3
Walch and Soesilo (1927)	447	0·2
Brug and Walch (1927)	221	0·4

Overbeek and Stoker (1937) state that in Macassar, Celebes, Rodenwaldt recorded an infectivity rate of 0·5 per cent.

SUBPICTUS VAR. *INDEFINITUS* LUDLOW, 1904

Distribution.—Philippine Islands; Formosa (Morishita, 1935); Indo-China (Toumanoff, 1934).

Breeding Places.—Clear, unshaded fresh water streams, rivers, pools, flowing irrigation channels, lakes and wells, sometimes in brackish or ocean salt water (Russell, Rozeboom and Stone, 1943).

Adult Habits.—Found frequenly in houses at night and will feed on man or animals. They are able to migrate at least one mile from their original source (Russell and Santiago, 1934*b*).

Relation to Malaria.—Not considered to be a vector in the Philippines (Russell, Rozeboom and Stone, 1943). Toumanoff (1934), however, considers that the *subpictus*, found in Indo-China, which is considered to be a secondary vector of some importance, is this variety. Farinaud (1938) recorded an infection rate of 4·4 per cent out of 68 specimens dissected at Poulo-Condore, in the coastal area of South Indo-China.

SUNDAICUS RODENWALDT, 1926

Distribution.—India (coastal areas of Bengal, Orissa, North Madras); Burma; Thailand; Indo-China; Southern China (Hainan, 1 specimen); Andamans; Nicobars; Malaya; Great and Little Sunda Islands as far east as Roma.

Breeding Places.—Saline water, usually (but not always) in the presence of algae. Favourite situations are behind embankments erected to protect ricefields from the sea, where mangrove has been cleared, and lagoons formed by the silting up of river mouths. Larvae are found in borrow-pits and hoof-prints and in any water collections in cleared mangrove where the influence of the daily tide has been impeded by the construction of roads, railway tracks, etc. This species will not breed in waters, subject to the influence of the daily tides, and the larvae are not usually found in virgin mangrove. It favours breeding places in which floating algae (Enteromorpha, Cladophora, Cyanophyceae) are present. In the vicinity of Calcutta it has most often been found in association with *Oscillatoria germinata*, *Lyngbya confervoides* and *Oedogonium sp.* It will also breed, however, in water in which no algae are present, or at any rate none visible to the naked eye (Covell, 1927*a*). Schuffner *et al.* (1919)recorded that in a locality in Sumatra the larvae were at first abundant in breeding places without algae, but that later on when algae appeared in certain water collections they were exclusively found in the latter. Senior White and Adhikari (1939) noted that in the Chilka Lake, Orissa, the favourite breeding place of *sundaicus* was among putrefying masses of sub-aqueous plants, *Potamogeton pectinatus* and *Halophila ovata*, which are uprooted by wave action at certain times of the year and become bound together into plaques by the algae *Lyngbya aestuarii*. Removal of weeds from breeding places results in a cessation of breeding (Covell and Pritam Singh, 1942). Rodenwaldt and Essed (1925) recorded

that this species breeds readily in water polluted with sewage. It prefers sunlit breeding places (Watson, 1921). The optimum salinity, according to Covell and Pritam Singh (1942) in Orissa, is from 600 to 800 parts per 100,000. Senior White and Adhikari (1939), from observations made in the same area, concluded that the optimum figure was 869 parts per 100,000 whilst the inhibitory salt value was 2,300 parts. Iyengar (1931*a*) in Bengal found that 1,500 to 2,500 mg. per litre was the optimum. He noted that Rodenwaldt and Essed (1925) in the Dutch East Indies had recorded a figure of 1·2 to 1·8 per cent as the most favourable, whilst Christophers (1912) had observed *sundaicus* breeding in a salt concentration of 0·4 per cent in the Andamans. The latter figure is in conformity with that recorded by Iyengar, but this is about one-tenth as high as that reported by Rodenwaldt and Essed (1925).

In the Dutch East Indies there is a fresh-water form of *sundaicus*, which breeds in fish ponds at the height of 1,500 feet above sea level, and has been found even as high as 3,250 feet. They are found in the narrow valleys among the mountains running parallel with the west coast of Sumatra.

Adult Habits.—The adults are strong fliers. Covell (1927*a*) and Porten (1924) have recorded that in their search for blood they can travel over one mile, and Breeman (1919) considered that they could fly as far as five kilometres. They are found in houses as well as cattlesheds, are exceedingly voracious feeders and have been known to bite by day as well as by night (Swellengrebel and Swellengrebel-de-Graaf, 1919). Overbeek and Stoker (1937) describe this species as a true house mosquito, with a strong tendency to feed on human blood even in the presence of cattle. Walch (1932) recorded 86 per cent with human blood out of 227 specimens caught in houses in a locality where cattle were present in normal numbers. Out of a small series of 17 caught in a locality where cattle were scarce the percentage with human blood was 94. Treillard (1934) alluded to the house-haunting habits of this species in Indo-China, and observed that it lived longer under laboratory conditions than either *minimus* or *candidiensis*.

Relation to Malaria.—This species is an important malaria carrier in India, the Andamans, Malaya, Indo-China and the Dutch East Indies. In India, Iyengar (1931*b*) dissected 838 specimens in Bengal of which 71 had gut and 169 gland infections (total infectivity rate 23·4 per cent). Covell and Pritam Singh (1942) reported 32 gut and 51 gland infections out of 10,714 specimens dissected in the vicinity of the Chilka Lake. In the Dutch East Indies very high infectivity rates have sometimes been recorded. Walch and Walch-Sorgdrager (1921) found 17·5 per cent infected out of 58, and Stoker is said to have reported a rate of 39 per cent in a locality in East Java (Overbeek and Stoker, 1937). Kuipers and Stoker (1934) during an epidemic at Brengkok, North Java, 1¼ miles from the sea, dissected 146 of which 32 had gut infections only, 24 gland infections only and the remainder mixed infections, gut plus gland. Stookes (1927) in Sarawak, Borneo, recorded 3 infections out of 22 dissected (13·6 per cent). But though highly susceptible to infection, *sundaicus* has also been found in large numbers in certain regions where the incidence of malaria is very slight indeed (Walch and Soesilo, 1929).

In South Indo-China, Farinaud (1938) recorded 50 dissections at Poulo-Condore, with an infection rate of 2 per cent; 126 at Gocong, infection rate 5·5 per cent; and 47 at Cape St. Jaques, infection rate 4·2 per cent.

Although *sundaicus* is rated as a major vector of malaria in the coastal regions of Orissa, Bengal, North Madras, Burma, the Andaman and Nicobar Islands, Malaya, Thailand, Indo-China and the Dutch East Indies, its distribution is extremely patchy, and it tends to appear and disappear in various localities depending on the variations in local conditions. This is well illustrated in the case of the villages in the vicinity of the Chilka Lake investigated by Covell and Pritam Singh (1942). Malaria occurs in these villages either in the spring or in the autumn or at both these periods in certain years, always depending on the presence or absence of *sundaicus*; whilst for several years it may be absent from particular villages altogether. In the same year there may be a spring epidemic in one village, an autumn epidemic in another, epidemics in both spring and autumn in a third and no transmission at all in a fourth. Another characteristic of *sundaicus* is its manner of infiltrating from one place to another. In recent years it has spread from the Sunderbans up to the outskirts of Calcutta, and along the whole stretch of the coast of Orissa from north to south, a distance of some hundreds of miles. Whether this represents an entirely new invasion is open to question, and it seems possible that there may be a permanent reservoir of breeding among the islands on the south-eastern margin of the Chilka Lake*.

SUPERPICTUS GRASSI, 1899

Distribution.—This is an Eastern Mediterranean species, found in Spain, Italy, Dalmatia, Yugoslavia, Albania, Macedonia, Greece, Bulgaria, Thrace, Anatolia, Cyprus, Caucasus, Trans-Caucasus, Syria, Palestine, Sinai, Iraq, Turkmenskaya Republic, Cossack Republic, Bokhara and Iran. It has been recorded in India in Baluchistan, Waziristan and North-West Frontier Province; does not occur east of the river Indus.

Breeding Places.—Usually in hill country in pools, beds of small streams, rivers and irrigation systems where there is flowing water. Wenyon (1921) recorded that in Macedonia it bred in pools beside streams at 4,000 feet, not in actual running water but in every tiny pool, especially those containing algae, and in backwaters protected from the direct current.

Mulligan and Baily (1936) stated that in Baluchistan this species showed a marked preference for running water, the larvae being found in greatest numbers along the edges of streams and rivers where dense masses of Spirogyra retarded the flow. Other favourite breeding places were seepages and marshy areas with water gently flowing, open irrigation channels, reservoirs, pools, wells, springs, subsoil water in cracks and fissures in the ground. The chief desiderate appeared to be exposure to direct sunlight, a gentle flow of water and plenty of vegetation, especially algae.

* There is a related sub-species, *parangensis* Ludlow, which occurs in the Philippines and Celebes, and breeds in fresh water. Its status as a vector is unknown, but adults have been observed feeding on cattle (Russell, Rozeboom and Stone, 1943).

Adult Habits.—The adults readily enter houses and tents (Buxton, 1924; Christophers and Shortt, 1921) and are said to prefer human blood (Russell, Rozeboom and Stone, 1943). Mulligan and Baily (1936) found large numbers in outdoor resting places, such as caves and karezes (a series of perpendicular shafts, the lower ends of which are connected by an unlined tunnel, forming what is to all intents and purposes a subsoil drain). These observers noted that there seemed no tendency for this species to disperse for more than half a mile, but Kligler (1930) stated that the range of flight may extend as far as 3·5 kilometres or more.

Relation to Malaria.—This species is an important vector in South Europe, Iraq and Baluchistan. Christophers and Shortt (1921) dissected 27 specimens in Iraq and found 2 with sporozoites (7·4 per cent). Mackie (1921) recorded 1 gut and 2 gland infections out of 105, also in Iraq. Mulligan and Baily (1936), in Baluchistan, dissected 1,412 with a total infection rate of 4·9 per cent (gut only 36, gland only 22, mixed gut and gland 11, total 69). This appears to be the only series of dissections published in India.

Distribution.—India (Assam, Bengal, Bihar, Bombay, Deccan, Central India, Central Provinces, Chota Nagpur, Delhi Province, Gujarat, Hyderabad State, Konkan, Madras Presidency, Malabar, Mysore State, Orissa); Maldive Islands; Ceylon; Burma; Thailand; Indo-China; Southern China; Formosa; Andamans; Malaya; Sumatra; Java; Borneo; Philippine Islands.

Breeding Places.—Swellengrebel and Swellengrebel de-Graaf (1919, 1920) describe this species as a 'dirty water mosquito', the larvae being found in buffalo wallows, etc., but that they also occur in clear overgrown pools, and in ricefields; and that they have been collected from buffalo hoof-prints containing strongly saline water, in company with those of *sundaicus*. Hacker (1920) classes it amongst the swamp fauna, its most frequent associate being *hyrcanus*, *kochi* and *leucosphyrus*, and states that in Malaya it is not dependent upon any special type of breeding place, the larvae being frequently found in quite dense jungle where there is no grass growing, but only decaying grass in the pools, whilst they also occur in pools completely in the open. In the Maldive Islands this species breeds in shallow fresh-water wells.

Adult Habits.—Venhuis (1942*a*) records the adults as being found with some frequency along stream banks but they also rest in cowsheds and in human habitations. In Indo-China, Marneffe, Gaschen and Tung (1938) stated that they were found more frequently in houses than in buffalo-sheds in the Tonkin Delta, but Toumanoff (1936) records them as being markedly zoophilic, attacking animals not only in shelters but also in the open, and only occasionally entering houses to feed on man. Raynal and Gaschen (1935) recorded precipitin tests on 120 specimens of which only 2·25 per cent contained human blood. On the other hand, in Formosa, this species is said to remain in houses after feeding and to prefer human blood (Yamada, 1926; Anazawa, 1931). Walch (1932), in a limited series of tests in the Dutch East Indies, found none with human blood out of 17 caught in houses where cattle were present in normal numbers, but 47 per cent human positive out

of 19 tested in a locality where cattle were scarce. Ave Lallemant, Soerono and Stoker (1932) in the Dutch East Indies showed that *tessellatus* could cover a distance of over 1,000 yards, whilst Russell and Santiago (1934*b*) in the Philippines recorded a flight range of nearly 2,000 yards for this species.

Relation to Malaria.—This species is considered to be responsible for malaria transmission in the Maldive Islands, where it is the only anopheline so far recorded. Two gland and 4 gut infections were encountered among 160 specimens dissected in 1944. It has been found naturally infected in the Dutch East Indies, Borneo, Indo-China, Southern China and Formosa, but is nowhere considered to play any part of importance as a vector. Records of natural infections are given below :—

Authority.	Locality.	Number dissected.	Number infected.	Per cent infected.
Soesilo (1929) ...	Nias, Dutch East Indies.	137	1 (gut)	0·7
Anazawa (1931) ...	Formosa.	?	?	1·9
Jackson (1936) ...	Hong Kong, Southern China.	40	1	2·5
Gaschen (1936) ...	Tonkin, Indo-China.	51	1 (gland)	2·0
Marneffe, Gaschen and Tung (1936) ...	Tonkin, Indo-China.	52	1 (gland plus gut)	2·0

Gaschen (1936) remarked that the scarcity of this species in Indo-China may be the only factor preventing it from being important as a vector.

UMBROSUS THEOBALD, 1903.

Distribution.—India (Assam only); Indo-China; Andamans; Malaya; Sumatra; Riouw; Java; Borneo; Banka; Celebes.

Breeding places.—Watson (1921) in Malaya recorded the larvae as being often found in wells of pure water at a foothill even in sunshine, but most frequently in stagnant pools of brown peaty water, or in the slowly streaming morasses of the virgin jungle*. Swellengrebel and Swellengrebel-de-Graaf (1919) stated that this species breeds in the Dutch East Indies in slow-running or stagnant clear water with or without vegetation, and in old cemented tanks overgrown with weeds, preferably under shade. Also in strongly brackish water in nipa and mangrove jungle. It does not breed in the hill-land jungle in Malaya (Strickland, 1916).

Adult Habits.—The adults are fierce biters and are found not only under shade in the jungle, but also in houses (Watson, 1921; Barbers, 1918; Covell, 1927*a*). Barber (1918) noted that whilst no exact experiments had been carried out to test its power or flight, the adults were often found in Malaya at some distance from its breeding places, indicating that it is a strong flier. Its effective flight range, however, probably does not exceed 1,000 yards. Manson and Ramsay (1933) state

* Hodgkin (1938*a*) states that *umbrosus*, contrary to general belief, does not breed in jungle. Also that the importance of *umbrosus* as a malaria carrier has probably been overestimated owing to its having been confused with the jungle breeding *novumbrosus*, which he considers to be a vector of importance.

that from investigations carried out in Assam it does not normally feed on man in Eastern India. In Malaya, Green (1934) recorded precipitin tests on 304 anophelines of various species, amongst which 95 per cent of *umbrosus* and *barbirostris* contained human blood. The corresponding figures for *maculatus* and *separatus* were 93 and 80 per cent respectively.

Relation to Malaria.—Barber (1918) working in Malaya noted that the susceptibility of this species to malarial infection under experimental conditions was relatively low, but that it may breed in immense numbers, and evidence from laboratory experiments as well as from the condition of adults caught in nature indicates that it is relatively long-lived. Natural infections have been recorded from Malaya, Borneo and the Dutch East Indies. Barber (1918) in Malaya dissected 261 and recorded 1 gut and 6 gland infections, with a total infectivity rate of 2·3 per cent. In the Dutch East Indies natural infections have been recorded by Walch and Soesilo (1929) in Banka (121 dissections, 6 gut infections, 5 per cent). According to Overbeek and Stoker (1937) this species was also found infected by Swellengrebel and Schuffner in Sumatra and by Stoker in Borneo. Soesilo *et al.* (1932) dissected 689 specimens caught indoors at Sanggau, Borneo, of which 3 were infected. Soesilo is also credited with having recorded an infection rate of 15·2 per cent in *umbrosus* out of 59 dissected in a locality in Pontiniak, Borneo (Overbeek and Stoker, 1937).

This species is probably too rare to play any part in the transmission of malaria in the Andamans. Covell (1927*a*) collected 2 adult specimens only and found no larvae, whilst Christophers (1912) encountered neither larvae nor adults.

VAGUS DONITZ, 1902

Distribution.—India (Assam, Bengal, Bihar, Bombay, Deccan, Central Provinces, Chota Nagpur, Gujarat, Hyderabad State, Konkan, Madras East and West Coasts, Malabar, Mysore State, Orissa); Ceylon; Burma; Thailand; Indo-China; Southern China; Formosa; Andamans; Malaya; Dutch East Indies; Moluccas; New Guinea; Philippine Islands.

Breeding Places.—The larvae are found typically in small pools and puddles, often in the midst of villages. Hacker (1920) notes that in Malaya they are most often found alone, but sometimes with those of *kochi*, *hyrcanus* and *barbirostris*; and that they have been found in brackish water. Covell (1927*a*) recorded them in considerable numbers in ricefields in the Andamans.

Adult habits.—The adults are found in large numbers both in houses and in cattlesheds. Venhuis (1942*a*) observed them resting on stream banks in the Dutch East Indies. Toumanoff (1936) stated that they almost always contain cattle blood, even when captured in human habitations. Raynal and Gaschen (1935) recorded precipitin tests on 839 specimens, of which more than 95 per cent contained cattle blood. Walch (1932) reported tests on 256 specimens caught in houses where cattle were present in normal numbers, of which only 1 per cent were human positive, whilst Ramsay, Chandra and Lamprell (1936) found less than 1 per cent

with human blood out of 593 tested. The species is thus obviously extremely zoophilic.

Relation to Malaria.—Natural infections of this species have been recorded in India, the Dutch East Indies and Indo-China, but it is nowhere considered to be an important vector. Russell and Rao (1940) dissected 6,874 at Pattukkottai, South India, of which one was found with degenerating sporozoites (0·015 per cent). Strickland, Choudhury and Choudhury (1933) reported 2 sporozoite infections among 10,452 dissected in Assam, whilst Viswanathan, Dass and Oommen (1941) also in Assam dissected 3,396 all of which proved negative.

In the Dutch East Indies, Swellengrebel and Swellengrebel-de-Graaf (1920) recorded 3,721 dissections with 3 infections (00·8 per cent). In Indo-China Toumanoff (1936) reported 1 gland infection out of 2,625 dissected in Tonkin (0·07 per cent.), and Marneffe, Gaschen and Tung (1938) at Binh-Ha in the Tonkin Delta dissected 216 with 1 gland infection during an epidemic ascribed to *sinensis*.

VARUNA IYENGAR, 1924

Distribution : India (Assam, Bengal, Bihar, Bombay, Deccan, Chota Nagpur, Gujarat, Konkan, Madras East Coast, Malabar, Mysore State, Orissa, United Provinces) ; Ceylon ; Burma ; Southern China.

Breeding Places : Stagnant water in pools, ditches, wells, slow-running streams and irrigation channels. Iyengar (1924) stated that it was most prevalent during and immediately after the monsoon rains, when it breeds in collections of storm-water by the roadside, often in company with *tessellatus* and *barbirostris*.

Adult Habits : The adults are found both in cowsheds and human habitations, and feed readily on man. Senior White (1941), in the vicinity of Vizagapatam, recorded that this species formed 65 per cent of the total catch in cattle-sheds in one area between February and August, but only 4·5 per cent of the whole year's catch in another. He ascribed this difference to the preference of *varuna* for a certain type of shed, a structure of palmyra thatch carried down almost to the ground, which is common in that district but not general. He notes that it appears to be purely a cattle feeder in this area.

Relation to Malaria : This species is a proved vector in certain areas though it appears to be innocuous in others. In Travancore, Mathew (1939) dissected 429 specimens with an oocyst rate of 2·3 and a sporozoite rate of 1·6 per cent and considered it a dangerous carrier. Iyengar (1928) reported 1 gland infection out of 25 specimens dissected in the Hooghly Delta and Roy (1939) dissected 113 specimens near Calcutta and recorded 4 gland infections (3·6 per cent). Senior White and his colleagues have reported a number of natural infections in this species in the Jaipur and Singhbhum Hills and Eastern Satpura Ranges as shown below :—

Authority.	Locality.	Number dissected.	Guts infected:		Glands infected:	
			Number.	Per cent.	Number.	Per cent.
Senior White (1937)	Jaipur Hills:					
	Chatikona Summit.	231	22	9·5	13	5·3
	Various villages.	317	26	8·4	15	4·7
Senior White (1938)	Jaipur Hills	225	20	8·9	9	4·0
Senior White and Das (1938)	Singhbhum Hills	189	8	4·2	2	1·1
Senior White and Narayana (1940)	Singhbhum Hills	153	8	5·2	3	2·0
Senior White and Adhikari (1940)	Eastern Satpuras	386	14	3·5	12	3·1

On the other hand in the vicinity of Vizagapatam, *varuna* appears to play little or no part in transmission, for though it is present in abundance, malaria incidence remains very low and all dissections have so far proved negative (Senior White, 1941). Covell and Pritam Singh (1942) recorded 450 dissections of this species in the Orissa coastal belt, all of which proved negative, and Russell and Rao (1940) dissected 505 at Pattukkottai, South India, with a like result. It seems possible that the form occurring along the eastern coast of India from Orissa to Madras and further south may belong to a different biological race from that found in the hills of Central India and in deltaic Bengal.

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APPENDIX XII.

ANTI-MALARIA UNIT—INDIAN WAR ESTABLISHMENT

	Head-quarters	Four sections (each)	Total
(i) PERSONNEL :			
Officer			
Captain or lieutenant (a)	1	...	1
Indian—IAMC			
Viceroy's Commissioned Officers			
Subedar (b)	1	...	1
Subedar (General Duty)	1	...	1
Totals VCOs	2	...	2
Havildars			
Stores section grade III	1	...	1
Ambulance section	...	1	4
Total havildars	1	1	5
Rank and file			
Ambulance section			
Naiks (c)	...	1	4
Sepoys (batmen)	3	...	3
Total rank and file, Indian	3	1	7
Total Indian, other ranks	4	2	12
Total all ranks, Indian	6	2	14
Non-combatants (enrolled)			
General Section—IAMC			
Cooks IT	2	...	2
Water carriers, grade II	2	...	2
Water servants, grade I (c)	...	6	24
Sweeper, grade II	1	...	1
Tinsmith, grade II	1	...	1
Labourers	25	...	25
Total non-combatants (enrolled)	31	6	55
Attached			
Drivers, MT, RIASC	4	...	4

APPENDIX XII (*Contd.*)

	Head-quarters	Four sections (each)	Total
NOT INCLUDED IN THE ABOVE :			
1st reinforcement (basic scale)			
Ambulance section sepoy—IAMC	1	...	1
Labourers	1	...	1
(ii) TRANSPORT :			
Bicycles	12	...	12
Jeeps (d)	1	...	1
Trucks, 15-cwt., GS	2	...	2
Total vehicles (excluding bicycles)	3	...	3

NOTES :

(a) May be a non-medical officer.

(b) May be replaced by a subaltern IAMC trained in anti-malarial duties.

(c) Trained in anti-malarial duties.

(d) For commanding officers. May be replaced by a truck, 15-cwt., GS.

APPENDIX XIII.

MALARIA BUREAU BULLETINS IN THE HEALTH BULLETIN SERIES*

No. 1. *Lectures on malaria.*—By G. Covell. 4th edition, 1948. As. 5 or 6d.

No. 2. *Synoptic table for the identification of anopheline mosquitoes of India.*—By S R. Christophers, J.A. Sinton, G. Covell and P.J. Barraud. 4th edition, revised, enlarged and illustrated by I. M. Puri. 1947. Rs. 1/6 or 2s. 6d.

No. 3. *Anti-mosquito measures with special reference to India.*—By G. Covell. 7th edition, 1946. As. 8 or 9d.

No. 4. *Table for the identification of Indian fresh water fishes with description of certain families and observations on the relative utility of the probable larvivorous fishes of India.*—2nd edition, revised and enlarged. By S. L. Hora and D.D. Mukerjee. 1938. As. 7 or 8d.

No. 5. *Instructions for collecting and forwarding mosquitoes.*—By J. A. Sinton. 4th edition, revised by I.M. Puri, 1949. As. 8 or 9d.

No. 6. *How to do a malaria survey?*—By S. R. Christophers, J. A. Sinton and G. Covell. 5th edition, 1944. Rs. 1/12 or 2s. 6d.

No. 7. *Synoptic table for the identification of the full-grown larvae of the India anopheline mosquitoes.*—By I. M. Puri. 6th edition, 1949. Rs. 3/6 or 5s. 6d.

No. 8. *The distribution of anopheline mosquitoes in India.*—By G. Covell. 2nd edition, revised and brought up to date by I.M. Puri, 1936. As. 6 or 8d.

No. 9. *A practical entomological course for students of malariology.*—By P. J. Barraud. 4th edition, revised by I. M. Puri, 1948. Rs. 1/12 or 2s. 6d.

No. 10. *Man-made malaria in India.*—By J.A. Sinton and Raja Ram. 3rd edition, revised by G. Covell, 1949.

No. 11. *Handbook of common water and marsh plants of India and Burma.*—By C.C. Calder and K. Biswas. 1937. Rs. 2/12 or 5s.

No. 12. *Prevention of malaria incidental to engineering construction.*—By H. W. Mulligan and M.K. Afridi, 1938. As. 7 or 8d.

No. 13. *What malaria costs India.*—By J. A. Sinton, 1939. As. 14 or 1s. 3d.

* These bulletins are obtainable from the Manager of Publications, Government of India, Publication Branch, Civil Lines, Delhi-8.

APPENDIX XIV.

PROGRAMME OF MALARIA COURSE FOR MEDICAL OFFICERS—FIRST TO FOURTH WEEK.

Week.	Monday.	Tuesday.	Wednesday.	Thursday.	Friday.	Saturday.
First week	10.15 to 11.15 hours *Lecture I* Introductory. Taking over and checking of equipment by students. Film—Life history of mosquito. 11.15 to 13.15 hours *Lecture II* Haematology.	08.00 to 10.00 hours *Practical I* Preparation and staining of blood films. 10.15 to 11.15 hours *Lecture III* Parasitology 1. The malaria parasites. 11.15 to 13.15 hours *Practical I* (Continued). 15.00 to 17.00 hours *Practical I* (Continued).	08.00 to 10.00 hours *Practical II* Study of malaria parasites. 10.15 to 11.15 hours *Lecture IV* Entomology 1. General characters of mosquitoes. 11.15 to 13.15 hours *Practical II* (Continued). 15.00 to 17.00 hours *Practical II* (Continued).	08.00 to 10.00 hours *Practical III* Study of general characters of mosquitoes. 10.15 to 11.15 hours *Lecture V* Entomology 2. Morphology. 11.15 to 13.15 hours *Practical III* (Continued) 15.00 to 17.00 hours *Practical III* (Continued).	08.00 to 10.00 hours *Practical IV* Morphology of adult mosquitoes 10.15 to 11.15 hours *Lecture VI* Entomology 3. Collection of larvae and pupae. 11.15 to 13.15 hours *Practical IV* (Continued) 15.00 to 17.00 hours *Practical IV* (Continued).	08.00 to 10.00 hours *Practical V* (Field) Collection of larvae and pupae. 10.15 to 11.15 hours *Lecture VII* Entomology 4. Examination of larvae. 11.15 to 13.15 hours *Practical V* (Continued). 15.00 to 17.00 hours *Practical V* (Continued).
Second week	08.30 to 09.30 hours *Lecture VIII* Entomology 5. Morphology and habits of larvae. General characters of pupae. 10.00 to 12.30 hours *Practical VI* General characters of larvae. 15.00 to 17.00 hours *Practical VI* (Continued).	08.30 to 09.30 hours *Lecture IX* Entomology 6. Collection and distribution of adults. General characters of eggs. 10.00 to 12.30 hours *Practical VII* General characters of larvae and pupae. 15.00 to 17.00 hours *Practical VII* (Continued).	08.00 to 11.00 hours *Practical VIII* (Field) Collection of adult mosquitoes. 11.00 to 12.00 hours *Lecture X* Entomology 7. Mounting and identification of adult mosquitoes. 15.00 to 17.00 hours *Practical VIII* (Continued).	08.30 to 09.30 hours *Lecture XI* Entomology 8. Identification of larvae. 10.00 to 12.30 hours *Practical IX* Mounting and identification of adult mosquitoes. 15.00 to 17.00 hours *Practical IX* (Continued).	08.30 to 09.30 hours *Lecture XII* Entomology 9. Internal anatomy and dissection of adult mosquitoes. Bionomics. 10.00 to 12.30 hours *Practical X* Mounting and identification of larvae. 15.00 to 17.00 hours *Practical X* (Continued).	08.30 to 09.30 hours *Lecture XIII* Entomology 10. Bionomics. 10.00 to 12.30 hours *Practical XI* Dissection of gut and salivary glands. 15.00 to 17.00 hours *Practical XI* (Continued).

APPENDIX XIV—(*Contd.*)

Week.	Monday.	Tuesday.	Wednesday.	Thursday.	Friday.	Saturday.
Third week	08.30 to 09.30 hours *Lecture XIV* Entomology 11. Reproductive organs. Determination of age of mosquitoes.	08 30 to 09.30 hours *Lecture XVI* Malaria Control 1. Basic Principles. Methods specially applicable in war. Measure of personal protection.	08.30 to 09.30 hours *Lecture XVIII* Epidemiology 1. Basic aetiology of malaria. Spleen census and methods.	08.30 to 09.30 hours *Practical XV* (Field) Examination of spleen and blood.	08.30 to 09.30 hours *Lecture XXI* Epidemiology 4. Effect of irrigation. Malaria surveys (Summary of methods).	08.30 to 09.30 hours *Lecture XXII* Malaria Control 3. Organisation of recurrent measures. Malaria problems in Delhi.
	09.30 to 11.30 hours *Practical XII* Dissection of adult mosquitoes. Salivary glands.	09.30 to 11.30 hours *Practical XIII* Dissection of mosquitoes.	09.30 to 11.30 hours *Practical XIV* Dissection of infected mosquitoes.	09.30 to 11.30 hours *Practical XV* (Field) Demonstration of DDT spraying in Okhla village.	09.30 to 11.30 hours *Practical XVII* (Laboratory) Examination of spleen and blood.	09.30 to 11.30 hours *Practical XVIII* Study of blood films. Demonstration of spray-killing of adult mosquitoes (two batches).
	11.30 to 12.30 hours *Lecture XV* Parasitology 2 and 3.	11.30 to 12.30 hours *Lecture XVII* Malaria Control 2. Measures against adult mosquitoes. Pyrethrum insecticide, DDT.	11.30 to 12.30 hours *Lecture XIX* Epidemiology 2. Significance of spleen rate. Endemic malaria. Degree of endemicity. Malaria Map of India. Course of an epidemic	11.30 to 12.30 hours *Lecture XX* Epidemiology 3. Influence of climate. Epidemic malaria.	11.30 to 12.30 hours *Practical XVII* (Continued).	11.30 to 12.30 hours *Lecture XXIII* Malaria Control 4. Chemical larvicides. DDT. Paris green. Cresol. Copper cyanide.
	15.00 to 17.00 hours *Practical XII* (Continued).	15.00 to 17.00 hours *Practical XIII* (Continued).	15.00 to 17.00 hours *Practical XIV* (Continued).	15.00 to 17.00 hours *Practical XVI* (Laboratory) Study of data and blood slides collected in field.	15.00 to 17.00 hours *Practical XVII* (Continued).	15.00 to 17.00 hours *Practical XVIII* (Continued).

APPENDIX XIV—(*Contd.*)

Week.	Monday.	Tuesday.	Wednesday.	Thursday.	Friday.	Saturday.
Fourth week	08.30 to 09.30 hours *Lecture XXIV* Malaria Control 5. Use of oil. Demonstration of oiling apparatus.	08.30 to 11.30 hours *Practical XX* (Field) Demonstration of oiling and drainage.	08.30 to 11.30 hours (Field) Demonstration of Delhi anti-malaria works.	08.30 to 12.00 hours *Practical XXIII* Revision.	09.00 to 10.30 hours Practical examination Part I.	09.00 to 12.30 hours Written and oral Examination.
	09.30 to 10.30 hours *Practical XIX* Demonstration—DDT residual and larvicidal.				10.30 to 12.30 hours Practical examination Part II.	
	11.30 to 12.30 hours *Practical XIX* (Continued).	11.30 to 12.30 hours *Lecture XXVI* Malaria Control 7. Naturalistic methods. Demonstration of flushing methods.	11.30 to 12.30 hours *Lecture XXVII* Malaria Control 8. Summary of control measures.			
	15.00 to 16.00 hours *Practical XIX* (Continued) (Laboratory) Demonstration of dustless Paris green methods.	15.00 to 17.00 hours *Practical XXI* (Laboratory) Revision.	15.00 to 17.00 hours Demonstration of spray-killing of adult mosquitoes in habitations.		15.00 to 16.15 hours Practical examination, Part III.	
	16.00 to 17.00 hours *Lecture XXV* Malaria Control 6. Drainage. Treatment of borrow-pits.				16.15 hours Handing over equipment by students.	

Note :—During lectures on entomology, students were required to have with them Malaria Bulletins No. 1, 2, 3, 6, 7, 9 and 10.

APPENDIX XV.

METHOD OF STAINING WITH J.S.B. STAIN

The usual precautions in the preparations of blood smears are necessary, and, to abtain satisfactory results, the slides should be as free from grease as possible. The thick smear should be spread evenly, otherwise de-haemoglobinisation and staining will not yield the desired results. The thin smear should preferably be one cell thick, so that the red blood corpuscles take up the stain uniformly. Old smears taken during the hot weather stained after some months gave consistently good results.

Thick and thin smears taken on the same slide can be easily stained.

Technique

(*i*) Fix the thin smear by dipping the slide in a jar containing methyl alcohol for a second or two. Do not fix the thick smear.

(*ii*) Dry thoroughly, preferably by waving the slide in the air.

(*iii*) Immerse the whole slide in Solution I for thirty seconds.

(*iv*) Wash in a jar containing acidulated tap water (pH 6·2 to 6·6). With tap water in Delhi (pH 7·6, indicator bromothymol-blue) approximately 50 mg. of sodium dihydrogen phosphate or five drops of 5 per cent acetic or citric acid solutions for each 100 c.c. of water are necessary.

(*v*) Stain with Solution II for one second.

(*vi*) Wash in the same jar for a couple of seconds.

(*vii*) Immerse in Solution I again for thirty seconds (*iii*).

(*viii*) Wash as above for ten seconds or till the smear gives a pink background (*iv*).

(*xi*) Dry and examine.

Thick smear alone

(*i*) Immerse the slide in Solution I for ten seconds.

(*ii*) Wash in a jar containing water acidulated (pH 6·2 to 6·6) for two seconds.

(*iii*) Stain with Solution II for one second.

(*iv*) Wash in the same jar (*ii*) for five seconds.

(*v*) Immerse in Solution I again for ten seconds.

(*vi*) Wash as above for two seconds or till the smear gives pink background (*ii*).

(*vii*) Dry and examine.

The total time taken for staining a combined thick and thin smear is one minute and twenty seconds, and for a thick smear only thirty seconds. The latter

period can be shortened to ten seconds but with old or very thick smears it is preferable to follow the above timings.

When a large number of blood smears have to be stained, special racks, holding 20, 25, 50 or 100 slides, and troughs of suitable sizes can be used to save time

The results achieved with this stain were striking, and the staining characteristics of the chromatin, cytoplasm, pigment and stippling can be differentiated in the same manner as with any of the Romanowsky's stains.

In a thick smear, the malaria parasites show the usual appearances of the parasites wi h a background made up of haemolysed red blood corpuscles. The parasites may appear either free or within the red cells. The young parasites usually appear as rings, semicolon or semilunar in shape, but they may be shrunken or distorted. The mature forms (trophozoites, schizonts and gametocytes) retain their shape well and the chromatin and cytoplasm stain intensely, with pigment showing up distinctly. Red blood cells infected with *P. vivax* show characteristic stippling. The Maurer's dots in *P. falciparum* infections can often be clearly seen.

In a thin smear, red blood corpuscles should appear faint pink or orange coloured, depending upon the extent of washing. The immature red corpuscles can be recognised by the polychromatic appearance of the cytoplasm and their characteristic nuclei. The other cellular elements of the blood stain in the same manner as with Giemsa or Leishman. Stippling in *P. vivax* and *P. falciparum* is seen better in a thin than in a thick smear. The stain is equally useful for the demonstration of sporozoites, trypanosomes, Leishman-Donovan bodies and oocysts in the stomach of infected mosquitoes.

Other additional advantages are :—

Wastage in staining solutions has been minimised. The same solutions can be used for a long period with occasional replenishment.

With age and use the stain does not deteriorate, and the technique of staining is simple.

There is no necessity for using distilled water at any stage. Ordinary tap water or boiled water slightly acidified is all that is required.

Thick and thin smears taken on the same slide can be stained simultaneously in a short time (within one minute and twenty seconds) and a thick smear alone in about thirty seconds. These timings can be varied slightly to suit individual requirements. The time taken in drying the film has not been taken into consideration as this will differ under varying atmospheric conditions.

Smears, several weeks old, can be stained satisfactorily, and the blood picture, obtained in both thick and thin smears, compares favourably with the original Romanowsky's stain and its various modifications.

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