Indian Journal of Malariology, **9**, 1, March 1955.

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Indian Journal of Malariology, **9**, 1, March 1955.

FILARIASIS IN MANGALORE (SOUTH INDIA).

BY

A. K. KRISHNASWAMI.

(*Malaria Institute of India, Delhi.*)

(July 2, 1954.)

An investigation into the incidence of filariasis in the municipality of Mangalore was carried out during the period March-April, 1954. The findings are recorded in this paper.

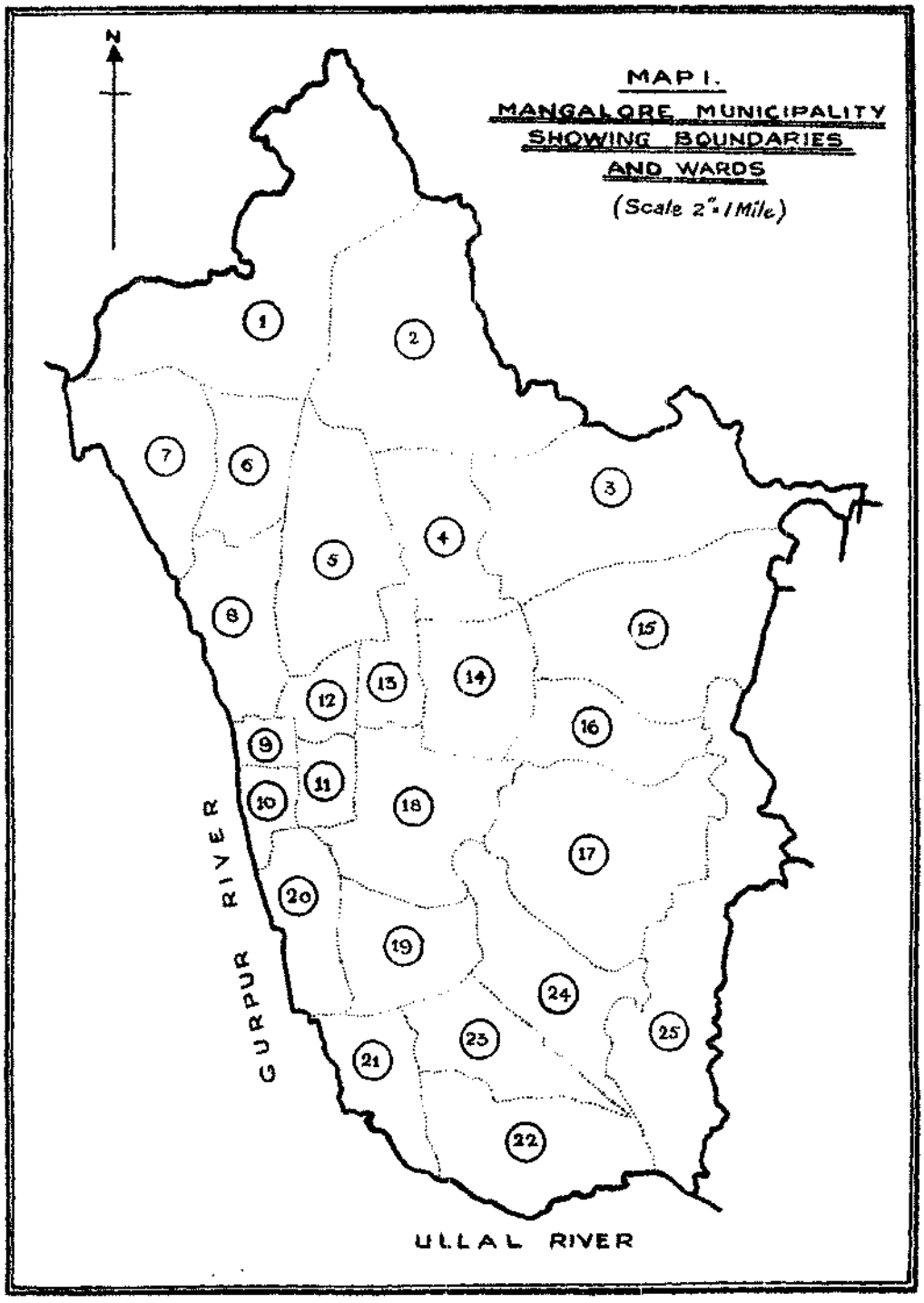
Mangalore is the headquarters of the South Kanara District of Madras State. The town, a terminus of the Southern Railways, is situated on the west coast of India and covers an area of about 18 sq. miles. The range of the western ghats bounds the town on its northern and eastern sides, while the Ullal River forms the southern limit. The Gurpur River runs parallel to the sea-coast on the western side of the town before joining the Arabian Sea.

For administrative purposes, Mangalore has been divided into 25 Wards (Map I). Extensive groves of coconuts and arecanuts abound in the town. In some places, fields with wet cultivaton are also present. The total population of the municipality according to last census (1951) is 1,17,095 and consists of Hindus, Christians and Mohammedans. The population of the different wards varies from 2,319 in Ward 10 to 9,094 in Ward 3.

The regional languages are Kanarese, Konkani and Tulu. A small proportion, mainly immigrants, speak Malayalam, Tamil and other South Indian languages. The literacy rate among the population is high.

METEOROLOGICAL CONDITIONS.

Temperature remains warm and the humidity high throughout the year. Rainfall is heavy (about 120-150 inches annually) and precipitation occurs mainly from June to September. Except during the height of the monsoon (July-August) favourable conditions appear to exist almost throughout the year both for the breeding and longevity of the local mosquitoes.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MAP I.
MANGALORE MUNICIPALITY
SHOWING BOUNDARIES
AND WARDS
(Scale 2"=1 Mile)
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GURPUR RIVER
ULLAL RIVER

WATER SUPPLY.

There is no protected water supply for the town, and wells are the main source of domestic water supply. There are a number of tanks, some of which form an additional source, especially after the monsoon. During summer, most of the tanks go dry and water supply from the wells becomes poor. A scheme for protected water supply for the town has been sanctioned and is expected to be implemented in the near future.

HISTORY OF FILARIASIS AND PREVIOUS SURVEYS.

There is an impression among the old residents of the town that cases of filariasis have been increasing alarmingly during recent years. It would appear that the number of cases were comparatively few about a couple of decades ago. Such cases appear to have been limited to the coastal areas. The cases at present are not only increased in numbers but have spread in the entire town. There is not a single ward free from it. A cause for worry to the citizens and to the city fathers is the occurrence of elephantiasis, particularly in girls of the younger age groups.

Filariasis surveys of the town have been carried out during the past four years by the staff of the Madras Public Health Organisation. The available data are presented in Table I. The disease rate among the community during these years has ranged from 2·2 to 4·7 per cent, while the microfilaria rate varied from 3·9 to 9·9 per cent.

TABLE I.

Previous filaria surveys of Mangalore Municipality 1950-53*.

Year.	Number of persons examined		Average per ward	
	for disease.	for Mf.	Disease rate.	Mf. rate.
1950	2501	1012	3·6	9·9
1951	2503	1179	2·2	7·4
1952	1489	1489	4·4	3·8
1953	1297	1297	4·7	4·6

*Data obtained from the unpublished records of the Regional Malariologist available from the Municipal Health Officer, Mangalore.

PRESENT WORK.

Investigations on the following lines were carried out to elucidate details regarding the epidemiology of filariasis in Mangalore.

(*a*) *Blood survey.*—Random representative samples of population from all the 25 wards, covering all age groups and both sexes among the different social

strata, were examined in order to determine the incidence of filarial infection and filarial disease in the community. Blood smears were collected during nights between 9·30 p.m. and 1 a.m. by house-to-house visits. Approximately 20 c.mm. of blood from a pricked finger of each person, was obtained on a clean glass slide and the blood spread into a thick oval smear, about one inch long and 0·5 inch broad. The slide was marked with the same number as appearing in the register in which other details *viz.*, name, parents' name, age, sex and external signs or symptoms of filarial disease, if any, were recorded. The slides were stained and examined on the following day, for microfilariæ. The entire smear was examined before declaring a slide negative. When a smear was positive, the species and total number of microfilariæ in the smear were recorded. The results have been analysed and presented in Tables II, III and IV.

TABLE II.

Filaria survey—Mangalore Municipality (March-April 1954).

Ward Number	Population	Number of persons examined	Percentage of population examined	With disease		With microfilaria		Endemicity rate per cent	Average infestation of microfilaria per 20 c.mm.
				Number	Percentage	Number	Percentage		
1.	7,285	370	5·1	25	6·8	61	16·5	23·0	34·0
2.	4,962	323	6·5	22	6·8	55	17·0	23·8	36·3
3.	9,094	603	6·7	32	5·3	67	11·1	16·2	51·1
4.	3,284	253	7·7	12	4·7	32	12·7	17·4	26·0
5.	5,748	237	4·1	40	17·0	25	10·6	27·0	25·1
6.	3,846	267	6·9	23	8·6	36	13·4	22·0	49·5
7.	4,542	296	6·5	25	8·4	53	17·9	26·3	34·0
8.	7,103	441	6·2	32	7·3	85	19·3	23·4	37·6
9.	2,976	326	10·9	53	16·2	64	19·6	35·6	42·4
10.	2,319	267	11·4	31	11·6	45	16·9	27·7	25·6
11.	4,445	300	6·7	39	13·0	62	20·7	32·7	69·5
12.	2,404	134	5·6	21	15·7	22	16·4	31·3	26·0
13.	4,098	314	7·7	34	10·8	52	16·5	26·7	52.1
14.	2,688	280	9·7	2	7·7	24	9·2	16·5	57·1
15.	5,441	273	5·1	15	5·5	29	10·5	16·0	38·8
16.	2,340	207	8·8	15	7·2	14	6·7	13·4	53·1
17.	6,617	428	6·4	55	16·8	69	21·0	37·5	45·6
18.	2,887	278	9·6	19	6·9	36	12·9	19·8	30·6
19.	3,356	232	6·9	21	9·0	30	12·9	21·9	39·1
20.	3,845	270	7·0	30	11·1	44	16·3	26·3	28·1
21.	3,907	288	7·3	41	14·2	47	16·3	27·8	43·8
22.	6,567	225	3·4	27	12·0	39	17·3	29·3	49·1
23.	4,607	260	5·8	28	10·4	40	15·0	24·9	34·0
24.	5,124	219	4·3	20	9·1	39	17·8	26·9	66·0
25.	7,610	322	4·2	24	7·4	42	12·0	19·4	61·5
Total	1,17,095	7,402	6·3	704	9·5	1,112	15·0	24·2	40·3

TABLE III.

Incidence of filariasis in different age groups and the two sexes.

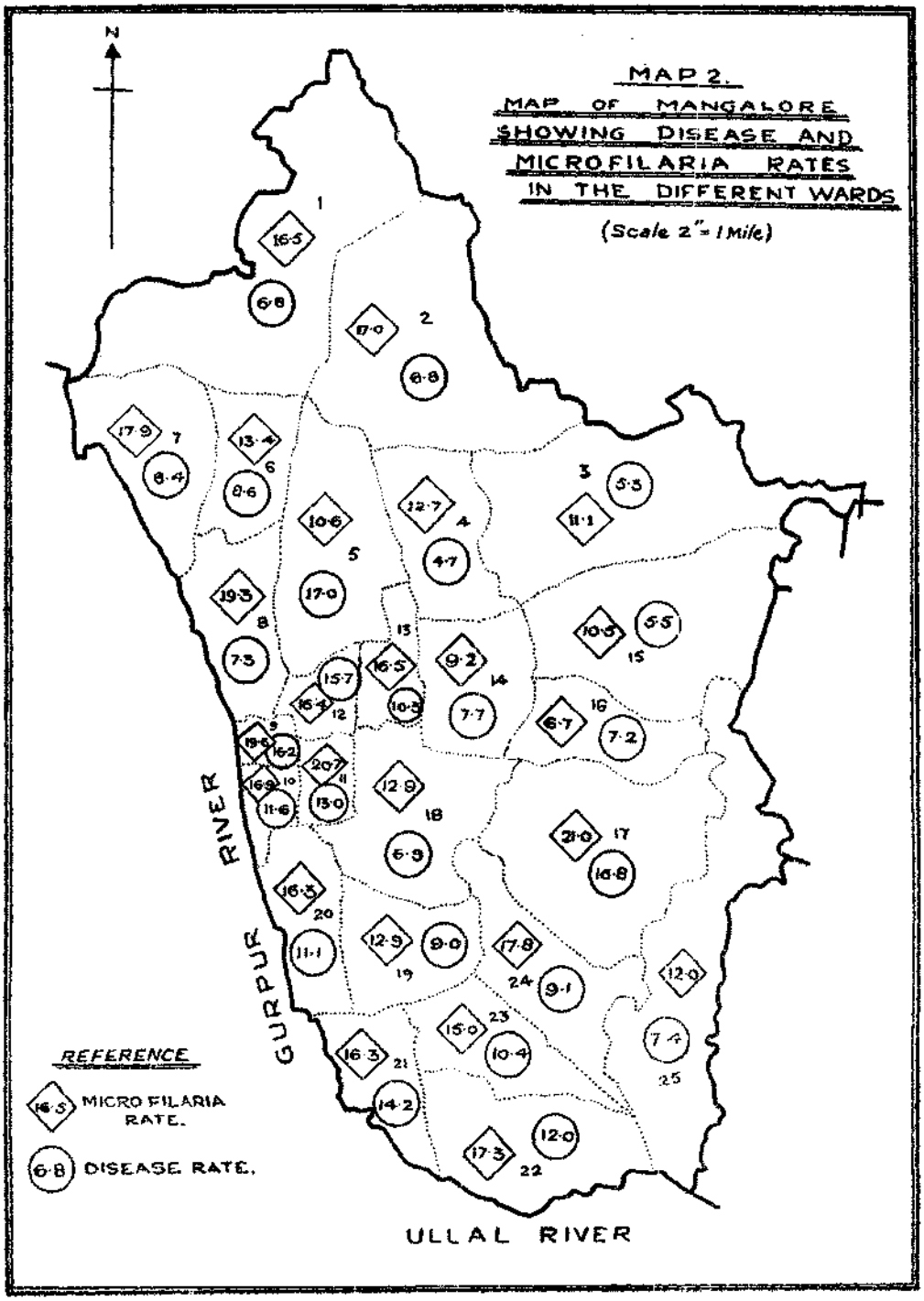
Age group (years)	Males					Females					Both sexes				
	Number examined	With disease		With microfilariæ		Number examined	With disease		With microfilariæ		Number examined	With disease		With microfilariæ	
		Number	Per cent	Number	Per cent		Number	Per cent	Number	Per cent		Number	Per cent	Number	Per cent
2–5	177	0	...	10	5·6	169	1	0·6	9	5·3	346	1	0·3	19	5·5
6–10	432	16	2·3	57	13·2	387	2	0·5	54	14·0	819	12	1·5	111	13·1
11–20	1310	108	8·2	204	15·6	992	81	8·2	166	16·7	2,302	189	8·3	370	16·1
21–30	933	73	7·8	146	17·6	733	107	14·6	109	14·9	1,666	180	10·8	255	15·0
31–50	528	48	9·1	90	17·3	492	81	16·4	61	12·4	1,020	129	12·7	151	14·8
11–50	389	42	10·8	71	18·2	334	64	19·2	45	13·5	723	106	14·7	116	16·0
Above 50	300	35	12·3	54	18·0	226	50	22·1	36	15·9	526	87	16·5	90	17·0
All Ages	4,069	318	7·8	632	15·6	3,333	386	11·6	480	14·4	7,402	704	9·5	1,112	15·0

TABLE IV.

Incidence of filarial disease manifestations.

Disease manifestations*	Percentage incidence among persons with filarial disease		Cases showing microfilariæ	
	Males (318 cases)	Females (386 cases)	Number	Per cent
Lower extremities ...	91	99·7	17	2·5
Upper extremities ...	5·7	5·2	2	5·2
Filarial scrotum ...	0·6	...	1	50
Hydrocele ...	3·0	...	5	50
Lymph scrotum ...	0·3	...	1	100
Chyluria ...	0·3	...	1	100

*Such individuals as had more than one type of manifestation have been shown in more than one place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MAP 2.
MAP OF MANGALORE SHOWING DISEASE AND MICROFILARIA RATES IN THE DIFFERENT WARDS
(Scale 2"= 1 Mile)
1
16·5
6·8
2
6·0
6·8
7
17·9
8·4
6
13·4
8·6
5
10·6
17·0
4
12·7
4·7
3
5·5
11·1
8
19·3
7·3
13
16·5
15·7
16·4
12
14
9·2
10·3
7·7
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10·5
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16
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19·5
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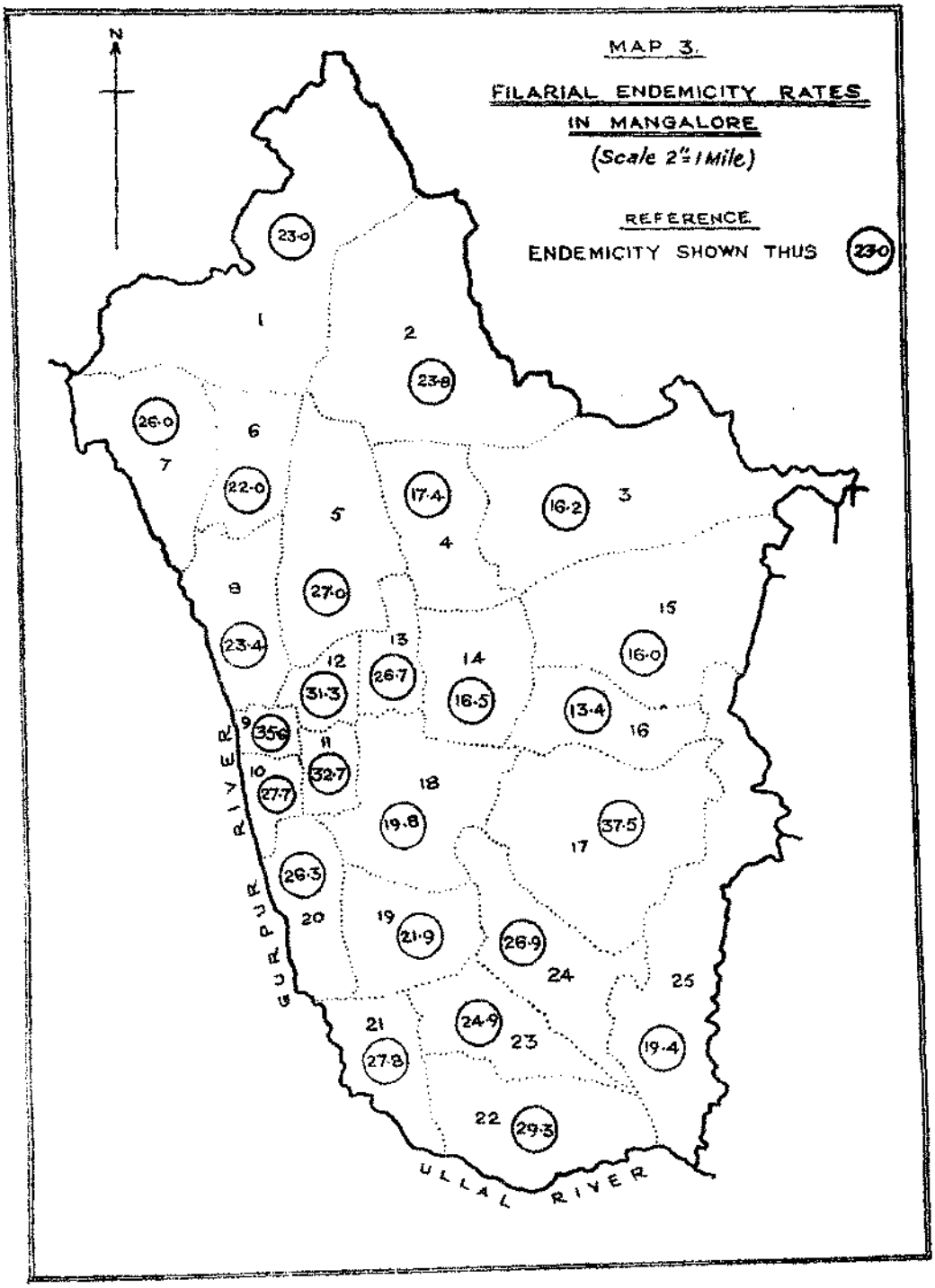
16·9
11·6
11
20·7
15·0
18
12·9
6·9
17
21·0
16·8
20
16·3
11·1
19
12·9
9·0
24
17·8
9·1
23
15·0
10·4
21
16·3
14·2
22
17·3
12·0
25
12·0
7·4
REFERENCE
16·5 MICRO FILARIA RATE.
6·8 DISEASE RATE.
GURPUR RIVER
ULLAL RIVER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MAP 3.
FILARIAL ENDEMICITY RATES
IN MANGALORE
(Scale 2"=1 Mile)
REFERENCE
ENDEMICITY SHOWN THUS 23·0
23·0
1
2
23·8
26·0
6
7
22·0
5
17·4
4
16·2
3
8
27·0
23·4
13
12
26·7
14
15
16·0
31·3
16·5
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35·6
11
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32·7
27·7
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19·8
37·5
17
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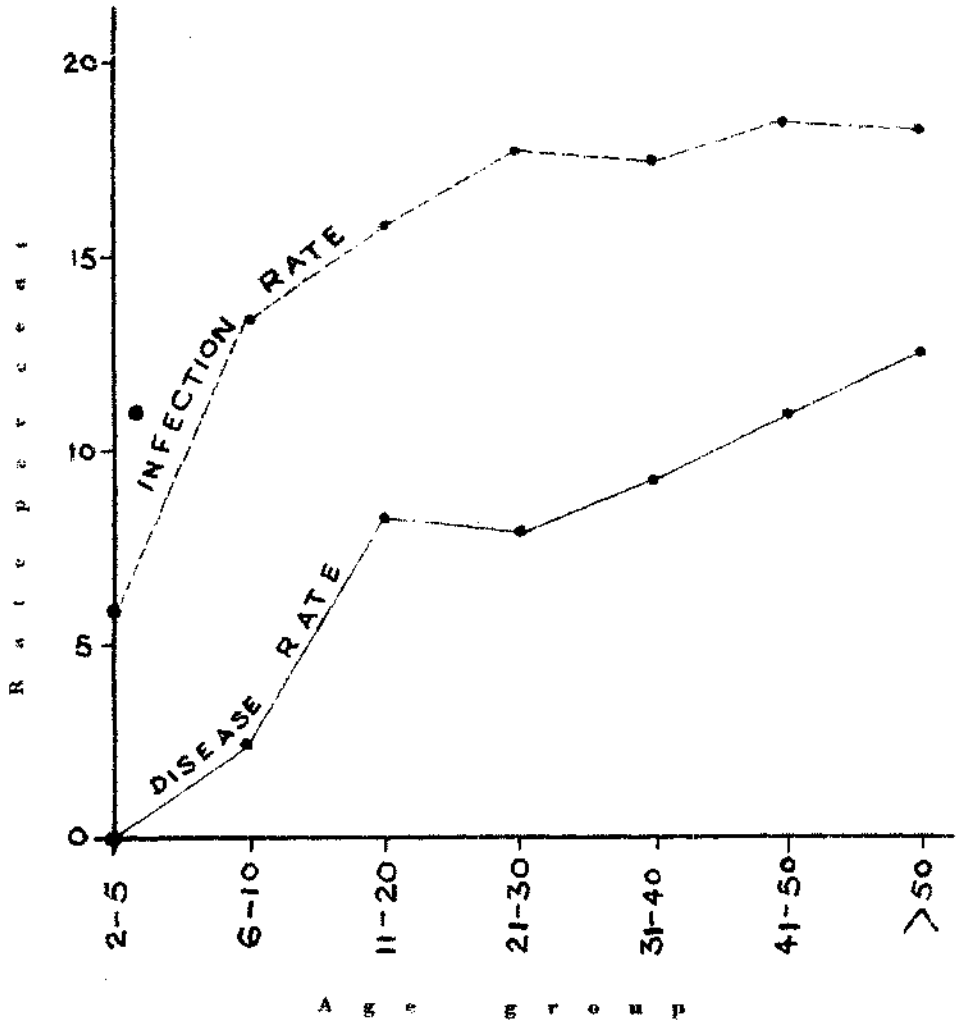
20
19
21·9
26·9
24
25
21
24·9
23
19·4
27·8
22
29·3
GURPUR RIVER
ULLAL RIVER

Seven thousand four hundred and two persons covering 6·3 per cent of the population were examined during the survey. External manifestations of filarial disease were encountered among 704 (9·5 per cent) while 1,112 persons (15·0 per cent) showed microfilariæ in their blood. The species of microfilariæ encountered during the survey was *W. bancrofti* only. The disease rate varied from 4·7 to 17 per cent while the infection rate was as low as 6·7 per cent in Ward 13 and as high as 20·7 per cent in Ward 8 (Map 2). The filarial endemicity rates in the different wards varied from 13·4 to 37·5 per cent (Map 3) ; the gross filarial endemicity rate for the town was 24·2 per cent (Table III).

GRAPH 1.

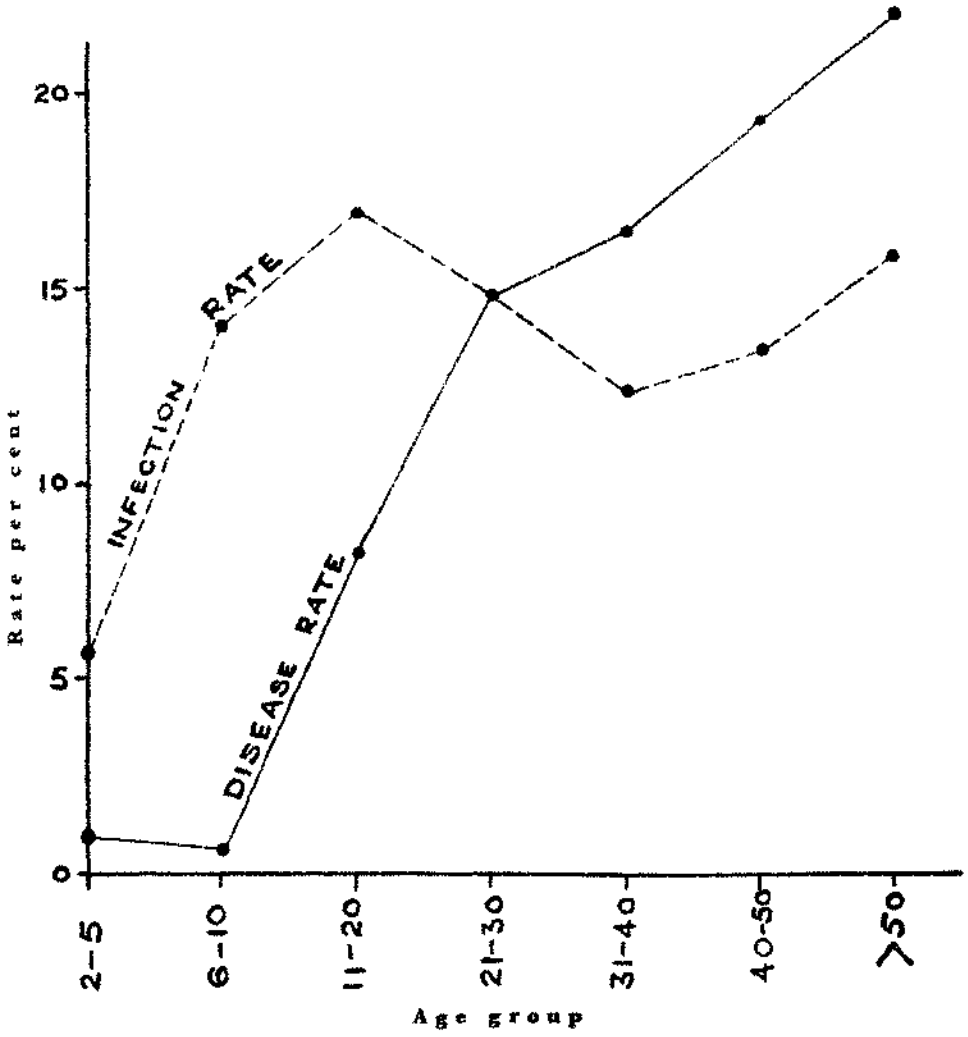
Incidence of filarial disease and infection among males.



The incidence of filarial disease and infection in the different age groups of the two sexes is presented in Table III and Graphs 1, 2 and 3. The occurrence of elephantoid condition appears to show a steady increase with age, this being more marked in females. The microfilaria rate shows a positive correlation with age in the earlier age groups (2 to 20 years), but beyond 20 years this index remains more or less steady without any appreciable rise.

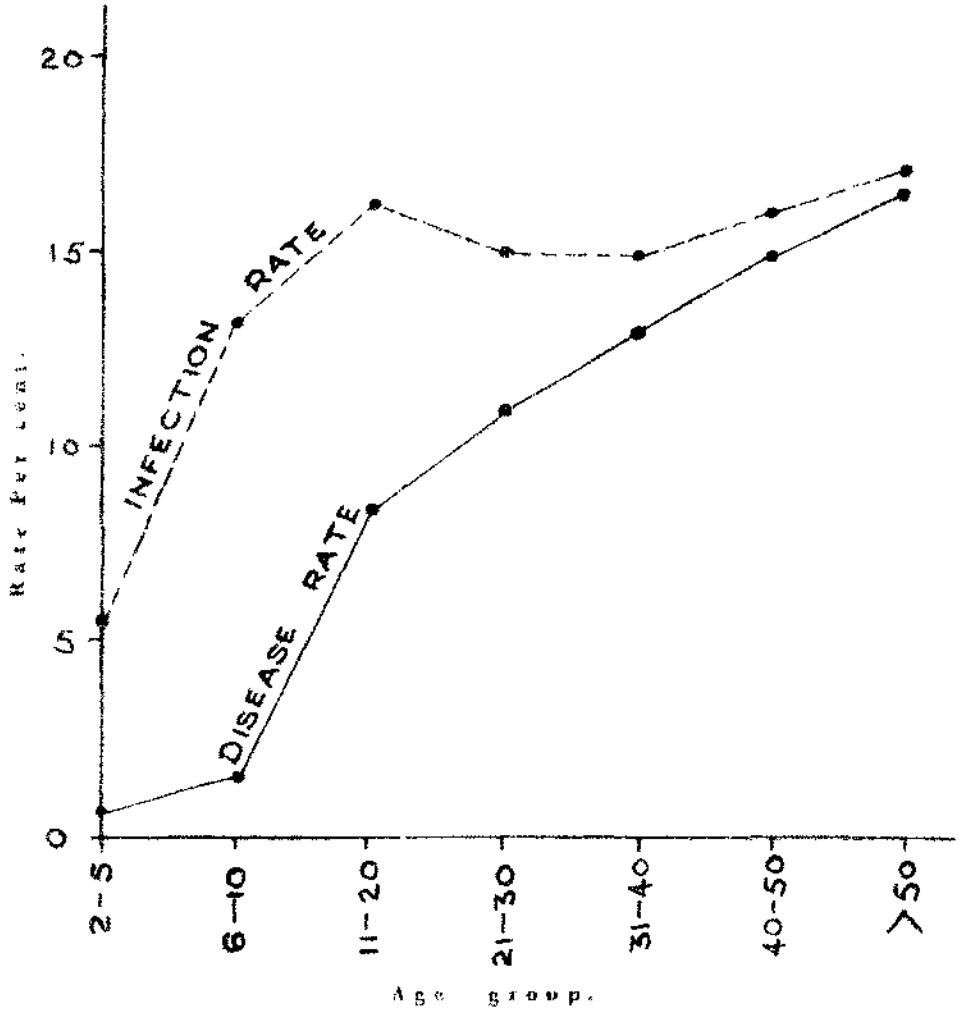
GRAPH 2.

Incidence of filarial disease and infection among females.



GRAPH 3.

Incidence of filarial disease and infection among both sexes.



The youngest age at which filarial infection was detected in the night blood was two years, while disease manifestations were observed in a child of five years—a case of swelling of the left leg of about six months duration.

The types of filarial manifestations recorded during the survey consisted of elephantoid swellings of the extremities, hydrocele, filarial scrotum and one case each of lymph scrotum and chyluria (Table IV). The disease rate would appear to be high (11·6 per cent) among females as compared to that (7·8 per cent) among males. This difference, however, is not statistically significant.

Concomitant occurrence of microfilariaemia was the least among those with swellings of extremities while both the cases of lymph scrotum and chyluria showed microfilariæ in their blood at night ; in the latter case the centrifuged deposit of the chylous urine passed in the morning also showed microfilariæ. Fifty per cent of the persons with hydrocele (ten cases) and filarial scrotum (two cases) showed circulating microfilariæ in their blood at night.

The density of microfilarial infestation in the positive persons was determined by enumerating the parasites in positive slides. The quantity of blood taken, though not measured every time, remained approximately the same in all smears and was about 20 c.mm. Whatever error there might have been in the sampling, it remained constant and negligible as the smears were collected by the same set of workers. Wide variations were noticed in the counts which ranged from 1 to 1,278 per smear. The average microfilarial infestation rate per 20 c.mm. of positive blood was 40·3 in the community examined (Table II).

Entomological survey.—Regular collections from all wards were made during the period for adult mosquitoes which were dissected for determining the vector (s) of filarial infection in Mangalore. The resting places searched included human dwellings, cattlesheds, a few mixed dwellings and outdoor shelters.

The following species of mosquitoes were recorded during the period :—

C. fatigans,
C. sitiens,
C. (Ficalbia) minima,
C. (Lophoceratomyia) sp.,
Armegeres obturbans,
Edes (Stegomyia) aegypti,
Anopheles barbirostris,
Anopheles jamesi,
Anopheles subpictus,
Anopheles vagus and
Mansonia (Mansonioides) sp. (one male specimen was collected).

The females were dissected on the same day they were collected and developmental stages of microfilariæ were looked for in the abdomen, thorax and head/proboscis. 3,646 specimens of different species of anophelines and culicines were dissected and the results are presented in Table V.

Developmental stages of microfilariæ were found only in *C. fatigans*. Of 3,387 specimens of this species dissected, 471 were found to be infected, giving an infection rate of 13·9 per cent ; 223 showed infections in abdomen, 358 in thorax and 44 in head. The infection rates during the three months, showed slight variation, being 12·1, 15·1 and 17·6 during the months of March, April, and May respectively.

TABLE V.

Mosquito dissections in Mangalore.

(*March-April* 1954).

Mosquito dissection.		March.	April.	May.
Species	Other details			
Culex fatigans	Number dissected ...	1784	1064	539
	Number positive ...	215	161	95
	Abdomen	85	85	53
	Thorax	166	119	73
	Head	35	7	2
	Infection rate (per cent) ...	12·1	15·1	17·6
Other culicines	Number dissected ...	68	9	3
	Number positive ...	nil	nil	nil
Anophelines	Number dissected ...	155	16	8
	Number positive ...	nil	nil	nil

BREEDING PLACES OF VECTOR.

An intensive survey of the different parts of the town revealed breeding of *C. fatigans* in the following types of water collections :

(*a*) *Domestic cess pools.*—There is no planned drainage and the waste water from houses either remains stagnating in cess pools which are kutcha in most cases or is led on to water a coconut palm in the neighbourhood. Such collections are sources of prolific breeding of *C. fatigans.*

(*b*) *Stagnant pools in drains.*—Pucca drains are few and far between, and even these are badly maintained in most places. The gradient is poor, and water either remains stagnating to form a chain of small collections or flows very sluggishly. These are perennial breeding places of the vector mosquito.

(*c*) *Tanks, disused wells and ponds.*—There are a number of tanks in the municipality, some of them attached to temples and mosques and some for pisciculture. The latter were almost dry during the time they were visited, and shallow stagnating pools in their bed showed heavy breeding of both anophelines and culicines. A few neglected wells adjoining some factories and the ponds in the coconut groves, also showed breeding. Many of the ponds and some of the tanks had heavy growth of *Pistia stratiotes* but no breeding of *Mansonioides sp.* was noticed.

(*d*) Apart from those enumerated above, a large number of potential breeding places exist in the municipality ; the numerous depressions in undulating areas in the northern and north western parts of the town are likely to become formidable sources of mosquito nuisance after the monsoon.

DISCUSSION.

Filariasis is prevalent throughout the municipality of Mangalore, though the intensity varies in the different localities. Iyengar (1933) observed a centripetal distribution of Bancroftian filariasis in Trivandrum, with endemicity rates over 25 per cent in the central areas, while in the peripheral parts of the town the endemicity rate was below four per cent. No such spatial distribution, however, is present in Mangalore as revealed by the present survey (Map 3). High rates in the peripheral wards as also low rates in the central wards, are equally common.

A high degree of positive correlation between microfilarial infection rates and the filarial disease rates was reported by Iyengar (*loc. cit.*). In the present studies a similar correlation has been observed in most wards (Graph 4).

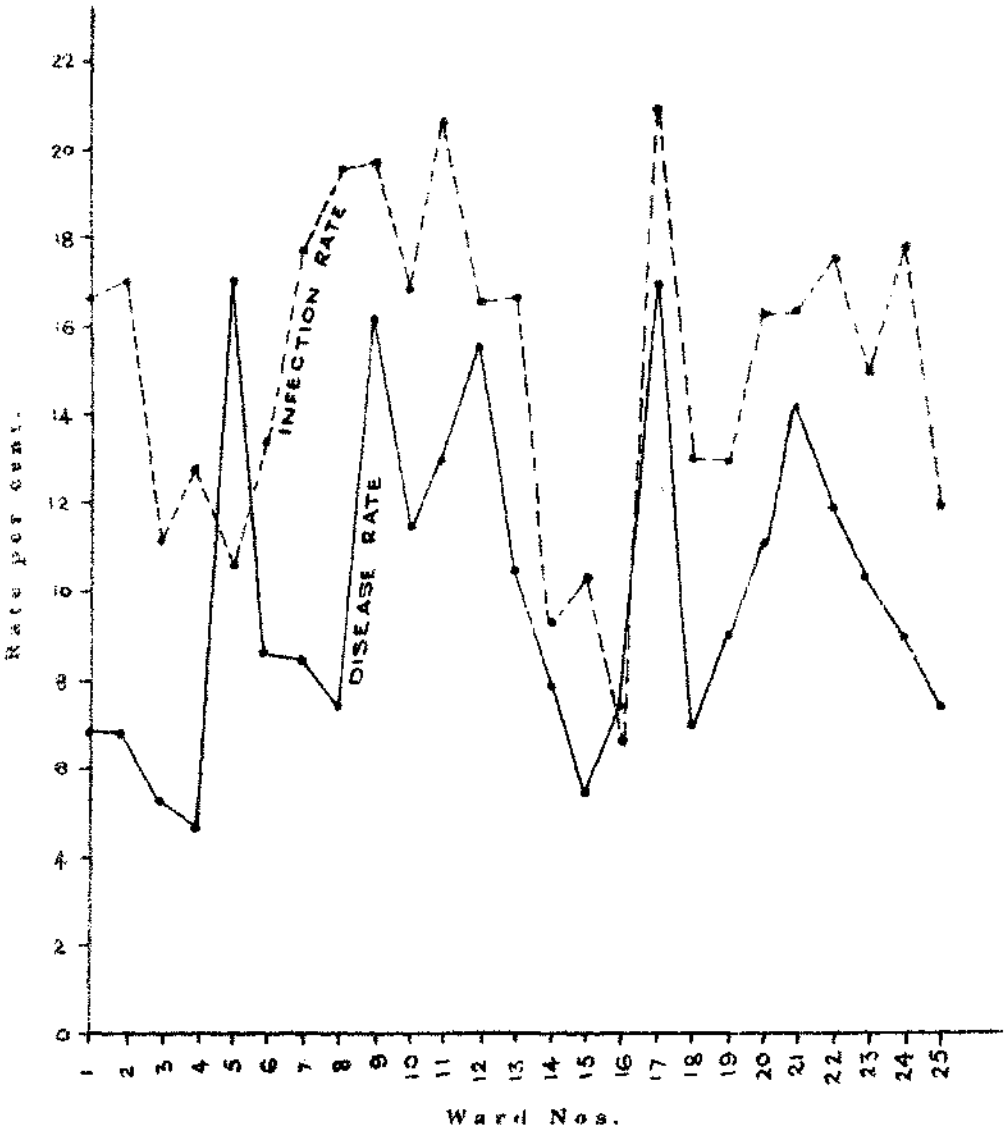
The disease rate, low in the younger age groups, shows a progressive increase with age, this being comparatively more marked during the first two decades (Table III and Graphs 1, 2 and 3). The incidence of disease appears to be comparatively higher among females than among males at all age groups. This variation, however, is not statistically significant.

The microfilaria rate, like the disease rate, shows a sharp rise up to the age of 20 years, but beyond that age, remains constant between 20 and 25 per cent, while the disease rate continues to show an upward trend. An analysis of those individuals with evidence of filariasis, i.e., having filarial disease and/or microfilaræmia shows, that the disease rate increases while the microfilaria rate declines with advancing age (Graph 5). Iyengar (*loc. cit.*) made similar observations in his studies in Trivandrum and explained the decrease in the microfilaria rate in higher age groups as due to the onset of filarial disease. It is likely, as has been observed by Pandit *et al.* (1929), that certain elements present in the sera of persons with elephantoid swellings tend to bring about a reduction in the microfilariæ. This observation is supported by the fact that among the 704 persons with external manifestations of filariasis examined in Mangalore, only 26 persons (3·7 per cent) showed circulating microfilariæ in their blood (Table VI).

Even among the symptomless persons, the filarial infection and infestation rates are lower among the older age groups. Iyengar (1938) pointed out that in the absence of any immunity one should normally expect both these indices to be higher in the older residents, who have been exposed to the infection for a larger number of years. Very little is known about immunity in filarial infections. It is likely that the lowering of infection and infestation rates in the older age groups is a result of long residence in the area, and exposure to repeated small doses of infection conferring a certain degree of tolerance.

GRAPH 4.

Filarial disease and infection rates in different wards of Mangalore.



GRAPH 5.

Variations in distribution of filarial disease and infection with duration of exposure.

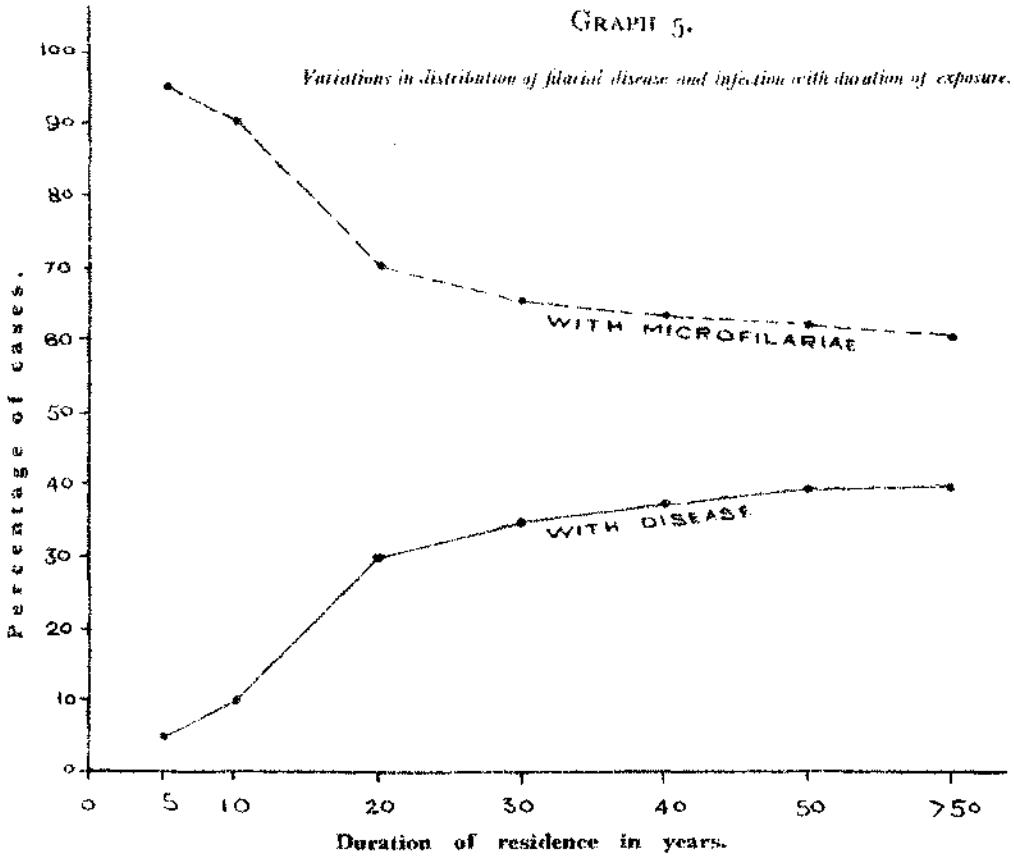


TABLE VI.

Incidence of filarial infection in persons with disease and those without disease.

Group	Number examined	Number showing microfilariæ	Infection rate per cent.
Persons with filarial disease ...	704	26	3·7
Persons without filarial disease ...	6,698	1,086	16·2

The youngest age, as stated above, at which microfilariæ were observed in the night blood was in a child of two years, while disease manifestations were evident at the age of five years. During his survey of Trivandrum, Iyengar (1933) observed the occurrence of microfilaria in a child of about two years while disease manifestations were not in evidence earlier than eight years. Acton and Rao (1930) analysed the data on filarial endemicity from different parts of India and

concluded that the site of lymphatic obstruction varied with the intensity of transmission under which the community was living. In areas where conditions favoured transmission of filariasis through a greater part of the year, the degree of endemicity was high and the age incidence of disease was low, from six to ten years. The predominant lesions in such areas were elephantoid swellings of extremities.

From the latter observations of Acton and Rao (*loc. cit.*), the available evidence that over 95 per cent of the external manifestations in Mangalore consisted of elephantiasis of the extremities, would appear to point to a prolonged transmission period in this town, probably during most months of the year. No direct evidence regarding the transmission period of filariasis in Mangalore is available ; the present observations having been restricted to only three months.

SUMMARY.

1. Observations recorded during March-April, 1954, on the incidence of filariasis in Mangalore have been reported.

2. The gross disease rate for the town was 9·5 per cent and the microfilaria rate was 15 per cent. The filarial endemicity rate in the town was 24·2 per cent.

3. An average microfilarial infestation of 40·3 per 20 c.mm. was recorded; the highest density of microfilaria enumerated being 1,278.

4. The youngest age at which microfilariæ were found in the night blood was in a child aged two years, while filarial swelling of the leg was noticed at the age of five years.

5. The disease manifestations of filariasis observed in Mangalore were swellings of extremities, filarial scrotum, hydrocele, lymph scrotum and chyluria.

6. *W. bancrofti* was the only species of microfilariæ recorded and natural infections were observed only in *C. fatigans*.

ACKNOWLEDGEMENT.

The author wishes to record his thanks to Shri K. S. N. Adiga, Chairman of the Mangalore Municipality, Dr. M. D. Edwards, the Municipal Health Officer and the officers and Councillors of the Municipality for their invaluable co-operation and help in the survey. Thanks are also due to Shri M. R. Visweswaran Thampi, Entomological Assistant for his technical assistance, and to Shri M. Viswanatha Rao, Sanitary Inspector, for his able assistance in the field.

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A NOTE ON THE DAYTIME RESTING HABITS OF *A. CULICIFACIES* IN CEYLON.

BY

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(July 5, 1954.)

THE fact that the habits of vectors of malaria, even belonging to the same species, vary a great deal from country to country, makes it necessary that they be thoroughly studied as part of any control scheme. In Ceylon, the vector is *A. culicifacies*. It was first incriminated as a vector in this country by James and Gunasekara (1913) and subsequently confirmed by Carter and Jacocks (1929). Rajendram and Jayewickreme (1951) report that in Ceylon *A. culicifacies* is domestic in habit and rests in dwelling houses after its blood feed ; that it is generally found in dark corners of houses and that if there is a fire burning inside, it is generally caught near the door.

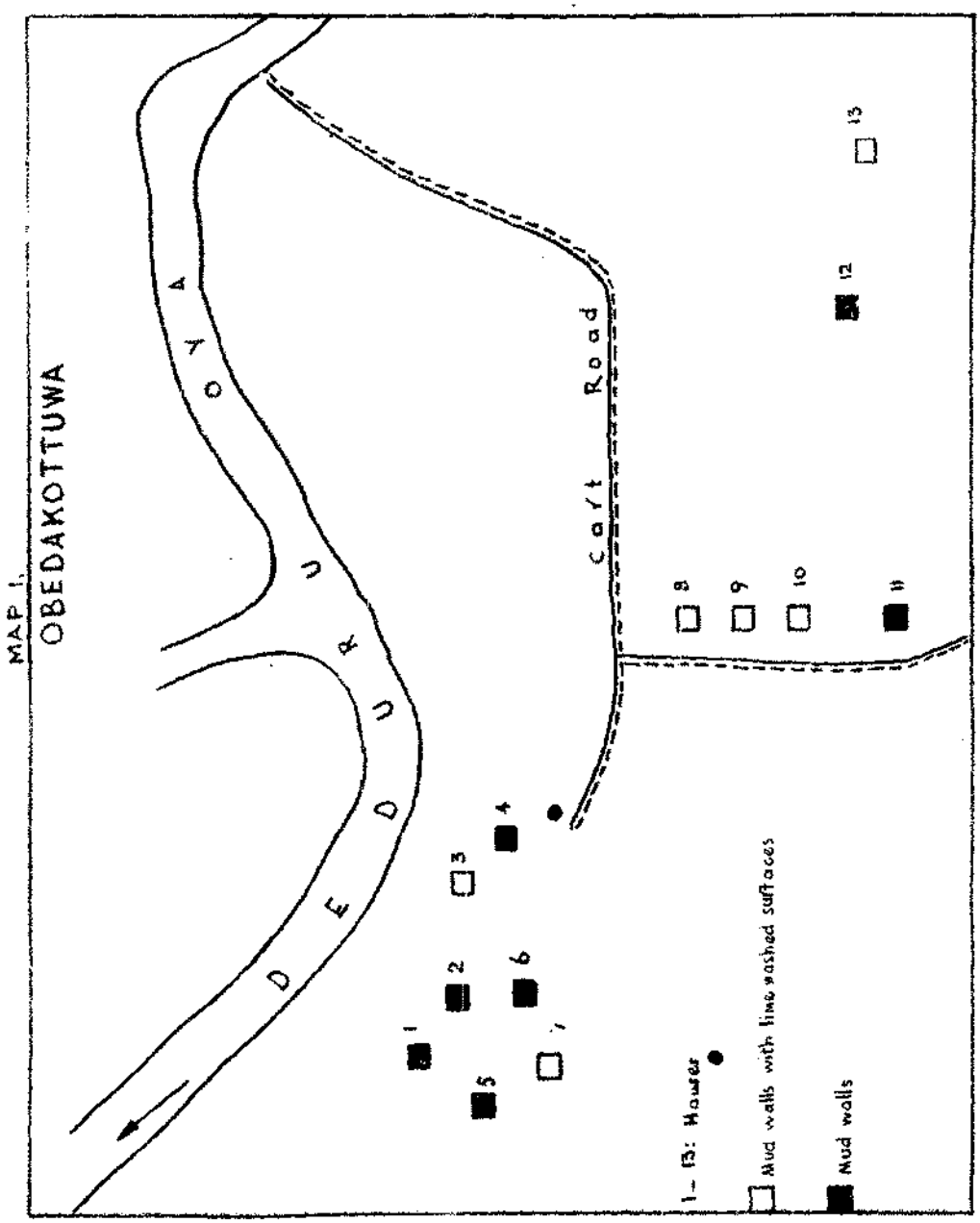
Rajendram *et al.* (1950) have found that the larvæ breed in shaded situations in streams in thick jungles up to a distance of six miles from human habitation and they could catch adults in human baited traps in the jungle far away from the nearest dwelling.

With a view to obtain precise information on the preferential daytime resting places of *A. culicifacies*, observations were carried out in two villages which had not been sprayed with residual insecticides.

EXPERIMENTAL AREA.

Two unsprayed villages, Obedakotuwa (Map 1) and Udugodagama (Map 2), situated in Hiripitiya in the Kurunegala District, were selected. The former village lies on the bank of River Deduru Oya, while the latter lies on the bank of Kimbulwana Oya, a tributary of the Deduru Oya. Each village is about a square mile in area. The locality receives scanty rain, the average monthly rainfall being 5·30 inches. A severe drought prevails during a great part of the year, the mean maximum temperature being 88°F. and the mean minimum 73°F. The average

humidity by day is 68 per cent and that by night is 92 per cent. The meteorological readings for the area for the year 1953 and the average readings for 30 years from 1911-1940 are given in Table I. As a result of the prevalent drought, pools are left in the river-bed and these afford favourable breeding places for *A. culicifacies.*



MAP 2.

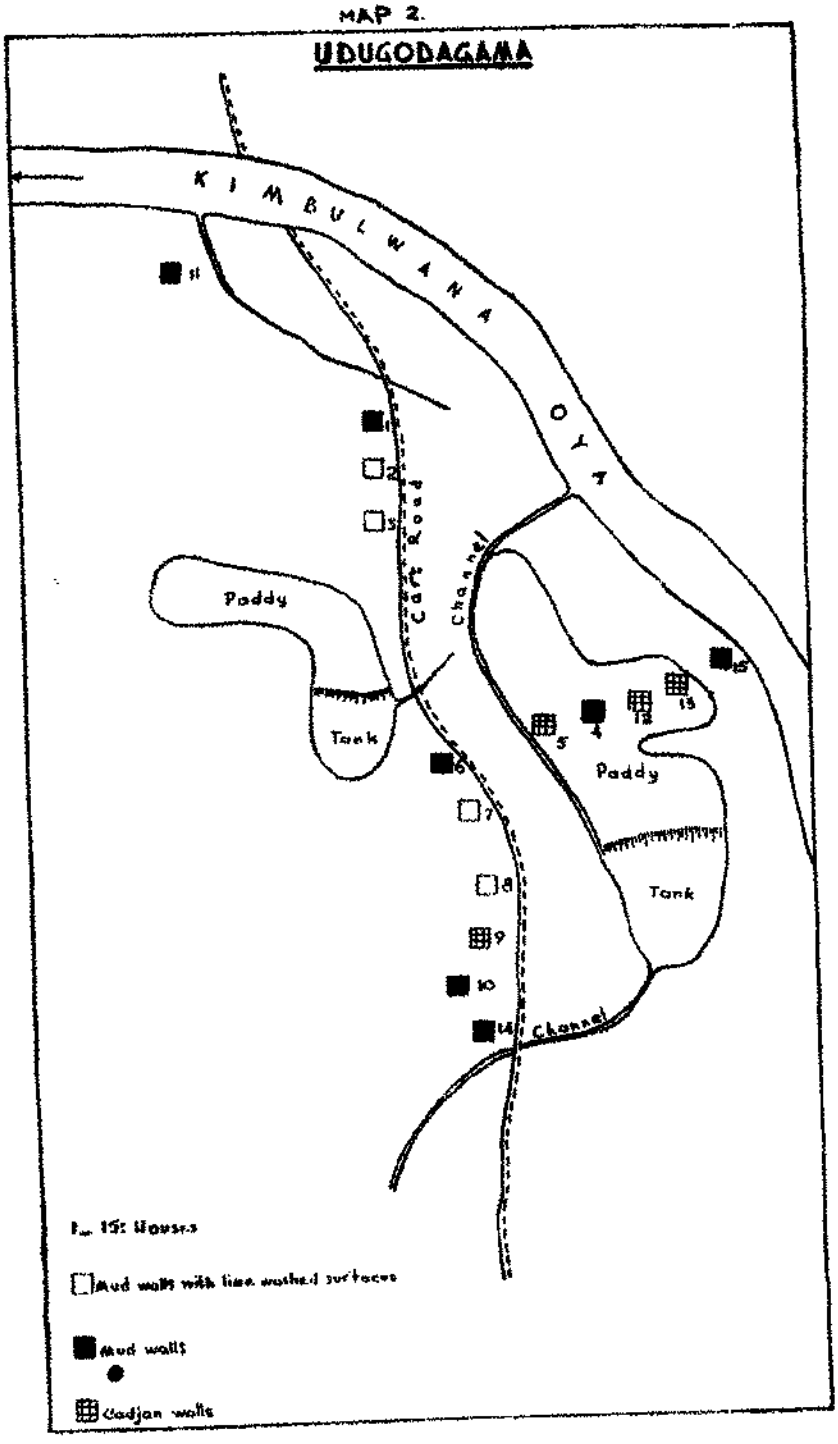

UDUGODAGAMA
KIMBULWANA OYA
Cart Road
Channel
Paddy
Tank
Paddy
Tank
Channel
Mud walls with lime washed surfaces
Mud walls
Cadjan walls

TABLE I.

Meteorological readings of the experimental area. Monthly averages for 30 years 1911-1940 and for the year 1953.

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Average
Rainfall (in inches)													
1911–1940 ...	1·50	2·79	5·70	5·71	0	0·74	11·91	0·12	2·76	21·25	6·32	4·90	5·30
1953 ...	1·84	2·51	2·07	9·91	0	0	...	0·27	4·04	18·84	10·72	3·67	
Maximum temperature (°F)													
1911–1940 ...	83	87	91	91	90	89	91	91	91	89	85	83	88
1953 ...	84·4	88·4	93·7	91·6	95·5	94·6	88·9	91·4	90·4	87·8	85·6	83·8	
Minimum temperature (°F)													
1911–1940 ...	69	69	71	74	76	76	76	75	75	73	71	70	73
1953 ...	68·4	67·5	72·4	73·0	76·9	77·2	74·3	75·1	74·2	73·4	70·1	69·8	
Day humidity (Per cent)													
1911–1940 ...	76	67	62	65	68	66	62	60	62	70	78	78	68
1953 ...	76	65	62	71	61	58	70	64	64	74	73	77	
Night humidity (Per cent)													
1911–1940 ...	95	94	94	93	92	90	88	88	90	93	95	95	92
1953 ...	95	95	95	95	91	84	88	86	88	93	93	93	

Out of a total of 28 houses in the two villages, 14 were mud-walled, ten mud-walled and surface lime-washed and four cadjan-walled.* The roofs were thatched either with cadjan or straw. Each house had a living room, where the inmates spend some part of the day and sleep at night, and a kitchen. Some houses had one or more 'other' rooms, which are non-living rooms, usually used as store rooms. Out-houses such as cattlesheds are rarely found as cattle are, as a rule, tethered in the open in these villages. The rivers were the main source of breeding, the farthest house being half a mile from the rivers.

MATERIAL AND METHODS.

Collections of mosquitoes were made separately from each house for half an hour every week between 7 a.m. and 12 noon. After the end of the half hour, further collections were continued in the houses till no more mosquitoes could be found. These two types of collections were kept separate and recorded as man-hour catches and total collections. These observations and collections

*Made from dry coconut leaves.

were continued from January to December 1953, with the help of an Entomological Assistant and a Field Attendant, all field determinations of species being checked in the laboratory. Male and female catches of *A. culicifacies* were recorded separately, notes being kept of the type of room each mosquito was found in, the type of resting place and its height. Collections, at heights above six feet, were carried out with the help of a ladder. The dimensions of various rooms in each house were measured and the areas of the rooms determined. The mosquito density per sq. ft. per room type was then calculated. This served as a convenient index for the comparison of the degrees of preference shown by *A. culicifacies* to the different types of room in a house. The average man-hour catch of *A. culicifacies* females per house type per month has also been determined to find out if there is any preferential selection of houses by them. As there was considerable fluctuation in the mosquito density in different seasons, the observations were extended over a whole year.

RESULTS.

The results are summarized in Tables II, III, IV and V.

TABLE II.

Man-hour catch of A. culicifacies *female adults in different types of houses (all cadjan roofed or straw thatched).*

Month.	Lime-washed, mud-walled.	Mud-walled.	Cadjan-walled.
1953 January ...	0·47	0·56	4·06
February ...	7·96	11·44	6·70
March ...	16·72	22·70	26·40
April	12·40	27·19	34·90
May	12·72	12·90	20·87
June	1·50	15·13	40·42
July	11·25	18·15	30·60
August ...	12·41	30·30	31·87
September ...	12·90	11·44	29·00
October ...	13·00	23·20	42·73
November ...	4·90	13·50	23·40
December ...	0·50	2·55	11·36
Total	108·73	189·06	302·31
Average ...	8·894	15·755	25·192

The number of male mosquitoes caught was extremely low as compared to that of females and, therefore, the conclusions have been drawn from the statistics of the latter only and do not apply to the males which are of no consequence so far as malaria is concerned.

1. The man-hour catch determined for the vector in the various types of houses (Table II) shows that during all months, except February, the prevalence of the vector was the highest in cadjan-walled houses. The next in order are houses with mud-walls and the last are those with the lime-washed walls.

2. As between rooms, the results clearly indicate that the highest number of mosquitoes per square foot of area examined (Table III) shelter in living rooms. The next in order is the kitchen, the mosquito density being the least in the 'other' rooms.

TABLE III.

Number of A. culicifacies *adults harboured by different types of rooms in houses.*

Month.	Living room.		Other rooms.		Kitchen.	
	♀	♂	♀	♂	♀	♂
1953 January ...	28	6	1	0	2	2
February ...	205	33	1	0	24	3
March ...	396	50	37	22	56	6
April	316	29	3	0	43	6
May	218	6	12	0	67	1
June	340	18	53	6	3	1
July	172	27	31	10	13	5
August ...	279	45	23	6	10	0
September ...	287	19	17	1	8	1
October ...	440	48	30	9	16	4
November ...	141	22	34	6	8	3
December ...	98	17	14	5	2	0
Total	2920	320	256	65	252	32

Total areas examined.

Living rooms — 7,682 sq. ft. Other rooms — 1,618 sq. ft.
Kitchens — 2,037 sq. ft.

Number of female mosquito adults per sq. ft.

Living rooms — 0·38 Other rooms — 0·059
Kitchens — 0·123

3. The majority of mosquitoes were found resting on the inner walls of the houses. Table IV shows that 65·5 per cent of the mosquitoes collected were found resting on walls and only 12·4 per cent on furniture and hangings. It is also worth noting that only one mosquito was caught on the eaves and none on the outer walls of the houses. Few were also found on the ceiling. Windows and doors also harboured a considerable number as they formed part of the wall surfaces.

TABLE IV.

Number of A. culicifacies *adults at various resting places in houses.*

Month.	Walls.		Windows and doors.		Furniture.		Hangings.		Roof.		Outer walls and eaves.	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
1953 January	22	5	5	1	1	0	0	0	0	2	1 (eaves)	0
February	99	5	85	21	15	3	23	1	8	5	0	0
March ...	294	48	102	18	33	4	34	0	37	5	0	0
April ...	276	24	32	4	17	0	18	1	23	3	0	0
May ...	232	3	29	1	18	0	7	0	12	1	0	0
June ...	193	6	69	1	45	1	74	3	3	2	0	0
July ...	129	27	48	8	25	0	1	0	13	7	0	0
August ...	214	38	71	11	9	1	5	0	11	1	0	0
September	222	16	44	0	31	2	2	1	13	1	0	0
October ...	347	54	70	2	19	2	30	1	17	2	0	0
November	126	19	44	10	6	0	6	1	4	6	0	0
December	83	18	18	4	4	0	1	1	1	0	0	0
Total ...	2237	263	617	81	223	13	201	9	148	35	1	0

4. Table V shows that the majority of *A. culicifacies* rested at heights ranging from two to six feet and considerable numbers below two feet ; comparatively few being found at heights above six feet.

TABLE V.

Number of A. culicifacies *adults at various heights in houses.*

Month.	0-2 ft.		2-4 ft.		4-6 ft.		6-8 ft.		Over 8 ft.	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
1953 January ...	10	1	9	2	6	3	8	2	1	0
February ...	31	3	85	14	90	13	16	6	0	0
March ...	79	8	75	9	223	38	92	18	16	4
April	86	8	78	5	149	17	49	5	4	0
May	71	2	118	2	95	2	12	1	3	0
June	101	5	189	7	98	9	5	0	3	2
July	68	11	61	12	86	10	0	2	2	0
August ...	70	13	88	7	142	32	12	1	0	0
September ...	99	4	132	13	67	4	10	1	0	0
October ...	99	14	200	28	172	18	17	2	0	0
November ...	35	6	91	11	49	13	4	6	0	0
December ...	20	0	52	10	39	12	2	1	0	0
Total	769	73	1178	120	1216	180	227	45	29	6

DISCUSSION.

Most observers agree that *A. culicifacies* is a domestic species. As to its relative prevalence in cattlesheds and houses, there are conflicting reports. Nursing *et al.* (1934) found that in Mysore, cattlesheds and houses yielded a higher catch than a human-baited tent, but in the region of the Western Ghats they found the reverse. Timbres (1935) working in Bengal found that the catch was higher in houses than in cattlesheds. Senior White (1937) reports the reverse from the Jeypore hill tracts.

James and Liston (1911) report that *A. culicifacies* hides in holes and crevices in walls and roofs of houses or may take shelter in heaps of straw and cow-dung cakes near stables and out-houses. Afridi and Puri (1940) state that the adults prefer cattlesheds to houses as daytime resting places. Pal (1945) observes that a room with unplastered walls having innumerable crevices which yielded a large number of adults of *A. culicifacies* showed a considerable reduction in mosquito population when the walls were replastered. He also states that in Indian villages, human beings and animals frequently occupy the same dwellings and strict comparison between the relative prevalence is not possible. As in Ceylon cattle are

usually tethered in the open and cattlesheds are seldom used, these observations were confined only to human dwellings. It was observed that on days when cattle were tethered within a few yards of houses, the catch in the houses was higher than on other days. It may be that the mosquitoes feed on the cattle and subsequently enter the dwellings for resting.

An analysis of the observations shows that *A. culicifacies* which normally tends to hide in dark corners, finds shelter mostly at lower levels on cadjan walls perhaps because cadjan provides more secure and unexposed recesses to hide in. Adult catches are higher in mud-walled houses than in houses with lime-washed walls, probably because the mosquitoes are repelled by the shine of the lime-washed walls and also because the mud-walls, without lime-washed surfaces, are darker and perhaps have more crevices and better resting places.

The highest prevalence of mosquitoes in the living room and kitchen, the two types of rooms most frequented by man, indicates that the mosquitoes are attracted by the presence of the inmates in these rooms which they enter in search of a blood meal, and subsequently rest there. Obviously, therefore, during residual spraying operations, more attention should be paid to the living room and kitchen than to other rooms in dwellings.

Muirhead-Thomson (1951) and Pal and Sharma (1952) have reported that *A. culicifacies* rests relatively more frequently on surfaces such as those of clothes, umbrellas, furniture, firewood, etc. However, the observations carried out in Ceylon show that as compared to the walls, fewer mosquitoes rest on furniture and hangings.

The analysis of the observations indicate that comparatively few moquitoes rest at heights above six feet. But further work will be necessary to show whether spraying up to six feet only will be sufficient to bring down the density of mosquitoes below the critical value for malaria transmission.

SUMMARY AND CONCLUSIONS.

Number of *A. culicifacies* found resting in various types of daytime resting places in Ceylon, were recorded for a whole year.

An analysis of the data collected showed that :

(1) *A. culicifacies* prefers cadjan-walled houses to mud-walled ones and mud-walled houses to those that are lime-washed.

(2) The living room and the kitchen of a house harbour comparatively large number of *A. culicifacies* than "other" rooms.

(3) *A. culicifacies* prefers to rest on the inner walls of houses than on other structures.

(4) The majority of *A. culicifacies* rest below six feet.

ACKNOWLEDGEMENTS.

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STUDY OF CHEMICAL AND PHOTODYNAMIC DETERIORATION OF DICHLORO-DIPHENYL-TRICHLORETHANE (D.D.T.) WHEN APPLIED ON SOLID SURFACES.

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(February 24, 1954.)

INTRODUCTION.

According to West and Campbell (1950), " Fleck and Haller (1944) found that hydrogen chloride was eliminated from D.D.T. (and several analogues) by the use of various catalysts including anhydrous ferric oxide, anhydrous ferric and aluminium chlorides, iron and other materials such as Fuller's earth and some mineral products—although this may have been due to the presence of small amounts of iron compounds. "

Alessandrini (1950) has stated that dechlorination of D.D.T. occurs in the presence of small traces of humic acid and other substances, which also act as catalysts.

The experience of some workers has shown that D.D.T. preparations sprayed on freshly lime-washed walls had a much shorter period of effectiveness than those sprayed on neutral surfaces (West and Campbell, *loc. cit.*).

Fleck and Haller (*loc. cit.*) studied the photodynamic deterioration of *pp*-isomer by spreading thin films of benzene solution of D.D.T. on pyrex glass dish and exposing it to ultraviolet and direct sun rays. They concluded that *pp*-D.D.T. undergoes profound chemical changes in ultraviolet light. Under direct sun rays, decompostion was less marked.

In order to study the above phenomena, in so far as they were applicable to Indian field conditions, a series of experiments were planned and systematically carried out in a selected area in the Tarai.

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EXPERIMENT I.

(*a*) *Preparation of test panels.*—Three cubicles, approximately 2 × 2 × 2 feet each, were made and placed under a thatched cover. Two of the cubicles, namely 'A' and 'B', were of mud proportionately mixed with cow-dung, and the third 'C' of cement. In two of the walls of each of these cubicles, small openings were made and screened with netting to maintain air circulation. On one of the external surfaces of each of the cubicles 'A' and 'C', clean glass plates, approximately 6 × 6 cm. each, were fixed in a line, spacing them at 6 cm. interval. The internal surfaces of the cement cubicle and its external surface on which the glass plates were fixed, were then treated once with lime-wash.

(*b*) *Spraying of cubicles.*—The inner surfaces of one of the mud cubicles, namely 'A', and those of the cement cubicle, namely 'C', were sprayed with an aqueous suspension of D.D.T. (Geigy malaria spray). The external surface of cubicles 'A' and 'C' on which glass plates were fixed, also received similar treatment, care being taken to see that the line of glass plates, alternated by bare wall surface, was covered by a single swath of spray. The second mud cubicle 'B' was not sprayed and kept as a 'control'.

(*c*) *Technique of bioassay.*—When the internal surfaces were dry, equal numbers (10-30) of laboratory-bred, unfed, females of *C. fatigans*, about 12 hours old, were released into each of the three cubicles and their 6-hour and 12-hour mortality rates recorded. Fresh specimens were released at regular intervals of 24 hours, one week, two weeks, three weeks and so on, and the usual mortality figures recorded.

(*d*) *Colorimetric assessment.*—After the sprayed external surfaces were dry, scrapings from about 25 sq. cm. of both the mud and cement cubicles from the glass plates and the adjacent bare walls were taken and subjected to colorimetric test (Alessandrini) to ascertain the *pp*-D.D.T. Subsequent scrapings were similarly taken and colorimetrically tested at intervals of 24-hours, one week, two weeks, three weeks and so on.

(*e*) *Results.*—From the bioassay results, as has been shown in Table I below, it appears that the lethal effect of D.D.T. against culicines does not last for more than a week after the spraying. Colorimetric test of samples of scrapings taken from the internal surfaces of the mud and cement cubicles at the close of second week after spraying, revealed a deposit of 22 mg. of *pp*-D.D.T. per square foot on the cement wall, whereas the mud surface showed much less. The dosage applied was approximately 100 mg. per square foot.

TABLE I.

Lethal effect of D.D.T. after spraying.

Days.	Number of female *C. fatigans* released.	Mortality percentage.					
		Control.		Mud wall.		Cement wall.	
		6th hour.	12th hour.	6th hour.	12th hour.	6th hour.	12th hour.
Immediate ...	30	...	6·67	6·67	93·33	...	93·33
24 hours ...	30	...	...	...	73·33	...	83·33
One week ...	20	...	...	5·00	70·00	...	50·00
Two weeks ...	10	...	...	...	...	...	...

Colorimetric test evinced that on the neutral glass surfaces, there was no evidence of deterioration of D.D.T. till the end of fifth week after spraying, whereas on the mud surface a fair amount of deterioration had occurred even 24 hours after spraying and complete deterioration (yellow colour) was seen at the end of fourth week. Cement wall undoubtedly showed progressively less deposit of *pp*-D.D.T. and the deterioration was less marked than on the mud-wall. In every case, immediate readings were taken.

(*f*) *Supporting test.*—A few field tests were performed by way of corroboration by spraying watery suspension of D.D.T. (Geigy malaria spray) and watery emulsion of D.D.T. in aromex in aimed dosages of 50 mg., 100 mg. and 200. mg. per square foot on previously unsprayed mud-walls and masonary walls. Colorimetric estimation of deposits and of ether and sulphuric ether extracts of deposit was undertaken soon after drying and subsequently at intervals of 24 hours, one week, two weeks, three weeks and so on.

The following observations were made :—

(*i*) Deterioration starts in the mud-walls soon or 24 hours after spraying, progressively becomes marked and complete between third and fourth weeks. On the masonry walls, the rate of deterioration is much slower and becomes marked after four weeks and it is completed between sixth and seventh weeks.

(*ii*) There is apparently very little difference in the rate of deterioration between suspension and emulsion.

(*iii*) Degree of deterioration is almost the same in aimed dosages of 50 mg., 100 mg. and 200 mg. per square foot as far as emulsion is concerned. In the case of suspension, however, it proportionately varies with the dosage. The higher the dose, the lower is the percentage of dechlorinated D.D.T. available on the sprayed surface.

EXPERIMENT II.

Preparation and technique. Four clean test tubes were selected. In each of them 0·005 gm. of D.D.T. (*pp.* content approximately 90 per cent), accurately weighed in a sensitive Oertling chemical balance, was taken. The technical D.D.T. was properly powdered in a pestle and mortar before weighing. One of these tubes was set aside as a 'control'. Into each of the remaining three tubes, 0·5 mg. of correctly weighed dry and powdered cow-dung, country mud, and lime scraping were poured, respectively, and thoroughly mixed by shaking. Into each of the four tubes, a jet of steam was then passed for a few seconds adequate to moisten the contents of the tubes. The tubes were then loosely plugged with cotton-wool and set aside for four hours, after which period they were subjected to colorimetric tests.

Results.—This experiment was repeated taking 0·5 gm. dry powdered cow-dung, powdered mud scraping from the wall of a village hut and dried mud from a river bank in separate tubes to each of which the same measured quantity (0·005 gm.) of D.D.T. was added. After subjecting the mixture to a steam jet as before, the contents were subjected to colorimetric tests.

An analysis of the tests in these two experiments showed that D.D.T. had appreciably deteriorated in all the tubes except in those in which it had not been mixed with any thing and had been kept as ' controls '.

EXPERIMENT III.

In the photodynamic series two sets of tests were carried out. One set was performed by uniformly spraying four clean plates (1 × 1 foot) with watery suspension of D.D.T. (Geigy malaria spray) and exposing three of these plates to sun rays, infra-red rays and ultraviolet rays, respectively, for 30 seconds, one minute, two minutes, five minutes, 15 minutes, 30 minutes, one hour and two hours at a time. The fourth plate was not exposed to any rays and was kept as a ' control '. After each exposure, samples from the unexposed plate were also colorimetrically tested to ascertain natural dechlorination under unexposed condition possibly due to other physical factors. Results showed that the plate exposed to ultraviolet rays only evinced signs of slight deterioration after two minutes of exposure, and complete deterioration after an exposure of two hours. Plates exposed to infra-red and sun rays and also the ' control ' plate did not evince any colour variation signifying deterioration. For exposures of infra-red and ultraviolet rays, a Westinghouse portable combined set of 110/125 volts having a 275 watt bulb for ultraviolet, and a 250 watt bulb for infra-red rays, was used.

The second set of tests was performed by accurately weighing seven clean petri dishes of nearly equal size and spreading in each 0·05 gm. of *pp*-D.D.T. dissolved in ether (extracted from technical D.D.T. in the laboratory of the second author). The ether was then allowed to evaporate. A pair of dishes were exposed respectively to sun rays, infra-red rays and ultraviolet rays, the seventh dish being kept unexposed as a ' control '. Of the pair of dishes under each type of radiation, one was given a continuous exposure of three hours and then tested colorimetrically, while the other was re-weighed at intervals of half an hour, one hour, two hours and three hours after continuous exposure. The ' control ' dish was also weighed along with others at the above intervals and ultimately subjected to colorimetric test at the end of three hours. It was found that, gravimetrically the weights of the ' control ' dish and the dish exposed to sun rays did not differ at the end of three hours from their initial weights, whereas the dish exposed to infra-red rays showed a decrease of 0·1 gm. and that exposed to ultraviolet rays a decrease of 0·0032 gm. Colorimetrically, except the ' control ' other dishes showed signs of slight deterioration.

DISCUSSION.

Analysis of mud-wall and masonrywall scrapings were done by two different organizations, namely, Laboratory of the Public Analyst to the Government, Uttar Pradesh, Lucknow, and the Regional Soil Laboratory, Rudrapur, District Nainital. The results of iron oxide content are given as follows :—

	Mud-wall.	*Masonry-wall.*
Public Analyst to the Government, U.P.	7·43 per cent	Traces
Regional Soil Laboratory ...	3·56 per cent	2·84 per cent

These experiments tend to show that in the field, D.D.T. very quickly deteriorates on mud surfaces which apparently is due to the high percentage of iron oxide catalysts present in the mud. Moreover, whatever preparation of D.D.T. is sprayed, whether suspension or emulsion, the rate of deterioration remains about the same, though in the case of suspensions the degree of initial deterioration is inversely proportional to the dosage.

In the authors' experience, on surfaces where the percentage of catalytic elements is high and where suitable anticatalysts are not provided, the recommended dose of application of D.D.T. suspension should not be massive. Emulsions should not be employed for such surfaces. But, as far as possile, the aim should be to provide a suitable anticatalyst, either by means of some inert material previously applied to the surface to prevent the D.D.T. deposit from coming into contact with the catalysts and also the sorption of D.D.T. from the surface or by mixing some fixing agents with the preparation of D.D.T. used, thereby reducing the dosage of application. Hadaway and Barlow (1951) are of opinion that sorbed D.D.T. is catalytically decomposed into inocuous ethylene derivative on surfaces having a high iron content. Kruse and Konchady (1950) recommend that a surface coating of starch and cow-dung carbon is a good material to check physical penetration and absorption of the insecticide.

SUMMARY.

Experiments were conducted in the field as well as in the laboratory to study the chemical and photodynamic deterioration of D.D.T. Soil analysis reports show high percentage of catalytic elements in mud.

The D.D.T. when applied on mud surfaces starts deteriorating almost immediately, and deteriorates completely in about four weeks.

The rate of deterioration of D.D.T. is the same in either emulsion or suspension, but the degree of deterioration in the case of suspension varies with the dosage employed. It has been indicated by the laboratory tests that cow-dung, by virtue of its organic elements, is a disintegrator of D.D.T. Ultraviolet rays considerably disintegrate D.D.T.

ACKNOWLEDGEMENT.

The authors express their sincere gratitude to the Public Analyst with the Government of Uttar Pradesh and the Regional Soil Chemist, Rudrapur, for their kindly analysing the soil samples with utmost care.

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FIELD STUDIES ON THE COMPARATIVE EFFECTIVENESS OF D.D.T., B.H.C. AND DIELDRIN RESIDUAL SPRAYS AGAINST ANOPHELINE MOSQUITOES.*

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July 5, 1954.)

INTRODUCTION.

The Malaria Advisory Committee of the Indian Council of Medical Research proposed that the effectiveness of certain new insecticides should be assessed under different conditions in various parts of the country against different vector mosquitoes, and the Director, Malaria Institute of India, be requested to co-ordinate this work in various States. In pursuance of these recommendations, field trials on the comparative effectiveness of D.D.T., B.H.C. and dieldrin residual sprays against anopheline mosquitoes were carried out in collaboration with the State Malaria Organizations in four States in India, namely, Punjab, Uttar Pradesh, Delhi and Bombay.†

*This comparative study was financed by the Indian Council of Medical Research, and the insecticides were supplied, free of cost, by Geigy Insecticides Ltd., Bombay (D.D.T. 50 per cent water dispersible powder and Hexidol 950) ; Imperial Chemical Industries (India) Ltd., Calcutta (Gammexane P. 520 manufactured in India and imported) and Shell Petroleum, London (Dieldrin 50 per cent water dispersible powder). Experiments were started in June 1953 and observations continued till March 31, 1954.

†Results of trials in the State of Bombay are being published separately by Viswanathan *et al.* (1955).

FORMULATION AND DOSAGES OF INSECTICIDES TESTED.

All the three insecticides namely, D.D.T., B.H.C. and dieldrin were tried in the form of water dispersible powders. Those of D.D.T. and B.H.C. formed fairly stable suspensions while that prepared from dieldrin was poor, necessitating the addition of lissapol-NX at the rate of 0·1 per cent (by volume of ready-to-use suspension) to improve its suspensibility to the same degree as that of the other two. The dosages employed were D.D.T. 50 mg., B.H.C. (from three separate samples : Gammexane P. 520-*A* manufactured in India and P. 520-*B* imported and hexidol 950) 10 mg. gamma isomer, D.D.T. and B.H.C. in combination* (25 mg. D.D.T. + 5 mg. gamma isomer) and dieldrin in three dosages 6·25, 12·5 and 25 mg. per sq. ft.

AREAS AND PLAN OF EXPERIMENTS.

In all the States, the areas selected were such in which *A. culicifacies* was known to be the main vector of human malaria. No malaria control measures had been previously carried out in these areas except in Delhi State in which, only the toxicity of dieldrin to spraymen and the inhabitants of the houses treated, was studied.

Although the recommendation of the Insecticide Sub-committee was to select areas with a population of not less than 10,000 for each test yet, in view of the limited supply of insecticides, very much smaller areas could only be taken up. Groups of villages were selected and each village treated with a different insecticide and dosage while some were left untreated for purposes of comparison.

The spraying in the Punjab and Uttar Pradesh was carried out by the trained staff of the Institute and in the Delhi area by the spraying squad of the Delhi Antimalaria Organization. In all cases, the spraying equipment used was double barrel stirrup pump. All structures, whether human dwellings or cattlesheds, were treated. The inner surfaces of the walls, and as much of the low roofs as could be conveniently reached, were sprayed. Majority of the houses in these villages have thatched roofs and the walls are made of sun-dried mud bricks and plastered with a mixture of cow-dung and mud.

Both in the Punjab and Uttar Pradesh only one round of spray with various insecticides was given. In Delhi area, only dieldrin and D.D.T. were used and two rounds of spray were given at six weeks interval in accordance with the routine spraying practice in this State.

DATA COLLECTED.

Density of mosquitoes per week.—In each of the experimental villages, two suitable catching stations were selected one of which was left untreated for purposes of comparison. Total mosquito collections were made weekly from these catching

*D.D.T. and B.H.C. combined spray has been found to be comparatively more effective than either D.D.T. or B.H.C. applied separately in equivalent biological doses (Pal, 1951*a* ; Jaswant Singh *et al.*, 1951 ; Sharma and Pal, 1952). These observations have been confirmed by Dicke and Paul (1951), van Tiel (1952) and Davidson (1953).

stations by atomising a weak solution of pyrethrum in the closed room and collecting the dead mosquitoes off the sheet spread on the floor. These collections were later recorded separately as total anopheline mosquitoes and *A. culicifacies*.

Window trap collection.—Separate catching stations were fitted with window traps of the type described by Jaswant Singh *et al.* (1951) and mosquitoes were collected from them daily. These mosquitoes were kept under observation in clean cages for 24 hours after which the mortality was recorded.* Both density of mosquitoes and window trap collections were also recorded from the comparison village.

Spleen and parasite rates.—Spleen and parasite rates, in children 2-10 years old, were determined before commencing spraying operations and after the transmission season. Infant parasite rate was also recorded but the number of infants of 0-12 months old was very small.

FIELD TRIALS IN KARNAL AREA, PUNJAB.†

Nine villages, scattered over an area of about ten square miles and with a total population of 7159, were selected in a water-logged, canal irrigated area near Karnal (Map 1).

A. culicifacies is the established malaria vector in this area (Hicks and Majid, 1937). Irrigation channels and seepages from them form ideal breeding places for *A. culicifacies* and other mosquitoes. From the extensive data already available for this area, it is known that the density of *A. culicifacies* gradually builds up some time after the onset of the monsoons in mid-July. It soon becomes fairly high and remains so till about the end of October. The survey carried out in May, 1953, revealed that the villages selected were moderately malarious with spleen rates ranging from 20 to 30 per cent. The area is liable to regional epidemics at regular intervals. The last epidemic was recorded about 5-6 years previously. Spraying of these villages was started on July 14, 1953, and twelve pumps took three days to complete the spraying of the whole area comprising about 1,100 houses.

The number of houses and total population, together with the insecticidal treatment given in each village, are shown in Table I. The observations recorded are discussed separately under each of the indices collected.

Mosquito densities.—An analysis of the data presented in Table II shows that in the comparison village the density of mosquitoes began to increase towards the end of July and reached its maximum in September, after which the decline started. In the case of the village treated with dieldrin 6·25 mg./sq. ft., the mosquito density began to increase from second week onwards whereas in the case of those treated at the rate of 12·5 and 25 mg., the density did not increase

*For lack of facilities to control the temperature and humidity conditions in the field, the mortality in mosquitoes even from the untreated villages was very high.

†These experiments were planned and executed in close collaboration with Dr. Dev Raj Mehta, Entomologist-cum-Malariologist, Punjab State, and his staff members Mr. K. G. Samnotra and Major Gobind Ram, without whose help it would not have been possible to conduct these investigations.

MAP 1.

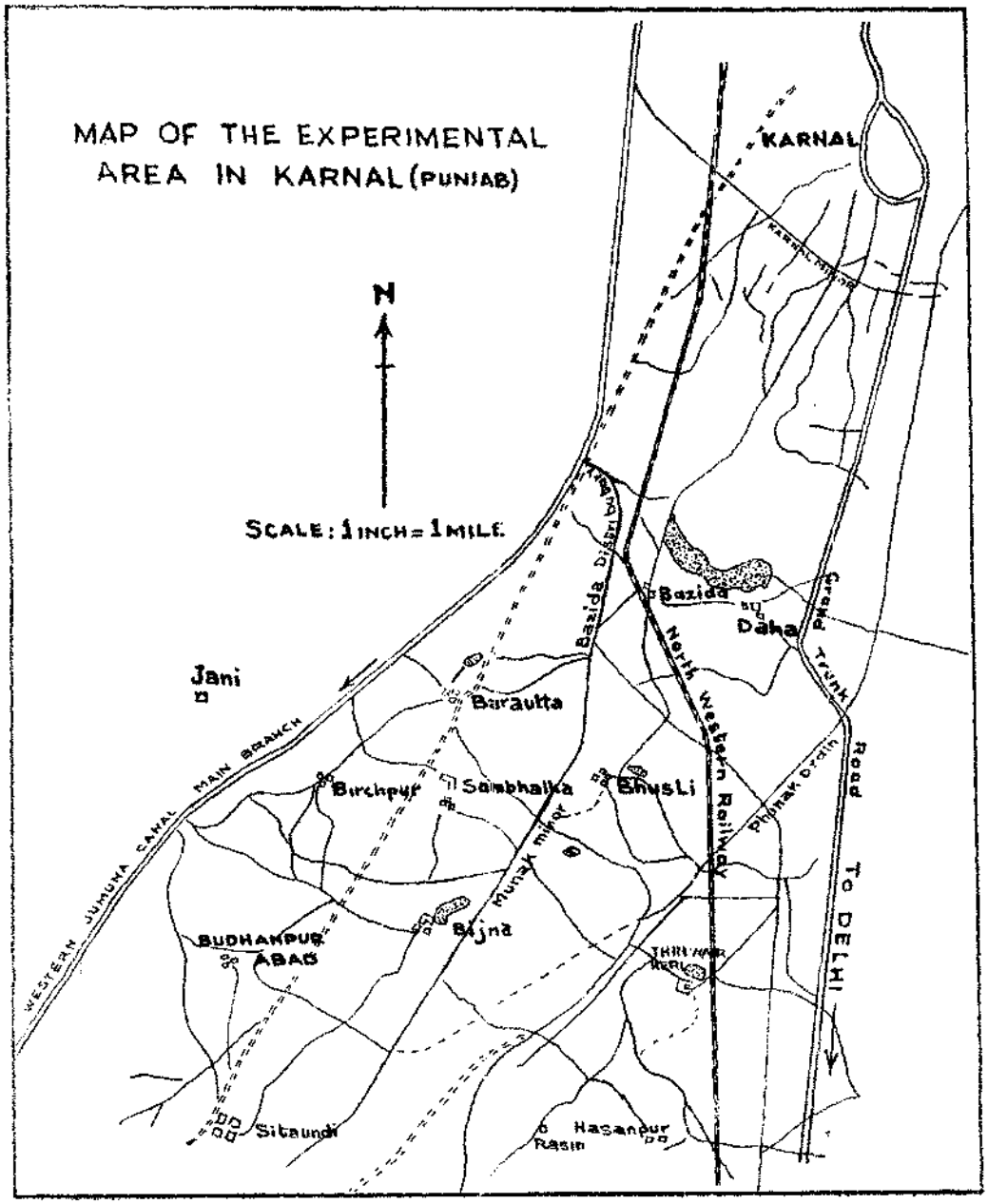
MAP OF THE EXPERIMENTAL AREA IN KARNAL (PUNJAB)
N
SCALE: 1 INCH = 1 MILE
KARNAL
Bazida
Daha
Jani
Barautta
Birchpur
Sambhalka
Bhusli
Bijna
BUDHANPUR ABAD
Sitaundi
Hasanpur Rasin
WESTERN JUMUNA CANAL MAIN BRANCH
Bazida Distributary
North Western Railway
Grand Trunk Road
Munak minor
Phuhak Drain
To DELHI

TABLE I.

Particulars of experimental villages and formulations and dosages of insecticides employed. Karnal area.

Village.	Number of houses.	Population.	Insecticides and formulations.	Dosage mg./sq. ft.	Date of spray.
Bijna ...	230	1196	1·25 per cent dieldrin suspension	25 mg. dieldrin	July 15, 1953
Birchpur ...	120	569	0·625 per cent dieldrin suspension	12·5 mg. dieldrin	,, ,,
Daha ..	100	600	0·3125 per cent dieldrin suspension	6·25 mg. dieldrin	July 17, 1953
Bhusli ...	82	398	2·5 per cent D.D.T. suspension	50 mg. D.D.T.	,, ,,
Samalka ...	88	404	1·25 per cent D.D.T. and 0·25 per cent B.H.C. gamma isomer combined suspension	25 mg. D.D.T. + 5 mg. B.H.C. gamma isomer	July 14, 1953
Budhanpurabad	53	474	0·5 per cent B.H.C. (P. 520-*A*) gamma isomer suspension	10 mg. B.H.C. gamma isomer	July 16, 1953
Baraotta .	224	1129	0·5 per cent B.H.C. (P. 520-*B*) gamma isomer suspension	10 mg. B.H.C. gamma isomer	,, ,,
Bazida Jatan ..	198	726	0·5 per cent B.H.C (Hexidol) gamma isomer suspension	10 mg. B.H.C. gamma isomer	July 18, 1953
Sitamdi ...	231	1673	Control	...	...

till about eight and nine weeks, respectively, after spraying. Where D.D.T. and B.H.C. combined spray had been applied, the density increased after eight weeks, whereas in the case of D.D.T. alone the increase in density was recorded after seven weeks, and in the case of B.H.C. after six weeks in the villages treated with gammexane P. 520-A and hexidol 950, but after seven weeks in the case of gammexane P. 520-*B*. After the effect of the insecticides had apparently worn off, the mosquito densities in the sprayed villages were about the same as in the unsprayed comparison village.

Pal (1951*b*) has already pointed out that if sprayed and unsprayed catching stations have been carefully selected, the ratio of the mosquito population in these two types of stations should give a useful index of the duration of effectiveness of insecticides. Under normal conditions, the ratio of densities, i.e., sprayed/unsprayed, is small and may increase slightly with time, but as soon as the effectiveness of the insecticide becomes lower, the ratio shows an abrupt increase.

There was an abrupt rise in the mosquito density ratio (sprayed/unsprayed) in the fourth week in the village sprayed with dieldrin 6·25 mg/sq. ft. and in the ninth week in the village sprayed with dieldrin 12·5 mg./sq. ft. and gammexane P. 520 -*B*. In the case of D.D.T., gammexane P. 520-*A* and hexidol, an abrupt rise in the mosquito catch ratio was noticed in the eighth week. In the

TABLE II.

Total number of anopheline mosquitoes caught, and ratio of catches (S./U.S.) from sprayed and unsprayed catching stations.

Karnal Area.

Weeks.	25 mg. dieldrin/sq. ft.			12·5 mg. dieldrin /sq. ft.			6·25 mg. dieldrin/sq. ft.			50 mg. D.D.T./sq. ft.			25 mg. D.D.T. +5 mg. B.H.C. gamma isomer/sq. ft.			10 mg. B.H.C. (P. 520—*A*) gamma isomer/sq. ft.			10 mg. B.H.C. (P.520—*B*) gamma isomer/sq. ft.			10 m.g. B.H.C. (Hexidol) gamma isomer/sq. ft.			Control
	S.	U.S.	$\frac{S.}{U.S.}$	S.	U.S.	$\frac{S.}{U.S.}$	S.	U.S.	$\frac{S.}{U.S.}$	S.	U.S.	$\frac{S.}{U.S.}$	S.	U.S.	$\frac{S.}{U.S.}$	S.	U.S.	$\frac{S.}{U.S.}$	S.	U.S.	$\frac{S.}{U.S.}$	S.	U.S.	$\frac{S.}{U.S.}$	
0	406	427	0·95	287	371	0·77	822	35	1·63	1224	806	1·5	3208	1060	3·03	569	629	0·9	268	276	0·97	548	421	1·3	315
1	0	120	0·00	0	141	0·0	0	41	0·0	0	52	0·0	0	81	0·0	0	106	0·0	0	105	0·0	0	63	0·0	300
2	3	9	0·33	0	31	0·00	46	163	0·28	9	167	0·05	3	95	0·03	0	0	0·00	07	7	0·00	26	191	0·18	350
3	34	78	0·43	2	31	0·07	73	181	0·4	12	58	0·21	0	48	0·00	0	36	0·00	0	1	0·00	5	77	0·07	971
4	14	24	0·58	17	292	0·06	123	156	0·79	28	219	0·13	15	146	0·10	14	253	0·06	9	122	0·07	33	272	0·12	1035
5	12	60	0·2	8	233	0·03	122	144	0·85	24	178	0·14	11	186	0·06	24	246	0·1	13	121	0·11	74	210	0·34	850
6	19	53	0·37	12	259	0·05	79	78	1·01	17	162	0·11	7	143	0·05	9	164	0·06	10	87	0·12	50	186	0·27	493
7	22	28	0·79	14	164	0·09	56	73	0·77	44	174	0·25	9	117	0·08	21	59	0·36	10	87	0·12	66	299	0·22	943
8	40	63	0·64	35	209	0·17	107	105	1·02	238	299	0·79	17	136	0·13	213	233	0·91	25	131	0·2	324	428	0·75	1243
9	11	116	0·68	295	449	0·66	263	324	0·81	238	285	0·84	280	604	0·46	306	486	0·63	181	302	0·6	261	676	0·39	789
10	282	294	0·96	357	174	2·05	196	179	1·1	288	309	0·93	343	484	0·71	660	486	1·36	208	340	0·61	427	413	1·03	806
11	295	269	1·1	304	312	0·81	182	240	0·74	278	389	0·82	326	422	0·77	588	738	0·8	191	230	0·83	293	462	0·63	629
12	360	511	0·7	315	355	0·89	217	271	0·8	303	364	0·83	246	457	0·54	454	548	0·83	129	138	0·93	377	498	0·78	485

S=sprayed. U.S.=unsprayed.

village sprayed with dieldrin 25 mg./sq. ft., the abrupt rise in the mosquito catch ratio was noticed in the seventh week. It may, however, be pointed out that in this village mosquito densities, both in the sprayed and unsprayed catching stations, remained low during seventh and eighth week after which a sudden rise in mosquito population in both sprayed and unsprayed catching stations was recorded. In the village sprayed with D.D.T. and B.H.C. combination, abrupt rise was recorded in the tenth week. It would appear that dieldrin 12·5 mg. and 25·0 mg./sq. ft., Gammexane P. 520-*B*, and D.D.T. and B.H.C. combined sprays, were effective on the whole for about eight to nine weeks; D.D.T., Gammexane 520-*A* and hexidol for about seven weeks; and dieldrin 6·25 mg./sq. ft. was ineffective as a residual spray. The results have been briefly summarised below :—

Treatment.	Density commencing to build up after weeks.	Sprayed/unsprayed ratio-an abrupt rise in weeks.
Dieldrin 6·25 mg/sq. ft. ...	2	4
Dieldrin 12·5 mg/sq. ft. ...	8	9
Dieldrin 25·0 mg./sq. ft. ...	9	7
D.D.T. 50 mg./sq. ft. ...	7	8
B.H.C. Gammexane P. 520-*B* 10 mg. gamma isomer/sq. ft. ...	8	9
B.H.C. Gammexane P. 520-*A* 10 mg. gamma isomer/sq. ft. ...	7	8
B.H.C. Hexidol 950 10 mg. gamma isomer/sq. ft. ...	7	8
D D.T. and B.H.C. combination (25 mg. D.D.T. plus 5 mg. B.H.C. gamma isomer/sq. ft.)	8	9

Window trap collections.—The results of window trap collections are interesting (Table III). In the villages sprayed with dieldrin 12·5 mg. and 25 mg/sq. ft., window trap collections are practically nil, whereas in the village sprayed with 6·25 mg./sq. ft., a few mosquitoes were collected from the window trap even during the first week after spray, and the number steadily increased in subsequent weeks. There was little difference in the mosquito catch from the room or from the window trap; in fact collections from window traps were more. In the absence of survival rate, the only interpretation which may be made of these results is that absence of mosquitoes in the window trap in the villages treated with dieldrin 12·5 mg. and 25 mg./sq. ft. may possibly be explained due to any of the following factors :—

(*i*) That the mosquitoes frequenting these catching stations were very few, (*ii*) they were not being repelled, and (*iii*) they did not survive through the night to escape to the window traps. In the case of other treatments, the distribution of mosquitoes in the room and window traps appears to be at random. In the comparison village, considerable number of mosqutioes were collected from the window traps and throughout the investigation the window trap collections were less than the room catch.

TABLE III.

Catches from window traps and rooms (Average for the week). Sprayed catching stations Karnal Area.

Weeks	25 mg. dieldrin/ sq. ft.		12·5 mg. dieldrin/ sq. ft.		6·25 mg. dieldrin/ sq. ft.		50 mg. D.D.T./ sq. ft.		25 mg. D.D.T. plus 5 mg. B.H.C. gamma isomer/ sq. ft.		10 mg. B.H.C. (P. 320—4) gamma isomer/sq. ft.		10 mg. B.H.C. (P. 520—B) gamma isomer/ sq. ft.		10 mg. B.H.C. (Hexidol) gamma isomer/sq. ft.		Control.	
	W.T.	R.C.	W.T.	R.C.	W.T.	R.C.	W.T.	R.C.	W.T.	R.C.	W.T.	R.C.	W.T.	R.C.	W.T.	R.C.	W.T.	R.C.
0	8	0	0	0	16	6	10	69	0	22	0	44	12	32	40	61	4	8
1	0	0	1	1	1	1	3	6	2	1	1	0	3	2	3	1	3	7
2	0	0	1	2	1	2	6	2	23	3	1	2	7	2	1	1	2	26
3	0	1	1	2	10	4	5	9	19	6	1	0	5	2	1	1	2	87
4	0	2	0	8	8	3	2	12	9	8	1	3	2	5	3	0	41	87
5	0	3	0	1	10	6	1	7	2	7	0	2	2	4	0	43		43
6	0	1	1	6	9	0	2	3	5	6	1	3	12	4	2	1	9	36
7	0	3	1	9	3	5	3	9	7	12	0	4	5	4	7	1	5	55
8	0	5	0	1	10	6	6	6	7	25	1	12	4	7	4	9	10	56
9	0	4	1	27	11	7	4	3	3	9	1	18	4	3	6	1	15	39
10	1	6	0	12	13	13	7	6	5	10	2	9	4	5	5	0	3	44
11	0	8	0	22	38	16	12	10	17	10	3	11	19	14	10	0	34	52
12	0	3	0	14	55	15	8	9	12	8	5	17	10	14	5	0	16	39

W.T. =window traps. R.C. =room catches.

The number of *Anopheles culicifacies* collected was very small. This species was absent for periods varying from six to seven weeks, except in the villages treated with dieldrin at the rate of 6·25 mg. and 12·5 mg./sq. ft. and D.D.T. 50 mg./sq. ft. where the species was encountered in the second, third and second week, respectively (Table IV).

Spleen and parasite rates.—Spleen and parasite rates taken in May and November are given in Table V.

Spleen rates showed a slight drop in almost all the treated villages while in the comparison village there was a slight rise. Dieldrin 12·5 mg./sq. ft. and 25 mg./sq. ft. show the largest drop in spleen rate followed closely by

TABLE IV.

Number of Anopheles culicifacies *caught from sprayed catching stations. Karnal Area.*

Weeks.	25 mg. dieldrin/sq. ft.	12·5 mg. dieldrin/sq. ft.	6·25 mg. dieldrin/sq. ft.	50 mg. D.D.T./sq. ft.	25 mg. D.D.T. plus 5 mg. B.H.C. gamma isomer/sq. ft.	10 mg. B.H.C. (P. 520—*A*) gamma isomer/sq. ft.	10 mg. B.H.C. (P. 520—*B*) gamma isomer/sq. ft.	10 mg. B.H.C. (Hexidol) gamma isomer/sq. ft.	Control.
0	20	11	21	20	13	12	5	21	12
1	...	...	...	...	...	...	...	...	9
2	...	...	2	1	...	...	...	...	24
3	...	2	3	1	...	...	...	...	49
4	...	1	7	3	...	...	...	...	46
5	...	2	2	1	1	...	...	1	25
6	1	1	1	...	...	...	...	2	12
7	1	...	5	1	...	1	...	2	25
8	...	2	4	3	...	2	1	4	40
9	1	8	2	3	8	6	4	3	15
10	6	9	13	5	9	15	6	5	19
11	7	9	8	11	4	13	5	7	3
12	8	1	4	3	2	3	3	9	5

TABLE V.

Spleen and parasite rates of children recorded in May and November, 1953. *Karnal Area.*

Village.	Insecticide used.	Dosage mg./sq. ft.	Spleen rate.		Parasite rate.	
			May.	Nov.	May.	Nov.
Bijna ...	1·25 per cent dieldrin suspension	25·0	20·0	8·5	1·7	0·0
Birchpur ...	0·625 per cent dieldrin suspension	12·5	23·2	6·8	4·7	0·0
Daha ...	0·3125 per cent dieldrin suspension	6·25	23·4	16·3	2·1	0·0
Bhusli ...	2·5 per cent D.D.T. suspension	50·0	23·8	13·3	4·8	8·9
Samalka ...	1·25 per cent D.D.T. and 0·25 per cent B.H.C. gammaisomer combined suspension	25·0+5·0	29·7	17·7	8·1	4·4
Budhanpurabad	0·5 per cent B.H.C. (P. 520-*A*) gamma isomer suspension	10·0	25·0	19·4	5·0	8·1
Barautta ...	0·5 per cent B.H.C. (P. 520–*B*) gamma isomer suspension	10·0	20·3	9·1	4·7	1·5
Bazida Jatan	0·5 per cent B.H.C. (Hexidol) gamma isomer suspension	10·0	25·3	11·6	4·8	8·3
Sitaundi ...	Control	...	23·3	27·7	3·1	6·7

Gammexane P. 520 imported from abroad. The parasite rates showed a drop in all cases except D.D.T., Gammexane P. 520-*A* and Hexidol 950.*

There is no dispensary attached to these villages ; hence morbidity data is not available.

FIELD TRIALS IN GANGA KHADAR AREA (UTTAR PRADESH).†

The Ganga Khadar tract lies in the north-east corner of Meerut District in Tehsil Mowana and in the adjacent area of Muzaffarnagar District, Uttar Pradesh. It is a lowlying land of Ganga Valley with marked characteristics of its own. The entire tract, from east to west, is divided in two distinct zones, namely, Khadar on the east and Bangar on the west separated by a well-marked raised sandy cliff, locally known as '*Kholas*'. In Khadar area, before extensive ploughing operations were undertaken, there were patches of cultivation interspersed with tall grasses, thorny bushes and date-palm trees. There are large number of ponds, lakes and nullahs covered with grasses and weeds, and water collections in lowlying areas after the recession of flood forming ideal breeding places for mosquitoes. The Bangar area lies west of '*Kholas*' and is cultivable. The whole of the tract is subject to annual inundation from the River Ganga which forms the eastern limit of the tract. The sub-soil water is fairly high. *A. culicifacies* is the vector in this area (Graham 1910 : 1911 ; Srivastava *et al.*, 1953). This area used to be highly malarious but following the activities of the antimalaria unit operating in Ganga Khadar tract, there has been a general decline in malaria incidence. For field trials, nine villages were selected in an uncultivated part of both Khadar and Bangar areas of Ganga Khadar tract (Map 2) within a radius of about ten miles. The number of houses and total population, together with the treatment given in each village, are shown in Table VI. Spraying of these villages was started on June 22, 1954, and eight pumps took three days to complete the spraying of 719 houses. Observations recorded are discussed below under each of the various indices separately.

Mosquito densities.—Mosquitoes collected from various catching stations are summarised in Table VII. An analysis of the data presented showed that, in the comparison village the density during the week prior to spraying operations was very high and further increase was noticed in the second week. There was a sudden drop, however, during the third week, and for the following six weeks the density remained low after which another slight rise was registered. The heavy rains and inundations from the river were responsible for this decline in mosquito prevalence in this area. In the village sprayed with dieldrin 6·25 mg./sq. ft., mosquito density dropped to low levels one week after spraying but thereafter

*The statistical analysis has shown that the drop in spleen and parasite rates is not significant. Apparently one round of spray in June of any insecticide was not enough to intercept the transmission of malaria during August-September.

†The authors sincerely thank Dr. R. S. Srivastava, the then Assistant Director of Public Health, Malariology, Uttar Pradesh, and the Medical Officer of Health, Muzaffarnagar, for providing all the facilities for these trials and for their active co-operation.

MAP 2.

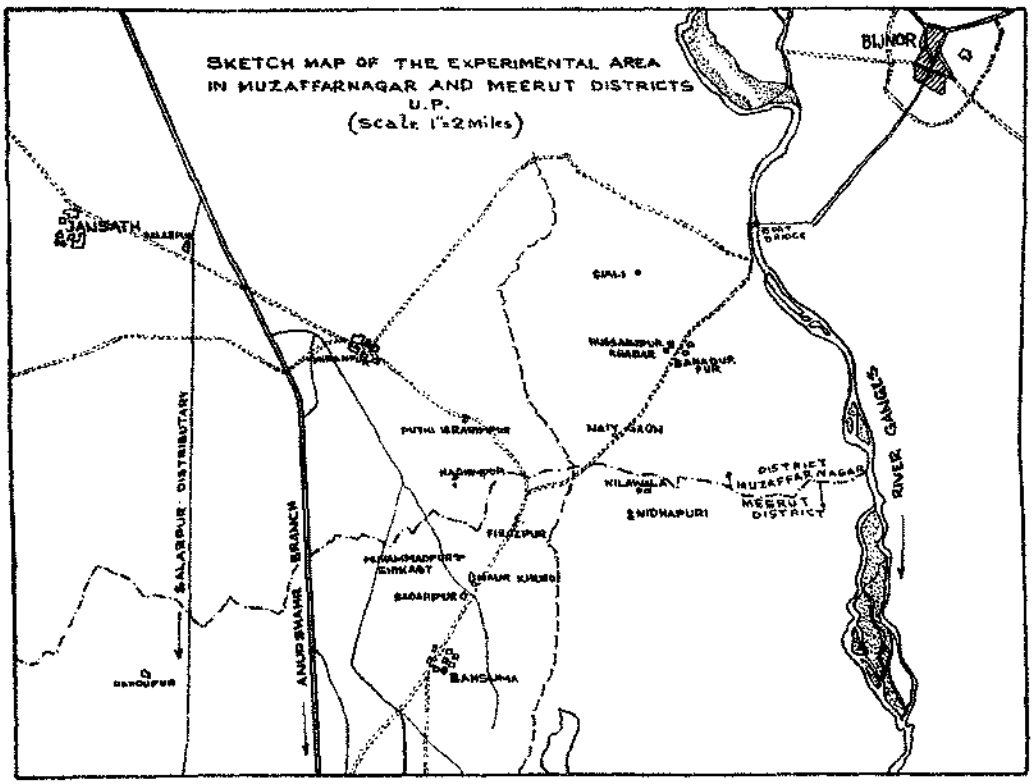

TABLE VI.

Particulars of experimental villages and of formulations and dosages of insecticides employed. Ganga Khadar Area.

Name of village.	Number of houses.	Population.	Insecticides and formulations used.	Dosage : mg./sq. ft.	Date of spray.
Nayagaon ...	36	130	1·25 per cent dieldrin suspension	25 mg. dieldrin	July 23, 1953
Samashpur	107	494	0·625 per cent dieldrin suspension	12·5 mg. dieldrin	July 25, 1953
Jamalpur	32	169	0·3125 per cent dieldrin suspension	6·25 mg. dieldrin	July 24, 1953
Bangar Puthi Ibrahimpur	170	1147	2·5 per cent D.D.T. suspension	50 mg. D.D.T.	July 26, 1953
Firozpur ...	52	227	1·25 per cent D.D.T. + 0·25 per cent B H.C. gamma isomer combined suspension	25 mg. D.D.T. + 5 mg. B.H.C. gamma isomer	July 22, 1953
Nilawala ...	62	233	0·5 per cent B. H. C. (P. 520–A) gamma isomer suspension	10 mg. B.H.C. gamma isomer	July 23, 1953
Sekrera ...	99	526	0·5 per cent B.H.C. (P. 520–B) gamma isomer suspension	10 mg. B.H.C. gamma isomer	July 24, 1953
Tajpura ...	121	506	0·5 per cent B.H.C. (Hexidol) gamma isomer suspension	10 mg. B.H.C. gamma isomer	July 25, 1953
Nidhapur ...	42	181	Control		

TABLE VII.

Total number of anopheline mosquitoes caught and ratio of catches ($\frac{S}{U.S.}$) from sprayed and unsprayed catching stations.

Ganga Khadar Area.

Weeks.	25 mg. dieldrin/sq.			12·5 mg. dieldrin/sq. ft.			6·25 mg. dieldrin/sq. ft.			50 mg. D.D.T./ sq. ft.			25 mg. D.D.T. +5 mg. B.H.S. gamma isomer/ sq. ft.			10 mg. B.H.C. (P. 520–A) gamma isomer/sq. ft.			10 mg. B.H.C. (P. 520–B) gamma isomer/sq. ft.			10 mg. B.H.C. (Hexidol) gamma isomer/ sq. ft.			Control.
	S.	U.S.	S./U.S.	S.	U.S.	S./U.S.	S.	U.S.	S./U.S.	S.	U.S.	S./U.S.	S.	U.S.	S./U.S.	S.	U.S.	S./U.S.	S.	U.S.	S./U.S.	S.	U.S.	S./U.S.	
0	595	702	0·85	440	532	0·86	925	1430	0·65	203	128	1·59	480	340	1·45	620	531	1·17	855	1067	0·8	665	950	0·7	2601
1	1	64	0·02	0	6	0·00	37	281	0·13	26	136	0·19	0	13	0·00	0	10	0·00	7	145	0·05	1	13	0·08	3697
2	5	26	0·19	3	14	0·21	56	175	0·32	6	44	0·14	2	238	0·01	8	33	0·24	9	64	0·14	2	44	0·05	104
3	2	31	0·07	3	29	0·1	31	202	0·16	22	127	0·17	8	39	0·21	19	28	0·68	35	108	0·32	3	37	0·08	283
4	12	23	0·52	5	13	0·38	63	84	0·75	47	50	0·94	12	165	0·07	5	10	0·05	13	75	0·17	2	16	0·13	120
5	7	15	0·47	5	22	0·23	68	133	0·51	23	38	0·61	14	102	0·14	7	13	0·54	11	20	0·55	6	13	0·46	78
6	5	20	0·25	4	16	0·25	42	3	14·0	8	15	0·54	40	100	0·4	13	17	0·76	10	29	0·35	6	15	0·4	87
7	18	34	0·53	5	5	1·0	102	140	0·73	30	50	0·6	4	20	0·2	19	30	0·63	11	92	0·12	9	16	0·56	58
8	16	24	0·66	6	6	1·0	16	37	0·43	26	28	0·75	31	75	0·41	8	15	0·54	4	9	0·44	6	4	1·5	27
9	20	26	0·77	5	14	0·36	48	69	0·69	21	44	0·48	3	24	0·13	10	30	0·33	50	76	0·72	41	38	1·68	149
10	39	117	0·34	31	13	2·38	97	132	0·74	63	94	0·67	9	19	0·47	29	44	0·66	58	81	0·78	52	37	1·41	98
11	132	189	0·69	26	28	0·86	79	112	0·71	60	63	0·95	23	28	0·82	33	42	0·78	69	114	0·61	71	48	1·48	93
12	90	75	0·53	37	23	1·61	262	205	1·28	49	32	1·5	101	69	1·58	10	43	0·23	127	132	0·96	4	14	0·29	102

S=sprayed. U.S.=unsprayed. S./U.S.=ratio of catches from sprayed and unsprayed catching stations.

steadily increased and was about the same as in the comparison village. In the case of dieldrin 12·5 and 25 mg./sq. ft., consistent rise in the density was noticed from eleventh week onwards. In the villages treated with D.D.T. and all the three formulations of B.H.C., the density steadily increased after 9-10 weeks and in the case of D.D.T. and B.H.C. combination, an increase in density of mosquitoes was noticed from eleventh week onwards.

Abrupt rise in the ratio of mosquitoes caught from sprayed and unsprayed catching stations (s./us.) was observed in the third and fourth weeks in the case of villages treated with dieldrin 6·25 mg./sq. ft., D.D.T., Gammexane P. 520–*A*. In the case of villages treated with dieldrin 12·5 mg. and 25 mg./sq. ft. and Hexidol 950, the abrupt rise was noticed in the seventh week. In the case of village treated with Gammexane P. 520–*B*, the abrupt rise was noticed in the ninth week, and in the case of village treated with D.D.T. and B.H.C. combination in the tenth week. These results may be briefly summarised as follows :—

Treatment.	Density commencing to build up after weeks.	Sprayed/unsprayed ratio—an abrupt rise in weeks.
Dieldrin 6·25 mg./sq. ft.	2	4
Dieldfin 12·5 mg./sq. ft.	11	7
Dieldrin 25·0 mg./sq. ft.	11	7
D.D.T. 50·0 mg./sq. ft.	9	4
B.H.C. Gammexane P. 520-*A* 10 mg. gamma isomer/sq. ft.	9	3
B.H.C. Gammexane P. 520-*B* 10 mg. gamma isomer/sq. ft.	10	9
B.H.C. Hexidol 950 10 mg. gamma isomer/sq. ft.	9	7
B.H.C. +D.D.T. (25 mg. D.D.T. +5 mg. B.H.C. gamma isomer/sq. ft.)	11	10

Window trap collections.—As in the Karnal area, practically no mosquitoes were captured from window traps in the villages treated with dieldrin 12·5 and 25 mg./sq. ft., whereas in the case of the village treated with 6·25 mg. dieldrin/sq. ft. mosquitoes were obtained from the window trap every week although collections from the room were few (Table VIII). In the case of D.D.T., and all other treatments, the number of mosquitoes collected from the window traps were comparatively low. The results are more or less identical to those obtained in the Karnal area.

Anopheles culicifacies.—There was more or less complete disappearance of *Anopheles culicifacies* for periods varying from two to four weeks with different insecticidal treatments, except in the case of dieldrin 6·25 mg./sq. ft. in which case this species was caught even in the first week after spray (Table IX).

Spleen and parasite rates.—An analysis of the spleen and parasite rates taken in May and December, 1953, showed that both these rates registered an increase

TABLE VIII.

Catches from window traps and rooms (Average for the week)—Sprayed Catching Stations. Ganga Khadar Area.

Weeks	25 mg. dieldrin/sq. ft.		12·5 mg. dieldrin/sq. ft.		6·25 mg. dieldrin/sq. ft.		50 mg. D.D.T. sq. ft.		25 mg. D.D.T. +5 mg. B.H.C. gamma isomer/ sq. ft.		10 mg. B.H.C. (P. 520-*A*) gamma isomer/sq. ft.		10 mg. B.H.C. (P. 520-*B*) gamma isomer/sq. ft.		10 mg. B.H.C. (Hexidol) gamma isomer/sq. ft.		Control	
	W.T.	R.C.	W.T.	R.C.	W.T.	R.C.	W.T.	R.C.	W.T.	R.C.	W.T.	R.C.	W.T.	R.C.	W.T.	R.C.	W.T.	R.C.
0	Not	done	Not	done	160	80	Not	done	10	1	88	170	Not	done	Not	done	127	60
1	2	...	...	...	65	...	5	4	...	7	...	79	...	...	...	1	45	104
2	...	...	...	...	52	...	6	3	2	20	4	112	...	...	5	3	18	114
3	...	...	...	...	14	...	4	3	...	21	1	69	...	...	7	3	9	91
4	...	4	...	...	16	...	3	6	...	4	4	34	...	3	1	8	3	49
5	...	3	...	1	14	...	1	2	...	14	3	25	1	3	1	2	9	38
6	1	4	...	...	12	2	1	7	1	20	8	65	...	2	...	7	2	36
7	1	2	...	1	8	2	1	3	1	2	15	69	...	...	...	10	...	28
8	1	2	5	3	1	6	...	2	13	1	26	8	...	1	...	18	8	34
9	...	3	...	2	1	2	...	3	1	1	18	39	1	2	...	15	...	17
10	1	8	...	1	8	3	...	7	6	3	15	37	1	6	...	21	2	23
11	...	12	1	6	3	5	...	9	3	1	19	18	1	9	...	25	2	27
12	...	7	...	3	2	5	1	13	2	2	30	19	1	10	...	21	2	17

W.T.=window traps. R.C.=room catches.

TABLE IX.

Number of Anopheles culicifacies *caught from sprayed catching stations. Ganga Khadar Area.*

Weeks.	25 mg. dieldrin/ sq. ft.	12·5 mg. dieldrin/sq. ft.	6·25 mg. dieldrin/sq. ft.	50 mg. D.D.T./ sq. ft.	25 mg. D.D.T. +5 mg. B.H.C. gamma isomer/ sq. ft.	10 mg. B.H.C. (P. 520–*A*) gamma isomer/sq. ft.	10 mg. B.H.C. (P 520–*B*) gamma isomer/sq. ft.	10 mg. B.H.C. (Hexidol) gamma isomer/sq. ft.	Control.
0	20	4	33	13	12	10	26	8	33
1	...	...	9	...	...	...	...	...	29
2	...	...	12	...	1	...	...	...	...
3	...	...	12	...	...	1	1	...	2
4	...	...	18	2	...	...	5	1	5
5	1	1	22	2	3	1	...	1	6
6	...	...	8	1	2	4	...	...	5
7	1	1	15	3	...	...	4	...	...
8	...	...	1	...	4	...	1	...	...
9	...	...	...	...	1	1	2	...	...
10	3	...	2	...	...	3	2	2	2
11	12	...	4	...	...	2	2	3	2
12	...	...	...	...	2	...	2	...	4

in the comparison village as well as in all the sprayed and unsprayed villages (Table X). This clearly shows that one round of spray with any of the three insecticides, namely, D.D.T., B.H.C. and dieldrin, given towards the third week of June, at the dosage levels used in the above experiment, was not enough to stop transmission of malaria which in this area commences towards the end of July or beginning of August and reaches its peak sometime in September or October.

There is no dispensary attached to these villages; hence morbidity data was not available.

TABLE X.

Spleen and parasite rates of children recorded in May and December, 1953.

Ganga Khadar Area.

Village.	Insecticide used.	Dosage mg./sq. ft.	Spleen rate.		Parasite rate.	
			May.	Dec.	May.	Dec.
Nayagaon	1·25 per cent dieldrin suspension	25·0	30·4	31·8	5·0	9·1
Samashpur	0·625 per cent dieldrin suspension	12·5	16·7	25·7	5·5	11·4
Jamalpur Bangar	0·3125 per cent dieldrin suspension	6·25	22·7	26·9	5·5	11·5
Puthi Ibrahimpur	2·5 per cent D.D.T. suspension	50·0	19·2	21·8	5·8	7·2
Firozpur	1·25 per cent D.D.T. 0·25 per cent B.H.C. gamma isomer combined suspension	25+5	23·3	35·3	6·7	11·7
Nilawala	0·5 per cent B.H.C. (P. 520-*A*) gamma isomer suspension	10·0	26·6	36·0	5·5	12·0
Sekrera	0·5 per cent B.H.C. (P. 520-*B*) gamma isomer suspension	10·0	14·0	21·9	2·4	19·5
Tajpura	0·5 per cent B.H.C. (Hexidol) gamma isomer suspension	10·0	17·2	24·2	3·4	6·1
Nidhapur	Control	...	34·6	53·6	7·7	14·3

RESULTS OF TOXICITY TESTS OF DIELDRIN IN SHAHDRA AREA, DELHI.*

Toxicity of D.D.T., B.H.C. and dieldrin to several species of laboratory animals like rabbit, rat, mouse, dog, guinea pig, when administered orally, has been worked out by various workers (Lehman, 1951). The experiments have

*The authors' sincere thanks are due to Capt. Dalip Singh, Asstt. Officer Incharge, Antimalaria Operations, Delhi, for his kind help and collaboration in this work.

shown that dieldrin is comparatively more toxic than D.D.T. and B.H.C. to warm-blooded animals. Mean lethal dose in mg/kg. for the three insecticides worked out to D.D.T. 250, B.H.C. 150 gamma isomer, and dieldrin 50-55.

Observations on toxicity of dieldrin to spraymen and inhabitants living in the sprayed houses, were carried out in a group of villages in the Shahdara area (Delhi State) with a total population of about 10,000. Dieldrin was sprayed twice at the rate of 25 mg./sq. ft. (a biological equivalent dose of D.D.T. 100 mg./sq. ft.) instead of the two rounds of D.D.T. applied at the rate of 100 mg./sq. ft., one in August and another in September.

Spraying was carried out by 18 persons of the spraying squads of Delhi State. They handled 380 lbs. and 290 lbs. of 50 per cent dieldrin water dispersible powder during the first and second rounds of spraying, respectively. Dieldrin 50 per cent water dispersible powder was diluted with water in the ratio of 1 to 19 parts of water, giving a concentration of 1·25 per cent.

The medical examination of each individual consisted of recording the weight, physical check-up which included besides routine examination of the circulatory and respiratory systems, examination of the nervous system for possible signs of peripheral neuritis and other nervous lesions.

No apparent abnormality, attributable to toxicity due to handling of insecticide, was detected. There was no significant loss of weight in any of the person and none of them complained of any symptoms. The persons engaged in spraying observed the usual precautions of washing the contaminated parts of the body after finishing the work of spraying at the end of each day.

In addition, two groups of about 100 children from each of the two areas treated with dieldrin and D.D.T. were also medically examined every six weeks. None of the children from both dieldrin and D.D.T. treated villages showed any apparent signs of toxicity and none of them complained of any symptom. Almost all the children, as would be expected in growing population, showed increase in weight.

These preliminary studies over a limited period of time, did not show apparent toxic manifestations amongst those actually handling the insecticide and amongst school children living in treated houses, when dieldrin was sprayed with the usual precautions observed in spraying of other insecticides like D.D.T. and B.H.C.

SUMMARY.

1. Field trials on the comparative effectiveness of D.D.T. and B.H.C. when applied separately and in combination, and of dieldrin, against anopheline mosquitoes, are described. Studies were conducted in the villages of Karnal District (Punjab) and Ganga Khadar area, Meerut and Muzaffarnagar districts (Uttar Pradesh). Toxicity tests were carried out in the villages of Delhi State.

2. The dosages employed were 50 mg. D.D.T./sq. ft., B.H.C. gamma isomer 10 mg./sq. ft. (three formulations, namely, hexidol of Geigy Insecticides Ltd., P. 520 of Imperial Chemical Industries—one manufactured in India and another imported), 25 mg. D.D.T. and 5 mg. B.H.C. gamma isomer/sq. ft. in a combined spray, and dieldrin (Shell Petroleum) at 6·25 mg., 12·5 mg. and

25 mg./sq. ft. All the insecticides were used in the form of suspension prepared from water dispersible powders.

3. Dieldrin 12.5 mg., D.D.T. 50 mg., and B.H.C. 10 mg. gamma isomer/sq. ft. have been found to be equally effective. The duration of residual effectiveness was observed to last for about seven to eight weeks.

4. Dieldrin applied at the rate of 25 mg./sq. ft. did not show an increase in the duration of residual effectiveness proportionately. Dieldrin 6·25 mg./sq. ft. was ineffective as a residual spray. Previous trials with D.D.T. have also shown that massive dosages of insecticides, when applied on mud plastered walls, do not seem to show proportionate increase in the duration of residual effectiveness.

5. D.D.T. and B.H.C. combined spray was comparatively more effective under some conditions than either of them applied separately.

6. Toxic hazards of dieldrin to spraymen or to the children living in sprayed houses, were studied in Shahdara near Delhi. No apparent toxic manifestations were encountered.

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FIELD TRIALS ON THE RELATIVE EFFICACY OF DIFFERENT DOSAGES AND FORMULATIONS OF D.D.T., B.H.C., COMBINATION OF D.D.T. AND B.H.C. AND DIELDRIN IN MALARIA CONTROL IN CERTAIN RURAL AREAS IN BOMBAY STATE.*

BY

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I. INTRODUCTION.

THERE are numerous references in literature to the successful establishment of malaria control in several countries of the world with the use of residual insecticides, principally D.D.T. The dosage of D.D.T. employed by different workers has varied within very wide limits from as low a figure as 4·6 mg./sq. ft. (Mandekos, 1945) to the common practice, particulaly in the Americas, of employing as high a dose as 200 mg./sq. ft. The number of applications of D.D.T. naturally varies with the dosage and the duration of transmission. Massive doses are applied only once during the malaria season in several countries, but in a few of them where the malaria transmission is prolonged and where the collateral benefits of D.D.T. application are sought for, three or four rounds of application of 200 mg./sq. ft. are also resorted to. By and large, in India, commencing with the first large-scale use of D.D.T. in Kanara and Dharwar Districts in Bombay State, the common practice is to apply two or three rounds of application of D.D.T. at

*The work, here reported, was carried out by the Bombay State Malaria Organisation with financial assistance from the Indian Council of Medical Research.

56 mg./sq. ft. It may be stated here that this dosage is arrived at from the more practical basis of spraying about a gallon of a fluid containing five per cent D.D.T. (Technical) over 4,000 square feet, (Viswanathan and Rao, 1947), and from an earlier pilot experiment by Viswanathan and Parikh (1946).

As regards formulations, wettable powders have, as a rule, been preferred by many workers to emulsions which had earlier completely supplanted solutions, the first formulation used in malaria control. In the past experience of the Malaria Organisation of Bombay State, no locally available preparations of wettable powders containing 50 per cent D.D.T. (Technical) were found to give better results than D.D.T. emulsions. Recent supplies imported from abroad of D.D.T. 75 per cent wettable powder, which have been in use since June 1953, have however been found to give as constant and as uniform results as emulsions, and they are obviously much more handy to use.

One of the earliest workers to sound a discordant note about the futility of D.D.T. was Muirhead Thomson (1947) who reported that *A. gambiæ* got irritated by exposure to sublethal doses of D.D.T. and left houses in sufficient numbers and lived long enough to reach the stage of infectivity. He (1949) and a few other workers have since reported that such a 'repellent' tendency was not so apparent with benzene hexachloride. Viswanathan *et al.* (1950) have shown that while B.H.C. may be used successfully in malaria control, its residual efficacy is of much shorter duration than D.D.T.

Pal (1951) has reported the synergistic effect in a mixture of D.D.T. and B.H.C. Cutkomp (1947), however, did not find any evidence of such synergistic effect, except for an increase in the speed of toxic action in the earlier period after application. One of the authors of the present paper (D.K.V.), likewise did not find any evidence of any increase in toxic or residual effect by the use of a combination of D.D.T. and B.H.C. against mosquitoes, nor even against flies.

More recently, dieldrin has been reported to have residual insecticidal efficacy to a greater extent than even D.D.T.

At the annual meeting of the Indian Council of Medical Research in 1952 it was decided that field trials on the comparative effectiveness of different insecticides in different dosages and formulations be carried out in different parts of the country against different known vector species in India in collaboration with the various State Governments. One set of such experiments was carried out by the Malaria Organisation of the Government of Bombay in which the effectiveness of different dosages and formulations of D.D.T., B.H.C., and a combination of these two insecticides and of dieldrin were tried in certain rural areas in Bombay State. This paper records the results of these trials which were carried out between May, 1953 and February, 1954.

II. AREA OF EXPERIMENT.

Songadh and Vyara talukas of Surat District, Bombay State, were selected for the experiment. They lie between 20° 50′ and 20° 10′ north latitude and 70° 25′ and 73° 50′ east longitude. The total area is 600 square miles comprising 260 villages with an approximate population of 120,000.

III. EPIDEMIOLOGY.

The area under experiment was highly malarious, the spleen rates in 1950 and 1951 varying from 27 to 89 per cent (Viswanathan, 1953). The childhood parasite rate varied from 5·9 to 40·0 per cent. The relative prevalence of plasmodial species was 70·0, 27·5 and 2·5 per cent for *falciparum*, *vivax* and *malariæ*, respectively. The tract had never been treated with any insecticide prior to the experiment. Although no dissections were carried out in the experimental area itself, judging from the results of dissection in a neighbouring part of the district, *A. culicifacies* is the sole vector in this area. The average annual rainfall is 60 inches most of which occurs from June to October. The tract is somewhat hilly and traversed by small rivulets. The malaria season is usually from August to December. The houses in the villages under experiment have bamboo walls plastered with a thin layer of a mixture of dung and mud and have thatched roofs. Renovation of the walls, with a mixture of dung and mud paste, is usually done once a year about a month before the start of rainy season, *viz.*, in the months of May-June.

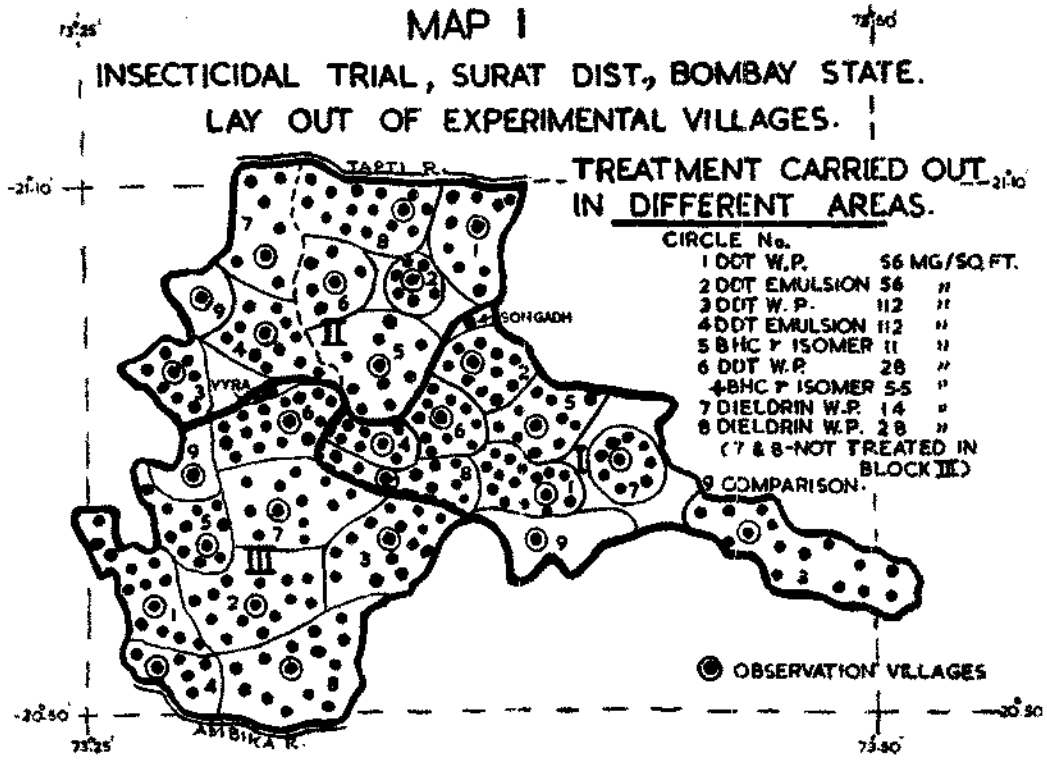
IV. INSECTICIDAL TREATMENTS ADOPTED.

The whole of the experimental area was divided into three blocks, each block consisting of nine circles with 6 to 16 villages in each. In each circle, one of the eight treatments mentioned below was carried out so that there were three replications of each type of experiment. In the case of dieldrin, however, on account of short supplies of the insecticide only two replications could be carried out. The two circles in the third block which were originally intended to be treated with dieldrin were, therefore, subsequently included in the district-wide malaria control scheme in which D.D.T. 75 per cent wettable powder is applied twice during the year in a dosage of 112 mg./sq. ft.

Treatments carried out.

1. D.D.T. wettable powder 56 mg./sq. ft.
2. D.D.T. emulsion 56 mg./sq. ft.
3. D.D.T. wettable powder 112 mg./sq. ft.
4. D.D.T. emulsion 112 mg./sq. ft.
5. B.H.C. wettable powder gamma isomer 11 mg./sq. ft.
6. Mixture of D.D.T. wettable powder 28 mg./sq. ft. and B.H.C. gamma isomer 5·5 mg./sq. ft.
7. Dieldrin wettable powder 14 mg./sq. ft.
8. Dieldrin wettable powder 28 mg./sq. ft.

The ninth circle in each block was kept for purposes of comparison. The selection of circles for each type of experiment was made purely at random by drawing lots. Map I shows the distribution of the experimental area into blocks and circles and the treatments carried out in each circle.



V. DETAILS OF SPRAYING.

All insecticides were sprayed with stirrup pumps provided with flat spray nozzles with an aperture of 3/64 inch. The spraying teams were trained to operate the pump at a more or less uniform pressure of 25 to 30 pounds per square inch. The speed of spraying was adjusted by prior training for ensuring an application of a gallon of fluid over 1,000, 2,000 and 4,000 square feet, respectively, in order to secure the desired dosage.

VI. RECORDING OF DATA.

One village in each circle in each block was selected for purposes of making entomological and malariometric observations. The data collected include spleen rates, parasite rates, infant parasite rates, and weekly catches of mosquitoes in daytime indoor resting places. In addition, mosquito collections were made every fortnight during the night. As there was a remarkable uniformity in the man-hour density of *A. culicifacies* collected from indoor resting places during the day and similar man-hour density in collections made at night, data regarding night collections are not presented in this paper. Viswanathan *et al.* (1950) have shown in another part of the Deccan Plateau that night collections of *A. culicifacies*, both in areas treated with D.D.T. and in the areas not so treated, are generally twice

the day-time collections in density per man-hour. In the present experimental area, night densities were almost the same as day-time densities. Men and cattle are housed in the same structures to a very much larger extent in the present experimental area, and as cattle are generally tethered inside the mixed dwellings during the night and as their presence puts a limitation on collection for fear of being gored by them, it is possible that the recorded night densities are an underestimate of the factual prevalence of mosquitoes.

VII. SECOND APPLICATION OF INSECTICIDES.

It was originally intended that the second application of any insecticide used in the experiment should be made if and when the day-time mosquito densities in indoor resting places in the observation villages exceed an artificially assumed critical density of 5 per man-hour, and either remain at the same level consecutively for two or three weeks or show a progressive rise. If there are fluctuations, at or even slightly above this level whereas in the comparison villages there is a progressive rise in mosquito densities, it was assumed that a second application was not necessary as, presumably, in such cases malaria control could still be achieved through the phenomenon of interception. It sometimes happened that the observation village selected in any particular circle showed a smaller density than the observation villages in similar circles in the other two blocks subjected to the same kind of treatment. In such cases, special efforts were made to determine *culicifacies* densities in a few nearby villages, and if the figures in these villages approximated to the densities in other circles, all the villages in each of the three circles in the three blocks were sprayed again with insecticide in the same dosage and formulation. In practice, however, on account of the limitation of labour, it has not been possible to carry out the second application as soon as the necessity was determined on the above basis.

VIII. RESULTS.

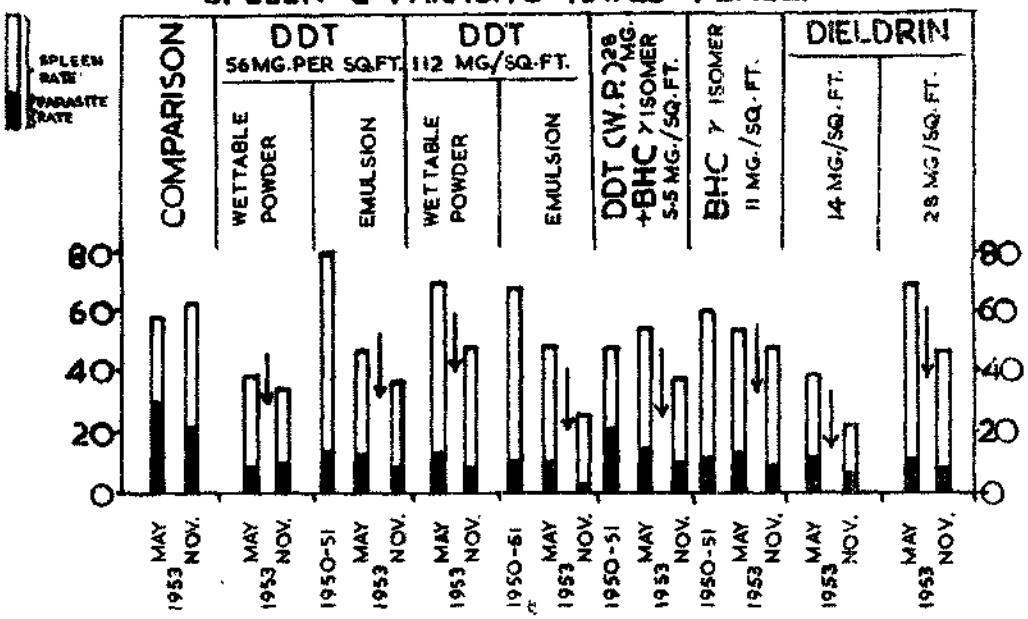
(*a*) *Spleen and parasite rates.*—Chart 1 shows the spleen rates and parasite rates in different groups of villages each of which was subjected to a particular kind of treatment. Similar data are also furnished for the comparison villages. The survey carried out in 1950-51 related to the entire district, and hence specific data regarding spleen rates and parasite rates were not compiled during that survey in all the villages in the present experimental area selected for observation. To the extent to which such data are available, they have been included in Chart 1. The spleen rates in 1950 were carried out at the end of the malaria season, and are therefore comparable to similar data in November 1953. In the comparison villages, the spleen rates have remained at the level of about 60 per cent and the parasite rates at the level of about 25 per cent without any significant change between May and November. The spleen rates show a significant reduction in the circles in which D.D.T. at a dose of 112 mg./sq. ft. was applied, either as an emulsion or as wettable powder and to a lesser but still significant extent in the case of D.D.T. emulsion at 56 mg./sq. ft. The reduction in the case of D.D.T. wettable powder 56 mg./sq. ft. is not statistically significant. In the case of parasite rates, the reduction is significant only in dosages of 112 mg./sq. ft. but

not in the smaller dosage. In the case of B.H.C. gamma isomer 11 mg./sq. ft., the reduction in the spleen rate is not significant. Reduction in the parasite rate is on the border line of statistical significance. With the combined use of D.D.T. and B.H.C., the reduction in the spleen rate is significant but not so in the case of parasite rate. The dieldrin circles show a significant reduction in spleen and parasite rates.

CHART I

INSECTICIDAL TRIAL, SURAT DIST., BOMBAY STATE.

SPLEEN & PARASITE RATES-PERCENT.



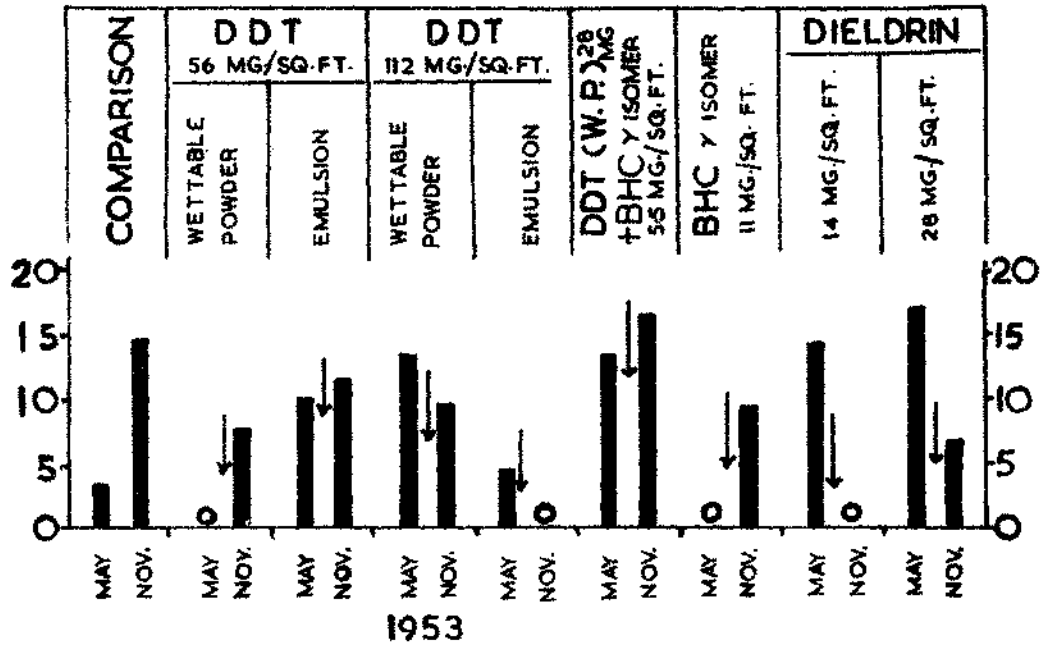
(*b*) *Infant parasite rates.*—Chart 2 shows the infant parasite rates in May and November 1953, respectively. In the comparison villages, the infant parasite rate is 14·7 per cent in November as compared to 3·2 per cent in May. The difference is perhaps the truest measure of the quantum of fresh transmission during the season. With 56 mg./sq. ft. of D.D.T., either as wettable powder or as an emulsion, the November infant parasite rates do not show a statistically significant reduction from similar figures for the comparison villages. The circles in which D.D.T. emulsion was applied in a dosage of 112 mg./sq. ft., *show a nil* infant parasite rate. In the case of wettable powder with similar dosage, however, though the infant parasite rate in November was smaller than in the comparison villages the difference is not statistically significant. In the circles treated with a combination of D.D.T. and B.H.C., the infant parasite rate in November was as high as 16·7 per cent which is even slightly larger, though not significantly, than in the comparison villages. In the circles treated with B.H.C. gamma isomer

11 mg./sq. ft., the November infant parasite rate was smaller but the difference is not statistically significant. The circles treated with dieldrin 14 mg./sq. ft. show a nil infant prasite rate, and in the circles treated with dieldrin 28 mg/sq. ft., one infant out of 16 examined showed parasites.

CHART 2

INSECTICIDAL TRIAL, SURAT. DIST., BOMBAY STATE.

INFANT PARASITE RATES - PERCENT.



(*c*) *Mosquito densities.*—Chart 3 shows the densities of *A. culicifacies* per man-hour in the comparison villages in each of the three blocks as well as the pooled average. Blocks II and III show a remarkable consistency in the seasonal numerical prevalence of this species. From about the middle of July there is a progressive and rapid rise and the peak density of nearly 70 per man-hour is reached in the third and fourth weeks of August. This peak is maintained till practically the end of the first week of October, that is, for as long as seven weeks. The mosquito densities undergo a decline nearly as steeply as they rose earlier in the year, and by the middle of November they are below five per man-hour. In the third block, the peak densities reached are less than 30 per man-hour and they generally tend to decline in numerical prevalence at a much earlier date than in the other two blocks.

CHART 3

INSECTICIDAL TRIAL, SURAT DIST., BOMBAY STATE.
WEEKLY DENSITY (PER MAN HOUR) OF A.CULICIFACIES.
(JUNE 1953-JAN. 1954)

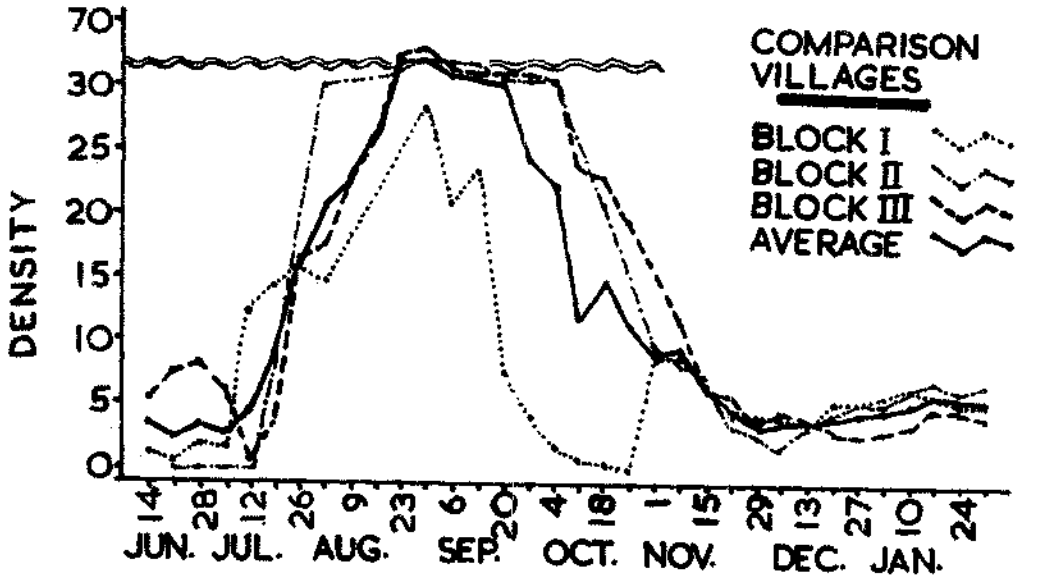


Chart 4 shows *A. culicifacies* densities in circles treated with D.D.T. wettable powder 56 mg./sq. ft. By the tenth week after the first application, the densities rise above five per man-hour. In Blocks I and III, the second application was made soon after this rise. In Block II, however, it took nearly three weeks after the rise before applying the second round. In all the three blocks, *culicifacies* densities remained extremely low after the second round of application. In the first and third blocks, the second round of application was made at the end of the first week of September. During this period, in the comparison villages the densities have remained much higher than five per man-hour, but there is a very perceptible downward trend.

CHART 4

INSECTICIDAL TRIAL, SURAT DIST., BOMBAY STATE.
WEEKLY DENSITY (PER MAN HOUR) OF A.CULICIFACIES.
(JUNE 1953 - JAN. 1954)

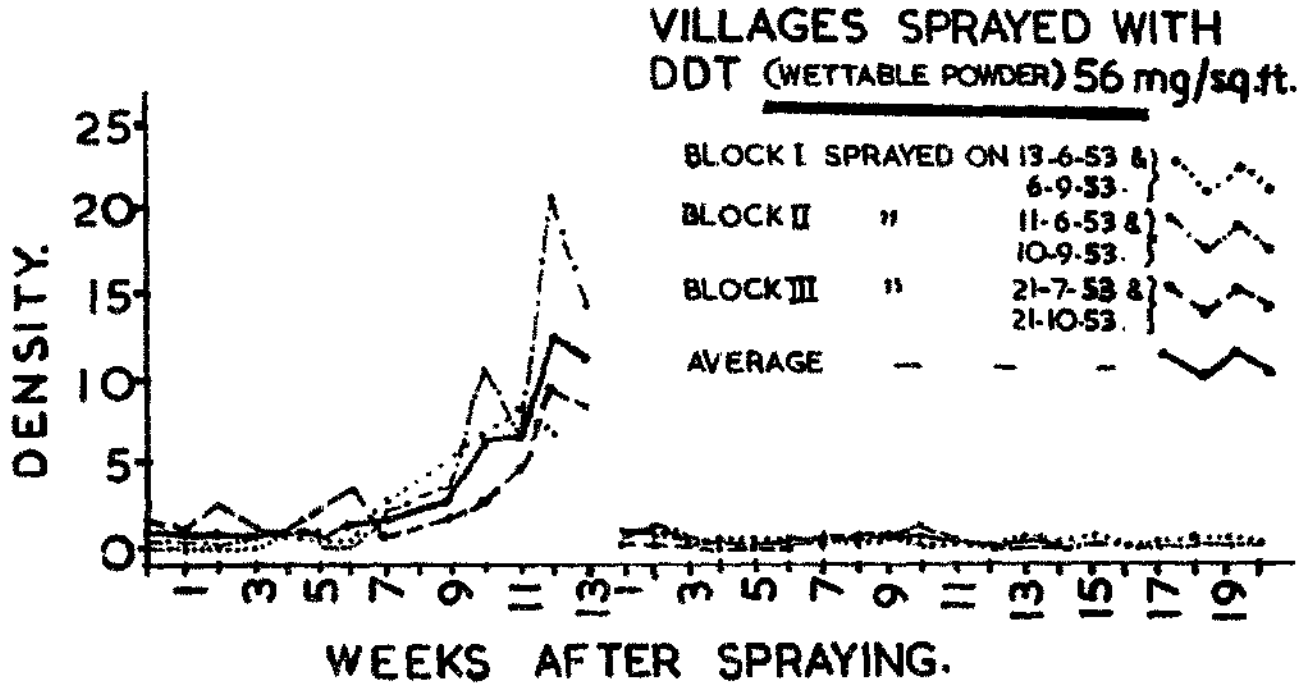


Chart 5 shows the densities in circles treated with D.D.T. emulsion 56 mg./sq. ft. Beginning with the ninth week, the average densities show a progressive rise. In the second block they show a rise as early as the fifth week, in the first block on the ninth week and in the third block only on the eleventh and twelfth weeks. The second round of application was carried out in the first two blocks only four weeks after the mosquito densities rose above five per man-hour. In these circles too, the mosquito densities remained extremely low after the second application.

CHART 5

INSECTICIDAL TRIAL, SURAT DIST., BOMBAY STATE.
WEEKLY DENSITY (PER MAN HOUR) OF A.CULICIFACIES.
(JUNE 1953-JAN. 1954)

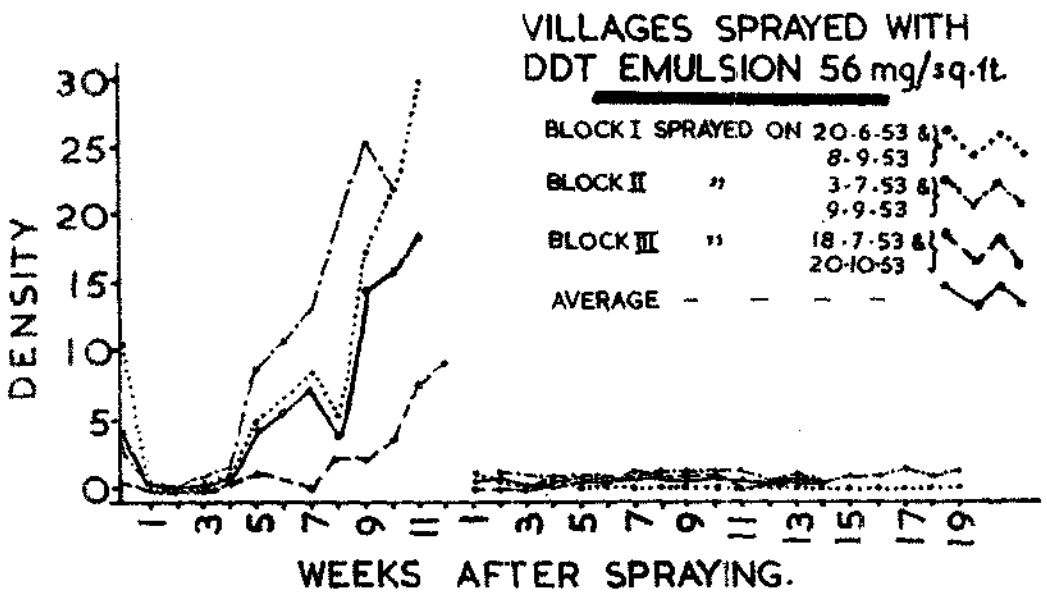


Chart 6 shows the *culicifacies* densities in circles treated with D.D.T. wettable powder 112 mg./sq. ft. There is a steady rise in the average mosquito densities from the eighth week onwards, and after the second round of application mosquito densities have remained extremely low.

CHART 6

INSECTICIDAL TRIAL, SURAT DIST., BOMBAY STATE.
WEEKLY DENSITY (PER MAN HOUR) OF A.CULICIFACIES.
(JUNE 1953 - JAN. 1954)

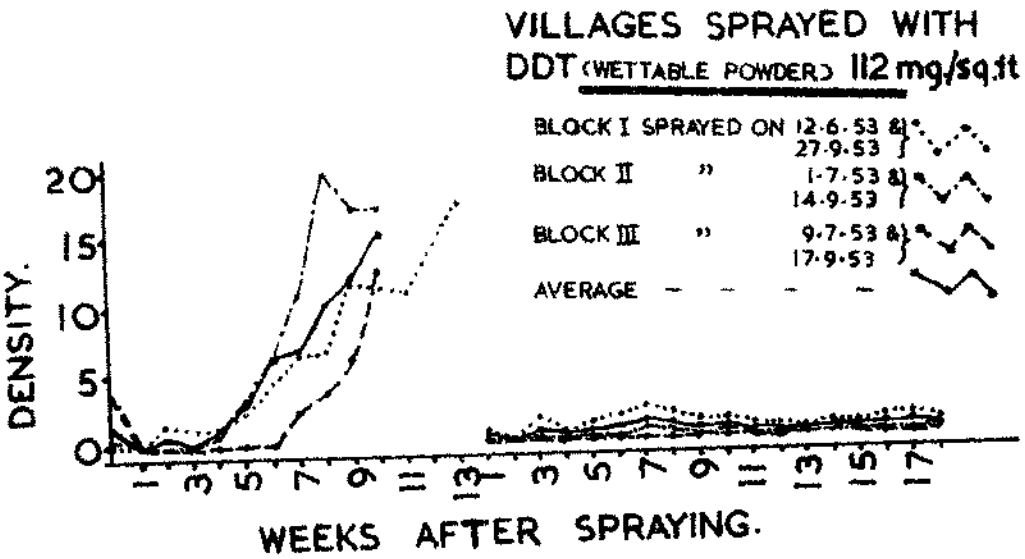


Chart 7 shows the *culicifacies* densities in the villages sprayed with D.D.T. emulsion 112 mg./sq. ft. The villages in Block II show a rise as early as six weeks after the first round of application. In the other two blocks, the rise in density is much later. But in Block I, although there was a rise in density in the ninth and tenth weeks, it was followed by a decline and hence a second round of application was not made. In the other two blocks, however, where a second application was carried out, mosquito densities remained extremely low later.

CHART 7

INSECTICIDAL TRIAL, SURAT DIST., BOMBAY STATE.

WEEKLY DENSITY (PER MAN HOUR) OF A.CULICIFACIES. (JUNE 1953-JAN 1954)

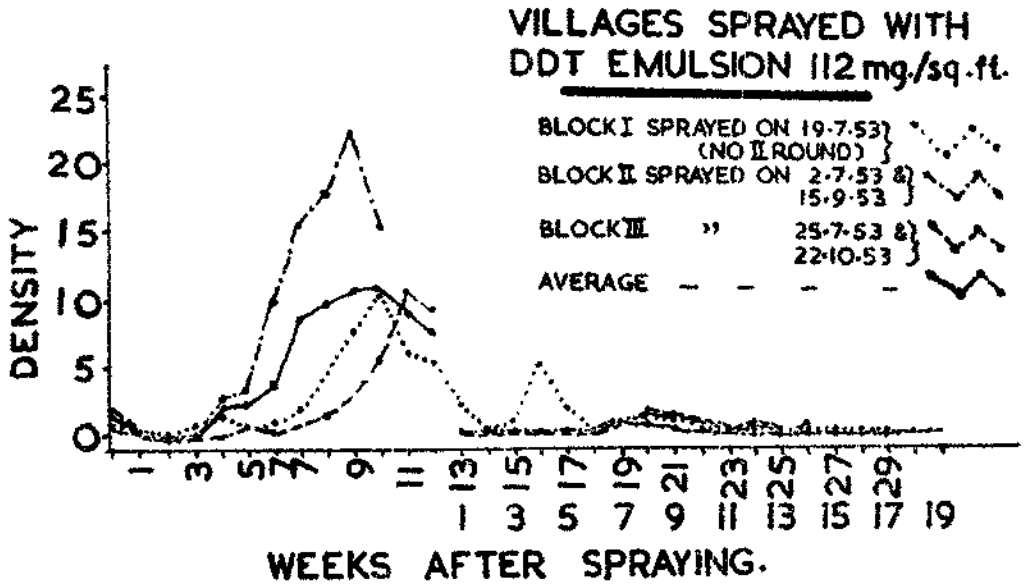


Chart 8, relating to treatment with D.D.T. and B.H.C. combined, shows that in Block III the mosquito densities started rising as early as fourth week, and the second round of application was made at the end of seven weeks. In Block I, the mosquito densities started rising in the ninth week and the second application was made at the end of the eleventh week. In Block II, the densities started rising in the seventh week and the second application was made at the end of the tenth week. After the second round of application, however, the mosquito densities in all the three blocks remained extremely low. In this type of experiment, the second application was carried out at a time when in the comparison villages the mosquito densities remained practically at their peak level, and yet

after the second application the densities have remained extremely low in all the three blocks.

CHART 8

INSECTICIDAL TRIAL, SURAT DIST., BOMBAY STATE,
WEEKLY DENSITY (PER MAN HOUR) OF A.CULICIFACIES
(JUNE 1953-JAN 1954)

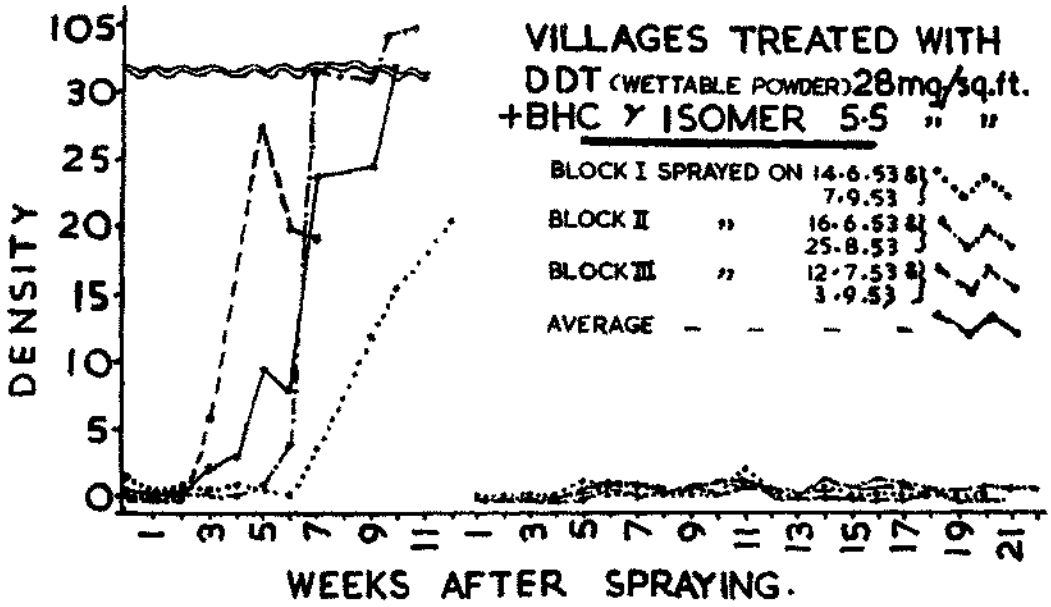


Chart 9 shows the mosquito densities in villages sprayed with B.H.C. gamma isomer 11 mg./sq. ft. In this group, in the first two blocks the density started rising abruptly during the sixth week. But in Block III in the observation village, it remained low even as late as ten weeks, but as mosquito densities in certain other villages showed higher figures, the second round of application was made in all the three blocks. In the first two blocks, the second application was carried out at a very much earlier date. In the third block, however, it was carried out only

on October 8, 1953. After the second application, mosquito densities have remained extremely low in all the three blocks.

CHART 9

INSECTICIDAL TRIAL, SURAT DIST., BOMBAY STATE.
WEEKLY DENSITY (PER MAN HOUR) OF A.CULICIFACIES.
(JUNE 1953-JAN.1954)

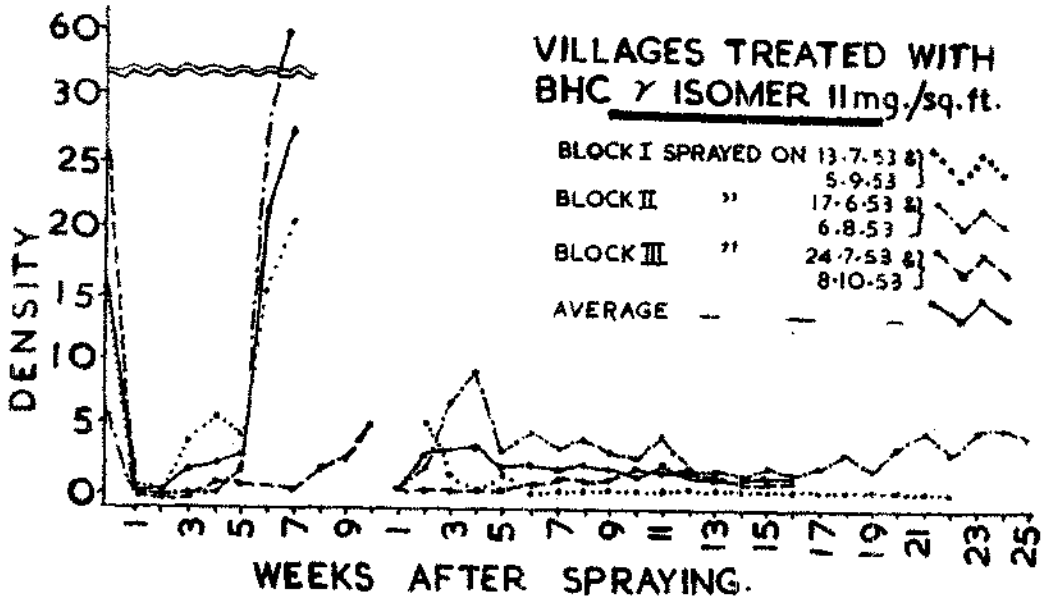


Chart 10 shows the mosquito densities in villages treated with dieldrin 14 mg./sq. ft. Although the mosquito densities tended to show a slight rise above five per man-hour in the eighth week, it showed a decline later and kept well below five per man-hour. There was therefore no occasion for applying the insecticide a second time.

CHART 10

INSECTICIDAL TRIAL, SURAT DIST., BOMBAY STATE.
WEEKLY DENSITY (PER MAN HOUR) OF A.CULICIFACIES.
(JUNE 1953–JAN. 1954)

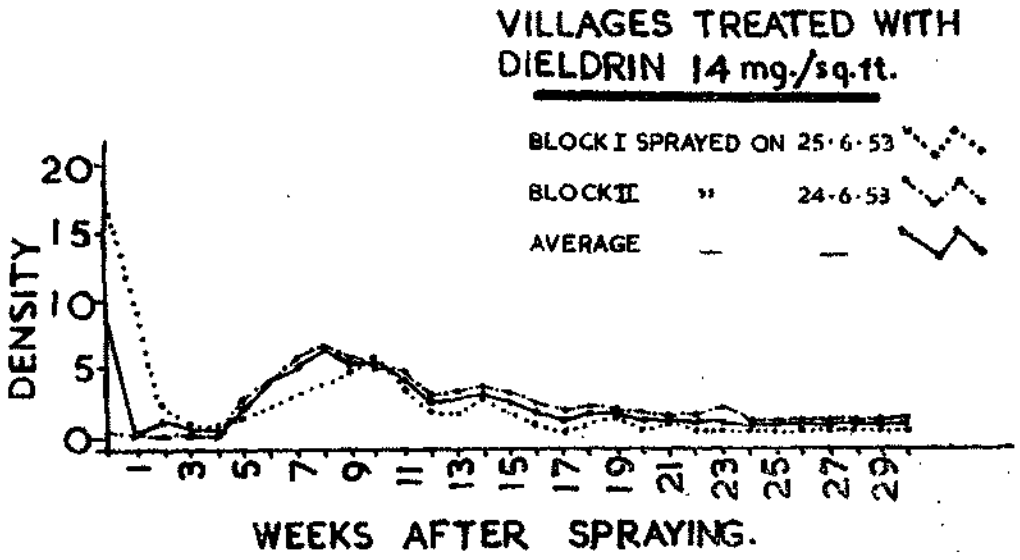
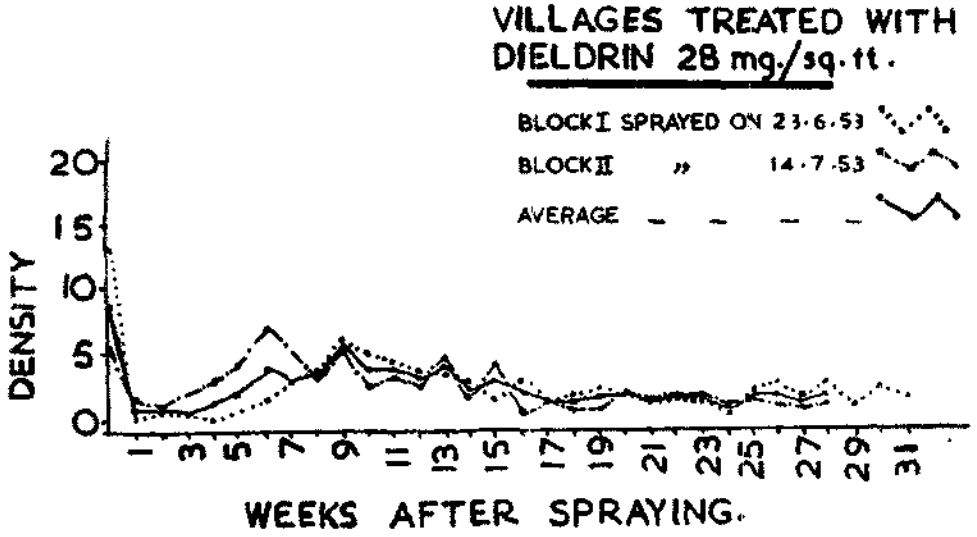


Chart 11 shows mosquito densities in villages treated with dieldrin 28 mg./sq. ft. The results are practically the same as shown in the circles treated with a smaller dosage of dieldrin.

CHART II

INSECTICIDAL TRIAL, SURAT DIST, BOMBAY STATE.
WEEKLY DENSITY (PER MAN HOUR) OF A.CULICIFACIES.
(JUNE 1953-JAN. 1954)



IX. DISCUSSION.

In human affairs it is often the end that justifies the means and the utility of any method employed is judged by the results achieved even if the manner of their attainment is not always manifest. Methods of malaria control are, therefore, to be judged by the resultant indices in malariometry.

The extent of malaria morbidity cannot be assessed in each village or group of villages due to paucity of medical personnel. Special canvass of malaria morbidity, by house to house visit made by the malaria staff, is likely to be biassed.

Spleen rates in the age group 2-10 provide a good measure but they are not often sensitive enough to demonstrate relative efficacy of different methods at the end of only one season of trial.

Parasite rates in the same age group generally run parallel to spleen rates but during the first season of successful malaria control they do not show in full the reduction achieved in transmission on account of relapses.

Taking childhood spleen and parasite rates together, significant reduction has been achieved with D.D.T. 112 mg./sq. ft. in either formulation and with dieldrin in either of the two dosages adopted. D.D.T. wettable powder had been

applied twice during the season in all the three blocks and D.D.T. emulsion in two out of three blocks. Dieldrin was applied only once. A more or less equal degree of successful malarial control was established with 14 mg./sq. ft. dieldrin applied only once early during the season as with D.D.T. applied in two doses of 112 mg./sq. ft. during the season at an interval of 10 to 12 weeks.

Infant parasite rates at the end of the malaria season are perhaps the truest measure of the extent of malaria transmission. They are however attended with a serious disability. In moderately endemic areas, infant parasite rates are usually low and unless a very large number of infants is examined, differences which may be noted cannot be proved to be of statistical significance. Thus taking the dieldrin experiment, none out of 30 infants showed parasite in November in the area treated with 14 mg./sq. ft., while in the area treated with the larger dose of 28 mg/.sq. ft., one infant out of 16 showed parasite. Actually the infant had been elsewhere during the season for some time. But that cannot definitely negative autochthonous infection, and anyway it is the purpose of statistical method to reckon for such variations which may be termed experimental errors. The following table shows the departure from expectation of infant parasite rates on the assumption that different treatments exercise no effect on them.

Number	Item.	Number examined.	Number infected.	Number expected.	$\frac{(T-0)^2}{T}$
1.	Comparison villages	34	6	2·65	4·3
2.	D.D.T. wettable powder 56 mg./sq. ft.	39	3	3·04	0·0
3.	D.D.T. emulsion 56 mg./sq. ft. ...	35	4	2·73	0·6
4.	D.D.T. wettable powder 112 mg./sq. ft.	43	4	3·35	0·1
5.	D.D.T. emulsion 112 mg./sq. ft. ...	69	0	5·38	5·4
6.	D.D.T. (28 mg.) plus B.H.C. gamma isomer (5·5. mg.) ...	36	6	2·80	3·7
7.	B.H.C. gamma isomer (11 mg./sq. ft.)	31	3	2·42	0·1
8.	Dieldrin 14 mg./sq. ft.	30	0	2·34	2·3
9.	Dieldrin 28 mg./sq. ft.	16	1	1·25	0·4
				$\chi^2=$	16·9

For n =8 the value of 16·9 for χ^2 is just significant at the level of P=0·05. This shows that the items in the above table do not represent homogeneous treatment. The principal contributions to nonhomogeneity are made by the comparison villages, D.D.T. emulsion 112 mg./sq. ft., D.D.T. plus B.H.C., and dieldrin 14 mg./sq. ft. Of these, the comparison villages naturally show a considerable excess of infection, and D.D.T. emulsion 112 mg./sq. ft. and dieldrin 14 mg./sq. ft. a gross deficiency in infections. D.D.T. plus B.H.C. also shows an excess of infections. This is a very stringent evaluation and on this basis D.D.T. emulsion 112 mg./sq. ft. applied once in some cases or twice in other cases during the season, helps root out fresh infections almost completely. So does dieldrin 14 mg./sq. ft.

do. By inference, dieldrin 28 mg./sq. ft. would also naturally bring about this result, the solitary infant infection in the experiment possibly being due to its acquisition elsewhere.

Mosquito densities have been made the basis for the second application of any insecticide as they constitute most readily ascertainable index in the field. The following table shows the build up of *culicifacies* densities to above five per man-hour, an artificially assumed critical density which, however, requires further examination for this area in the different experimental areas.

Number.	Item.	Block I.	Block II.	Block III.	Average.
1.	D.D.T. wettable powder 56 mg./sq. ft.	About	ten	weeks	10 weeks.
2.	D.D.T. wettble powder 112 mg./sq. ft.	About	eight	weeks	8 weeks
	(Note : The above observation shows the nonsuperiority of 112 mg. D.D.T. wettable powder over 46 mg./sq. ft. but other indices show superiority of the higher dose).				
3.	D.D.T. emulsion 56 mg./sq. ft. ...	9th week.	5th week	11th week	9 week
4.	D.D.T. emulsion 112 mg./sq. ft. ...	9th to 10th week but later declined. Hence no second application.	6th week	9th to 10th week.	9 weeks
5.	D.D.T. 28 mg./sq. ft. plus B.H.C. gamma isomer 5·5. mg./sq. ft. ...	9th week	7th week	4th week	7 weeks
6.	B.H.C. gamma isomer 11mg./sq. ft.	6th week	6th week	9th week	7 weeks
7.	Dieldrin 14 mg./sq. ft. ...	One application was found adequate to keep mosquito density low throughout the season.			
8.	Dieldrin 28 mg./sq. ft. ...				

The above experiments show that in this experimental area a single application of dieldrin in a dosage of 14 mg./sq. ft. at the beginning of the season would establish as much malaria control as the application of D.D.T. 112 mg./sq. ft. once or even twice during the season. There have been no untoward symptoms noticed in men and domestic birds and animals on account of any of the insecticides used. B.H.C. when applied in a dosage of 11 mg./sq. ft. (gamma isomer) has a residual efficacy of not longer than six weeks (Note: One of the authors of the present paper (D.K.V.) reported earlier (1949) that its residual efficacy is only four weeks). Combination of D.D.T. and B.H.C. has not shown in these experiments to be of any advantage over either insecticide used singly.

X. SUMMARY AND CONCLUSIONS.

A field trial to test the relative efficacy of different dosages and formulations of D.D.T., B.H.C. and dieldrin was carried out in 260 villages in an area of about 600 square miles in Songadh and Vyara talukas of Surat District, Bombay State, India.

Eight different treatments were adopted in eight groups of villages and this was replicated into two more blocks of similar groups of villages. A ninth group was kept for comparison in each block. D.D.T. was applied as an emulsion and as water dispersible powder in dosage of 56 and 112 mg./sq. ft. in either case. B.H.C. was applied as a water dispersible powder in a dosage of 11 mg./sq. ft. of gamma isomer. D.D.T. (28 mg./sq. ft.) was combined with B.H.C. (5 mg. of gamma isomer sq. ft.) in one experiment. Dieldrin was sprayed as a water dispersible powder in dosages of 14 and 28 mg./sq. ft., respectively.

Observations were recorded regarding childhood spleen and parasite rates and infant parasite rates before and at the end of transmission and weekly densities of *A. culicifacies* throughout the season.

A second application of insecticide was made only when the density of the vector, *A. culicifacies*, exceeded five per man-hour and remained at that level for two to three weeks.

The conclusions arrived at broadly are :—

(1) A single application of dieldrin in a dosage of 14 mg./sq. ft. applied at the beginning of the season would establish as high degree of successful malaria control as an application of D.D.T. 112 mg./sq. ft. once or twice during the season.

(2) B.H.C. when applied in a dosage of 11 mg./sq. ft. (gamma isomer) has a residual efficacy of not longer than six weeks.

(3) Combination of D.D.T. (28 mg./sq. ft.) and B.H.C. (5·5. mg. gamma isomer/sq. ft.) has not shown any advantage over D.D.T. (56 mg./sq. ft.) or B.H.C. (11 mg. gamma isomer/sq. ft.) used singly.

(4) There have been no untoward symptoms noticed in man, domestic birds and animals on account of any of the insecticides used.

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SUSCEPTIBILITY OF *CULEX* (CULEX) *BITÆNIORHYNCHUS* GILES, 1901, TO *PLASMODIUM RELICTUM* BUT NOT TO *PLASMODIUM GALLINACEUM* AND *PLASMODIUM FALCIPARUM*.

BY

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INTRODUCTION.

THE present paper reports the results of feeding trials with *C. bitæniorhynchus* on gametocytes of *P. relictum** in sparrows, *P. gallinaceum* in domestic fowls and *P. falciparum* in human volunteers. In all the trials, *C. bitæniorhynchus* females were fed simultaneously with at least one known vector of Plasmodium species concerned. This procedure was adopted to rule out the possibility, if any, of the gametocytes being present in inadequate numbers or being otherwise unsuitable. The control vectors were *C. fatigans* for *P. relictum*, *Aedes ægypti* for *P. gallinaceum* and *A. fluviatilis* and *A. stephensi* (type) for *P. falciparum*. All the species of mosquitoes were insectary-bred. The colony of *C. bitæniorhynchus* (type-form) was about four years old (Mohan, 1950), *C. fatigans* and *Aedes ægypti* each about five years, *A. fluviatilis* about three years (Mohan, 1952) and *A. stephensi* (type) about fifteen years old (Russell and Mohan, 1939*b*).

Mosquitoes were kept reasonably hungry prior to feeding on gametocyte carriers for increasing their biting potential. *C. bitæniorhynchus* and *C. fatigans*

*Almost all Coonoor sparrows were found to be infected with a species of Plasmodium which was provisionally identified as *Plasmodium præcox* by Mulligan *et al.* (1940) ; *J. Mal. Inst. Ind.*, 3, pp. 513-524 (footnote page 514). *P. præcox* (Grassi and Feletti, 1890) is the synonym of *P. relictum* (Grassi and Feletti, 1891).

needed relatively longer period of starvation than *A. fluviatilis* and *A. stephensi* (type) and in *Aedes ægypti* it was least.

The method of feeding the mosquitoes on a sparrow was to confine the infected bird in a small wire-cage which was then kept overnight inside a bobbinet cage containing *C. bitæniorhynchus* and *C. fatigans* females. The following morning, fully engorged females were removed into separate cages for dissections later on.

The method of feeding the mosquitoes on an infected fowl adopted in the present experiments was slightly different. The feathers of the donar fowl were clipped on the back and legs bandaged ; it was then exposed to the bites of *C. bitæniorhynchus* and *Aedes ægypti* in a standing position, the head remaining outside the cage through the sleeve which was wound round its neck and tucked in to the wire-frame. The movements of the fowl were thus greatly restricted. To prevent the fowl from damaging or otherwise soiling the cage, a thick sheet of paper was placed on the bottom for the fowl to stand on. Feeding was done usually towards evening and fully fed females were removed immediately into separate cages.

For feeding on human carriers, mosquitoes were caged in screened wooden or bamboo rings and applied simultaneously to the donors.

In all the trials, only one infective blood meal was given and only fully engorged females were selected. The mosquitoes were handled exactly alike under identical conditions.

TRIALS WITH *P. RELICTUM.*

Table I shows the results of infection with *P. relictum* in *C. bitæniorhynchus* versus *C. fatigans.*

TABLE I.

Susceptibility of C. bitæniorhynchus *to* P. relictum (=*P.* praecox) *in sparrows* (*with* C. fatigans *as control*).

Lot number.	*C. BITÆNIORHYNCHUS*					*C. FATIGANS*					Remarks.
	Number.				Per cent total positive.	Number.				Per cent total positive.	
	Dissected.	Gut positive.	Glands positive.	Total positive.		Dissected.	Gut positive.	Glands positive.	Total positive.		
1	21	21	14	21	100	7	7	2	7	100	Heavy infections in almost all specimens.

Both the species were infected to the extent of 100 per cent. This high infection index compensates for the relatively small number of dissections. The findings are in agreement with those of Russell and Mohan (1942) who found complete exogenous development of this species of avian malaria in *C. bitæniorhynchus.*

TRIALS WITH *P. GALLINACEUM.*

The results of comparative feedings of *C. bitæniorhynchus* and *Aedes ægypti* on infected fowls are summarised in Table II.

TABLE II.

Resistance of C. bitæniorhynchus *to* P. gallinaceum *in domestic fowls (with* Aedes ægypti *as control.)*

Lot number.	*C. BITÆNIORHYNCHUS.*					*AEDES ÆGYPTI.*					Remarks.
	Number.				Per cent total positive.	Number.				Per cent total positive.	
	Dissected.	Gut positive.	Glands positive	Total positive.		Dissected.	Gut positive.	Glands positive.	Total positive.		
1	203	0	0	0	0	21	Not examined.	19	19	90	In all the three lots, dissections of *C. bitæniorhynchus* were commenced after the development of sporozoites in the salivary glands of *Aedes ægypti.*
2	100	0	0	0	0	23	,,	23	23	100	
3	115	0	0	0	0	21	,,	12	12	57	
Total	418	0	0	0	0	65	,,	54	54	83	

No infection, either of gut or of salivary glands, was encountered in any of 418 *C. bitæniorhynchus*, although *Aedes ægypti* which fed simultaneously on the same fowls on three occasions showed infection in 57, 90 and 100 per cent of the specimens. It would appear that *C. bitæniorhynchus* is highly resistant to *P. gallinaceum.*

TRIALS WITH *P. FALCIPARUM.*

The results of dissections of *C. bitæniorhynchus* and of the control mosquitoes, *A. stephensi* (type) and *A. fluviatilis*, fed on crescent-carriers, are recorded in Table III.

TABLE III.

Resistance of C. bitæniorhynchus *to* P. falciparum. *(With* A. stephensi *(type) and* A. fluviatilis *as controls).*

Lot number.	*C. BITÆNIORHYNCHUS*					*A. STEPHENSI* (TYPE)					*A. FLUVIATILIS*				
	Number				Per cent total positive	Number				Per cent total positive	Number				Per cent total positive
	Dissected	Gut positive	Glands positive	Total positive		Dissected	Gut positive	Glands positive	Total positive		Dissected	Gut positive	Glands positive	Total positive	
1	22	0	0	0	0	23	4	1	5	22	39	13	12	14	36
2	129	0	0	0	0	55	15	7	20	36	144	26	51	55	38
Total	151	0	0	0	0	78	19	8	25	32	183	39	63	69	38

Gametocytes : Lot 1, ten crescents per 100 leucocytes : Donor, male, 15 years.
Lot 2, fifteen crescents per 100 leucocytes : Donor, male, 20 years.

It will be seen that none of 151 *C. bitæniorhynchus* was found infected either with gut or salivary glands infection in contrast to *A. stephensi* (type) and *A. fluviatilis*, both of which showed an infection index of 32 and 38 per cent, respectively. Majority of the mosquitoes were dissected long after the formation of sporozoites in the control species. It is, therefore, reasonable to conclude that *C. bitæniorhynchus* is highly refractory to infection with *P. falciparum*. The results corroborate those of Russell and Mohan (1939*a*) but not those of Williamson and Zain (1937).

SUMMARY AND CONCLUSIONS.

The results of dissections of comparative feedings of *C. bitæniorhynchus* and the control species on gametocytes of two species of avian malaria and one species of human malaria are recorded. *C. bitæniorhynchus* is highly susceptible to *P. relictum* but refractory to *P. gallinaceum* and *P. falciparum* under laboratory conditions.

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COMPARATIVE SUSCEPTIBILITY OF SOME *AËDES* MOSQUITOES TO *PLASMODIUM GALLINACEUM*.

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[March 5, 1955.]

VARIOUS species of culicines have been experimentally infected with *Plasmodium gallinaceum* up to the sporozoite stage (Russell and Mohan, 1942; Russell and Menon, 1942). It was to evaluate quantitatively the susceptibility of some of the common culicine species occurring along the eastern slopes of the Nilgiris (South India) that experiments were carried out under laboratory conditions with the following six species :—

1. *Aedes* (*Stegomyia*) *ægypti* Linnæus, 1762.
2. *Aedes* (*Stegomyia*) *albopictus* Skuse, 1894.
3. *Aedes* (*Stegomyia*) *vittatus* Bigot, 1861.
4. *Aedes* (*Finlaya*) *pseudotæniatus* Giles, 1901.
5. *Aedes* (*Aedimorphus*) *jamesi* Edwards, 1914.
6. *Armigeres* (*Armigeres*) *obturbans* Walk, 1860.

Experience extending over several years has shown that in the case of *P. gallinaceum* infections, a higher density of oöcysts per positive gut is almost always associated with a higher production of sporozoites in the salivary glands after the incubation period. Consequently the different species of *Aedes* were first compared, only on the incidence of gut infections and subsequently on the density of oöcysts per infected gut.

MATERIALS AND METHODS.

The strain of *P. gallinaceum* used in these experiments was brought originally from Colombo and has been maintained in the local domestic fowls in the laboratories of the Malaria Institute of India for the past several years. *Aedes* (*S.*)

ægypti and *Aedes* (*S.*) *albopictus* used were taken from the insectary colonies, while the remaining species were reared from larvæ collected from their natural habitats. For feeding the mosquitoes on an infected fowl, feathers on the back of the bird were clipped and its legs gently tied together with a piece of cloth bandage. The fowl was then made to stand inside the mosquito cage with its head protruding out through a sleeve which was rolled and tucked so as not to let any mosquitoes escape. The movements of the fowl were thus restricted. A thick paper was used to cover the bottom of the cage to prevent the fowl from damaging or soiling it. Fully engorged females of the various species were removed separately into different cages and kept under identical conditions until dissection.

RESULTS.

In the first set of experiments the relative susceptibility of six different species of *Aedes* were determined on the basis of qualitative infections, that is, on the number of specimens showing gut infections. The results of dissections are presented in Table I.

TABLE I.

Comparative infectibility of six species of Aedes *with* P. gallinaceum *in domestic fowls.*

Species.	Number.		Per cent positive.	Number of guts examined for oöcyst counts.	Average number of oöcysts per positive gut.
	Examined.	Gut positive.			
Aedes (*Stegomyia*) *albopictus*	28	26	93·0	8	Heavy intensity of oöcysts.
Aedes (*Stegomyia*) *ægypti* ...	17	14	82·0	11	22
Aedes (*Stegomyia*) *vittatus* ...	71	18	25·0	4	14·5
Aedes (*Finlaya*) *pseudotæniatus*	10	4	40·0	4	Fairly heavy intensity of oöcysts.
Aedes (*Aedimorphus*) *jamesi*	15	15	100·0	11	43
Armigeres (*Armigeres*) *obturbans*	2	2	100·0	2	87·5

An analysis of the data shows that 100 per cent infection was found in the case of *Aedes* (*Aedimorphus*) *jamesi* and *Armigeres* (*Armigeres*) *obturbans*, although there were only two specimens of the latter species. The infection index of the remaining species was in a descending order as follows : *Aedes* (*Stegomyia*) *albopictus* (93 per cent), *Aedes* (*Stegomyia*) *ægypti* (82 per cent), *Aedes* (*Finlaya*) *pseudotæniatus* (40 per cent) and *Aedes* (*Stegomyia*) *vittatus* (25 per cent).

COMPARATIVE SUSCEPTIBILITY OF *AËDES* (*S.*) *ÆGYPTI* AND *AEDES* (*S.*) *ALBOPICTUS.*

The two species *Aedes* (*S.*) *ægypti* and *Aedes* (*S.*) *albopictus* which are common all over India and which had shown a very high rate of infection in the previous experiments were subjected to further comparative tests on their susceptibility. Three lots of mosquitoes of these two species were fed as in the previous experiment. All the mosquitoes in Lots 1 and 3 and most of them in Lot 2 were dissected before sporozoites reached the salivary glands. The results of dissections in this second series of tests are presented in Table II.

TABLE II.

Comparative infections in Aedes (S.) ægypti *and* Aedes (*S.*) albopictus *with* P. gallinaceum *in domestic fowls.*

Lot number.	*AEDES* (*STEGOMYIA*) *ÆGYPTI*					*AEDES* (*STEGOMYIA*) *ALBOPICTUS*				
	Number.				Per cent total positive.	Number				Per cent total positive.
	Examined.	Gut positive.	Glands positive.	Total positive.		Examined.	Gut positive.	Glands positive.	Total positive.	
1	5	4	...	4	80·0	8	7	...	7	87·5
2	46	46	11	46	100·0	36	36	7	36	100·0
3	64	26	...	26	40·6	29	19	...	19	65·5
Total	115	76	11	76	66·0	73	62	7	62	85·0

An analysis of the data shows that while in Lot 2, all *Aedes* (*S.*) *ægypti* and *Aedes* (*S.*) *albopictus* were found infected, in Lots 1 and 3, the percentage of infection was higher in *Aedes* (*S.*) *albopictus* than in *Aedes* (*S.*) *ægypti* as observed in the previous experiment.

The incidence of the quantitative infection was determined by counting the oöcysts after the guts had been properly stained and mounted in canada balsam. The results obtained are shown in Table III.

It would be observed from the above that, the higher the index of infection the greater was the average density of oöcysts per infected gut and that this average was significantly higher in *Aedes* (*S.*) *Albopictus* than in *Aedes* (*S.*) *ægypti* in all the three series of tests.

COMPARATIVE SUSCEPTIBILITY OF *AËDES* (*S.*) *ÆGYPTI* AND *ARMIGERES* (*A.*) *OBTURBANS.*

Experiments similar to those carried out with *ægypti* and *albopictus* were repeated with females of *ægypti* and *obturbans*. The results of simultaneous feedings on infected fowls are shown in Table IV, and the intensity of gut infection before the invasion of salivary glands by sporozoites in Table V.

Table III.

Quantitative intensity of oöcysts in Aedes (S.) ægypti *and* Aedes (S.) albopictus.

Lot number	Species.	Per cent positive.	Number of guts examined for oöcyst counts.	Average number of oöcysts per positive gut.	Oöcysts per positive gut examined.						
					-10	11-50	51-100	101-200	201-300	301-400	401+
1	*Aedes (Stegomyia) ægypti*	80·0	4	47·5	...	2	2	...	...	...	...
2	*Aedes (Stegomyia) ægyptia*	100·0	31	77·6	8	9	4	8	1	...	1
3	*Aedes (Stegomyia) ægypti*	40·6	26	3·6	25	1	...	...	...	...	...
1	*Aedes (Stegomyia) albopictus* ...	87·5	7	99·0	2	2	...	2	1	...	...
2	*Aedes (Stegomyia) albopictus* ...	100·0	23	215·0	1	1	3	7	5	5	1
3	*Aedes (Stegomyia) albopictus*	65·5	19	8·5	14	5	...	...	...	...	...

Table IV.

Comparative infections in Aedes (*S.*) ægypti *and* Armigeres (*A.*) obturbans with P. gallinaceum *in domestic fowls.*

Lot number	*Aedes (Stegomyia) ægypti.*					*Armigeres (Armigeres) obturbans.*				
	Number.				Per cent total positive.	Number.				Per cent total positive.
	Examined.	Gut positive.	Glands positive.	Total positive.		Examined.	Gut positive.	Glands positive.	Total positive.	
1	18	18	5	18	100	15	15	5	15	100

Table V.

Quantitative intensity of oöcysts in Aedes (S.) ægypti *and* Armigeres (A.) obturbans.

Species.	Per cent positive.	Number of guts examined for oöcyst counts.	Average number of oöcysts per positive gut.	Oöcysts per positive gut examind.				
				-100	101-200	201-300	301-400	400+
Aedes (Stegomyia) ægypti ...	100·0	13	135·46	3	9	...	1	...
Armigeres (Armigeres) obturbans ...	100·0	11	324·81	...	2	4	3	2

All the mosquitoes of both the species were found infected. The average number of oöcysts per gut in infected *Armigeres* (*A.*) *obturbans* was more than twice as high as in *Aedes* (*S.*) *ægypti*.

DISCUSSION AND SUMMARY.

On the basis of gut infections, *Aedes* (*S.*) *vittatus* and *Aedes* (*F.*) *pseudotæniatus* have been found to be less susceptible than the remaining four species, namely *Aedes* (*S.*) *ægypti*, *Aedes* (*S.*) *albopictus*, *Aedes* (*A.*) *jamesi* and *Armigeres* (*A.*) *obturbans*.

Aedes (*A.*) *jamesi* although highly susceptible, has a very restricted distribution. It has so far been recorded from peninsular India and Ceylon and extends as far north and east as Bihar (Barraud, 1934).

Aedes (*S.*) *ægypti* and *Aedes* (*S.*) *albopictus*, the two highly susceptible species, are common practically all over India. They are easy to rear and colonize and feed very readily on man as well as on laboratory animals. The results of comparative feedings have consistently indicated that of these two species, *Aedes* (*S.*) *albopictus* is a more suitable host to *P. gallinaceum* than *Aedes* (*S.*) *ægypti*, both qualitatively and quantitatively.

Armigeres (*A.*) *obturbans* has shown to be the most efficient carrier of *P. gallinaceum*. According to Barraud (*loc. cit.*) it is prevalent in the area extending from Punjab to Assam and Burma, and through peninsular India to Ceylon. It is a very hardy mosquito, easy to colonize. Unlike other *Culex* and *Aedes* mosquitoes, the salivary glands of this species can be easily dissected out almost free from any encumbering tissue. Consequently of all the species tried, *Armigeres* (*A.*) *obturbans* appears to be the most suitable for experimental infections with *P. gallinaceum*.

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COMPARATIVE EXPERIMENTAL INFECTIONS IN *ANOPHELES FLUVIATILIS* AND *ANOPHELES STEPHENSI* (TYPE) WITH *PLASMODIUM FALCIPARUM* WELCH, 1897.

BY

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A. fluviatilis is one of the most efficient vectors in South India where it has been found infected in nature in several places, often with high infectivity rates. Covell (1944) in his review of the malaria vectors of S. E. Asia observed that *A. fluviatilis* comprised at least two biological races. According to him, the race prevalent in South India was strongly anthropophilic while the race in the north and north-east India fed mainly on cattle and was not considered a malaria carrier. Recently, however, *A. fluviatilis* has been found infected in nature in Terai, Nainital District, Uttar Pradesh (Srivastava and Chakrabarti, 1952 ; Issaris *et al.*, 1953). Different antimalaria organisations working since 1947 in Uttar Pradesh Terai had found that *A. minimus*, which according to earlier investigations was the chief vector in that area, had become virtually non-existent and that *A. fluviatilis* and *A. culicifacies* were now responsible for malaria transmission in Uttar Pradesh Terai (Issaris *et al.*, *loc. cit.*).

Although several records of experimental infections relating to *A. stephensi* (type)* have been reported (Iyengar, 1933 ; Strickland *et al.*, 1933; Russell and Mohan, 1939*a* : 1939*b* : 1939*c* : 1940 : 1941), no attempts have apparently so far been made to infect *A. fluviatilis* under laboratory conditions. With a view to determine the comparative susceptibility of *A. fluviatilis* and *A. stephensi* (type) to *Plasmodium falciparum* infections, feeding experiments were carried out under laboratory conditions at the field station at Mettupalayam (Nilgris, South India). Insectary specimens of these two species were used in these investigations.

**A. stephensi* is known to be an important vector under rural conditions in western and north-western India and under urban conditions in certain localities in peninsular northern India (Covell, *loc. cit.*).

The use of insectary *A. stephensi* (type) as the control mosquito to gauge the significance of experimental infections in other mosquitoes in India, was suggested by Russell and Mohan (1939*b*).

The colony of *A. fluviatilis* was established with eggs laid by a few wild-caught females from Kallar, eastern Nilgris, where it is responsible for hyperendemic malaria (Russell and Jacob, 1942). At the time of investigations, it was about four years old and had been maintained as inbred, the females being given rabbit blood feed throughout (Mohan, 1952).

The colony of *A. stephensi* (type) was established in 1938 (Russell and Mohan, 1939*d*) with 25 larvæ collected from house-wells in Madras City. This colony had been throughout inbred as in the case of *A. fluviatilis*. Although these two colonies were fairly old and had throughout subsisted on rabbit blood, the adults showed no apparent deviation in their eagerness to feed on human beings. The *A. stephensi* colony was 17 years old and it is of interest to note that it showed no abatement in its susceptibility to experimental infections. Females selected for the experiments were three to five days old and had occasion to subsist on glucose solution during this period. They were confined in screened wooden rings and applied simultaneously to the gametocyte carriers. Out of these, fully engorged were taken, the two species being kept separate under indentical environmental conditions in cages (6×6×6 inches). Dissections of these specimens were begun a few days afterwards. The results of dissections of three separate lots of both *A. fluviatilis* and *A. stephensi* (type) are presented in Table I.

TABLE I.

Susceptibility of A. fluviatilis *and* A. stephensi (*type*) *to infection with* P. falciparum.

Lot number.	Species.	Gut infections alone			Glands alone or both gut and glands infections			All infections (gut alone and glands or both gut and glands)		
		Number.		Per cent positive.	Number.		Per cent positive.	Number.		Per cent positive.
		Examined	Positive.		Examined.	Positive.		Examined.	Positive.	
1	*A. fluviatilis* ...	11	2	18	28	12	43	39	14	36
2	,, ,, ...	14	4	29	130	51	39	144	55	38
3	,, ,,	30	30	100	39	34	87	69	64	93
1	*A. stephensi* (type)	18	4	22	5	1	20	23	5	22
2	,, ,,	29	13	45	26	7	27	55	20	36
3	,,	26	25	96	36	31	86	62	56	90

Gametocytes : Lot 1, ten crescents per 100 leucocytes : Donor, male, 15 years.
Lot 2, fifteen crescents per 100 leucocytes : Donor, male, 20 years.
Lot 3, twenty-two crescents per 100 leucocytes : Donor, male, 18 years.

An analysis of the results shows that the infection index of the salivary glands was higher in *A. stephensi* (type) in Lot 2 and almost equal in Lot 3. In Lot 1, sporozoite infection in *A. stephensi* (type) was again lower, but the numbers dissected were small. On the basis of gut infections before the sporozoite invasion of the

salivary glands, the index was in favour of *A. stephensi* (type) in Lots 1 and 2 and about equal in Lot 3. If all infections, *viz.*, gut infections alone and glands infections with or without gut infections are combined, both species appear to be about similar in their susceptibility to experimental infection.

Mosquitoes in Lot 3 showed the highest and the heaviest infection. It was thought desirable to study comparative infection in this lot both quantitatively and qualitatively. The latter were made by making counts of oöcyts before the infection of sporozoites in the salivary glands. All the preparations were stained.

The average number of oöcysts per gut was 50·3 in 19 positive *A. fluviatilis* and 60·3 in 16 positive *A. stephensi* (type). The quantitative incidence of infection, that is, the number of oöcysts per positive gut was well scattered in both the species.

Among the factors concerning the susceptibility to the species and strains of plasmodia, the density of gametocytes is important, but is by no means the only factor involved. In the present experiments, the higher content of gametocytes per 100 leucocytes coincided with higher index of infection in both *A. fluviatilis* and *A. stephensi* (type).

SUMMARY.

Comparative susceptibility of *A. fluviatilis* and *A. stephensi* (type) to infections with *plasmodium falciparum* was studied. Both these species were found to be highly susceptible.

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EXPERIMENTAL STUDIES ON REPRODUCTIVE CAPACITY OF *ANOPHELES FLUVIATILIS* AND *ANOPHELES STEPHENSI* (TYPE) AFTER EXPOSURE TO SUBLETHAL DOSES OF D.D.T. IN DIFFERENT STAGES OF GONOTROPHIC CYCLE.

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[March 5, 1956.]

INTRODUCTION.

Since the introduction of D.D.T. for the control of malaria, the study of the effect of different dosages of this insecticide on mosquitoes has been attracting the attention of a number of workers. As a result of intensive studies by different workers all over the world, a number of papers have been published dealing with its residual toxicity, excito-repellent action and the development of resistance amongst the mosquitoes of various species, exposed to lethal and sub-lethal dosages of D.D.T.

In order to study the effect, if any, of exposure to sublethal doses of D.D.T. on the reproductive capacity of *A. fluviatilis* and *A. stephensi* (type) at different stages of the gonotrophic cycle, namely, unfed, blood fed, partially gravid and gravid females, experiments were undertaken under laboratory conditions. During the course of these experiments a few observations on the swarming and mating behaviour of these two species after exposure to D.D.T. were also made.

MATERIALS AND METHODS.

Both males and females of *A. fluviatilis* and *A. stephensi* (type) used in these experiments were taken from the colonies maintained at Mettupalayam (South India).*

*The colony of *A. fluviatilis* was about four years old (Mohan, 1952) and that of *A. stephensi* (type) about 17 years old (Russell and Mohan ,1939).

As there is a definite correlation between fertilization and the maturation of ovaries in both the species, males and females of each species after hatching out, were kept together in the same cage and fed on 10 per cent glucose solution for four to five days, during which period a major proportion of the females of each species became fertilized.*

In the experiments described herein, all mosquitoes were given an opportunity to feed on a rabbit during night and only fully engorged females were selected for the trials. In all the experiments, only a single full blood meal was given.

Exposure of mosquitoes to D.D.T.—Exposure chambers were boxes made of ply wood (about seven inches cube) with total inside surface area of two square feet. The top cover had a central circular aperture for the introduction of mosquitoes. The cover was held in position by rubber bands which were strung across it from small nails fixed at each corner at the base. The boxes were lined on the inside, including the cover, with glazed kraft paper. The entire exposed surface of the paper lining was smeared with 4 c.c. of 0·625 per cent D.D.T. solution in acetone by means of a camel hair brush to give a deposit of 12·5 mg. of D.D.T. per sq. ft. The solution was coloured with Sudan III to facilitate complete and uniform coverage of the entire surface. To prevent evaporation, the solution was kept in a glass stoppered cylinder, care being taken to expose it as little as possible at the time of application.

Each of these exposure chambers was used only for two or three exposures on the same day. The kraft paper was then removed and replaced by a clean one. The exposure boxes smeared with D.D.T. solution were usually prepared one or two days prior to use.

About 15 mosquitoes were picked up in a sucking tube and when four such tubes were ready, the mosquitoes were gently introduced into the exposure chamber. After the required exposure to D.D.T. deposits, mosquitoes of each species were released separately into a bobbinet cage (2 × 2 × 2 feet). From these large cages, the survivors were removed after a few hours and those of different species were kept separately under observation in 6 × 6 × 6 inches cages and dissected as and when required.

In all the experiments mentioned in this paper, the dosage of D.D.T. tried was 12·5 mg. per sq. ft. and time of exposure was five or ten minutes.†

A number of mosquitoes unexposed to D.D.T. were also kept simultaneously under identical conditions for purposes of comparison.

Dissections for the examination of the ovarian stage and of the presence or absence of sperms in spermatheca were carried out and completed as soon as permissible.

*Jaswant Singh and Mohan (1951) have shown that in a majority of *A. fluviatilis* mating precedes blood feed, which is equally true of *A. stephensi* (type) under laboratory conditions (Russell and Mohan, 1939).

†In order to observe the total contact period of the mosquitoes released in an exposure chamber, lower wall of the box was made of cellophane paper. The box was placed in such a way that one could watch the movements of the mosquitoes from below. It was observed that the mosquitoes on getting excited after contact with the D.D.T. deposit, kept dashing against the sides of the box and did not fly in the space enclosed by the walls. In other words, mosquitoes in such a chamber were in contact with D.D.T. deposits practically throughout the period of exposure.

DEVELOPMENT OF OVARIES IN *A. FLUVIATILIS* AND *A. STEPHENSI* (TYPE) WHICH WERE FIRST EXPOSED TO D.D.T. AND THEN GIVEN BLOOD FEED.

Unfed females were exposed to D.D.T. in the late afternoon and then released in the evening into a cage in which a rabbit was kept for the mosquitoes to feed upon during the night. One series* of *A. fluviatilis* and two of *A. stephensi* (type) were exposed to D.D.T. for five minutes and two series each of *A. fluviatilis* and *A. stephensi* (type) were given an exposure of ten minutes. In a separate cage, mosquitoes unexposed to D.D.T. were similarly released for purposes of comparison. The following morning, fully engorged females of each species, exposed and unexposed, were picked out and kept in separate cages. These were allowed to feed on glucose solution until dissection.

In another set of experiments, specimens of each species were divided beforehand into two separate groups, one consisting of virgin females and the other of fertilized females (confirmed by dissection later) and were exposed to D.D.T. Females which were intended to be kept unfertilized were removed into a separate cage soon after emergence.† Others were allowed to remain with the males, which were in a prepondering ratio. Four series of experiments each with virgin and mated *A. stephensi* (type), and three series of experiments each with virgin and mated *A. fluviatilis*, were carried out, the time of exposure to D.D.T. deposit being five minutes. The combined results of dissections of both the sets of (fertilized and unfertilized) *A. stephensi* (type) and *A. fluviatilis*, together with those of the unexposed females, are set out in Table I.

An analysis of the data shows that regardless of whether they were exposed to D.D.T. or not, the fertilized females of both the species showed a significantly higher percentage of mature ovaries than the unfertilized females.

From the above results, a close correlation between fertilization and maturation of ovaries in both *A. fluviatilis* and *A. stephensi* (type) is very apparent. In a relatively small proportion of exposed and unexposed fertilized females, ovaries failed to reach maturity. This is in conformity with the earlier findings of Jaswant Singh and Mohan (1951) who reported that one full blood meal was not adequate for complete ovarian development in all cases of fertilized *A. fluviatilis*.

DEVELOPMENT OF OVARIES IN *A. FLUVIATILIS* AND *A. STEPHENSI* (TYPE) WHICH WERE FIRST BLOOD FED AND THEN EXPOSED TO D.D.T.

Females of both species which became fully engorged overnight, were used for these experiments.

*As the mortality among the unfed females exposed to D.D.T. was very high, a large series had to be exposed so as to get a fair number of survivors for feeding and subsequent maturation of the ovaries.

†Under laboratory conditions, *A. fluviatilis* and *A. stephensi* (type) hatching out towards the evening, have seldom been observed to indulge in any biological activity during that night. This was confirmed in experiments especially designed to throw light on how soon after emergence, do mosquitoes begin to swarm and mate, and females commence to take blood meal.

TABLE I.

Maturation of ovaries in fertilized and unfertilized A. fluviatilis *and* A. stephensi *(type) which were first exposed to D.D.T. (12·5 mg. per sq. ft.) for 5 and 10 minutes, respectively, and then blood fed, together with the results of unexposed controls.*

Species.	Number series combined.	Period of exposure (Minutes)	Exposed to D.D.T. (12·5 mg. per sq. ft.)						Unexposed (Control)					
			Fertilized.			Unfertilized.			Fertilized.			Unfertilized.		
			Number examined.	Ovaries.		Number examined.	Ovaries.		Number examined.	Ovaries.		Number examined.	Ovaries.	
				Mature.	Immature.		Mature.	Immature.		Mature.	Immature.		Mature.	Immature.
A. fluviatilis	4	5	98	77	21	201	34	167	143	124	19	158	41	117
Per cent				78·6	21·4		16·9	83·1		86·7	13·3		26·0	74·0
A. fluviatilis	2	10	34	29	5	29	10	19	52	48	4	27	12	15
Per cent				85·3	14·7		34·5	65·5		92·3	7·7		44·4	55·6
Total			132	106	26	230	44	186	195	172	23	185	53	132
Per cent				80·3	19·7		19·1	80·9		88·2	11·8		28·6	71·4
A. stephensi (Type)	6	5	74	50	24	319	42	277	113	102	11	330	76	254
Per cent				67·6	32·4		13·2	86·8		90·3	9·7		23·0	77·0
A. stephensi (Type)	2	10	19	16	3	11	4	7	45	43	2	32	16	16
Per cent				84·2	15·8		36·4	63·6		95·6	4·4		50·0	50·0
Total			93	66	27	330	46	284	158	145	13	362	92	270
Per cent				71·0	29·0		14·0	86·0		91·8	8·2		25·4	74·6

One batch of *A. fluviatilis* and two batches of *A. stephensi* (type) were exposed to D.D.T. for five minutes and two of *A. fluviatilis* and four of *A. stephensi* (type) for ten minutes each. All exposures were given early in the morning except in the case of one batch of each of the two species in the second series in which the females were exposed to D.D.T. soon after feeding, about midnight. Since the development of the ovaries was about the same in specimens exposed at midnight or in the morning, the data for all the specimens exposed have been combined in Table II.

An analysis of the results clearly shows that exposure to D.D.T. did not seem to inhibit in any way the development of ovaries* in *A. fluviatilis* or in *A. stephensi* (type).

*In all the experiments wherever full development of ovaries was observed, the fertilized females laid viable eggs.

TABLE II.

Maturation of ovaries in A. fluviatilis *and* A. stephensi *(type) which were first blood fed and then exposed to D.D.T. (12·5 mg. per sq. ft.) for 5 and 10 minutes, respectively, together with the results of unexposed controls.*

Species.	Number series combined.	Period of exposure in minutes.	First blood fed and then exposed to DDT. Dosage: 12·5 mg. per sq. ft.						Unexposed (Control).					
			Fertilized.			Unfertilized			Fertilized.			Unfertilized.		
			Number examined.	Ovaries.		Number examined.	Ovaries.		Number examined.	Ovaries.		Number examined.	Ovaries.	
				Mature.	Immature.		Mature.	Immature.		Mature.	Immature.		Mature.	Immature.
A. fluviatilis	1	5	24	19	5	11	6	5	18	16	2	1	1	0
Per cent				79·2	20·8		54·5	45·5		88·9	11·1			
A. fluviatilis	2	10	19	4	15	25	2	23	22	5	17	27	1	26
Per cent				21·0	79·0		8·0	92·0		22·7	77·3		3·7	96·3
A. fluviatilis Total			43	23	20	36	8	28	40	21	19	28	2	26
Per cent				53·5	46·5		22·2	77·8		52·5	47·5		7·1	92·9
A. stephensi (type)	2	5	35	25	10	8	3	5	26	23	3	2	1	1
Per cent				71·4	28·6		37·5	62·5		88·5	11·5			
A. stephensi (type)	4	10	80	62	18	36	13	23	54	46	8	23	8	15
Per cent				77·5	22·5		36·1	63·9		85·2	14·8		34·8	65·2
A. stephensi (type) Total			115	87	28	44	16	28	80	69	11	25	9	16
Per cent				75·7	24·3		36·4	63·6		86·2	14·6		36·0	64·0

DEVELOPMENT OF EGGS IN PARTIALLY GRAVID *A. FLUVIATILIS* AND *A. STEPHENSI* (TYPE) AFTER EXPOSURE TO D.D.T.

A. fluviatilis and *A. stephensi* (type) females which had taken a blood meal and had become half gravid or nearly so were selected for exposure to D.D.T., a number being kept unexposed for comparison. All the females were examined individually and those which showed progressive digestion of the blood meal without the corresponding development of ovaries, were discarded.

In the first series of experiments, one batch each of *A. fluviatilis* and *A. stephensi* (type) was given five minutes exposure to D.D.T. and one batch of *A. stephensi* (type) only ten minutes exposure. The results, together with those of the unexposed batches, are presented in Table III.

TABLE III.

Maturation of ovaries in partially gravid A. fluviatilis *and* A. stephensi (*type*) *after exposure to D.D.T. (12·5 mg. per sq. ft.) for 5 and 10 minutes, respectively, together with the results of unexposed controls.*

Species.	Number series combined.	Period of exposure in minutes.	Partially gravids exposed to D.D.T. Dosage: 12·5 mg. per sq. ft.						Unexposed (Control).					
			Fertilized.			Unfertilized.			Fertilized.			Unfertilized.		
			Number examined.	Ovaries.		Number examined.	Ovaries.		Number examined.	Ovaries.		Number examined.	Ovaries.	
				Mature.	Immature.		Mature.	Immature.		Mature.	Immature.		Mature.	Immature.
A. fluviatilis	1	5	44	39	5	6	6	0	39	34	5	5	5	0
Per cent				88·6	11·4		100·0			87·2	12·8		100·0	
A. fluviatilis Total			44	39	5	6	6	0	39	34	5	5	5	0
Per cent				88·6	11·4		100·0			87·2	12·8		100·0	
A. stephensi (type)	1	5	66	54	12	11	10	1	41	38	3	9	6	3
Per cent				81·8	18·2		90·9	9·1		92·7	7·3		66·7	33·3
A. stephensi (type)	1	10	28	26	2	5	3	2	8	8	0	2	2	0
Per cent				92·9	7·1		60·0	40·0		100·0				
A. stephensi (type) Total			94	80	14	16	13	3	49	46	3	11	8	3
Per cent				85·1	14·9		81·3	18·7		93·9	6·1		72·7	27·3

Note.—Females in which abdomen was collapsing without the corresponding ovarian development, were excluded.

In another series, definitely half gravid females were exposed to D.D.T. for five minutes. In these the abdomens were distended with partially digested blood and developing ovaries occupied four to six segments dorsally and three to four ventrally. All the surviving females, 80 *A. fluviatilis* and 104 *A. stephensi* (type), were found to have become fully gravid.

The results clearly show that once the ovaries have commenced developing and the female has reached nearly the half gravid stage, exposure to D.D.T. does not in any way inhibit the development of the ovaries, and viable eggs are laid.

OVIPOSITION BEHAVIOUR OF *A. FLUVIATILIS* AND *A. STEPHENSI* (TYPE) AFTER EXPOSURE TO D.D.T.

For these experiments, only those females which had taken a blood meal two nights earlier and had become gravid were selected. Two series of experiments with each species were carried out. Females exposed to D.D.T. for five minutes

were released separately into bobbinet cages (2 × 2 × 2 feet). The bottom of each cage was completely covered with a dark cloth. Provision for oviposition was made by keeping inside the cage an earthenware dish, partially filled with water taken from the larval rearing basins. A rabbit was kept in the cage throughout the night so that mosquitoes could feed on it after oviposition. Control experiments were run simultaneously under identical conditions.

A number of females of both the species exposed to D.D.T. behaved normally in that the eggs were deposited on water and after oviposition they fed the same night on rabbit. In the case of others, in spite of the availability of water for oviposition, eggs were dropped at random on the dark cloth. The females kept unexposed to D.D.T. for purposes of comparison oviposited normally. It seems that as a result of exposure to D.D.T., the faculty of selecting a proper place for oviposition is somewhat impaired in a certain number of females or that they are physically incapacitated to oviposit in a normal manner. Observations also showed that a small proportion of the females had taken a blood meal after partial oviposition.

SWARMING AND MATING BY *A. FLUVIATILIS* AND *A. STEPHENSI* (TYPE) AFTER EXPOSURE TO D.D.T.

A. fluviatilis and *A. stephensi* (type) males were separately exposed in the evening to 12·5 mg. D.D.T. per sq. ft. for three minutes and released with normal unfed females of their respective species. No clear-cut swarming was seen but attempts to swarm were apparent. This was presumably due to the incoordinated flight of the males. The males attempted to mate but most frequently unsuccessfully. Some in-coupla pairs were observed and separated after about half to one minute. Russell and Mohan (1939) have reported having observed a live and active *A. stephensi* (type) female coupled to a dead male. Such instances were not rare when males, after exposure to D.D.T., were released with the normal females.

When unfed females were exposed to 12·5 mg. D.D.T. per sq. ft. for five minutes and then placed with normal males* in separate cages, males were seen to swarm and also to mate successfully with the females, though to a somewhat lesser extent.

When both sexes were exposed to D.D.T., the instinct to swarm and mate was apparent but attempts to mate were mostly abortive. When these mosquitoes were fed on glucose solution, the surviving males and females were found to swarm and mate the following evening, particularly if present in fairly large numbers.

DISCUSSION.

Rajindar Pal *et al.* (1952) while dealing with *A. stephensi* (type) at Delhi observed that "an exposure to D.D.T. (12·5 mg./sq. ft.) for ten minutes inhibited the development of eggs in unfed *A. stephensi* (type)." They further

*Russell and Mohan (1939) many times observed two undoubted *A. stephensi* males in what appeared to be attempts to mate. Similar attempts on the part of *A. fluviatilis* males have also been observed.

observed, "Fully fed mosquitoes exposed to D.D.T. for five minutes and afterwards kept under normal conditions, also failed to lay eggs, whereas completely gravid mosquitoes laid viable eggs after exposure to D.D.T. deposits for five minutes". The experiments carried out at Mettupalayam, South India, as described above, do not conform to their findings except with regard to the laying of viable eggs by completely gravid mosquitoes after exposure to D.D.T. deposits for five minutes. The complete failure of development of the ovaries in the experiments at Delhi, however, might have been due to the fact that the *A. stephensi* females used were probably unfertilized.

The correlation between fertilization and maturation of ovaries has also been referred to by Muirhead Thomson (1951). He has further referred to the far-reaching secondary physiological effects of fertilization. Virgin females may continue to feed without their ovaries developing. This phenomenon is of great importance in the transmission of malaria. He also observed that a single blood meal might be sufficient for the maturation of ovaries in virgin *A. stephensi* (var.?) but not in virgin *A. minimus*. The experiments described in this paper, however, indicate that usually both virgin *A. stephensi* (type) and virgin *A. fluviatilis* behave the same way as recorded by Muirhead Thomson (1951) for *A. minimus*.

Observations on swarming and mating of *A. stephensi* (type) and *A. fluviatilis* with both sexes or either sex exposed to sublethal doses of D.D.T., indicate that while partially paralysed males with incoordinated movements could not swarm or dance, they did attempt to mate with the normal females, some successfully. Normal males inseminated partially paralysed females. When both sexes were partially paralysed, there was no swarming, and mating activity was considerably curbed during that evening.

SUMMARY AND CONCLUSIONS.

1. Experiments with *A. fluviatilis* and *A. stephensi* (type) after exposure to sublethal doses of D.D.T. in different stages of the gonotrophic cycle, namely, unfed, blood fed, partially gravid and gravid females, have been described.

2. A single sublethal contact with D.D.T. deposits was found to have had no effect on the development of eggs when the females were (*i*) first exposed to D.D.T. and then blood-fed, (*ii*) first blood fed and then exposed to D.D.T. and (*iii*) exposed to D.D.T. as half gravids.

3. There was close correlation between fertilization and maturation of ovaries irrespective of the exposure to D.D.T. Fertilized females showed a significantly higher percentage of mature ovaries than the unfertilized females.

4. Exposure to D.D.T. affected a fair number of gravid *A. fluviatilis* and *A. stephensi* (type) in that eggs were laid at random although suitable water for oviposition was available.

5. Males of *A. fluviatilis* and *A. stephensi* (type), exposed to sublethal dosage of D.D.T. at dusk, could not swarm or dance, but attempted and succeeded to mate with the normal females of their own kind in some instances; mating did not appear to be significantly affected between normal males and females exposed to D.D.T. When both the sexes were partially paralysed, swarming and mating activity was

observed the following evening, particularly, if the surviving mosquitoes were in fairly sufficient numbers.

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OBSERVATIONS ON A NATURAL (CRYPTIC) INFECTION OF TRYPANOSOMES IN SPARROWS (*PASSER DOMESTICUS* LINNAEUS).

Part I.

Susceptibility of birds and mammals to the trypanosomes.

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[March 2, 1955.]

THE presence of trypanosomes and sporozoites of *Plasmodium relictum* in the salivary glands of a laboratory bred *Culex fatigans* fed on a house-sparrow, was reported by Jaswant Singh, Ramakrishnan and David (1950). Examination of the blood film from the sparrow did not reveal any trypanosomes. Accidental contamination of the laboratory reagents was excluded. The authors concluded that mosquito infection of the trypanosomes, in all probability, was derived from the sparrow. Attempts to maintain the strain of trypanosomes for further study, by inoculating a pigeon, proved unsuccessful.

On March 29, 1954, one of the authors (A.D.) once again observed trypanosomes under identical conditions described above in a laboratory bred *C. fatigans* fed on a sparrow with a patent natural infection of *P. relictum*. The strain has since been successfully maintained to date in *C. fatigans* mosquitoes fed on birds inoculated with the trypanosomes. This paper describes the results of inoculation of trypanosomes from mosquitoes to different kinds of birds and a few mammals.

MATERIALS AND METHODS.

House-sparrows, nightingales (*Molpastes cafer* and *M. leucogenys*), black-headed mynas (*Temenuchus pagodarum*), seven sisters (*Crateropus canorus*), crows (*Corvus splendens*), red munias (*Amandava amandava*), fowls (white leghorn), parrots (*Palaeornis torquatus*), pigeons (*Columba livia Gmelin*), quails (*Coturnix coturnix*), grey

partridges (*Francolinus pondicerianus*), chicks, mammals like monkeys (*M. mulatta mulatta*) and albino rats (*Mus norvegiens albinus*), were used in these experiments. All the birds, except chicks and fowls, were wild-caught and obtained from the local bird market. The rats and chicks were obtained from the reared stock in the Institute and the fowls from the local Government Poultry Farm. Monkeys were trapped from the surrounding areas of Delhi. The blood smears of all these animals were examined on three consecutive days before actual use for the susceptibility test and all of them proved negative for trypanosomes. In addition, prior to inoculation with trypanosomes, sufficiently large number (not less than 50 per animal/bird) of laboratory bred *C. fatigans* were fed on them and dissected six to ten days later. None of the above mosquitoes showed any form of trypanosomes—a fact which was taken to mean freedom of these animals from natural (cryptic) trypanosome infection.

The developmental forms of the parasite (trypanosome), maintained in *C. fatigans* by feeding them on sparrows known to have the infection, were inoculated in heavy doses to these animals either intravenously or intraperitoneally or subcutaneously or a combination of these routes. Thereafter, laboratory-bred *C. fatigans* were fed on them at varying intervals commencing from 12 hours after inoculation up to over two months. These, after feeding, were kept in the insectarium under controlled conditions of temperature (20 to 25° C.) and humidity (70 to 90 per cent) and dissected one and a half to 104 days later. If developmental forms of trypanosomes could be traced in these mosquitoes either in gut or gland or both, the particular animal on which they were fed, was considered as a susceptible host to this strain of the parasite.

RESULTS.

Infection was detected in mosquitoes fed on sparrows, nightingales, crows, parrots, pigeons, seven sisters, black-headed mynas, quails, chicks, fowls, red munias, grey partridges, monkeys, and albino rats all of which were previously inoculated with trypanosomes from mosquitoes. The rate of gut infection in the lots of mosquitoes fed on different birds and animals, varied from the least rate of 0·7 per cent to the highest rate of 74 per cent in those fed on grey partridges and parrots, respectively. The details are given in Table I.

DISCUSSION.

An important limitation experienced during the course of these experiments was the complete absence of the blood forms of trypanosomes in infected birds on microscopic examination, even though they were able to impart infection to a great majority of mosquitoes. On this account, the susceptibility of the different vertebrate hosts had to be judged by the presence of developmental forms in mosquitoes. In this respect, this strain of trypanosomes resembles *T. noctuae* of the little owl (Minchin and Woodcock, 1911) and *T. fringillinarum* of the chaffinch (Woodcock, 1910), because in these cases also the parasites were not patent in the blood.

TABLE I.

Susceptibility of various birds and mammals to a trypanosome species of avian origin.

Name of animal.	Number of animals used.	Route of inoculation.	Lapse between infecting the bird and feeding mosquitoes on it. (Days)	Lapse between the blood feed and dissection of mosquitoes. (Days)	Number dissected.	Percentage positive.		
						Gut.	Gland.	Total.
House-sparrows.	2	I.P.	½-1	4–19	145	0·7	*Nil*	0·7
	9	I.P., I.V. & S.C.	1–5	2–46	338	15·0	*Nil*	15·0
	11	I.P. & I.V.	6–10	1½–76	480	25·0	1·6	26·6
	4	I,P., I.V. & S. C.	11–20	4–49	194	16·5	0·5	17·0
	3	I.P. & I.V.	21–60	9–52	164	6·7	0·6	7·3
Nightingales	5	I.P. & I.V.	1–10	3–40	218	4·0	2·5	6·5
	3	I.P. & I.V.	11–20	2–22	60	30·0	10·0	40·0
	3	I.P.	21–30	3–45	97	35·0	4·0	39·0
	5	I.P.	31–67	6–44	178	13·5	*Nil*	13·5
Crows	2	I.P.	5–10	8–33	114	7·0	*Nil*	7·0
Seven sisters	2	I.P.	27	5–29	100	8·0	1·0	9·0
Black-headed mynas	2	I.P.	5–7	4–11	50	54·0	*Nil*	54·0
Domestic fowls	2	I.V.	4–5	2–5	65	4·5	*Nil*	4·5
Red munias	2	S.C.	13	11–16	66	*Nil*	1·5	1·5
Parrots	1	I.P.	2–5	5–40	50	74·0	*Nil*	74·0
Pigeon	1	I.P., I.V. & S.C.	6–68	7–10	60	15·0	*Nil*	15·0
Quail	1	I.P. & I.V.	2–3	2–26	70	24·3	*Nil*	24·3
Chick	1	I.V.	4–5	7–10	90	4·4	*Nil*	4·4
Grey partridge	2	I.P.	3–6	5–26	288	0·7	*Nil*	0·7
Monkey	1	I.V.	2–40	5–13	36	11·0	*Nil*	11·0
Albino rat	2	I.P.	4–10	7–14	143	3·5	*Nil*	3·5

I.P.=Intra-peritoneal, I.V.=Intra-venous, S C.=Sub-cutaneous.

Although all the animals used in the experiments proved to be susceptible to trypanosomes, yet the degree of susceptibility varied greatly in terms of the percentage of mosquitoes that became positive after feeding on infected animals.

Of all the animals used, parrots proved to be the most susceptible, then black-headed mynas, nightingales, quails, sparrows, etc. Red munias, on the other hand, were only very slightly susceptible.

The wide range of susceptibility of this strain of trypanosomes to various species of birds locally available in Delhi, indicates the possibility that there may be several hosts harbouring the natural infection. Similar observations were made by Thiroux (1905) while working with *T. paddae*. He showed that this trypanosome was inoculable to other birds, such as, *Serinus serinus*, *S. canarius*, *Lagonosticta minima*, *moriposa phoenicotis* and *Estrilda cinerea* (Wenyon, 1926).

It has been determined that the infected birds are able to impart infection to *C. fatigans* fed on them as early as 12 hours after they have been inoculated with the trypanosomes. The period over which the birds harbour the infection, has not been worked out fully, but from the results so far obtained it can be safely said that the infection can last for at least over two months.

The complete life cycle of the parasite in mosquito is under detailed study. In the meantime, it can be pointed out that the forms found in the invertebrate host as early as two and a half days after the infective feed, are capable of infecting other normal vertebrate hosts, and that when once the mosquito is infected the infection remains, perhaps, throughout the life of the insect because in the authors' experiments trypanosomes could be found in mosquitoes dissected as late as 104 days.

SUMMARY.

Trypanosomes in the salivary glands of *C. fatigans* fed on house-sparrows, were observed, for the second time, in the laboratories of the Malaria Institute of India. The susceptibility of birds such as nightingales, parrots, crows, pigeons, seven sisters, black-headed mynas, quails, fowls, chicks, red munias, grey partridges, and mammals such as monkeys and rats, to this strain of trypanosome, was determined. As the trypanosomes were not to be seen in the blood of any animal, the criterion followed in all the cases was to look for the developmental forms in the mosquitoes after they were fed on the respective infected hosts.

All the animals proved to be capable of taking up the infection but among these, parrot appeared to be most susceptible, and grey partridges the least.

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STUDIES ON NURI STRAIN OF *P. KNOWLESI*.

Part VI.

Some observations on hæmatology and temperature reaction in blood-induced infection.

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THE morphology, periodicity and virulence of Nuri strain of *P. knowlesi* (Jaswant Singh, Ray and Nair, 1953 ; Edison and Davey, 1953) have been reported by Jaswant Singh, Nair and Ray (1954*a* : 1954*b*). In this paper, observations are recorded on the red and white blood cell counts, hæmoglobin content, reaction of red cells in hypotonic saline solution (fragility test) and anal and axillary temperatures, of *M. mulatta mulatta* monkeys infected with this strain.

METHODS AND MATERIALS.

A total of 29 *S. rhesus* monkeys belonging to both sexes and weighing between 3·2 and 8·2 kg. body weight were utilized for these studies. After initial studies on total erythrocyte and leucocyte counts, estimation of hæmoglobin, etc., the animals were inoculated intravenously with *P. knowlesi*. The inoculum used in all the cases was five million parasitized cells per kg. body weight of the animal. The investigations mentioned above were continued once a day during the prepatent periods and thereafter until death. The observations were made generally between 11 a.m. and 4 p.m. (between two principal feeds). In some cases, to avoid emotional disturbances, the animals were anæsthetized before drawing the blood and recording the body temperature. Intravenous kemithal (Jaswant Singh, Nair and Ray, 1952) was used for the purpose. In the rest of the cases, they were caught gently without exciting them too much.

Blood smears were collected from the monkeys both in the morning and evening, and the daily average parasite density estimated during the entire period of observation. The parasites were counted in relation to 10,000 R.B.C., and for this Ehrlick's eyepiece was used.

For total erythrocyte and leucocyte counts, the usual standard procedures were adopted.

Hæmoglobin was estimated using sahli hæmometer and the values were expressed in terms of grammes of hæmoglobin per 100 c.c. of blood.

The qualitative method for testing red cell fragility, as described by Napier and Das Gupta (1942), was adopted. For this, one or two drops of blood were added to small test tubes, containing saline at various dilution. After shaking the tubes, they were centrifuged for about 10 minutes and readings taken. The highest concentration of saline showing any red or pink contour on the supernatant, was taken as beginning of hæmolysis and the highest concentration showing no cells was recorded as complete hæmolysis.

Anal and axillary temperatures were taken every day and these were recorded in Fahrenheit scale.

The data obtained from the above investigations were subjected to statistical analysis and compared to the initial findings.

RESULTS.

The mean values of the different observations made in the monkeys prior to inoculation, during prepatent and patent periods, are given in Tables I and II.

The daily average parasite count reached from $41{\cdot}1 \pm 4{\cdot}9$ per 10,000 erythrocytes on the first day to $5{,}360 \pm 210$ on the last day of infection, i.e., on the fifth day.

Normal values of the erythrocyte counts were found to be $4{\cdot}63 \pm 0{\cdot}15$ (range 3 to 6·3) million per c.mm. of blood. This count gradually decreased to $2{\cdot}8 \pm 0{\cdot}33$ million on the last day of infection (fifth day). The gradual decline in the total erythrocytic count noted on the different days of the infection as compared to that prior to inoculation was found to be statistically significant according to Fisher's '*t*' test. However, the slight decline in R.B.C. count noted during the prepatent period, was not insignificant.

Normal mean hæmoglobin value of the monkeys was $14{\cdot}35 \pm 0{\cdot}36$ gm. per 100 c.c. blood. There was no appreciable change in this figure during the prepatent period but subsequently as the infection increased progressively each day, this value gradually decreased and finally on the last day of infection it was found to be only $8{\cdot}5 \pm 1{\cdot}22$. This decline was also statistically significant at every stage, similar to the R.B.C. count mentioned above.

The colour index calculated from the mean values of R.B.C. and hæmoglobin before inoculation, was 1·083. The same during the prepatent period was 1·053 and during the last day of infection 1·047.

Normal white cell count showed an average of $4{,}629 \pm 357$ (range 2,080 to 8,200) per c.mm. of blood. A leucopenia to the order of $3{,}500 \pm 388$ to $4{,}266 \pm 469$

TABLE I.

Hæmatological observations in monkeys infected with P. knowlesi *(nuri strain).*

Period.	Daily average parasite count per 10,000 R.B.C. (mean)	R. B. C.		HÆMOGLOBIN:		W. B. C.		FRAGILITY:			
								Beginning.		Complete.	
		Number of observations.	Millions per c.mm. (mean)	Number of observations.	Grammes per 100 c.c. (mean)	Number of observations.	Thousands per c.mm. (mean)	Number of observations.	Percentage sodium chloride (mean)	Number of observations.	Percentage sodium chloride (mean)
1	2	3	4	5	6	7	8	9	10	11	12
Preinoculation	...	29	4·63 ±0·155	28	14·53 ±0·36	24	4·829±0·357	23	0·44 ±0·0065	23	0·35 ±0·009
Prepatent ...	...	12	4·45 ±0·25	14	13·6 ±0·414	11	3·66 ±0·423	11	0·416±0·018	11	0·344 ±0·021
Infection											
First day ...	41·125±4·9	20	*4·052±0·171	20	*13·395±0·24	18	3·94 ±0·344	16	0·426±0·011	16	0·3612±0·0098
Second day	393±80	21	*3·76 ±0·163	20	*12·68 ±0·283	18	3·954±0·52	16	0·435±0·009	16	0·358 ±0·015
Third day ...	1,523±66	19	*3·43 ±0·19	19	*11·836±0·482	17	3·3 ±0·388	15	0·43 ±0·014	15	0·35 ±0·015
Fourth day	4,290±395	12	*3·30 ±0·24	12	*10·58 ±0·4	12	*4·266±0·464	10	0·44 ±0·0158	10	0·35 ±0·021
Fifth day ...	5,360±216	4	*2·8 ±0·33	4	* 8·50 ±1·22	3	8·89 ±5·56	2	0·43 ±0·01	2	0·36

*Difference from the mean values for the preinoculation is statistically significant according to Fisher's "t" test.

TABLE II.

Temperature reaction in monkeys infected with P. knowlesi (*nuri strain*).

Period.	Daily average parasite count per 10,000 R.B.C. (mean.)	Axillary temperature.		Rectal temperature.		Difference between the mean axillary and rectal temperatures. °F.
		Number of observations.	°F.	Number of observations.	°F.	
Preinoculation ...	...	30	102·34±0·34	26	103·6 ±0·31	*1·32
Prepatent	...	15	102·27±0·351	20	102·987±0·436	0·717
Infection						
First day ...	41·125±4·9	22	101·6 ±0·362	20	103·019±0·47	*1·42
Second day ...	393±80	24	102·22±0·065	21	103·679±0·412	*1·5
Third day ...	1,523±66	23	101·8 ±0·44	21	103·8 ±0·36	*1·4
Fourth day ...	4,290±395	14	102·2 ±0·56	15	103·2 ±0·39	*1·7
Fifth day ...	5,360±216	3	102·2 ±1·095	3	103·661±0·875	1·7

* Difference is statistically significant according to Fisher's "*t*" test.

occurred during the prepatent and first four days of the disease period. But this reduction in leucocyte count, excepting on the fourth day of parasitæmia, was not, however, statistically significant when compared with the pre-inoculation figure. On the fifth day of infection, an apparent increase in leucocyte count amounting to 8890 ±5,560 was observed but this again was not found significant when subjected to statistical tests.

Normal fragility in the monkeys under study was indicated by hæmolysis beginning at 0·44 ± 0·0065 per cent. During the prepatent and disease periods, no commencement of hæmolysis at a concentration greater than 0·44 per cent was observed but complete hæmolysis occurred at 0·3612 ± 0·0098 on the first day of infection and 0·36 per cent on the last day but none of these changes was statistically significant.

Normal axilla and anus temperature, prior to inoculation, was 102·34 ± 0·34 and 103·6 ± 0·31, respectively. There was no definite increase in the two temperatures either during the prepatent or the infection period. In all cases there was slight increase in the mean anal temperature as compared to that taken in the axilla ranging from 0·7 to 1·7 °F. This difference was found to be rather real than due to any chance.

DISCUSSION.

A progressive and rapid reduction in R.B.C. count and hæmoglobin concentration was observed with the daily increase in parasite counts in untreated *P. knowlesi* (Nuri strain) infection. On the last day of infection, there was a mean reduction of about 40 and 41 per cent of R.B.C. and hæmoglobin, respectively. Working with the same strain, Ray (1953) had observed that the reduction of hæmoglobin was by 46 per cent. Observations by Maegraith (1948) and others show that the anæmia produced with this infection, is similar to that seen in respect of the strain isolated by Sinton and Mulligan (1932) prior to its loss of virulence

(Jaswant Singh *et al.*, 1949). The colour index, both during the preinoculation and the infection period, remained slightly higher than one (1·05 to 1·19). A similar observation of the colour index, slightly higher than one, has been recorded by Malamos (1934) at the terminal stage of fatal *P. knowlesi* infection. The anæmia, observed in the present studies during acute infection, would thus appear to be more of a megaloblastic type. Such megaloblastic anæmia in malaria with colour index greater than one, has also been reported especially during acute malignant tertian infection (Bianchi, 1940 ; Schretzenmayr, 1938 ; Seelig and Hemming, 1944). It is even said that there is some similarity between pernicious anæmia and malarial anæmia but according to Maegraith (*loc. cit.*) this similarity is only a superficial one. From the hæmatological studies on the Nuri strain, Ray (1954) had observed that though during the early phase of the infection the M.C.V. was slightly lower than normal, during the terminal stage it was somewhat higher. In other words, at this stage there was a tendency towards macrocytosis. The same worker made similar observations in respect of fatal infection due to *P. berghei* in white mice. According to Wintrobe (1951), anæmia is usually normocytic in malaria but " when blood destruction is very rapid it may become macrocytic ".

Though in many cases, it could not be proved statistically, a slight but constant leucopenia, both before the appearance of parasites in the peripheral blood and during the first four days of infection, was evident. On the last day of infection, a leucocytosis in the mean count is apparent but on account of the smallness of the observations, the very high figure of standard error of the mean and lack of statistical significance, this particular data is not considered adequate for discussion here. Many workers have observed a mild leucopenia in ordinary malarial infection in man (Maegraith, *loc. cit.*), similar to the present investigations. In monkeys infected with *P. knowlesi* (original strain), Malamos (*loc. cit.*) recorded a mild leucocytosis shortly after experimental infection but soon it was followed by leucopenia and return to normal value after treatment. Kehar (1936) while dealing with a similar observation in monkeys, observed leucocytosis during the incubation and disease period, and a fall in this number when the number of parasites increased considerably. In *P. berghei* infection in albino rats, Ramakrishnan *et al.* (1953) showed a mild leucocytosis in acute stage of the infection and early latency. Thus it would appear that no uniform standard can be laid down with regard to changes in leucocyte count during malarial infections.

Normal fragility in healthy monkeys, as judged by the pre-inoculation figures, indicated hæmolysis beginning at a mean concentration of 0·44 ±0·0065 per cent and complete at 0·35 ±0·009 per cent sodium chloride. There was no proof to show any alteration in saline fragility during infection with *P. knowlesi* (Nuri strain). This indicates that hæmoglobinuria, which frequently occurs as a terminal event in *M. mulatta mulatta* infected with *P. knowlesi*, is not due to increased fragility of the non-parasitized erythrocytes but perhaps mainly due to the excessive destruction of erythrocytes by plasmodia as suggested by Rigdon and Quattlebaum (1950).

Normal axillary temperature of monkeys varied between 98·6 and 105·8° F. and anal temperature between 100·4 and 107·6° F. There was definite increase in anal temperature by 0·7 to 1·7° F. as compared with the axillary temperature and the same in majority of cases was statistically significant. Such differences between normal, oral and rectal readings (0·8° F.—Thomson, 1953; 1·8° F.—Selle,

1953) occur in human beings also. A comparison of the pre-inoculation anal and axillary temperature with the post-inoculation figures indicated no correlation whatsoever between temperature and severity of malarial infections in monkeys. This is in complete agreement with the findings of Kehar (*loc. cit.*).

SUMMARY.

Total R.B.C. and W.B.C. counts, hæmoglobin concentration, saline fragility of cells, and body temperature were studied in a total of 29 *S. rhesus* monkeys during normal conditions and also during prepatent and infection periods following intravenous inoculation of blood forms of *P. knowlesi* (Nuri strain) parasites.

Marked reduction of R.B.C. and hæmoglobin concentration occurred during the infection.

Leucocyte counts revealed a mild leucopenia throughout the course of infection.

There was no real change in saline fragility at any time during the observation period.

Body temperature was not found to be of any use to indicate the severity or otherwise of malarial infection in these animals.

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STUDIES ON NURI STRAIN OF *P. KNOWLESI*.

Part VII.

Comparative efficacy of the active metabolite and the precursor (M. 3349) of proguanil.

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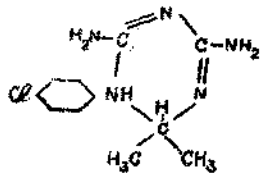
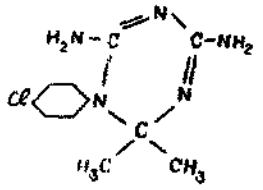
[March 7, 1955.]

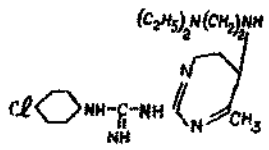
FOLLOWING the observations that proguanil (I), though active *in vivo*, was inactive *in vitro* when tested against *P. gallinaceum* (Hawking, 1947 ; Hawking and Perry, 1948) and *P. cynomolgi* (Madinaveitia and Raventos, 1949), the question of the metabolic fate of the drug interested many workers in the field of malaria chemotherapy. This ultimately led to the isolation by Carrington *et al.* (1951) and Crowther and Levi (1953) of the active metabolite of proguanil (II) from the urine of rabbit and man receiving proguanil. Preliminary studies of this compound against avian and rodent malarias showed high degree of activity (Jaswant Singh *et al.*, 1954 ; Krishnaswami *et al.*, 1953). In this paper, the results are recorded of the testing of this drug against *P. knowlesi* (Nuri strain) which is highly pathogenic to *M. mulatta mulatta*, and the same is compared to the activity of M. 3349 (III), one of the precursors of proguanil. The structural similarity of these compounds (II and III) to proguanil (I), is represented below : —



Proguanil (I) (N¹-p-chlorophenyl-N⁵-isopropyl-biguanide).



Active Proguanil Metabolite (II) 1-p-chlorophenyl-2:4-diamino-1:6-dihydro-6:6-dimethyl-1:3:5-triazine.



M 3349 (III) 2-p-chlorophenyl-guanidino-4-β-diethylamine-ethyl-mino-6-methyl-pyrimidine.

MATERIALS AND METHODS.

The details of the methods followed for the testing of the two drugs were on the same lines as already described by Nair *et al.* (1953). *M. mulatta mulatta* monkeys used for the experiments were young and healthy (weighing between 2·5 and 5·0 kg.) and negative for tuberculin test (Nair and Ray, 1954). These animals were inoculated intravenously with five million parasitized erythrocytes per kg. body weight. Blood smears were collected both in the morning and evening, stained with J.S.B. (Jaswant Singh and Bhattacharji, 1944) and examined for malaria parasites. Drug in suitable concentrations in liquid form (solution or suspension) was administered orally for seven days in single daily doses when the density of parasites was 0·1 to 1·0 per cent cell infection. Different dosage schedules were tried and all of them were in terms of the base of the drug per kg. body weight. The minimum dose that was effective in clearing parasites completely from the peripheral blood by the day following the cessation of treatment, was taken to be the minimum affective dose—Class II effect of Shannon (Wiselogle, 1946). If a monkey did not relapse for a period of one month after the cessation of treatment, splenectomy was performed. If subsequently, no parasites appeared in the peripheral blood for a period of another month, it was assumed that the drug was effective in sterilization of blood-induced infection—Class III effect of Shannon (Wiselogle *loc. cit.*). Likewise, if the drug could only bring about a significant deceleration of the parasites with the prolongation of the survival period of animals for at least three days after the cessation of treatment, the activity was considered as Class I effect of Shannon. On the other hand, dosages that were insufficient to control the infection and to prevent death of the animal during treatment period, were recorded as inactive (ineffective).

RESULTS.

In the trials with M. 3349, the dosages used ranged from 1·0 mg. to 25·0 mg. per kg. body weight of the monkey (Table I). Out of these, no appreciable difference could be observed in the results obtained with the different regimes upto 20 mg./kg., as even with the highest dose, there was no effect in one of the monkeys tried. In comparison, 25·0 mg. produced Class II effect

in three out of five monkeys. Of these, one died before and another immediately after the completion of drug administration, and the third relapsed ten days after the cessation of treatment. Regarding the remaining two monkeys, one succumbed during treatment before parasites could disappear from the peripheral blood and the other showed Class I effect. Death in all the cases was probably due to toxic effects of the drug. In those monkeys in which the clearance of parasites was obtained during the course of treatment, the speed of action of the drug had no direct correlation with the dosage. Parasites disappeared, in general, from the peripheral blood of these monkeys within 48 to 144 hours, the average being 97·5 hours. Relapses in all these cases occurred within two to twelve days.

TABLE I.

Effect of M. 3349 against P. knowlesi (*Nuri strain*).

Dosage mg./kg. (base) body weight.	Number of monkeys employed.	Effect								Remarks.
		Ineffective.	Class I	Class II				Class III		
						Relapse.				
		Number.	Number.	Number.	Clearance of parasites (hours).	Number.	Interval (days)§	Number.	Clearance of parasites (hours).	
1·0	2	1	1	...	...	...	...	...	...	
2·0	2	1	...	1	60	1	2	...	...	
5·0	3	2	1*	...	...	...	...	...	...	
7·5	2	2	...	...	..	...	...	...	..	
10·0	4	2	1	1	108	1	5	...	..	
15·0	4	1	1	2	48, 144	2	7, 12	...	...	
20·0	4	1	2	1	96	1	4	...	...	
25·0	5	...	1†	3‡	84, 96, 144	1	10	...	...	One died due to toxic effect during treatment.

*Parasites were cleared initially within 72 hours but reappeared on the last day of drug administration.

†Parasites cleared from the peripheral blood initially within 60 hours, but reappeared 24 hours later.

‡Two died, one before and another after the cessation of treatment (toxic effect).

§From the cessation of treatment.

Dosages ranging from 0·2 to 35·0 mg. were employed in the case of the active metabolite of proguanil for which a total of 54 monkeys were utilized. As could be seen from Table II, the results obtained with dosages up to 5·0 mg. were very poor when compared to those obtained with higher doses. Out of the 39 monkeys utilized for testing the lower doses, the drug proved ineffective in 26 animals. On the other hand, dosages from 10·0 mg. upwards were effective in producing either

TABLE II.

Effect of the active metabolite of Proguanil against P. knowlesi (*Nuri strain*).

Dosage mg./kg. body weight (base).	Number of monkeys employed.	Effect.								Remarks.
		Infective.	Class I.	Class II.				Class III.		
						Relapse.				
		Number.	Number.	Number.	Clearance of parasites (hours).	Number.	Interval (days).	Number.	Clearance of parasites (hours).	
0·2	2	1	...	1	96	1	3	...	...	
0·25	2	2	...	...	...	...	...	...	...	
0·3	3	...	1	1	72	1	4	1	72	
0·325	2	1	...	1	48	1	5	...	...	
0·35	2	2	...	...	...	...	...	...	...	
0·375	2	2	...	...	...	...	...	...	...	
0·4	3	2	...	1	48	1	2	...	...	
0·425	2	2	...	...	...	...	...	...	...	
0·5	3	1	1	1*	84	...	...	...	...	*Died during observation.
0·6	3	3	...	...	...	...	...	...	...	
1·0	3	1	1	1	132	1	5	...	...	
1·5	3	...	1	2	60, 84	2, 5	...	...	...	
2·0	3	3	...	...	...	...	...	...	...	
2·5	3	3	...	...	...	...	...	...	...	
5·0	3	3	...	...	...	...	...	...	...	
10·0	2	...	1	1†	60	1	1	...	...	†Indication of resistance and reappearance of parasite during treatment.
15·0	2	...	1	1	168	1	3	...	...	
20·0	2	...	1‡	1	96	1	1	...	...	‡Parasites disappeared in 72 hours but reappeared 48 hours later.
25·0	2	...	1	1	132	1	...	...	...	
30·0	2	...	2§	...	...	...	...	...	...	§Initial disappearance of parasites in both within 60 hours and reappearance before the cessation of treatment.
35·0	5	...	...	5¶	60, 60, 84, 84, 96	4	3	...	...	¶Died during drug administration after the clearance of parasite.

Class I or II effect. In some cases, the parasites disappeared initially in the early stages of drug administration but they reappeared before the completion of treatment. Thus there was mere deceleration but not complete clearance. The lowest dose that was found effective in producing a Class II effect in all the monkeys tested, was 35·0 mg. This dosage, therefore, is taken as the M.E.D. of the drug. The speed of action of the drug, as in the case of M. 3349, was not accelerated with the increase in dosage. Parasites, in general, disappeared within 48 to 132 hours with an average of 86·1 hours. All the monkeys that responded well to this drug, relapsed within a period of one to eleven days after the cessation of treatment irrespective of the dosage used.

DISCUSSION.

Minimum effective doses (M.E.D.) of quinine and proguanil to effect Class II effect, against Nuri strain of *P. knowlesi*, have been recorded by Nair, Ray and Jaswant Singh (1953) as 30·0 and 0·2 mg. of the base per kg. body weight of the monkey, respectively. In the present investigation, it was found that the M.E.D. of the active metabolite of proguanil was 35·0 mg. Death occurred, in one monkey that received 35.0 mg. of the proguanil metabolite, after the actual clearance of parasites from the peripheral blood but it was not possible to ascertain whether this was due to any intercurrent disease or toxic effect of the drug. In the case of M. 3349, even with a dosage of 25·0 mg., which appeared toxic to many of the monkeys, Class II effect was not produced in all.

The results obtained indicate that the active metabolite of proguanil is 175 times less active than the parent drug proguanil, and 0·86 times as active as quinine against Nuri strain of *P. knowlesi*. With regard to M. 3349, it is even less effective than proguanil metabolite and in addition more toxic.

Proguanil metabolite (II) demonstrated a very flat dose response curve. A similar defect was observed in the case of M. 3349 (III) also. These two compounds, therefore, had to be tested with a large number of dosage regimes. This fact, more than anything else, establishes the desirability of having large samples in the initial chemotherapeutic trials in animals and men.

Another peculiarity applicable to both the drugs, though more evident in the case of proguanil active metabolite, was the initial clearance and subsequent reappearance of parasite during the treatment period in some of the monkeys receiving the drug in doses approximating to their minimal effective doses. The question of correlating this feature with the potentiality of acquiring resistance by the parasites, had already been discussed by Nair, Ray and Jaswant Singh (1953).

The therapeutic properties of the active metabolite of proguanil vary with the particular host and the species of the parasite against which it is used. For example, this drug was reported to be 16 times as active as proguanil when tested against *P. gallinaceum* in chicks (Jaswant Singh *et al.*, 1954) and six times as active as quinine when tested against *P. lophuræ* (Modest *et al.*, 1952). Krishnaswami *et al.* (1953) observed that the drug on the basis of the dosage required to produce Class I effect against *P. berghei*, was just the same as proguanil in activity. Its effect against simian malaria, *P. cynomolgi*, was much less, being only half to one quarter as active as the parent drug (Schmidt *et al.*, 1952). The findings of the

present investigation indicate that against Nuri strain of *P. knowlesi*, the parent drug (proguanil) is 175 times as active as its active metabolite. Thus it becomes apparent that however effective the latter drug (II) may appear to be against avian and rodent plasmodia, its action against simian plasmodia is extremely tardy. It will be interesting to know in this connection the degree of effectiveness of the active metabolite of proguanil against human infection and to learn whether human malaria behaves like avian or simian infection with regard to its response to antimalarial treatment.

SUMMARY.

M. 3349 (the precursor of proguanil) and the active metabolite of proguanil, have been tested against the Nuri strain of *P. knowlesi* using 80 monkeys.

While the M.E.D. of the active metabolite of proguanil (II) was worked out as 35·0 mg. of the base per kg. body weight of the animal, the M.E.D. of M. 3349 (III) could not be exactly estimated due to the extremely tardy effect of the drug and its comparatively higher toxicity. A dose of 25·0 mg. which was toxic to some monkeys was not found sufficient to produce a Class II effect in all the monkeys treated.

Both the drugs showed a marked flat dose response curve and a tendency to acquire resistance by the plasmodia when used in dosages approximating to the M.E.D. of the drugs.

ACKNOWLEDGMENT.

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MALARIA IN TRIPURA STATE.

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[March 5, 1955.]

RAY AND BASU (1952), after a rapid survey, reported the high incidence of malaria in the Tripura State and emphasized the need of adequate control measures. Before establishing control as a part of the National Malaria Control Programme, it was considered necessary to collect further information about the extent and distribution of malaria in the State, and the vector or vectors transmitting the infection. The data collected during the present survey undertaken during January to May, 1954, are presented here.

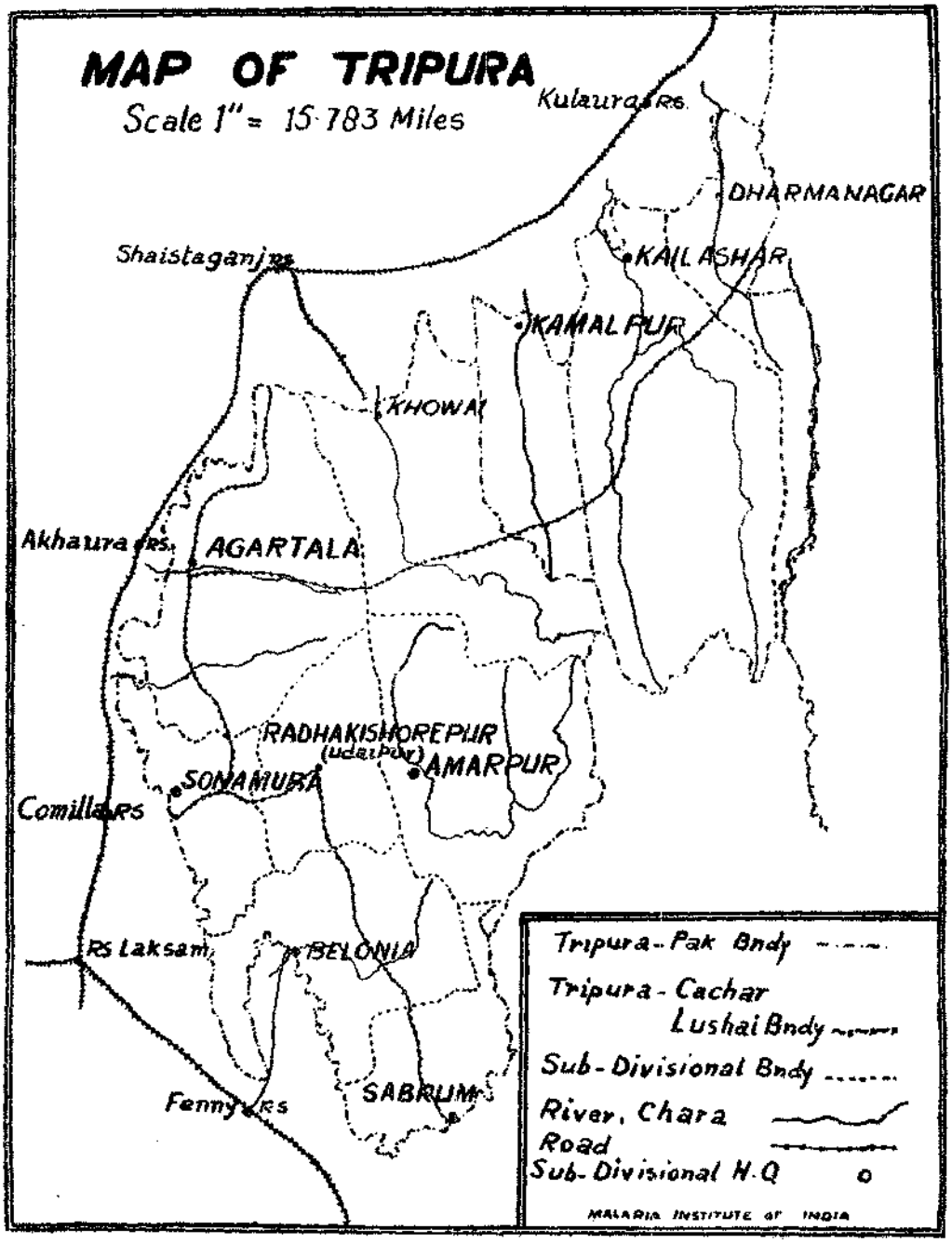
TOPOGRAPHY.

Tripura is a Part 'C' State in the Indian Union, situated between 22° 56′ and 24° 31′ N. and 91° 10′ and 92° 21′ E. It occupies an area of 4,116 square miles with a population of 639,029 (Census Report, 1951). About 1/6th of the total population is formed by displaced persons.

The State is bounded by Sylhet and Cachar districts to the north ; Sylhet, Tripperah and Noakhali districts to north-west and the west; Chittagong and Noakhali districts to the south ; and Sushai and Chittagong hills to the east. The State is practically land-locked by Pakistan on all sides except to the north, where it is connected with the Cachar District in Assam by a narrow strip of land.

The State is mostly hilly and gradually slopes down from 2,234 ft. above sea level on the eastern sector to between 250 and 486 feet above sea level to the west and south-western sector. It is divided into a number of valleys by a series of high

MAP 1.


MAP OF TRIPURA
Scale 1" = 15·783 Miles
Kulaura PS.
DHARMANAGAR
Shaistaganj PS.
KAILASHAR
KAMALPUR
KHOWAI
Akhaura PS.
AGARTALA
RADHAKISHOREPUR
(Udaipur)
AMARPUR
SONAMURA
Comilla PS
PS Laksam
BELONIA
Fenny PS
SABRUM
Tripura - Pak Bndy
Tripura - Cachar
Lushai Bndy
Sub - Divisional Bndy
River, Chara
Road
Sub - Divisional H.Q
MALARIA INSTITUTE OF INDIA

hill ranges running north and south. The hill slopes are heavily wooded and traversed by numerous streams draining into the main water courses running along the valleys.

The State is divided into 10 sub-divisions for administrative purposes (Map 1). Agartala Town in Sadar subdivision forms the capital of the State. Road communication within the State is poor. Except for one all-weather road, running across the State connecting Agartala with Karimganj in Assam, there are just a few others which are only fair weather roads. Hence any road transport during the rainy months is practically impossible.

Inhabitants.—The population is scattered along the valleys and the hills. The hills are inhabited by several tribes, *viz*. Gonds, Khasias, Nagas, Kukis, etc. There is very little intercourse between the hill tribes and the people living in the valleys. The general health of the people in the State is poor and the hill tribes are relatively more backward all round.

Housing conditions.—The housing conditions, except in Agartala Town, are extremely primitive and insanitary. Houses are built of split bamboo mattings which form the walls, and the roofs are thatched with hay. Most of the population in the State lives in scattered villages and there is only one town, Agartala, the capital of the State. The village houses have hardly any windows. Ventilation, however, is provided for by a gap of about six to twelve inches on all sides in between the walls and the roofs.

Cultivation.—Rain-fed paddy is the main agricultural crop raised in the valleys and along the hill slopes. Tea, cotton, and jute are also grown but to a much less extent. The forests are exploited for timber and fire-wood.

Climate.—Most of the year, it is hot and humid with a short mild winter lasting for about two months in December and January. April and May are oppressive owing to excessive heat and humidity. The average rainfall is 101·92 inches, and varies from 46·90 to 152·10 inches. The rains are spread out from March to October and are mostly received from the S. W. monsoon.

Morbidity rates.—Table I shows the number of malaria cases treated in a few dispensaries of the State for the last two years for which figures were available. They are tabulated by sub-divisions.

It will be seen that the peak is reached during June and July. A slight rise is also noticeable during April and May, thus giving the usual appearance typical of malarious districts of Assam and Bengal duars.

RESULTS OF THE PRESENT INVESTIGATION.

Spleen survey.—A preliminary spleen survey of 48 villages amongst 2,603 children from two to ten years of age in various sub-divisions of the State, was carried out in two stages, the first during January to March 1954 of 20 villages and the second during April and May 1954 of the remaining 28 villages. The results are presented in Table II. Spleen rates varied from 14 to 100 per cent. Sub-divisional headquarters and a few semi-urban areas showed low spleen rates which varied from 14 to 25 per cent. The average enlarged spleen was found to vary from 1 to 3·8.

TABLE I.

Total number of malaria cases treated, month by month, during the years 1953 and 1954.

	Name of the sub-division.	Jan.	Feb.	Mar.	Apr.	May.	June.	July.	Aug.	Sep.	Oct.	Nov.	Dec.	Total.
	1953													
1.	Sadar ...	2,821	2,038	2,427	3,325	3,158	4,377	5,035	4,791	3,163	2,462	2,302	2,014	37,913
2.	Kailash Shahar ...	300	356	540	771	689	694	655	412	371	292	328	357	5,756
3.	Dharamnagar ...	715	684	773	1,500	2,049	2,565	2,366	1,430	1,099	847	878	888	15,794
4.	Kamalpur ...	654	794	554	531	576	481	392	382	364	447	345	332	5,852
5.	Khowai ...	416	118	515	1,564	1,277	1,239	1,504	804	898	732	795	600	10,762
6.	Belonia ...	1,419	1,061	1,542	2,046	2,052	1,777	1,398	1,199	1,250	1,023	1,090	1,111	16,968
7.	Sonamura ...	219	216	349	497	680	637	608	601	608	577	380	323	5,757
8.	Udaipur ...	78	52	91	133	142	144	142	206	116	128	116	108	1,456
9.	Amarpur ...	108	96	333	645	646	665	677	536	406	392	626	564	5,692
10.	Sabroom ...	699	427	682	1,436	1,279	1,043	757	644	553	477	509	411	8,917
	1954													
1.	Sadar ...	3,824	2,836	6,324	4,256	1,372	1,986	2,261	1,452	...	...	...	...	...
2.	Kailash Shahar ...	393	253	436	344	362	405	572	214	...	...	...	...	...
3.	Dharamnagar ...	829	687	721	1,029	1,416	1,533	1,843	1,195	...	...	...	...	...
4.	Kamalpur ...	146	139	240	918	319	331	420	259	...	...	...	...	...
5.	Khowai ...	552	329	506	456	787	785	1,014	566	...	...	...	...	...
6.	Belonia ...	674	554	829	1,006	1,036	1,008	1,111	757	...	...	...	...	...
7.	Sonamura ...	59	147	227	689	415	509	659	623	...	...	...	...	...
8.	Udaipur ...	108	86	128	645	729	503	412	519	...	...	...	...	...
9.	Amarpur ...	171	131	183	394	458	694	771	818	...	...	...	...	...
10.	Sabroom ...	258	177	278	460	552	442	494	339	..	...	...	...	...

TABLE II.

Spleen indices amongst children.

Name of sub-division.	Serial number.	Names of the villages.	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Average enlarged spleen.
Sadar	1.	Champaknagar	125	107	85·6	3·0
	2.	Bisalgarh	142	20	14	2·3
	3.	Kamalghat	123	99	80·5	2·5
	4.	Krishnajoy Kobra	16	16	100	3·5
	5.	Raj Gobrapara	12	12	100	3·3
	6.	Ballam Thakurpara	4	4	100	1·5
	7.	Champaknagar Bazar	24	13	54·1	2·8
	8.	Ananda Nagar	56	42	75	2·4
	9.	Cherilam	258	124	48·07	1·1
	10.	Jirania Colony	111	76	68·5	1·9
	11.	Nagaripara	11	11	100	2·8
	12.	Harish Nagar T. E.	29	23	79·3	1·9
	13.	Madhupur area	30	20	66·6	1·8
	14.	Berinara	39	9	23	1·9
Khowai	15.	Telimura	89	33	37	1·8
	16.	Hawaibari	22	20	91	2·0
	17.	Khowai Proper	112	28	25	1·4
	18.	Singherchra	92	54	58·6	2·4
	19.	Gonkey	88	82	95·4	2·3
	20.	Pormura and Gour Nagar	33	25	75·7	1·8
Kamalpur	21.	Kamalpur	110	17	15·4	2·0
	22.	Fulchhri Maya Chhri	61	47	77·9	2·5
	23.	Kalachhara	54	37	72·5	1·8
	24.	Manik Bhandar	54	38	70·3	2·3
	25.	Harer Khola	44	28	63·6	1·6
Kailash Shahar	26.	Kailash Shahar Proper	117	31	26·9	1·8
	27.	Sri Ram Pur	37	22	59·4	1·3
	28.	Rangrung Tea Estate	45	34	75·5	2·0
	29.	Kailash Shahar Tea Estate	47	40	85·1	2·6
Kailash Shahar	30.	Hallarpur	21	17	82·2	2·4
	31.	Bhagwan Nagar	12	9	75	1·3
	32.	Bhadranagar	33	24	72·7	2·3
	33.	Bidya Nagar	11	9	81·8	1·8
	34.	Chandrapur	26	7	26·9	2·0
	35.	Kanakpur	37	23	62	1.4
	36.	Golokdharpur	45	29	64·4	1·6
Dharamnagar	37.	Chandrapur Colony	19	3	16	1·3
	38.	Yakub Nagar	14	3	21·4	1·3
	39.	Dewan Pasha	95	44	46·3	1·5
	40.	Dharmapur	48	14	29·1	1·3
	41.	Joy Nagar	19	17	89·4	3·8
	42.	Tilthoi Bazar	29	7	24·1	1·0
	43.	Chamtila	17	13	76·5	2·2
	44.	Pearchra Tea Estate	75	70	93·3	3·2
	45.	Rani Bari Tea Estate	52	42	80·8	2·9
Sonamura	46.	Sonamura village	14	10	71·4	1·2
	47.	Rangamati	20	16	80	1·4
	48.	Bartoli	34	20	58·8	1·0
		Total	2,403	1,49	57·2	2

The villages located along the foot-hills and the hilly regions are generally hyperendemic whereas those in valleys are moderately to highly endemic. Again, amongst the villages along the foot-hills, those in close proximity of streams showed spleen rates as high as 80 to 100 per cent.

Parasite survey.—A parasitic survey was simultaneously carried out amongst the children examined for spleen. Table III records the parasite rates and the

TABLE III.

Parasitic indices amongst children.

Subdivisions.	Serial number.	Villages.	Number examined.	Number positive of parasites.	Parasite rate per cent.	Species			
						P. falciparum	*P. vivax*	*P. malariae*	Mixed *P. vivax* and *P. falciparum*
Sadar	1.	Champak Nagar	125	43	34·4	20	22	1	...
	2.	Bishal Garh	142	4	2·8	1	3	...	...
	3.	Kamal Ghat	123	52	42·2	31	14	4	3
	4.	Krishnajoy Kobra	16	8	50	5	2	1	...
	5.	Raj Gobrapara	12	6	50	4	2	...	...
	6.	Ballam Thakurpara	4	2	50	2	...	...	...
	7.	Champa Nagar Bazar	24	4	16·7	4	...	...	...
	8.	Ananda Nagar	56	16	28·6	9	7	...	...
	9.	Cherilam	258	63	24·4	48	14	1	...
	10.	Jirania Colony	111	37	33·3	21	15	...	1
	11.	Nagaraipara	11	2	18·2	1	1	...	...
	12.	Harish Nagar Tea Estate	29	9	31	8	1	...	...
	13.	Madhupur area	30	11	36·6	7	4	...	...
	14.	Berimara	39	1	2·5	...	1	...	...
Khowai	15.	Teliamura	89	7	7·7	4	3	...	...
	16.	Hawaibari	22	13	59	13	..	...	...
	17.	Khowai Proper	112	5	4·4	2	3	...	...
	18.	Singherchra	92	12	13	7	5	...	...
	19.	Gonkey	88	23	26·1	14	9	...	...
	20.	Pormura and Gaur Nagar	33	6	18·1	4	2	...	...
Kamalpur	21.	Kamalpur	110	11	10	6	5	...	...
	22	Fulchhri Mayachhri	61	11	18	5	6	...	...

TABLE III—*Concld.*

Sub-division.	Serial number.	Villages.	Number examined.	Number positive for parasites.	Parasite rate per cent.	Species			Mixed *P. vivax* and *P. falciparum*.
						P. falciparum.	*P. vivax*.	*P. malariæ*.	
	23.	Kalachari	51	10	19·6	6	4	...	...
	24.	Manik Bhandar	54	9	16·6	5	4	...	...
	25.	Harer Khola	44	7	15·9	2	5	...	...
Kailashahar	26.	Kailashahar Proper	117	13	11·1	1	12	...	...
	27.	Sri Ram Pur	37	5	13·5	2	3	...	...
	28.	Rangrung Tea Estate	45	14	31·1	9	5	...	...
	29.	Kailashar Tea Estate	47	22	46·8	17	5	...	...
Kailashahar	30.	Hallarpur	21	4	19	4	...	...	...
	31.	Bhagwan Nagar	12	3	25	1	2	...	...
	32.	Bhadra Nagar	33	6	18·1	3	3	...	...
	33.	Baidya Nagar	11	3	27·2	1	2	...	...
	34.	Chandrapur	26	4	15·3	1	3	...	...
	35.	Kanakpur	37	5	13·5	1	4	...	...
	36.	Golokdharpur	45	12	26·6	6	6	...	...
Dharamnagar	37.	Chandrapur Colony	19	0	...	...	...	...	...
	38.	Yakub Nagar	14	0	...	...	...	...	...
	39.	Dewan Pasha	95	11	11·5	9	2	...	...
	40.	Dharmapur	48	5	10·4	5	...	...	...
	41.	Joy Nagar	19	7	36·8	7	...	...	...
	42.	Tilthoi Bazar	29	1	3·8	1	...	...	...
	43.	Chamtila	17	1	6	1	...	...	...
	44.	Pearachra Tea Estate	75	33	44	20	13	...	...
	45.	Rani Bari Tea Estate	52	13	25	9	3	1	...
Sonamura	46.	Sonamura village	14	1	7·1	1	...	...	...
	47.	Rangamati	20	3	18·7	2	1	...	...
	48.	Bortali	34	6	17·6	6	...	...	...
		Total ...	2,603	544	20·9	336	196	8	4
		Per cent ...				(61·7)	(36)	(1·5)	(0·8)

distribution of different species of malaria parasites. The highest parasite rate recorded was 59 per cent. *Plasmodium falciparum* was found to be the predominant species and constituted 61·7 per cent, whereas *P. vivax*, *P. malariæ*, and mixed infections of *P. vivax* and *P. falciparum*, accounted for 36·0, 1·5 and 0·8 per cent respectively.

Infant blood examination.—Blood smears from as many infants as possible, between one month and two years of age, were collected and examined.

The results of the examination of 394 infant blood smears from 35 villages are recorded in Table IV. Infant parasite rates varied from 0 to 100 per cent. The villages in the foot-hill regions, adjacent to streams, showed the highest rates. The lowest rates were recorded from villages in the plains. Here again *P. falciparum* was the most predominant species with 57·3 per cent while *P. vivax*, *P. malariæ*, and mixed infection of *P. falciparum* and *P. vivax*, came to 37 per cent, 3·4 percent, and 2·3 per cent, respectively.

TABLE IV.

Infant parasite rate.

Sub-division.	Serial number.	Villages.	Number examined.	Number positive.	Parasite rate per cent.	Species of parasites			
						P. falciparum.	*P. vivax.*	*P. malariæ.*	Mixed *P. falciparum* and *P. vivax.*
Sadar	1.	Champak Nagar	9	6	66·6	4	2	...	...
	2.	Bishal Garh	46	5	10·8	2	3	...	...
	3.	Kamal Ghat	9	1	11·1	1	...	...	...
	4.	Krishnajoy Kobra	2	2	100	1	1	...	..
	5.	Rajgobrapara	3	2	66·9	1	1	...	...
	6.	Ballam Thakarpara	3	0	...	...	...	...	...
	7.	Champak Nagar Bazar	17	2	11·8	2	...	...	...
	8.	Ananda Nagar	22	5	22·7	2	3	...	...
	9.	Cherilam	78	17	21·8	12	5	...	...
	10.	Jirania Colony	10	1	10	1	...	...	...
	11.	Nagraipara	8	1	12·5	1	...	...	...
	12.	Harish Nagar T.E.	4	...	...	...	...	...	...
	13.	Madhupur area	5	1	20	1	...	...	...
	14.	Berimara	13	1	7·7	...	1	...	...

TABLE IV—(*Concld.*)

Sub-divison.	Serial number.	Villages.	Number examined.	Number positive.	Parasite rate per cent.	Species of parasites			
						P. falci-parum.	*P. vivax.*	*P. malariæ.*	Mixed *P. falciparum* and *P. vivax.*
Khowai	15.	Howai bari	12	7	58·3	4	3	...	...
	16.	Singhichra	2	...	...	...	...	...	...
	17.	Gonkey	6	2	33·3	1	1	...	...
	18.	Gournagar and Dealia Tilla	8	...	...	...	...	...	...
Kamalpur	19.	Manik Bhandar	4	...	...	...	...	...	...
	20.	Harrer Khola Colony	10	1	10	...	1	...	...
Kailashahar	21.	Rungrung T.E.	4	3	75	3	...	...	...
	22.	Kailashahar T.E.	11	3	27·3	1	2	...	...
	23.	Bhagwan Nagar	6	1	16·6	...	1	...	...
	24.	Bhadra Nagar	5	...	...	...	...	...	...
	25.	Bidhya Nagar and Chandrapur	7	...	...	...	...	...	...
	26.	Kanakpur	7	...	...	...	...	...	...
Dharamnagar	27.	Chandrapur Colony	4	...	...	...	...	...	...
	28.	Dharampur	7	1	14·3	1	...	...	...
	29.	Joy Nagar	3	2	66·6	2	...	...	...
	30.	Tilthoi Bazar	3	...	...	...	...	...	...
	31.	Chamtila	9	2	22·2	1	1	...	...
	32.	Piarachra T.E.	35	19	54·3	9	7	1	2
	33.	Rani Bari T.E.	17	4	23·5	1	1	2	...
Sonamura	34.	Sonamura	3	...	...	...	...	...	...
	35.	Bortali	2	...	...	...	...	...	...
		Total ...	394	89	22·6	51	33	3	2
		Per cent				(57·3)	(37)	(3·4)	(2·3)

ANOPHELINE SURVEY.

Adult mosquito collections were made in almost all the villages visited for spleen and parasite surveys. Routine weekly collections of anopheline adults were, however, made from fixed catching stations in a number of villages where simultaneous studies on the period of residual activity of different dosages of D.D.T. on locally prevalent different types of wall surfaces, were in progress.

The following adult anopheles mosquitoes were collected and identified :—

A. aconitus	*A. hyrcanus*
A. annularis	*A. jeporiensis*
A. barbirostris	*A. minimus*
A. culicifacies	*A. subpictus*
A. fluviatilis	*A. vagus*

A. fluviatilis and *A. hyrcanus* were not recorded previously by Ray and Basu (*loc. cit.*) whereas *A. kochi* reported by them, was not found in the present collection. Thus the anopheline fauna of the State so far recorded consists of eleven species. Of the total collection of 1,508 anopheline mosquitoes, the percentage of different species caught is represented graphically in Chart 1. *A. minimus* formed 13 per cent of the total catches.

Adult collections were made from human dwellings and cattlesheds. The human dwellings, most of which were of one or two room tenement type, had smoke from the oven as an ever-present deterrent factor and in the case of the cattlesheds, they were mostly open on the sides. A number of those best suited was, however, selected as catching stations for routine mosquito catches. All *A. minimus* mosquitoes caught were only from human dwellings.

BREEDING PLACES.

Though systematic larval collections were not made during the present survey, the types of breeding places met with are enumerated below :—

1. *Tanks or ponds.*—They were present round about some villages and were all rainfed. These were mostly meant for household washings, etc., though a few appeared to be used for irrigation purposes as well.

2. *Swamps and seepages.*—These were plentiful and usually contained clear slow moving water. A great deal of grass and swamp vegetation, affording very little shade, was found in those areas. These constitute ideal places for the breeding of *A. minimus*.

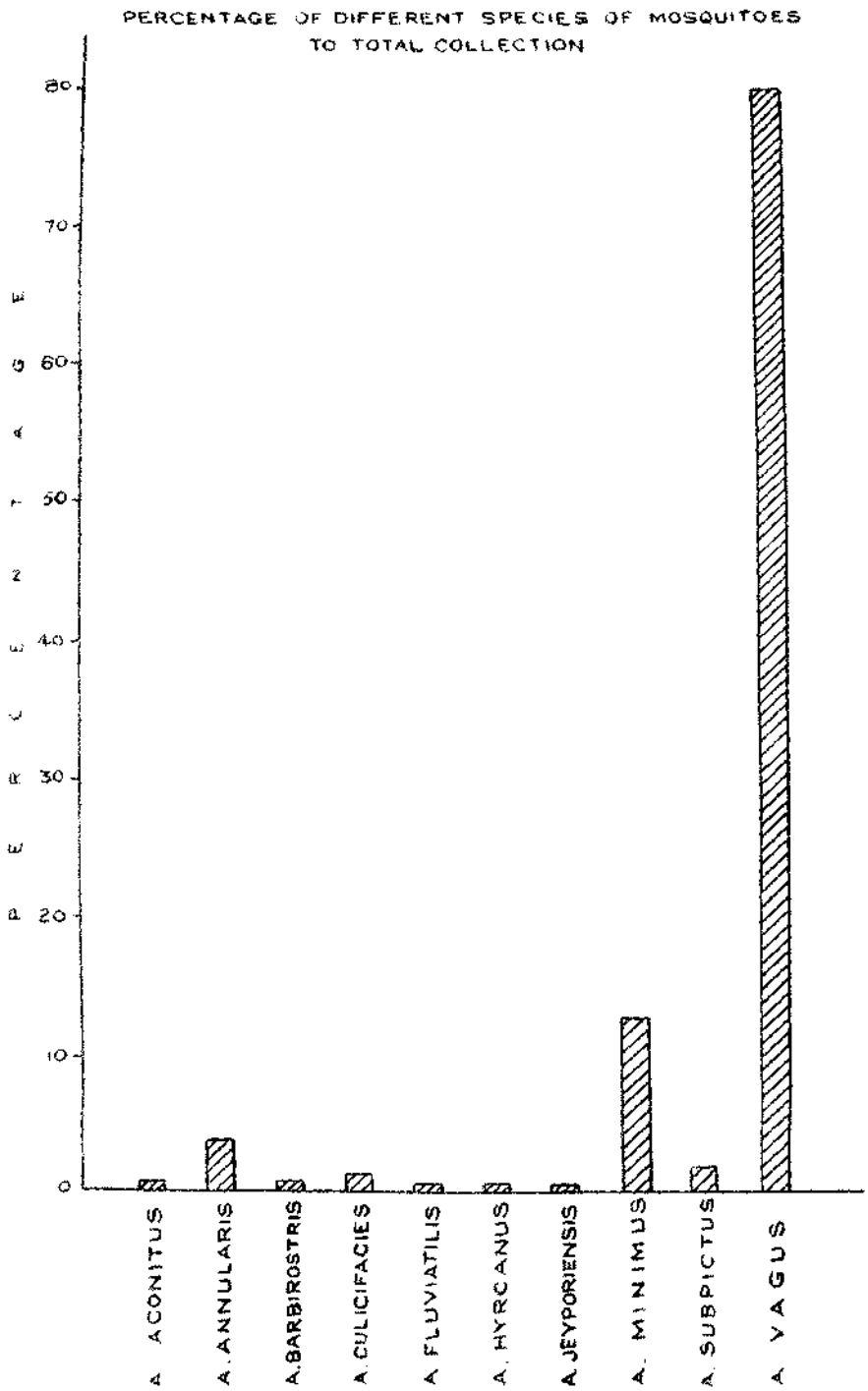
3. *Rivers and streams.*—Innumerable streams emerge out of hill ranges and drain into the bigger rivers. These streams with slow running water are known as the favoured breeding places of *A. minimus*.

4. *Borrowpits.*—These were encountered mostly along the road sides and were in formations. A few of them were examined and *A. vagus* was the most predominant species found breeding.

5. *Bamboo reserves.*—Various bamboo reserves were searched for larvæ but no evidence of breeding was encountered.

6. *Water collections in dense jungle.*—A few of them were searched for anopheline larvæ. One stream with partial shade showed larvæ of *A. barbirostris* and *A. hyrcanus* only.

CHART 1.


PERCENTAGE OF DIFFERENT SPECIES OF MOSQUITOES TO TOTAL COLLECTION
PERCENTAGE
80
70
60
50
40
30
20
10
0
A. ACONITUS
A. ANNULARIS
A. BARBIROSTRIS
A. CULICIFACIES
A. FLUVIATILIS
A. HYRCANUS
A. JEYPORIENSIS
A. MINIMUS
A. SUBPICTUS
A. VAGUS

DISSECTION OF MOSQUITOES.

Mosquito density during the period of the year the survey was carried out, was very low. Moreover as the places from which the mosquitoes were collected were at a great distance from the field laboratory, a number of those caught, died during transit. This accounts for the small number of mosquitoes dissected, results of which are presented in Table V.

TABLE V.

Results of dissection.

Species.	Number dissected.	Number positive		Percentage.	Results.
		Gut.	Gland.		
A. annularis	43	...	...	...	...
A. subsicifacies	13	...	...	...	...
A. fluviatilis	1	...	...	...	...
A. jeyporiensis	2	...	...	...	...
A. aconitus	6	...	...	...	...
A. minimus	99	...	2	...	...

Of the 99 *A. minimus* dissected, 81 specimens were caught at Hawaibari, a village at the foot of Buramurra Range of hills, on two consecutive days. Of the 52 mosquitoes dissected from the first day's collection, two specimens were found positive for sporozoites (Misra, 1954).

PRECIPITIN TEST.

The source of blood meal in 21 *A. minimus* has been determined and the results are presented in Table VI.

TABLE VI.

Result of precipitin tests of mosquito blood meals collected at Tripura.

Mosquito species.	Place of capture.	Date of capture.	Number of blood meals treated.	Man+	Cow+	Remarks.
A. minimus	Hawaibari	May 5, 1954	21	9	2	

DISCUSSION.

From the data presented, it can be seen that the area as a whole is endemic for malaria. There appears to be a close relation between the degree of endemicity and the proximity to the running water courses. Slow running streams being the favoured breeding places of *A. minimus* which is the local vector species, such a distribution is to be expected.

In view of the low density of all Anopheles population during the period of survey, it was not possible to carry out extensive dissection of all species of mosquitoes locally prevalent. The terrain in the State is closely similar to the hill ranges and foot-hill regions in the adjoining States like Chittagong hill tracts, Assam and Bengal Duars. *A. minimus* has been established as the principal vector in these areas, and the finding of two specimen of this species *A. minimus* with positive salivary glands, is indicative of the rôle played by it in Tripura. Whether any other species is also responsible for malaria transmission, particularly in the areas along the valleys, has yet to be determined and this is possible only when large scale dissections of all the probable vector species present in the State are carried out for at least one full season.

Control measures.—No concerted effort to control malaria in the State was previously attempted. With the inauguration of the National Malaria Control Programme (Jaswant Singh, 1953) in April 1953, the State has been allotted one unit to protect five out of six lakhs of population. The unit started functioning from the end of 1953 when only a few villages in the vicinity of Agartala Town were taken up for D.D.T. spraying. During the current year, the entire State is proposed to be sprayed with D.D.T. Since effective control of *A. minimus* has been achieved (Puri and Krishnaswami, 1947 ; Krishnaswami, 1952) with D.D.T. in India, it is hoped that malaria in the State will be effectively controlled.

SUMMARY.

1. The topography of Tripura State is similar to that of the adjoining States like Chittagong hills tracts, Assam and Bengal Duars, and naturally the fauna are more or less the same.

2. The State is poorly developed in so far as road communication is concerned. There are a few fair weather roads and hence inter-communication during wet months, is extremely difficult.

3. Except for a few thickly populated villages in the plains, the entire State is more or less hyperendemic for malaria as determined by spleen and parasite survey.

4. Eleven species of anophelines have so far been recorded.

5. *A. minimus* has been incriminated vector for malaria in the state.

ACKNOWLEDGMENT.

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Indian Journal of Malariology, 9, 2, June 1955.

MALARIA CONTROL IN LOWER BHAVANI PROJECT HEADWORKS, COIMBATORE DISTRICT, MADRAS STATE.

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[March 11, 1955.]

INTRODUCTION.

THE paper summarizes the results of malaria control measures adopted in the Lower Bhavani Project Headworks area during the years 1949 to 1953 when the Lower Bhavani Dam was under construction. The entire cost of the antimalaria scheme was borne by the Lower Bhavani Project Engineering Authorities.

Arising from the eastern slopes of the Western Ghats, the River Bhavani meanders through Coimbatore District of the Madras State, and joins the River Cauvery below the Mettur Dam. It is a perennial river, draining an area of about 1,620 square miles subjected to both the monsoons (Map 1).

The Lower Bhavani Project comprises the construction of a dam across the River Bhavani just below its confluence with the Moyar River and taking of a new irrigation canal, about 124 miles long, from its right flank. The object of construction of the dam is primarily to impound flood water for irrigation purposes, and the development of electric energy to the extent of 10,000 kilowatts is only secondary and seasonal.

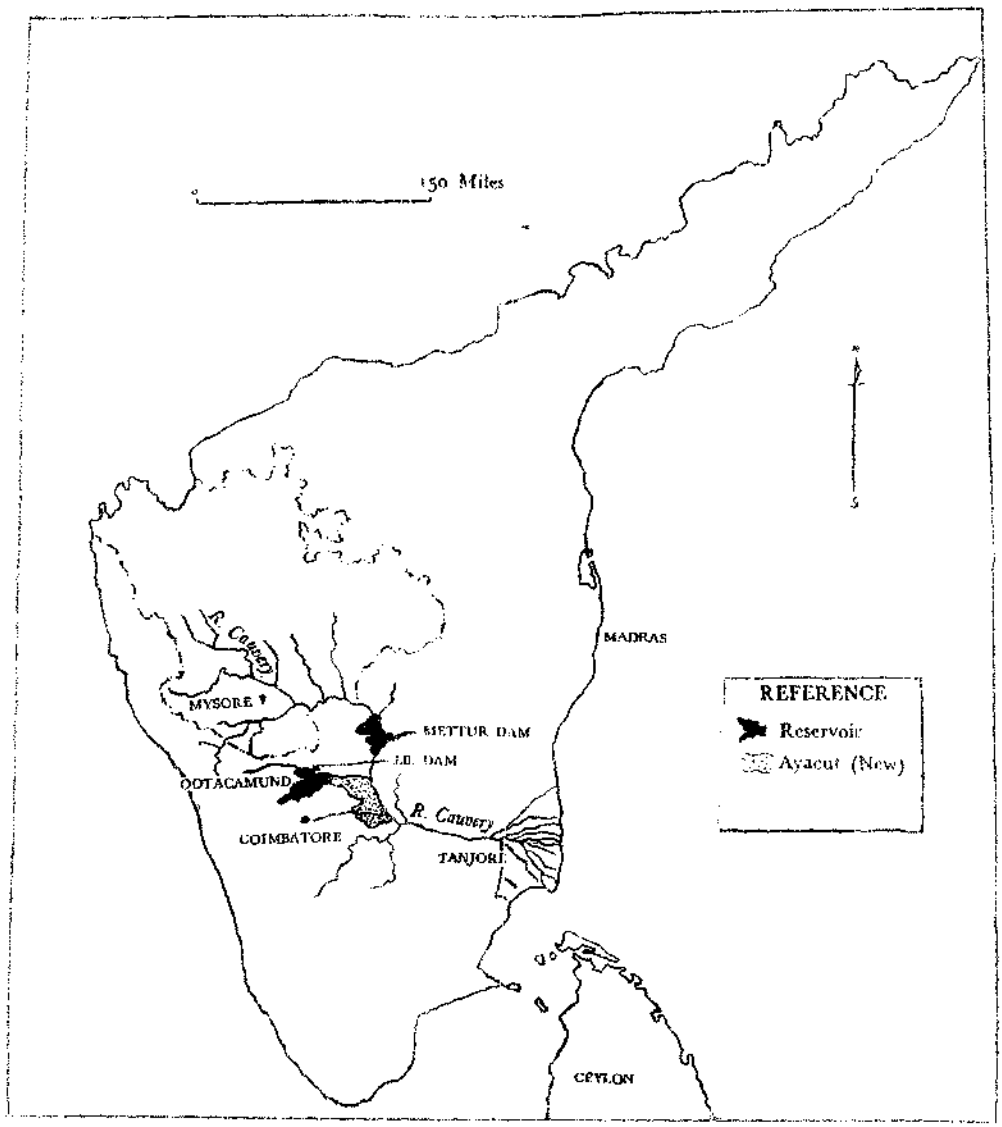
The site chosen for the construction of the dam is in Gobichettipalayam Taluk of Coimbatore District about 22 miles from Mettupalayam Railway Station on the Southern Railway. The dam is over 5½ miles long with a capacity to impound about 28,000 million cubic feet of water and a water spread of about 30 square miles.

The new canal taking off from the dam traverses parts of Gobitchettipalayam, Bhavani, Erode, and Dharapuram taluks of Coimbatore District and Karur Taluk of Trichinopoly District, and will bring 2,07,000 acres of land under irrigation. About 10,000 acres will be under paddy while in the rest of the area, dry irrigated crops and cotton will be raised (Map 2).

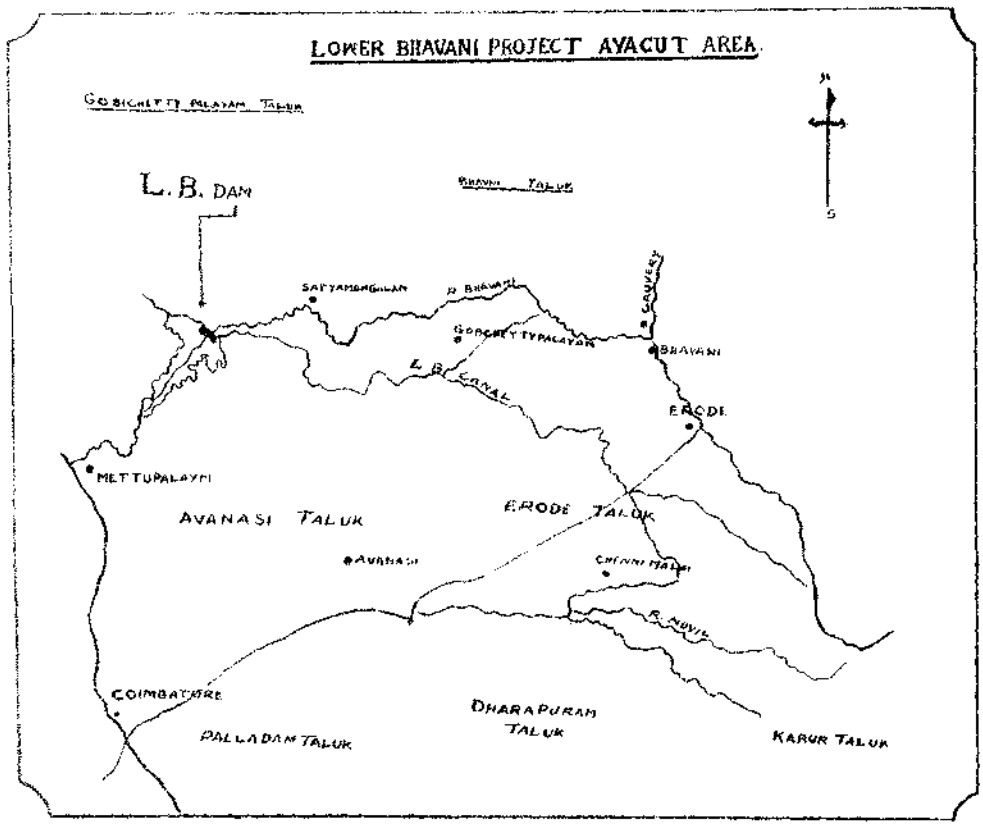
MADRAS STATE

MAP I.

Lower Bhavani dam reservoir and ayacut area.



MAP 2.



It is expected that as a result of this project, the annual yield of food-grains will increase considerably besides producing large quantities of cotton. Further, the canal will afford relief to portion of Dharapuram Taluk of Coimbatore District which is at present frequently affected by famine.

The estimated cost of the scheme is 9·5 crores of rupees.

PREVIOUS HISTORY OF MALARIA IN THE AREA.

During the year 1947—a year prior to the commencement of construction of the dam—the area was surveyed by the Regional Malaria Organisation, Coimbatore. The spleen rates, recorded in the villages around the dam site, were as follows :—

Kothamangalam	...	...	12·5	per	cent.
Pungar ...	...	...	11·5	,,	,,
Bahaduthurai	...	...	10·0	,,	,,
Vadavalli ...	...	...	14·3	,,	,,
Karapuram	...	...	21·0	,,	,,
Peerkadavu	...	...	22·7	,,	,,

In a village, Pungar, the Regional Malariologist found 100 per cent adult spleen rate, the eight adults examined all showed large hard spleens. On enquiry by him, it was found that these people contracted malaria in Thalamalai, near the Kollegal Hills, where they had migrated for work. These persons on return constituted the main reservoirs of infection. As *A. culicifacies*, the vector in these areas, was prevalent in large enough numbers, the Regional Malariologist emphasized that epidemics were liable to occur if malarial control measures were not taken in the area.

An antimalaria scheme was consequently sanctioned by the State Government to protect the health and welfare of the staff working in the Project Headworks, and the scheme was put into operation in 1949.

TOPOGRAPHY OF THE HEADWORKS AREA.

The dam site is close to a small village on the eastern slopes of the Nilgiris or the Blue Mountains. The altitude of the place is about 850 feet above the mean sea level. To an extent of three or four miles all around this site, there are some seven or eight villages and they are very sparsely populated. Main occupation of the people in the locality is agriculture, grazing of cattle in the hills, and the crops raised there are usually millets, chillies and tobacco.

SOIL AND DRAINAGE.

The soil is generally gravely loam in the area under study. The country on either side drains into the river. A few small streams join the Bhavani River in this area after this river is itself joined by its main tributary, the Moyar River. Pool formations are common in the small streams during summer months. The subsoil water table in these localities varies from 10 feet to 35 feet, as ascertained from a number of agricultural wells over different months.

POPULATION.

The local population in all the villages round about the dam site was negligible when compared to the large influx of labourers who come in connection with the dam construction work right from the beginning. After reaching a peak figure of about 40,000 during the year 1950, the strength gradually came down as the work drew to its final phases.

A very large number of non-skilled and skilled labourers from this and the surrounding districts, found employment mainly in the first two or three years. The '*Oddars*' and '*Boyars*', the noted stone workers from Coimbatore and Salem districts, and the fine chisel dressers from Madura and Tinnevelly districts, helped in the dam construction work. People from the adjoining district of Malabar and a large number of ex-army men found occupations as drivers of heavy and other earth-moving machinery, mechanics, fitters, etc. Many of them were people from highly malarious tracts.

HOUSING.

To accommodate the various classes of workers, very rapidly a town came into being—the township of Bhavanisagar—with a large number of pucca buildings and numerous other semi-permanent and temporary quarters. Besides, many of the labourers constructed their own huts with whatever materials they could get and in close proximity to various work spots. Usually these were put up near some water-sources like streams or agricultural wells. Some of the workers lived in the existing villages in the neighbourhood.

The type of houses may be broadly grouped into three classes :—

(*a*) Pucca houses in the main camp.

(*b*) Temporary sheds put up departmentally, both in the main camp area and near several work spots. These had mud walls with either thatched roofings or roofs with galvanised iron sheets.

(*c*) Huts put up by labourers themsleves. These were just conical structures made of twigs and leaves with practically no walls.

METEOROLOGICAL DATA.

Rainfall.—In the area under the project, there were three rainfall recording stations and the avarage rainfall, recorded during the different seasons over a number of years, was as follows :—

Station.	Average rainfall (inches)			
	S.W. monsoon.	N.E. monsoon.	Other months.	Total.
1. Sathyamangalam ...	8·63	12·65	6·94	28·22
2. Gobichettipalayam ...	10·37	12·15	7·34	29·86
3. Erode	10·96	11·52	6·57	29·05

After the commencement of dam construction, observations were made and recorded locally, and are furnished in Table I.

Temperature and humidity.—The climate of the area is not subject to extremes of heat or cold and the seasons are not well marked. The monthly mean maximum and minimum temperatures and mean relative humidity are given in Table II. The maximum temperature is usually recorded in May or April, and the lowest in December or January.

TABLE I.

Rainfall in inches.

Month.	Year				
	1949	1950	1951	1952	1953
January	Not received	0·0	1·70	0·0	0·20
February		2·81	0·06	0·62	0·85
March		0·0	0·50	0·22	0·0
April ...		0·35	0·40	·05	1·31
May	8·58	2·56	3·47	0·78	2·05
June	0·33	0·0	0·06	0·10	2·03
July	2·64	0·17	0·86	1·11	2·34
August ...	1·45	4·09	0·91	0·12	0·97
September ...	0·55	0·58	0·57	1·15	0·84
October ...	6·26	4·55	4·83	3·78	17·13
November ...	1·72	4·64	7·73	0·14	1·78
December ...	0·60	0·02	0·75	5·01	0·14
Total ...		19·77	27·78	14·08	32·64

TABLE II.

Temperature and humidity.

Month.	1950 Monthly mean.			1951 Monthly mean.			1952 Monthly mean.			1953 Monthly mean.		
	1	2	3	1	2	3	1	2	3	1	2	3
January ...	91·0	77·0	73·7	91·8	80·8	65·2	87·0	68·0	73·1	88·6	69·9	77·6
February ...	92·6	82·3	75·2	94·5	71·1	57·4	91·8	73·2	75·5	91·2	68·7	74·5
March ...	97·5	85·8	67·1	97·9	74·8	65·3	94·5	75·9	77·1	98·6	77·2	70·6
April ...	103·2	87·1	59·7	96·2	78·1	72·7	99·5	80·2	67·7	100·4	76·0	74·0
May ...	101·5	86·8	61·0	98·7	78·1	70·3	100·0	81·3	59·3	101·3	80·0	61·4
June ...	93·9	85·0	69·9	93·8	79·2	62·2	96·7	79·2	59·1	95·5	78·7	66·8
July ...	91·5	82·8	66·7	92·9	78·6	62·2	93·0	78·3	63·3	91·5	76·2	64·6
August ...	92·0	83·1	67·5	94·9	79·4	61·5	94·8	78·1	62·6	93·7	78·4	64·5
September	92·0	83·6	64·4	93·3	77·4	70·2	96·1	78·6	58·6	90·13	82·5	64·6
October ...	90·1	81·1	70·4	92·4	76·4	83·0	92·2	75·9	77·7	89·7	75·9	85·8
November	90·4	79·6	72·8	87·5	74·9	89·3	91·5	73·5	70·1	73·0	79·8	71·8
December	90·7	79·5	65·1	86·7	66·8	81·9	85·8	70·9	81·5	83·6	76·7	67·1

Column 1 = Maximum degree F. Column 2 = Minimum degree F. Column 3 = Relative humidity, per cent.

ANOPHELINES OF THE AREA.

The anopheline fauna of the area was very limited being only *A. culicifacies*, *A. subpictus* and *A. vagus*. Rarely a few specimens of *A. stephensi*, *A. hyrcanus* and *A. pallidus* were met with.

The main breeding places were small pools in several small streams which join the two main rivers—Bhavani and Moyar. The rest of the breeding places included borrowpits created for the earth dam, quarry pits, stagnations around and over masonry dam, ornamental and cement curing tanks, wells, etc. This dam has two peculiar features, namely, it is mainly an earthern dam and is practically the longest dam now completed in India. As much as 161 million cubic feet of earth has gone into its construction, and as this huge quantity of earth was mostly taken from the submergible area of the dam, it is not likely to leave many borrowpits as a future problem. Subjected as the area was to D.D.T. control, only a small number of *A. culicifacies* was dissected with no infection either in gland or gut.

EPIDEMIOLOGY OF MALARIA IN THE PROJECT AREA.

Large engineering works, especially those connected with the river valley projects, are usually associated with explosive outbreaks of malaria, sometimes paralysing the works, as the following new additional factors come into play, namely (*i*) a sudden and enormous increase in the local vectors (if not adequately controlled) as a consequence of the increase in the breeding places—borrowpits, quarry pits, diversions of rivers and streams in several different ways to suit works, etc.—which are incidental and unavoidable to such engineering works; (*ii*) introduction of human malaria carriers and susceptible population amongst the several thousands of labourers imported from various places; (*iii*) conditions of hard work, physical strain, over-crowding and poor housing conditions.

Based on a previous study of malaria epidemiology in the area, the local vector is presumably *A. culicifacies*. It is an ubiquitous breeder occurring in most type of waters. Gametocyte carriers too were not wanting in the imported population. Out of the 258 blood smears from adult labourers down with fever, 72 showed malaria parasites, mostly *P. falciparum*. Local transmission season for malaria appears to be the wet cold months of the year when rainfall and atmospheric conditions are favourable for breeding and longevity of the vector mosquitoes.

CONTROL OF MALARIA IN THE PROJECT AREA.

As no risks could be taken with the population employed in the speedy execution of the dam construction works, an all-out effort was made from the very beginning to prevent and control malaria by adopting antiadult, antilarval and antiparasitic measures. The Health Officer was assisted in this work by one health inspector trained in malaria work, a laboratory assistant and one or two field assistants (insect collectors), a malaria mistry and six to eight field workers. Indoor residual spraying with D.D.T. was the sheet anchor of malaria control.

Greater attention was paid to actual control measures than to investigation of malaria in the area. The main camp and the immediately adjoining smaller camps were given six to eight rounds of spray at intervals of about six weeks all round the year, while the peripheral groups of villages were given four or less rounds during the transmission season only. The area under control was about 25 square miles and extended wherever new huts were put up or new breeding conditions were created, like extensive quarrying, etc. Twentyfive per cent D.D.T. (70-80 para para) in aromex soap emulsion, was used. In all, a total of 66,834 structures were sprayed till the end of December 1953. Cattlesheds, disused and partially dismantled houses were also sprayed. Whenever there was a breakdown in the supply of commercial D.D.T., Geigy's water wettable powder 50 per cent was used. By the middle of the year 1953, free supply of D.D.T. under the National Malaria Control Programme was made available. The number and frequency of sprays that each structure received varied according to its nearness to breeding grounds and based also on entomological observations. A dosage of 50-60 mg. of D.D.T. per sq. ft. was adopted. The " Little Giant " pumps supplied by Myers Co. Ltd., Madras, were used during the later period of the project and were found useful for application of the correct dose.

With a maximum of eight field workers, employed on a monthly basis, the entire area was covered with no wastage of manpower or any undue haste.

Antilarval measures, mostly against *culicines*, were also adopted and intensified whenever there happened to be any breakdown in the continuity of supply of D.D.T. for residual spraying. Waste engine oil, available in good quantities locally, was used with one to two per cent D.D.T. Paris green and gammexane P. 520 were used as larvicides in places where oil could not be used.

Proguanil, in selected areas and under certain conditions, was administered mostly for clinical prophylaxis and occasionally for clinical cure.

RESULTS OF MALARIA CONTROL.

The area remained remarkably free from any local transmission of malaria throughout the period.

(*a*) *Incidence of malaria.*—Hospital figures (Lower Bhavani Project Camp Hospital) relating to the incidence of malaria in the main camp and adjoining areas for the years 1949 to 1953, are furnished in Table III.

TABLE III.

Lower Bhavani Project Camp Hospital figures relating to incidence of malaria.

Year.	Total admission under all causes.	Cases treated for malaria.	Percentage of Column 3 to Column 2.
1949	11,109	356	3·2
1950	18,803	429	2·3
1951	18,489	297	1·6
1952	15,235	158	1·04
1953	15,773	148	0·9

As the hospital itself came into being only in connection with the project, the figures for malaria cannot be compared with any previous figures.

The percentage of malaria cases to total hospital admission is a more sensitive index under project conditions where labour strength is a widely fluctuating one. This figure was always low. There were no cases admitted as inpatients in the hospital nor any death was recorded due to malaria or its complication.

(*b*) *Spleen rates.*—A quarterly survey of all the children, between two and ten years of age, was conducted and the results are furnished in Table IV. When viewed against the background of 10 to 20 per cent spleen rate that prevailed in the area before the engineering work and malaria control works were started, the zero spleen rate at the tail end of the project work during 1953 speaks the efficacy of the control measures.

TABLE IV.

Spleen rates.

Year.	Number of children examined.	Number found with enlarged spleen.	Spleen rate per cent.
1949	876	23	2·6
1950	765	29	3·8
1951	1,465	7	0·5
1952	1,159	1	0·09
1953	243	*Nil*	*Nil*

(*c*) *Parasite rate.*—A total of 758 blood smears of children in the project area, between two and ten years of age, were examined during 1949 to 1953. The parasite rate has been getting progressively low as seen in Table V. In most of these cases, the parasite was *P. falciparum.*

TABLE V.

Parasite rate.

Year.	Number examined.	Number found positive with plasmodia.	Parasite rate per cent.
1949	231	15	6·5
1950	216	11	5·1
1951	120	1	0·8
1952	180	*Nil*	*Nil*
1953	48	*Nil*	*Nil*

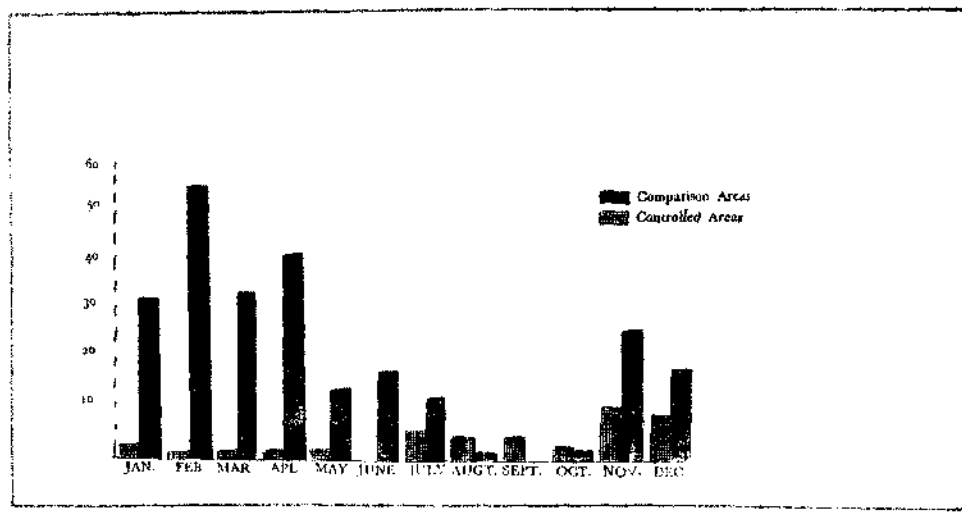
(*d*) *Anopheline density.*—Though not strictly comparable in all respects, a village on the other side of the Bhavani River, opposite the main camp, was selected for observations on the anopheline densities and was left unsprayed. Observations carried out during the year 1952, both in the controlled area and in the comparison village, are given in Chart 1. Density of *A. culicifacies* in human and mixed dwellings per 5 man-hours are also given. Chart 1 well illustrates the remarkable reduction in *A. culicifacies* density in the sprayed area.

CHART 1.

Lower Bhavani Headworks

1952.

Density of A. culicifacies in controlled and comparison areas.



FINANCE AND COST OF MALARIA CONTROL MEASURES.

The health officer was in charge of general health and sanitation measure and also responsible for malaria control work. The total cost for public health measures including malaria, for the period 1948-49 to December 1953, was about Rs. 1,51,653 which forms 0·003 per cent of the estimated cost of the dam. This cost is given in Table VI, together with the population benefited and (*i*) cost per capita per year for malaria control and (*ii*) annual per capita cost for other general health measures, epidemic control, etc.

TABLE VI.

Cost of public health measures and of malaria control.

Year.	Population benefited.	Cost of malaria control. (Rupees).	Cost of general health measures and other epidemics, etc. (Rupees)	Cost per annum per capita for malaria control only. (Rupees)
1948–49	Details not available		Total Rs. 3,984/-.	
1949–50	40,000	11,885	10,634	0·26
1950–51	42,000	16,198	20,544	0·39
1951–52	30,000	17,004	18,620	0·56
1952–53	20,000	14,843	19,055	0·68
1953–54 (Up to December 1953 end.)	11,000	8,316	19,480	...

The annual per capita cost varied from Rupees 0·26 during 1949-50 to Rupees 0·68 during 1952-53 and has secured full and complete freedom from malaria.

SUMMARY.

1. An account is given of the malarial conditions and control measures adopted in Lower Bhawani Project Headworks area, Coimbatore District, Madras State.

2. Prior to the commencement of work, malaria was moderately endemic.

3. The results of malaria control measures, primarily with indoor residual sprayings with D.D.T., are discussed.

4. The annual per capita cost for malaria control in the project, under conditions existing there, is furnished.

ACKNOWLEDGMENT.

The author thanks Sri S. S. Krishnamurthy, Senior Health Inspector, for the valuable work rendered both in actual field work and in the compilation of this report.

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OBSERVATIONS ON THE TRANSMISSION OF MALARIA IN BABINA AREA, JHANSI DISTRICT, UTTAR PRADESH.

BY

MAJOR PRITAM SINGH, A.M.C.

[March 11, 1955.]

THE observations recorded were carried out during the period June to September 1953. The area surveyed is 4 by 1½ miles and includes Babina, Hirapur and Raipur villages. As these villages have been under partial malarial control for the last ten years, the investigtions were extended to Manpur and Chakarpur villages which are 2½ and 4 miles, respectively, from Babina. The former village depicts the true picture of Babina and the latter that of Hirapur and Raipur villages.

TOPOGRAPHY.

Babina village is 16 miles from Jhansi on the Jhansi—Saugor road. The military camp area is three miles long and on an average half a mile broad. The terrain is uneven and is bounded on the south and east by a range of low rocky hillocks. Rain water is impounded by the construction of earth dams in the gaps between these hillocks. The reservoirs thus formed are utilized for irrigation purposes through channels which traverse the area. Below the dams are seepage outcrops. On the north of the area flows the Gurari Nala which runs a tortuous course and has abundant rank vegetation along its banks.

In addition to tanks, the source of water supply is from shallow wells, a large number of which are situated in the cultivated fields. The water from these wells is lifted either by a primitive type of earthen pot persian wheels or by *charsa*.

The main crops during the rainy season are paddy, sesame, maize, Kodo (*Paspalum scrobiculatum*), pulses, etc. Kodo is the chief common food.

METEOROLOGICAL DATA.

The maximum and minimum temperatures, relative humidity and rainfall were recorded from June 1953 to January 1954. In June there is extreme dry heat, the highest maximum temperature of 113° F. was recorded on June 8 and 11. The coldest months are December and January when the temperature falls to 50° F.

The total rainfall in 1953 from June to September was 31·12 inches, out of which a precipitation of 17·94 inches occurred during July. The average relative humidity during these months ranged between 70 and 80 per cent.

SPLEEN AND BLOOD EXAMINATIONS.

These examinations were carried out in June and in the last week of September. The results are shown in Table I and Map 1.

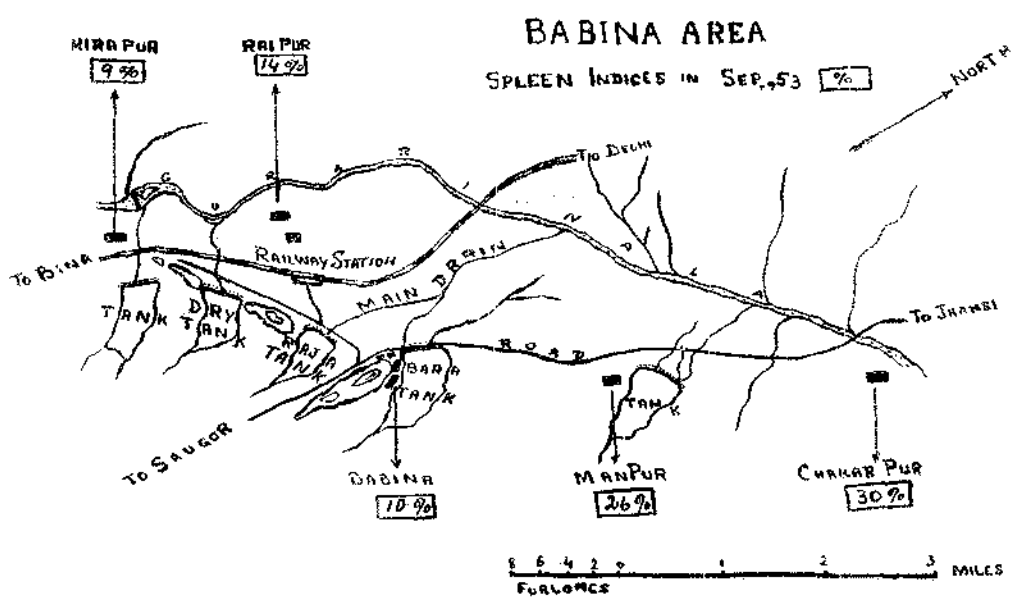
TABLE I.

Results of spleen and blood examinations of children between two and ten years.

Name of the village.	June 1953.				September 1953.			
	Number examined for spleen.	Number with enlarged spleen.	Spleen rate (Per cent).	Parasite rate.	Number examined for spleen.	Number with enlarged spleen.	Spleen rate (Per cent).	Parasite rate (Per cent).
Hirapur ...	35	1	3	30 blood smears examined. All negative.	35	3	9	25*
Raipur ...	33	0	0		29	4	14	
Chakarpur	22	3	14		30	9	30	Not done
Babina ...	100	2	2		120	12	10	
Manpur ...	45	5	11		58	15	26	20†

* Twenty blood smears examined. † Fifty blood smears examined.

MAP 1.



The low spleen indices in Hirapur, Raipur and Babina villages are due to malaria control (indoor D.D.T. spraying) practised in this area for the last ten years. The indices in Manpur and Chakarpur villages, which are not affected by this factor, reveal the true malarial endemicity in the area which can be classified as highly malarious. The rise of indices in September is obvious.

The species of malaria parasites found were three *P. vivax* and twelve *P. falciparum* out of 70 blood slides examined.

ADULT ANOPHELINE COLLECTIONS.

The average weekly catches of *A. culicifacies*, *A. annularis* and the total of all species are shown in Table II. The other species, except *A. subpictus* appeared in small numbers. Three *A. turkhudi* were collected during June and one *A. hyrcanus* during September. Specimens of *A. stephensi* and *A. pallidus* appeared off and on. One specimen of *A. fluviatilis* was also caught.

In Hirapur, Raipur and Chakarpur, the villages on the Gurari Nala, *A. culicifacies* was the predominant species, and *A. annularis* was found in very small numbers. In Babina and Manpur, near the tanks having cultivation of *trapa*, *A. annularis* dominated the catch. In Babina, *A. culicifacies* was a rarity, due to larval control particularly applied against this species. In Manpur Village, the adult density of this species was approximately one-third of that in habitations located on the Gurari Nala.

It was interesting to note that a spate in the Gurari Nala on August 14 and 15, caused a marked reduction in adult *A. culicifacies* density during the week ending August 22, 1953. This provides corroborative evidence of the source of this species from the nala.

Precipitin tests carried out on 199 *A. annularis* by courtesy of the Director, Malaria Institute of India, showed a human positive percentage of 0·5.

DISSECTIONS.

Results of dissection are shown in Table III. Both *A. culicifacies* and *A. annularis* were found with gland infections and are the vector species of the area. In Hirapur, Raipur and Chakarpur villages, *A. culicifacies* is the principal vector species and *A. annularis* is not considered to be an effective carrier due to low density. In Babina and Manpur villages, *A. annularis* is the important vector species. The part played by *A. culicifacies* in these villages depends on the numerical prevalence of this species.

It is noteworthy that *A. culicifacies* kept in cages in laboratory for dissection died in large numbers. During the first 24 hours of captivity, mortality rate of over 90 per cent was repeatedly observed among *A. culicifacies* whereas among *A. annularis* kept under similar conditions, the rate varied from 27 to 43 per cent, thus proving that *A. annularis* is a longer-lived species. This fact would appear to be of importance for the species as a carrier of malaria.

Table II.

Average weekly anopheline collections for July, August and September, 1953.

Week ending	Babina			Hirapur			Raipur			Manpur			Chakarpur		
	All species.	*A. annularis.*	*A. culicifacies.*	All species.	*A. annularis.*	*A. culicifacies.*	All species.	*A. annularis.*	*A. culicifacies.*	All species.	*A. annularis.*	*A. culicifacies.*	All species.	*A. annularis.*	*A. culicifacies.*
July															
6	2	2	0	3	1	0·5	5	0	2	5	4	0	...	...	...
13	5	5	0	3	0·3	0	7	0	2	6	5	0·7	...	...	...
20	13	9	1	6	0	3	7	0	3	8	5	2	...	...	...
27	23	18	2	14	0	10	8	0·3	3	17	10	6	...	...	...
August															
1	22	20	2	20	1	15	8	0	6	32	15	8	...	...	...
8	28	22	0·7	25	2	14	22	2	14	39	19	14	...	...	...
15	30	30	0	35	0·5	30	28	2	17	44	18	14	...	...	...
22	21	16	0	9	3	8	13	1	9	35	16	9	...	...	...
29	47	45	2	49	4	45	35	0	31	35	22	7	31	1	25
September															
5	37	37	0	48	2	38	38	1	27	29	15	8	36	0·5	27
12	23	18	2	35	4	28	33	1	19	21	12	6	24	4	15
19	18	7	2	46	3	36	41	1	38	19	8	6	29	5	20
26										17	8	6	35	1	32
October															
3	Indoor D.D.T. spraying done. Collection stopped.									17	9	5	40	3	33

Table III.

Dissections of A. culicifacies *and* A. annularis.

Month and year	Hirapur, Raipur and Chakarpur villages				
	A. culicifacies.				
	Number dissected for gut infections.	Number with gut infections.	Number dissected for salivary gland infections.	Number with sporozoites in salivary glands.	Infectivity rate.
1953					
July	23	0	64	0	0
August	28	0	130	1	0·77
September	0	0	214	2	0·94
Total	51	0	408	3	0·74

TABLE III—(*Concld.*)

BABINA AND MANPUR VILLAGES

Month and Year	*A. culicifacies*		*A. annularis*				Infectivity rate.
	Number dissected for salivary gland infections.	Number with sporozoites in salivary glands.	Number dissected for gut infections	Number with gut infections.	Number dissected for salivary gland infections.	Number with sporozoites in salivary glands.	
1953							
July ...	0	0	103	0	109	0	0
August ...	38	0	181	1*	229	1*	0·87
September	74	0	63	0	207	1†	0·48
Total	198	0	347	1	545	2	0·55

*Specimens collected from Babina. †Specimens collected from Manpur.

ANOPHELINE BREEDING PLACES.

Gurari Nala. During June, the bed of the nala had a series of pools in which *A. subpictus* was profusely breeding. Rare specimens of *A. culicifacies* and *A. stephensi* were also found. From July to September, the nala continued to flow and out of 1,300 larvæ examined, 98 per cent were *A. culicifacies* and two per cent *A. subpictus*. This nala is the biggest source of *A. culicifacies breeding*.

Tanks.—These can be divided into two groups.

(i) Those with cultivation of watermut of the genus *trapa* (*Singhara*) and also naturally growing aquatic weeds. The surface area of two such tanks in Babina Village is 300 acres. Along the banks of the tanks, larvæ of *A. culicifacies, A. subpictus*, and *A. annularis* are found, while away from the banks only *A. annularis* breeds profusely. Out of 500 larvæ examined from these tanks, 92 per cent were *A. annularis*, two per cent *A. subpictus*, four per cent *A. culicifacies*, one per cent *A. hyrcanus* and one per cent *A. pallidus*. The largest output of *A. annularis* is from these tanks.

(ii) Along the banks of tanks containing a little or no aquatic weeds only scanty breeding of *A. culicifacies* and *A. subpictus* is found. In the last week of September, a few *A. annularis* larvæ were also collected along the portions of banks with weeds.

Paddy fields.—The species found breeding were *A. annularis, A. culicifacies, A. subpictus, A. hyrcanus* and *A. pallidus*. *A. culicifacies* was found either when the plants were less than nine inches in height or along the margins of fields. *A. annularis* was completely replaced by *A. hyrcanus* when the plants grew to a height of two and a half feet.

The other important breeding places of *A. culicifacies* are seepage area drains, irrigation channels, small nalas and wells, and those of *A. annularis*, borrowpits and channels with aquatic vegetation.

DISCUSSION.

Prior to this survey, no dissections were carried out to incriminate the vector species in this area but *A. culicifacies* was presumed to be the vector. *A. annularis* was never suspected to be responsible for transmission of malaria. The dissection results confirm that both *A. culicifacies* and *A. annularis* are vectors of malaria in this area. The former is proved as the important vector species in habitations located on the Gurari Nala, and the latter in those near water reservoirs in which *trapa* is cultivated.

A. annularis has been considered to be the principal malaria carrier in coastal plains of Orissa by Senior White (1943). Out of 9,183 specimens dissected, 14 gut and 7 gland infections were found giving infectivity rate of 0.23 per cent. In Birbhum District, Bengal, Timbre (1935) found a sporozoite rate of 0·02 per cent after dissecting 49,698 specimens. In Goalpara District, Assam, Viswanathan *et al.* (1941) dissected 7,481 specimens and found infectivity rate of 0·15 per cent. They considered this species as the sole vector in that locality. In the rest of India, it was not considered to be playing any significant part in malaria transmission, as no salivary gland infections were met with except one infection, by Adie (1905) at Ferozepore. In the vicinity of the Chilka Lake, Covell and Pritam Singh (1942) dissected 20,844 specimens and found only one gut infection. Senior White and Adhikari (1939) recorded three gut infections in 1,048 specimens in the same area. Senior White (1943) dissected 1,930 *A. annularis* in Hazaribag and found one gut infection. But in Babina area, two salivary gland and one gut infections out of 545 *A. annularis* dissected during July, August and September 1953, were found, giving an infection rate of 0·55 per cent. Consequently, this species is considered to be an important vector species in places where its density is high due to cultivation of *trapa* in tanks. The breeding of this species in paddy fields, borrowpits etc., does not give rise to high density. The part played by *A. culicifacies* in these places also depends on the numerical prevalence of the species.

The degree of malarial endemicity in Manpur and Chakarpur villages does not show much difference, in spite of the density of *A. culicifacies* being far less in the former village. This state of endemicity in Manpur Village is maintained by the preponderance of *A. annularis* in that village. The rise of spleen index in Babina from two per cent in June 1953 to ten per cent in September 1953, corroborates the validity of the above statement.

CONTROL OF MALARIA.

Gurari Nala and tanks with *trapa*, the principal breeding places of *A. culicifacies* and *A. annularis*, respectively, are extensive in area and will need collosal expenditure for control. As both the vector species are domestic in habit, the method of choice is anti-adult mosquito measures. Indoor D.D.T. spraying has been practised and found effective.

Routine antilarval control will prove beneficial as an auxiliary measure. The stoppage of cultivation of *trapa* will render *A. annularis* an ineffective carrier of malaria by reducing its density to a very low level ; but is not recommended, as it will not be economical and will render several families destitute. Dusting the tanks with paris green can be instituted. This will entail the provision of a boat and motor dust sprayer. In these tanks, exists a species of beetle in large numbers, which causes considerable damage to *trapa* plants. People kill it by beating with brooms, which is laborious and not very effective. Trials carried out in the laboratory showed that the larvæ of the beetle are killed by paris-green, in the quantity usually used as a larvicide.

SUMMARY.

1. Both *A. culicifacies* and *A. annularis* are the vector species of the area. The former forms the major portion of the catch in habitations near Gurari Nala, and the latter in those near tanks with cultivation of *trapa*.

2. The largest output of *A. culicifacies* is from Gurari Nala, and of *A. annularis* from tanks with *trapa*.

3. The intensity of malaria in the area is high. The main transmission occurs during August and September.

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PYRIMETHAMINE IN THE TREATMENT OF MALARIA.

BY

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THE therapeutic doses of pyrimethamine (Daraprim—Burroughs Welcome & Co. Ltd.) so far used have been rather small. With few exceptions, 25 to 100 mg. in all only have been administered, and in such doses the drug has not been considered suitable for the treatment of acute malaria. Clearance of pyrexia and parasitæmia has been slow in many cases and in some the drug has been a failure. However, a study of results shows that in some cases clearance occurs in the shortest expected time. It was, therefore, felt that larger doses than hitherto used may be more satisfactory. With this end in view, a comparative clinical trial using larger doses was carried out between June 1952 and May 1954 and a dosage scheme of 150 mg. daily × 2 was found to give quite satisfactory results. The details are as below.

SUBJECTS AND METHODS.

Most patients had acquired recent infection after variable stay in endemic and hyperendemic malarial zones in various parts of India. Some had relapses. Probably all were premunised subjects. A few had been on suppressive treatment with paludrine or mepacrine.

Thick and thin blood smears were examined six-hourly till they were persistently negative for ten days. This prolonged examination was necessary as in some cases, after initial clearance, parasites reappeared after five or more days.

R.B.C. counts, Hb. estimations and total and differential W.B.C. counts were done before and two and ten days after administration of the drug for evidence of toxicity, if any.

Temperature was recorded four-hourly. Many cases had low periodic fever, about 100°F., which occurred with specific malarial periodicity after the main paroxysms had been controlled and four-hourly temperature was necessary to bring out these little pyrexial peaks. Before administration of the drug, the previous range of temperature was 101° to 107° F.

Only pure *P. vivax* and *P. falciparum* infections were selected for trial. The parasite count varied from 0·3 to 64 per 1,000 R.B.Cs. Antimalarial therapy was given to those who showed almost a persistent parasitæmia for twenty-four hours and were not expected to have natural pyrexial remissions without treatment. Such remissions occurred in nearly 24 per cent of all cases and weeding them out was an important aspect of selection.

Therapy was begun at the onset of a paroxysm to ensure uniformity in assessing the duration of pyrexia and parasitæmia. In a few cases vomiting occurred within two hours of administering the drug and in these half to full dose was repeated.

In all, there were seven groups of cases. Groups I to IV received a single dose of 50, 100, 200, and 300 mg. daraprim, respectively, and Groups V to VII received 50, 100, and 150 mg. daraprim daily × 2, respectively. The patients were all adults between the ages of 18 and 34 years. Their mean weight was 118 and the range from 108 to 132 pounds.

All patients were treated under identical conditions. They were hospitalized for about a month and subsequently followed up by post for evidence of relapse.

EFFECT ON ASEXUAL PARASITÆMIA.

(*a*) *Action on preschizonts and early schizonts.*—Rings and early amœboid forms which were present at the onset of a paroxysm, when the drug was administered, continued to recur subsequently. Their probable development into mature schizonts and merozoites was observed only in five cases, but even then, these forms rapidly cleared within 48 hours. Likewise adult schizonts initially present in ten cases of *P. vivax* malaria, rapidly disappeared. The action of pyrimethamine (daraprim) thus appeared to be on the preschizont and early schizont phases of the asexual parasite and was a consistent finding. It occurred in all dosage schemes and no additional advantage was obtained with larger doses.

(*b*) *Modes of clearance of asexual parasitæmia.*—In 15 out of 42 cases of *P. falciparum* malaria and 28 out of 86 cases of *P. vivax* malaria, the clearance of asexual parasitæmia, when it occurred, was almost sudden and complete and no further parasites were seen in the peripheral blood. In 10 out of the 15 cases of *P. falciparum* malaria, such clearance occurred when the dose of the drug was 200 or 300 mg. daily ×1 or 100 or 150 mg. daily ×2 and in 21 out of the 28 such cases of *P. vivax* malaria, clearance occurred when the dose was 150 mg. daily ×2. In *P. falciparum* infections, the clearance had a tendency to correlate itself to the normal expected withdrawal from the peripheral circulation prior to schizogony in internal organs. In *P. vivax* malaria, preschizonts and schizonts, if present in peripheral blood, appeared to be destroyed and rings were hardly ever seen to develop subsequently into schizonts (*vide supra*).

Similarly when parasites were not present in the peripheral circulation just before administration of drug in previously positive cases, schizogony appears to have been completely suppressed and no subsequent asexual parasitæmia occurred in 15 out of 42 cases of *P. falciparum* malaria and 13 out of 86 cases of

P. vivax malaria. In 7 out of 15 cases of *P. falciparum* infection and in 12 out of 13 *P. vivax* cases, clearance occurred when the dose was 150 mg. daily × 2.

Due to the absence of subsequent recurrence, the duration of asexual parasitæmia was the shortest in both the above series of cases.

In the remaining cases there was irregular recurrence after initial withdrawal for varying periods or parasites were found at irregular intervals only.

There was no evidence that previous immunity affected the mode of clearance.

Table I gives the incidence of various modes of clearance.

TABLE I.

Incidence of various modes of clearance of asexual parasitæmia in P. vivax *and* P. falciparum *malaria.*

Type of malaria.	Total number of cases.	Complete clearance.	Clearance with irregular recurrence.	Irregular recurrence.	Remained negative.
P. vivax	86	28	25	20	13
P. falciparum	42	15	7	5	15

(*c*) *Rate of clearance.*—The clearance on the whole was slow and uncertain. Generally, however, the 150 mg. daily × 2 dosage schedule was most effective in both *P. falciparum* and *P. vivax* malaria. This is obvious in Tables II and III, especially when the rates of clearance of asexual parasitæmia and high initial pyrexia are studied together. It will be noticed that 5 out of 42 cases in *P. falciparum* malaria and 16 out of 86 cases in *P. vivax* malaria did not clear in eight days. The longest duration in *P. falciparum* malaria was 34 days and in *P. vivax* malaria 39 days.

TABLE II.

Rate of clearance of asexual parasitæmia and high initial pyrexia in P. vivax *malaria.*

Dosage scheme.	Total number of cases.	Number cleared in days.											
		Asexual parasitæmia.						Pyrexia.					
		2	4	6	8	10	10+	1	2	3	4	5	5+
50 mg. × 1	9	4	2	1	2	0	0	3	2	1	2	0	1
100 mg. × 1	23	7	2	7	3	2	2	10	6	5	1	0	1
200 mg. × 1	10	1	1	1	3	2	2	3	0	4	3	0	0
300 mg. × 1	9	3	1	0	1	2	2	3	0	6	0	0	0
50 mg. × 2	6	4	0	2	0	0	0	1	2	3	0	0	0
100 mg. × 2	5	2	1	0	0	0	-	1	0	4	0	0	0
150 mg. × 2	24	12	8	2	0	0	2	12	7	5	0	0	0

TABLE III.

Rate of clearance of asexual parasitæmia and high initial pyrexia in P. falciparum *malaria.*

Dosage scheme.	Total number of cases.	Number cleared in days: Asexual parasitæmia.						Pyrexia.					
		2	4	6	8	10	10+	1	2	3	4	5	5+
50 mg. × 1	4	4	0	0	0	0	0	2	0	1	0	1	0
100 mg. × 1	5	3	1	1	0	0	0	2	2	0	1	0	0
200 mg. × 1	4	1	0	0	0	0	0	1	2	0	1	0	0
300 mg. × 1	2	0	1	0	0	0	1	0	0	2	0	0	0
50 mg. × 2	4	3	3	1	0	0	0	0	0	1	1	-	1
100 mg. × 2	5	3	1	1	0	0	1	1	1	1	0	1	1
150 mg. × 2	18	9	6	0	0	0	3	4	5	8	1	1	0

GAMETOCYTE CLEARANCE.

The vast majority of cases which did not reveal gametocytes initially, showed these forms (not heavy) within four days of administration of daraprim (Table IV).

TABLE IV.

Appearance of gametocytes.

Type of malaria.	Total number of of cases who developed gametocytes.	Number who developed gametocytes on days after daraprim administration.					
		1	2	3	4	5	5+
P. vivax ...	21	0	5	3	9	2	1
P. falciparum	13	0	3	4	3	1	2

The appearance of gametocytes was closely related to the mode of clearance of asexual parasitæmia. In Table V, it will be noticed that most cases of *P. vivax* malaria, in which clearance of asexual parasitæmia was associated with irregular recurrence, developed gametocytes subsequently, whereas the incidence was appreciably low in cases where the clearance was sudden and least in cases who remained altogether negative after administration of daraprim. Except in the last group in *P. falciparum* malaria, however, gametocytes were detectable as a rule. The incidence was generally, but not invariably, less in the relatively more immune cases.

TABLE V.

Relation between mode of clearance of asexual parasitæmia and appearance of gametocytes in previously negative cases for gametocytes.

Type of malaria.	Type of clearance of asexual parasitæmia.	Number of negative cases.	Number who developed gametocytes.	Number who did not develop gametocytes.
P. vivax	Complete clearance	18	5	13
	Clearance with irregular recurrence	18	12	6
	Irregular recurrence ...	6	2	4
	Remained negative ...	9	1	8
P. falciparum	Complete clearance ...	5	5	0
	Clearance with irregular recurrence ...	4	4	0
	Irregular recurrence ...	3	3	0
	Remained negative ...	4	1	3

The appearance of gametocytes was also related to the dosage of daraprim used. In both *P. falciparum* and *P. vivax* malaria, cases who received 150 mg. daily ×2, had the lowest incidence of gametocytes. Thus the greater effectiveness of this dosage scheme against asexual parasitæmia (*vide supra*) indirectly reflected itself here (Table VI).

TABLE VI.

Effect of daraprim dosage on the appearance of gametocytes.

Type of malaria.	Total number who developed gametocytes.	Number out of each daraprim group who developed gametocytes.						
		50 mg.	100 mg.	200 mg.	300 mg.	50/50 mg.	100/100 mg.	150/150 mg.
P. vivax ...	20	5/6	4/12	5/8	2/4	2/5	1/5	1/11
P. falciparum ..	13	2/2	2/2	1/1	0/1	2/2	3/3	3/5

The rate of clearance of gametocytes did not show any relation to the mode of clearance or the duration of asexual parasitæmia or the daraprim dosage in both types of malaria. In *P. vivax* malaria, the clearance itself was definitely slow and in three cases of *P. falciparum* malaria a dosage of 150 mg. daily ×5 was tried but crescents were not eradicated. Table VII summarizes the rate of clearance in both types of malaria.

TABLE VII.

Rate of clearance of gametocytes.

Type of malaria.	Number of cases with gametocytes.	Number cleared in days.								
		5	10	15	20	25	30	35	40	40
P. vivax ...	54	23	20	8	1	1	0	0	0	1*
P. falciparum	37	5	5	9	5	5	3	3	0	2†

* This took 56 days to clear. The dose of daraprim given was 300 mg. daily × 1 and the duration of asexual parasitæmia was 11 days.

† These took 41 and 46 days to clear. Both cases received 200 mg. × 1 dose of daraprim and the duration of asexual parasitæmia in each of them was less than one day.

PYREXIA.

(*a*) *Control of main paroxysms.*—In Tables II and III, it will be noticed that no dosage was particularly effective in controlling main paroxysms in *P. falciparum* malaria. Repeated doses were, however, definitely more effective in *P. vivax* malaria and this correlates generally with clearance of asexual parasitæmia. On the whole the 150 mg. daily × 2 dose appears to have been the most effective in both types of malaria. With this dose, 12 out of 24 cases of *P. vivax* malaria subsided within 24 hours and 9 out of 18 cases of *P. falciparum* malaria subsided within 48 hours. Almost all cases in both types subsided within 72 hours.

The pyrexial patterns, after daraprim administration, are summarized in Table VIII. When there were two paroxysms, the second paroxysm was often surprisingly higher than the first one. Multiple paroxysms were successively lower or lower only at the tail end. Some of these patterns are shown in Charts 1, 2, 3, 4, 5 and 6.

(*b*) *Low Periodic fever.*—In 54 out of 86 cases of *P. vivax* malaria and 21 out of 42 cases of *P. falciparum* malaria, there was low periodic fever from 99° to 100° F. following control of main proxysms. The duration of periodic fever varied from 3 to 34 days during which the number of spikes of periodic temperature were 1 to 7. Details are summarized in Table IX.

At the same time, blood was positive for asexual parasites, generally at irregular intervals, in 28 out of 54 cases of *P. vivax* malaria and in 8 out of 21 cases of *P. falciparum* malaria. In 20 out of the 28 positive *P. vivax* cases and in 6 out of the 8 positive *P. falciparum* cases, parasitæmia was present throughout the course of periodic fever. In the remaining cases of both types of malaria, however, blood was negative towards the end. Symptoms suggestive of latent malaria were present during the course of periodic fever and in cases where this was prolonged, patients remained in poor health and did not gain weight.

TABLE VIII.

Pyrexial patterns after daraprim administration.

Pyrexial pattern.	Type of malaria.	
	P. vivax	*P. falciparum.*
One paroxysm only	17	7
Daily intermittent paroxysm for two days ...	7	6
Daily intermittent paroxysm for three days	7	10
Daily remittent paroxysm for three days ...	6	7
Two paroxysms on alternate days	36	0
Three paroxysms on alternate days	1	0
Four paroxysms on alternate days	1	0
Daily intermittent paroxysm for two days and third paroxysm on fourth day	3	1
Daily intermittent paroxysm for three days and fourth paroxysm on fifth day	0	3
Daily intermittent paroxysm for five days and sixth paroxysm on seventh day	3	2
One paroxysm followed by daily paroxysms on third and fourth day	1	0
Low fever paroxysms only	1	6
Total number of cases	86	42

TABLE IX.

Incidence and details of periodic fever.

Type of malaria.	Total number of cases treated.	Number with periodic fever.	Incidence of periodic fever in weeks.					Incidence of spikes of periodic fever.						
			1	2	3	4	5	1	2	3	4	5	6	7
P. vivax ...	86	54	28	13	9	3	1	20	9	8	2	2	4	1
P. falciparum ...	42	21	3	14	4	0	0	4	4	3	2	2	0	1

The periodic fever occurred irrespective of the dose of daraprim administered and previous immunity in relapse cases.

The pathogenicity of parasites during this stage could not be tested. It will be noticed from Tables IX and X, however, that only two such cases out of 54

in *P. vivax* malaria and three out of 21 cases in *P. falciparum* malaria, subsequently relapsed.

(*c*) *Relation between duration of pyrexia and duration of parasitæmia.*—The duration of pyrexia, main paroxysms plus periodic fever, was more than the duration of asexual parasitæmia in 65 out of 86 cases of *P. vivax* malaria and in 31 out of 42 cases of *P. falciparum* malaria. With main paroxysms alone, this incidence was 28 out of 86 and 27 out of 42, respectively. *In P. falciparum* malaria, therefore, asexual parasitæmia cleared earlier than pyrexia twice as commonly as in *P. vivax* malaria.

RELAPSES.

During a follow up of four to nine months there were no delayed relapses. Eleven relapses, details of which are summarized in Table X, however, occurred within the first 80 days. These relapses occurred irrespective of previous immunity in both fresh and relapse cases. But in the groups which received daraprim 300 mg. daily × 1, and 150 mg. daily × 2, there were no relapses at all. Similarly a relapse, following use of a smaller dose, was cured subsequently by a bigger dose. Thus, in Table X, Serial Number 2 of *P. vivax* malaria, made a dramatic response to a 300 mg. daily × 2 dose. Both pyrexia and asexual parasitæmia in this attack were controlled within 24 hours and no subsequent relapse occurred during seven months follow up.

TABLE X.

Details of relapses.

Type of malaria.	Total number of cases.	Serial number of relapses.	Dose of daraprim mg. in days.	Duration of asexual parasitæmia in hours.	Duration of main paroxysms in hours	Periodic spikes on days after treatment.	Relapse on day after treatment
P. vivax ...	86	1	50 × 1	43	44	0	43
		2	50 × 1	172	76	7, 9, 18	20
		3	100 × 1	120	6	0	14
		4	200 × 1	192	76	5, 8, 10	80
		5	100 × 2	0	54	0	15
P. falciparum	42	1	50 × 1	18	58	30, 37	39
		2	100 × 1	0	40	0	7
		3	100 × 1	0	45	0	11
		4	200 × 1	0	48	4, 10	21
		5	200 × 1	0	6	8, 12	13
		6	100 × 2	98	70	0	20

RESISTANCE.

The following clinical types of resistance were encountered.

1. Asexual parasitæmia persisted after treatment for an unusually long time.—In 16 out of 86 cases of *P. vivax* malaria and in 5 out of 42 cases of *P. falciparum* malaria, asexual parasitæmia persisted for more than eight days, the longest duration in the former was 39 days and in the latter 34 days (*vide supra*).

2. The low periodic fever, following control of main paroxysms by treatment, merged into an overt relapse.—Serial Number 2 of *P. vivax* malaria cases and Serial Number 5 of *P. falciparum* series in Table X.

3. After a sharp control of the initial paroxysm, there was an abrupt relapse within one to three weeks—Serial numbers 3 and 5 of *P. vivax* malaria cases and serial numbers 2, 3 and 6 of *P. falciparum* series in Table X.

4. The patient developed resistance to repeated daraprim treatments.—Relapse on twenty-first day of Serial Number 4 of *P. falciparum* malaria cases in Table X was treated with a single dose of daraprim 300 mg. but another relapse occurred after 20 days. This time a dose of 150 mg. daily × 2, altogether failed to control the attack. On the fifteenth day, the patient's condition became serious and the attack was terminated within 18 hours by one 1 g. dose of camoquin. No subsequent relapse occurred during the following six months. The strain of parasite was from Jammu.

Another case of *P. falciparum* malaria acquired at Agra had a first attack which subsided without any treatment after six days. Scanty rings and crescents, however, persisted and symptoms suggestive of latent malaria were present. He was given a single 100 mg. dose of daraprim nine days after the previous attack had subsided, but he relapsed after 14 days. This time another single dose of 100 mg. brought no signs of remission in ten days but a single 1 g. dose of camoquin terminated the attack within 12 hours. No subsequent relapse occurred during the following eight months.

CROSS RESISTANCE TO OTHER ANTIMALARIAL DRUGS.

Six cases had been on suppressive paludrine, 300 mg. weekly, for four to ten months and four cases had been on suppressive mepacrine, 100 mg. daily, for three to fourteen months. In all of them, an overt attack of malaria had occurred within three weeks of cessation of such treatment. None of them showed any evidence of cross resistance to daraprim. Similarly daraprim relapses were successfully treated by single dose of camoquin.

TOXICITY.

The drug was generally well tolerated. The main symptoms of intolerance are summarised in Table XI.

TABLE XI.

Incidence of toxic symptoms.

Symptoms.	Type of malaria.	
	P. vivax.	*P. falciparum.*
Headache	1	0
Giddiness	3	5
Nausea	1	3
Abdominal pain	2	3
Vomiting	8	6
Chest pain	5	4
Muscular weakness	3	4
Hæmolytic anæmia	0	1
Total number of cases	86	42

These symptoms usually occurred only when the dose of daraprim was 200 mg. or 300 mg. In one case, however, severe vomiting occurred within half an hour each time a dose of 50 mg. was given and daraprim treatment had to be abandoned.

The case of acute hæmolytic anæmia had received a dose of 150 mg. daily ×2 and his parasite count had been two per 100 R.B.Cs.

Three cases who were given 150 mg. daily × 5 (*vide supra*) developed light-headedness, mental confusion and muscular weakness. These symptoms took a week to subside after the drug was discontinued.

DISCUSSION.

The results of daraprim treatment appear to vary with different geographical strains. Apparently premunity does not alter the course to a significant extent.

Schneider, Canet and Dupoux (1952) treated 132 cases of *P. vivax* and *P. falciparum* malaria in Tunisia and Indo-China and found daraprim in single doses of 50 or 100 mg. or two doses of 50 mg. to be as rapid in its action as chloroquine, and reported only one failure out of 36 cases with the Tunisian strain.

Field, Edeson and Wilson (1952) treated 112 cases of *P. vivax* and *P. falciparum* malaria in Malaya with 50 mg. daily × 1, 50 mg. daily × 2, and 100 mg. daily × 1 plus 50 mg. daily × 4 dosage schedules and found its action to be slow. Some cases had symptoms persisting for three days and others had symptoms for a day or two after asexual parasitæmia had cleared. In *P. falciparum* malaria there were failures in each dosage schedule and incidentally most numerous, 7 out of 26, in the 100 mg. daily × 1 plus 50 mg. daily × 4 dosage schedule.

Gilroy (1952) and Norman (1952) from Assam, and Hay Arthur (1952) from Bengal, reported good results in most of their cases. The incidence of failures, however, was rather high. In *P. falciparum* malaria on a daraprim dosage of 50 mg. daily × 1, Gilroy had two failures out of 11, and Norman one out of 21 cases. With 20 mg. daily × 1, the incidence of failures was three out of 15 cases in *P. falciparum* malaria in Norman's series and one out of 6 cases in *P. vivax* malaria in Hay Arthur's group.

To 29 cases of fresh and relapse-induced *P. vivax* malaria, due to Chesson strain, Coatney *et al.* (1948) gave a single dose of 25 mg. daraprim and found the immediate results to be almost as good as with a single dose of 0·6 g. chloroquine. The asexual parasitæmia, however, tended to be longer, and all cases unless placed on suppressive treatment, subsequently relapsed.

McRobert (1952) treated seven cases of *P. vivax* malaria (Korean strain) who previously had been on suppressive paludrine and found the clinical response to 25 mg. daraprim b.d. very slow. Asexual parasitæmia persisted over 90 hours in one case and over 150 hours in two cases.

Jaswant Singh *et al.* (1953) treated 30 cases of *P. vivax* malaria with a single dose of 25 mg. daraprim. All cases were afebrile within 72 hours and in 96·6 per cent, asexual parasitæmia cleared within the same period. The symptoms and asexual parasitæmia seemed to clear more quickly in those cases who gave a previous history of malaria. Earlier the same authors (Jaswant Singh *et al.*, 1952) using a dosage scheme of 25 mg. ×2 in 84 cases of *P. falciparum*, recorded parasite clearance within 72 hours in 93 to 100 per cent of cases. Clinical response during the same period was observed in all the 22 cases treated at the Police Hospital Delhi. However, the drug failed to respond in two cases (Chaudhuri and Chakravarty, 1953).

In monkeys infected with *P. cynomolgi*, Schmidt and Genther (1953) found that asexual parasitæmia cleared in seven to eight days. A dose of 0·6 mg. per kg. was required for eradication.

By giving the drug in repeated, increasing, but inadequate quantities, Rollo (1952) found that resistance developed after a relatively small number of treatments in mice heavily infected with *P. berghei*. Similary a thousand-fold resistance was reported in monkeys infected with *P. cynomolgi* by inadequate treatment by Schmidt and Genther (1953). Jaswant Singh *et al.* (1954) made similar observations in *P. knowlesi* and the resistance developed was found to be many thousand-fold. In two cases of *P. falciparum* malaria described above, similar resistance with repeated but bigger doses of daraprim appears to have developed.

In *P. falciparum* malaria, Wilson and Edeson (1952) found that daraprim remained effective in normal doses in patients resistant to paludrine. Similar findings have been reported by Hawking and Thurston (1951) and Hawking (1952) in animal experiments. Jaswant Singh *et al.* (1953), however, have reported high degree of cross-resistance between paludrine and daraprim in simian malaria.

Schmidt and Genther (1953) in their animal experiments found that the same total dosage was more effective given in small daily doses spread over a week than as a single dose in *P. cynomolgi* infections which are closely allied to *P. vivax* infection in man. In *P. falciparum* malaria in man, Field *et al.* (1952), however, found that daraprim dosage of 100 mg. daily ×1 plus 50 mg. daily ×4, was no

more effective than single doses of 50 mg. or 100 mg. In fact, with the repeated doses the number of failures were more numerous.

Although single doses of 25 mg. or 50 mg. have been reported to be quite effective by some of the workers mentioned above, experience in this trial indicates that to get the maximum number of pyrexial clearances in the least time, prevent development of resistance and failures, and reduce the incidence of early relapses, the dose should be 150 mg. daily ×2. There is evidence of a cure of frequently relapsing case of *P. vivax* malaria with a dosage of 300 mg. daily ×2. Absence of delayed relapses indicates that much importance cannot be attached to the slow clearance of asexual parasitæmia. It appears that ring forms, not destroyed early, are incapable of completing the asexual cycle in the blood or the internal organs. Further, Foy and Kondi (1952) have shown that although gametocytes exposed to daraprim continue to circulate and are normal in appearance, they fail to develop to maturity when injected by the mosquito as was shown by Shute and Maryon (1948) in case of paludrine. In this respect, the drug is superior to other antimalarials. However, delayed clearance perhaps handicaps its use in cerebral malaria and other pernicious forms of malaria.

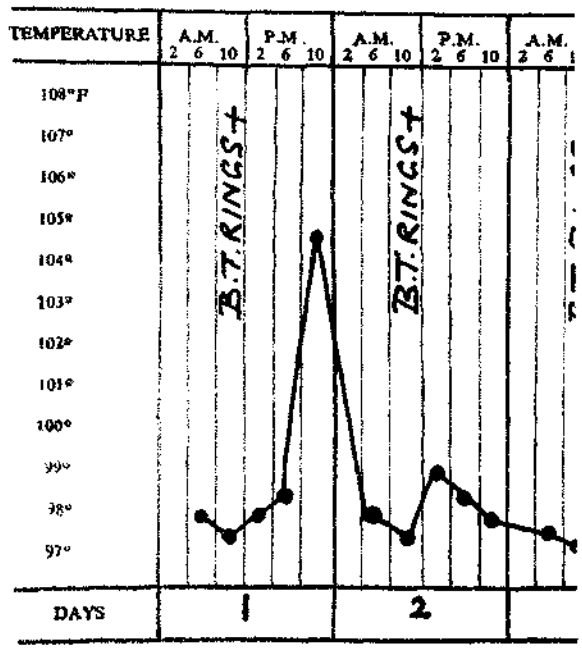
The drug appears to be parasitostatic rather than parasiticidal. This by itself may account for the prolonged parasitæmia made worse by asynchronicity in development. Thus synchronicity which is more marked in *P. vivax* malaria than in *P. falciparum* malaria, probably accounts for better results in the former.

The drug is on the whole well tolerated. Giddiness, abdominal pain, vomiting, muscular weakness, vague chest pains which may occur in patients after daraprim, cannot unequivocally be attributed to the drug as these may be due to malaria itself. Individual susceptibility, however, may play a part in their causation as indicated in the case who vomited after each 50 mg. dose of daraprim. Large doses, not therapeutically indicated, have caused temporary mental confusion and light-headedness. Blood changes have also been reported. Field (1952) reported fall in the white cell count in some patients who received 100 mg. daily ×1 plus 50 mg. daily × 4. McGregor and Smith (1952) found a significant rise of eosinophils with a peak about the fifth day after treatment in some cases. Schmidt *et al.* (1953) have quoted hæmoglobinæmia in one case, slight anæmia in seven cases and degenerative changes in 5 of 12 volunteers who took 25 mg. daraprim daily for seven weeks. The occurrence of hæmoglobinæmia suggests that as is the case with other antimalarials, blackwater fever may be precipitated by daraprim.

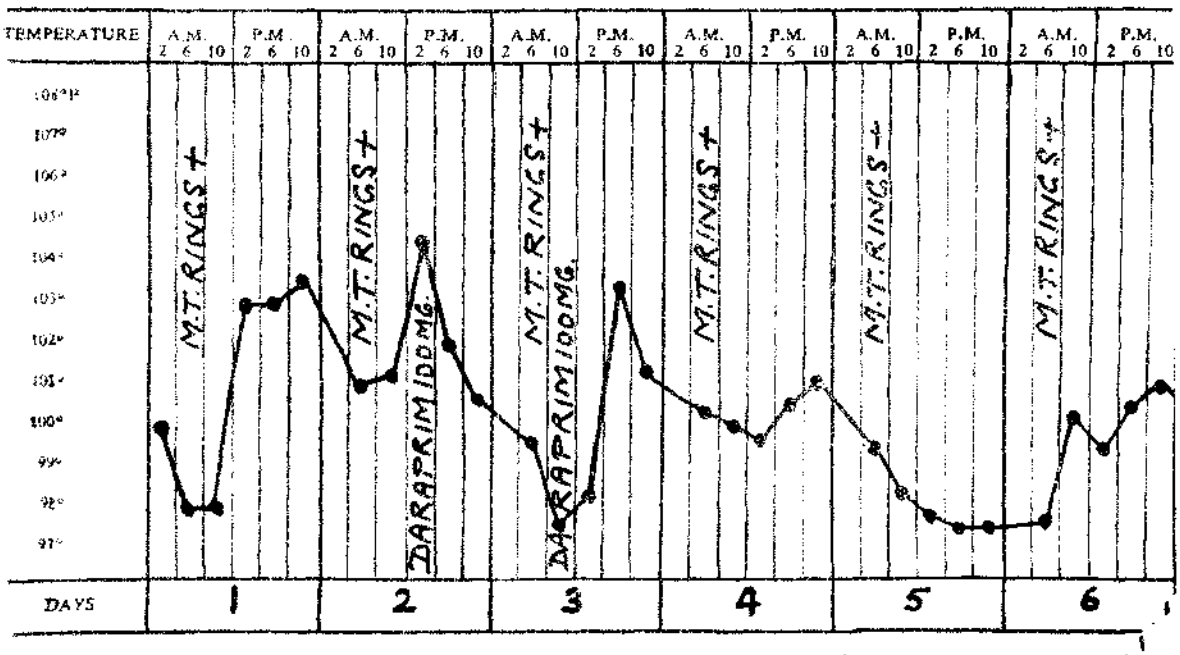
SUMMARY.

Eighty-six cases of *P. vivax* malaria and 42 cases of *P. falciparum* malaria were treated with daraprim 50, 100, 200, and 300 mg. daily ×1 and 50, 100, 150 mg. daily ×2, respectively.

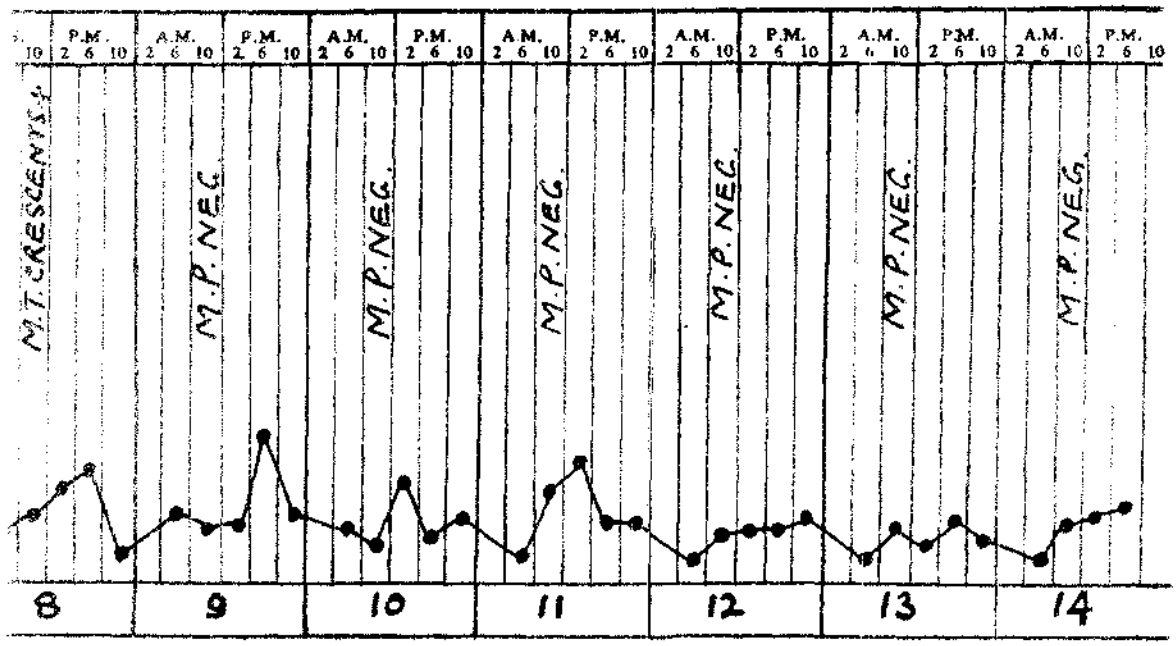
Based on majority of pyrexial clearances in a relatively quick time, mode of development of resistance, frequency of failures and early relapses, the most effective dose was found to be 150 mg. daily ×2 for both *P. vivax* and *P. falciparum* malaria. In 79 per cent of *P. vivax* malaria cases and in 50 per cent of *P. falciparum* cases, fever subsided within 48 hours and almost in all the cases of both types of



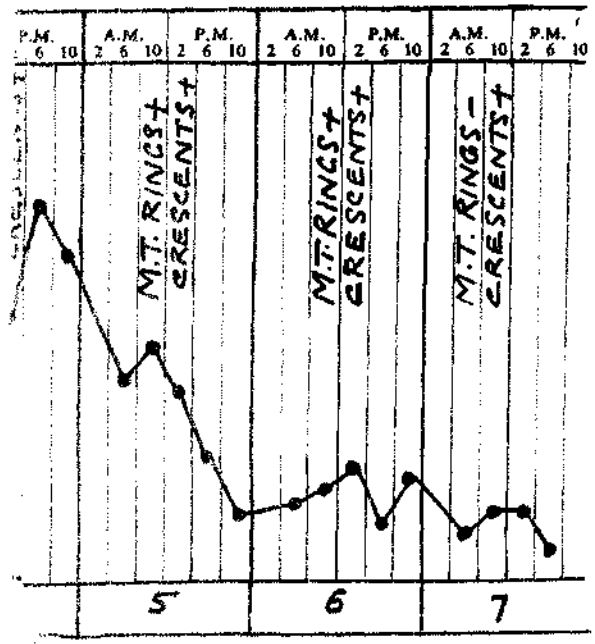
Ideal response in a case of *P. vivax* n
of pyrexia and asexual parasitæmi



Slow response in a case of *P.* ... forms were present in the peripheral ... on the seventeenth day after daraprim ... and low periodic fever was 152 h ... months.



... case of *P. falciparum* malaria
...exual parasitaemia was 66 hours
...r was 248 hours.

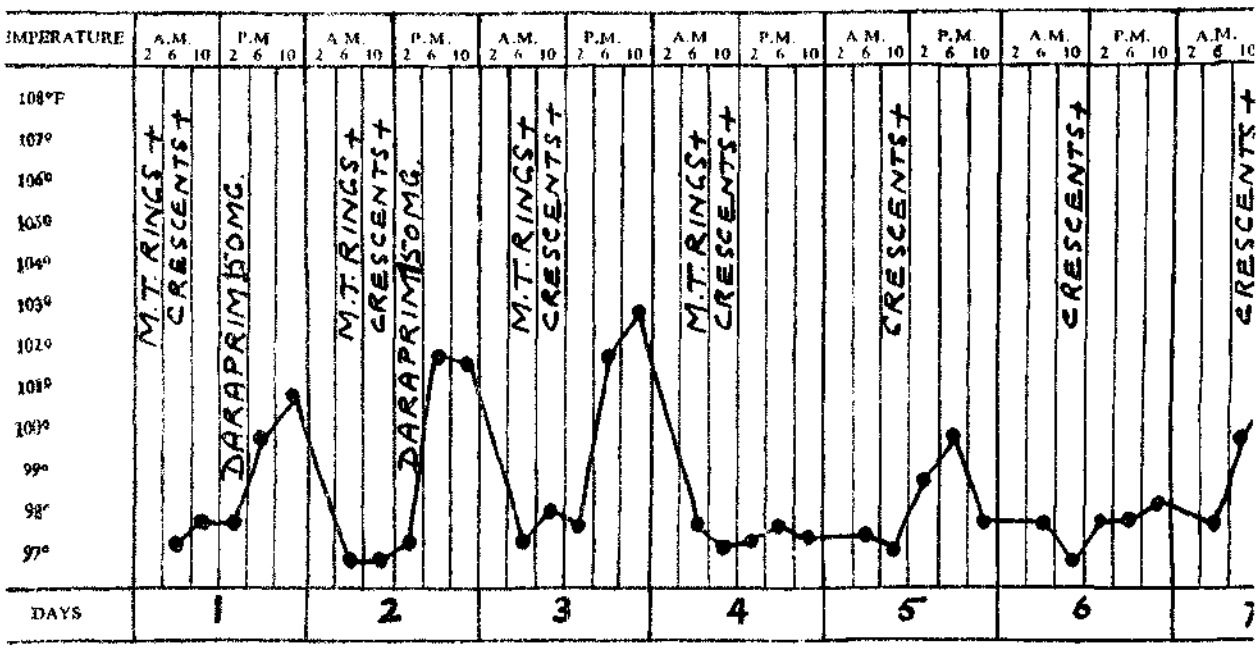


...the onset of which a single 100 mg.
...malaria. The duration of sexual
...The duration of main paroxysms

TEMPERA

108°F
107°
106°
105°
104°
103°
102°
101°
100°
99°
98°
97°

DAYS



malaria it subsided within 72 hours. Sixty-three per cent cases in *P. vivax* malaria and 50 per cent cases in *P. falciparum* malaria had periodic spikes of temperature subsequently which, if numerous, delayed convalescence.

In 19 per cent of *P. vivax* malaria cases and 12 per cent of *P. falciparum* malaria cases, asexual parasitæmia persisted longer than eight days.

Effect on gametocytes of *P. vivax* malaria was rather slow and in two cases these persisted for 24 and 56 days, i.e., as long as the crescents in some cases.

Effect of previous immunity on results was not significant.

In *P. falciparum* malaria relapses, repeated treatments with daraprim favoured development of resistance to the drug even though big doses were used in two cases.

Immediate relapses were more common in *P. falciparum* malaria than in *P. vivax* malaria. The incidence was 14 per cent and six per cent, respectively.

The drug was fairly well tolerated. Only one individual was really sensitive to the drug in doses of 50 mg.

ACKNOWLEDGMENT.

The author is grateful to Brigadier Sarup Narain, A.M.C., Director of Research and Health, Office of the Director-General, Armed Forces Medial Services, Ministry of Defence, India, and Messrs Burroughs Welcome and Co., Ltd., for supplies of daraprim and to Captain A. R. Subramanian, A.M.C., for his kind help in carrying out this trial.

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Indian Journal of Malariology, **9**, 2, *June 1955*.

ANNOUNCEMENT.

INDIAN COUNCIL OF MEDICAL RESEARCH.

COLONEL AMIR CHAND TRUST PRIZES FOR MEDICAL RESEARCH.

LIEUT.-COLONEL AMIR CHAND, ex-Principal, Lady Hardinge Medical College, New Delhi, has made a donation of Rs. 50,000/- to the Indian Council of Medical Research for the purpose of awarding prizes for the best published research work in the field of 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 Funds.

SIX PRIZES, of almost equal value, of which some may be in the form of medals, are awarded annually on an All-India basis for the best published research work in any subject pertaining to all fields of medical sciences in general including clinical research. The term, 'Clinical research' will imply research into the mechanism and causation of disease, including its prevention and cure. It covers not only work in patients in hospitals, but also field studies in epidemiology and social medicine and observations in general practice.

THREE of the Prizes are known as, 'BASANTI DEVI AMIR CHAND PRIZE' and the other three 'SHAKUNTLA AMIR CHAND PRIZE'.

TWO OUT OF THE SIX PRIZES shall be awarded to graduates of not more than ten years standing counting from the date of graduation, provided that the work for which the prizes are to be awarded is of approved merit.

THE COMPETITORS for the prizes may be MEDICAL or NON-MEDICAL graduates.

THE SELECTION of candidates for the award of the prizes will be made by a Selection Board appointed for the purpose.

IN A JOINT PUBLICATION the prize shall be divided between the joint workers in such proportion as the Selection Board may recommend.

IT has been decided to award during 1955 six prizes of the value of Rs. 300/- each for the best research papers in medical science published by workers during the year 1954 (1st January to the 31st December, 1954).

THE AWARD of the prizes will be announced at the annual meetings of the Scientific Advisory Board and the Advisory Committees of the Indian Council of Medical Research, to be held at Nagpur in November/December, 1955.

THE CANDIDATES are required to submit 15 REPRINTS of their papers published during 1954. These should be sent to the SECRETARY, INDIAN COUNCIL OF MEDICAL RESEARCH, 'P' BLOCK, RAISINA ROAD, NEW DELHI, so as to reach him NOT LATER THAN THE 1st SEPTEMBER, 1955.

THE PAPERS should be accompanied by a short biographical sketch and two copies of PASSPORT SIZE PHOTOGRAPHS of the worker or workers concerned.

Indian Journal of Malariology, **9**, 3, *September 1955*.

THE BIOCHEMISTRY AND NUTRITION OF *PLASMODIUM BERGHEI*.*

BY

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AND

D. F. SPOONER.

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[May 11, 1955.]

For a long time the attack on many malaria problems of fundamental importance was hampered by the absence of suitable laboratory infections. In the earlier experiments on chemotherapy the avian malaria parasites, *P. relictum*, *P. cathemerium* and *Hæmoproteus orizivoræ* were most commonly used. Much useful information was also furnished by experiments on therapeutic malaria in man. It soon appeared that many of the outstanding problems, including that of the mode of action of antimalarial drugs and the development of new ones, might be solved through studies of the metabolism of the parasite. Such studies were initiated by Christophers and Fulton (1938) using the monkey parasite *P. knowlesi*. Similar studies were later intensively pursued in America for which pure strains of other monkey parasites such as *P. inui* and *P. cynomolgi* were also available as well as those of the avian parasites *P. gallinaceum* and *P. lophuræ*. The human parasites have been relatively little studied because of the difficulty in obtaining them regularly in sufficient numbers.

Since the discovery of the malaria parasite, *P. berghei* (Vincke and Lips, 1948), which infects a wide variety of rodents, numerous studies on this plasmodium have been reported in recent years. Up to the present, however, little is known of its biochemistry and nutritional needs. In this article an attempt has been made to summarize the known facts for the first time.

*This paper forms part of the Symposium on *Plasmodium berghei* published in the December 1954 issue of the Indian Journal of Malariology.

BIOCHEMISTRY OF *P. BERGHEI.*

Investigations into the chemical composition and metabolism of the malaria parasite have, in the past, been mainly confined to the erythrocytic forms of avian and simian species due to the technical difficulty in obtaining sufficient amounts of material for study from other sources. As *P. berghei* can be transmitted to laboratory rodents, a species of malaria is now readily available for biochemical studies. The number of publications concerned with this aspect of the parasite is limited however; this is probably due in part to some of its characteristics which complicate such researches. Any study of the metabolism of malaria parasites is, of course, complicated by their situation within another living cell—the erythrocyte. In interpreting results obtained from studies of parasitized whole blood or washed parasitized cells, allowance must be made for contributions by the host cell to the metabolic activities measured. It is not known to what extent the metabolism of the host cell is altered by the presence of the parasite within it, and so control experiments using normal erythrocytes are only of limited value. The problem is made technically more difficult with *P. berghei* as it is now established that this species preferentially invades the reticulocyte (Ramakrishnan and Prakash, 1950; Corradetti and Verolini, 1951). This is a metabolically active blood cell in which the synthesis of protein (hæmoglobin) is occurring (Borsook *et al.*, 1952), accompanied by marked respiration and glycolysis (Ponder, 1948). Ribosenucleic acid, a constituent of other active cells (Caspersson, 1947; Claude, 1949), is present in high concentration within immature erythrocytes (Dustin, 1944; Burt *et al.*, 1951). Reticulocytes of varying age, from the nucleated erythroblast to cells which have almost lost the characteristic reticulum, are found circulating in the blood of rats in the later stages of infection with *P. berghei* and all the developmental stages appear to be invaded by the parasite. Thus the host cell in this infection has not only a high but a variable metabolic rate. In *P. berghei* infections too, multiple infection of reticulocytes frequently occurs and this makes an exact determination of the number of parasites present in a stained smear difficult. The infection is asynchronous and the simultaneous presence of many developmental stages of the parasite in a sample of infected blood complicates a study of any particular asexual stage. Little pigment is formed by *P. berghei* and even after prolonged centrifugation, it is often difficult to determine the line of demarcation between parasitized red cells and non-parasitized cells which was so characteristic of *P. knowlesi* infected blood on account of the large amount of pigment present in that parasite. In any case, good separation of normal and parasitized cells is not achieved by this means due to the wide range of specific gravities of the formed elements of the blood in *P. berghei* infections. These characteristics tend to outweigh the advantages of a readily available source of material for *in vitro* biochemical study.

CHEMICAL COMPOSITION OF *P. BERGHEI.*

Kruszynski (1952) has applied the technique of microincineration to demonstrate the localization of inorganic substances within *P. berghei* infected cells. This procedure has been used with some success to demonstrate inorganic material in a few other parasitic protozoa (Scott and Horning, 1932; Horning and Scott,

1933). Such methods are of value in the study of the composition of the malaria parasite as it may then be studied *in situ* without the necessity of separating it from the host cell for chemical analysis. Kruszynski (1951) had previously found that as *P. gallinaceum* developed within the avian erythrocyte, the mineral content (Na, K, Ca and P) of the parasite increased. The amount of calcium and potassium in the cytoplasm of mature erythrocytes is small and so Kruszynski's observations indicated that the parasite obtained these minerals either directly from the plasma through the host cell membrane, or from the host cell nucleus. To rule out the latter alternative, the author used the mammalian species, *P. berghei*. Smears of infected blood were made on cover slips, incinerated, and the resulting preparation, in which the cells were found to retain their normal shape, examined by phase contrast microscopy. An increase in the mineral content of the parasitized cells occurred synchronously with the development of the parasite and the presence of calcium was demonstrated. The residue of normal mature cells after incineration was found to contain little or no calcium and so the author concluded that the parasite obtained calcium directly from the plasma through the host cell membrane. However, the host cell in *P. berghei* infections is chiefly the reticulocyte and no mention is made of the calcium content of the cytoplasm of this cell. Little appears to be known of its mineral content and, as it is a more complex cell than the mature erythrocyte, it is possible that it may contain significant amounts of calcium which may serve as a source for the parasite.

Little is known of the composition of the nucleic acids of malaria parasites. As already mentioned, the metabolism of these substances appears to be closely associated with the processes of protein synthesis and, as the plasmodia rapidly grow and divide in the blood of the host, much protein synthesis and, therefore, a marked nucleic acid metabolism would be expected to occur in them. Deane (1945) has studied the formation of desoxyribose nucleic acid (DNA) in *P. knowlesi* by using the specific Feulgen histo-chemical technique. She found a marked increase in DNA as the parasite developed, the highest concentrations being within the merozoites. Ball *et al.* (1948) followed nucleic acid-phosphorus changes of monkey erythrocytes infected with *P. knowlesi* and calculated that each parasitized red cell contained 13·5 times as much nucleic acid phosphorus as the normal cell. Recently, Lewert (1952) has demonstrated a marked ribose nucleic acid (RNA) metabolism in *P. gallinaceum*. It is thus evident that there is an appreciable synthesis of nucleic acids by malaria parasites and Whitfeld (1952:1953*a*) has used *P. berghei* in an attempt to learn more about the composition of these cellular constituents. In his experiments the blood of infected mice was collected before the parasitæmia was acute to avoid marked changes in its composition. The author found it difficult to separate infected from normal cells and, therefore, hæmolysed washed cells from infected blood with saponin and carried out his analyses on the solid residue washed free of hæmoglobin with saline. Blood from normal mice was treated in the same way and the figures from the analysis of the residue were used as a control. The nucleic acid content of a known number of mouse leucocytes was also determined and allowance thus made for the increased number present in *P. berghei* infections. In this way a marked increase in RNA and DNA content of 10^{10} red blood cells associated with the presence of the parasite was determined, the increase in RNA being twice that of DNA. By counting the number of

parasitized cells in a stained sample of blood before hæmolysis, Whitfeld (1952: 1953*a*) also calculated an approximate value for the RNA and DNA content of a single parasite. Spectrophotometric examination of the nucleic acids of parasites freed from the host cell revealed an absorption spectrum corresponding exactly with yeast nucleic acid. The purine and pyrimidine content of the DNA and RNA from isolated parasites was determined spectrophotometrically after the separation of these bases (prepared from the nucleic acids by hydrolysis with perchloric acid) by paper chromatography. The nucleic acids were found to contain the same bases as yeast and thymus nucleic acids, the DNA being of the "AT type" (the concentration of adenine and thyamine exceeding that of cytosine and guanine) which, according to Chargaff's theory (1951), would suggest that it is of animal rather than bacterial origin. Wyatt (1951) has described the presence of 5-methyl cytosine in the DNA from certain microbial sources but Whitfeld (1952:1953*a*), without having recourse to the extensive procedure of that author, was unable to establish its presence in *P. berghei*. The quantitative results from these experiments must be interpreted with reserve. Although attempts were made in Whitfeld's work (1952:1953*a*) to use controls for the presence of nucleic acids from other formed elements in infected blood, no allowance was made for the large number of reticulocytes which are known to occur in the blood during infection and which are invaded by the parasite. As we have previously noted, these cells contain much nucleic acid and it is doubtful if the freed parasites can be washed completely free from this host-cell material. The reticulocyte RNA was probably responsible for the unduly high RNA content found by the author in infected blood. As he points out, too, the calculated nucleic acid content for a single parasite must only be regarded as very approximate due to the varying number of parasites in each erythrocyte, the loss of parasites after counting and the simultaneous presence of many different developmental forms in the same sample of infected blood. The uptake of radioactive phosphorus by erythrocytic stages of *P. berghei* was later investigated by this author (Whitfeld, 1953*b*). He inoculated infected mice with 32_P and sacrificed them at intervals of several hours. The distribution of phosphorus within the isolated parasites was followed using standard methods. In the RNA fraction the 32_P content remained fairly constant. The highest concentration of the isotope was found in the lipid fraction but the greatest turnover was in the DNA fraction. The rate of incorporation of the isotope varied at different periods during the infection.

In some strains of *P. berghei*, difficulty is frequently experienced in observing pigment in stained smears (Thurston, 1952). However, Fulton and Rimington (1953), using a strain in which pigment was easily detected by microscopic examination, have examined the chemical nature of this substance. The pigment was obtained from the blood of young hooded rats with a seven day infection by laking the cells with saponin and washing the residue free of hæmoglobin. The pigment was then extracted with phenol and characterized spectroscopically and by the preparation of a crystalline derivative. By these means it was identified as hæmatin, which has been shown to be the pigment occurring in all other species of plasmodia so far investigated (Ghosh and Sinton, 1934; Morrison and Anderson, 1942; Rimington and Fulton, 1947). Fulton and Rimington noted that cells infected with *P. berghei* contained less pigment than an equivalent volume of cells

infected with *P. gallinaceum* or *P. knowlesi*. This fact may possibly be correlated with the situation of the parasite within the immature erythrocyte, in which the concentration of hæmoglobin (from which the hæmatin is derived) is variable as it is still being synthesized. The small quantity of pigment in the parasite suggests that *P. berghei* obtains amino acids from sources other than hæmoglobin. It is known that other malaria parasites obtained these nutrients both from globin and from plasma, (Geiman and McKee, 1950).

RESPIRATION OF *P. BERGHEI*.

The malaria parasite lives in an oxygen rich environment within the red blood cell and can draw on the oxygen supply of oxyhæmoglobin as can readily be seen by the darkening in colour of infected blood when examined *in vitro*. The oxygen uptake of living cells is an overall measure of oxidative enzyme systems functioning in the material under observation. Since the initial experiments of Christophers and Fulton (1938) the respiratory activity of parasitized blood, washed parasitized cells and malaria parasites freed from the host cell by hæmolysis, has been observed by several workers. Success has attended the use of this procedure in studying the metabolic steps occurring within the parasite, but as a means of evaluating new antimalarial drugs the method has not given encouraging results, except with a series of naphthoquinones (Wendel, 1946). It has been shown that the *in vitro* respiratory rate of the infected erythrocyte is markedly greater than that of the normal erythrocyte and experiments with freed parasites have demonstrated that the parasite itself possesses a respiratory mechanism and oxidizes glucose by the same steps as do mammalian tissues (Speck *et al.*, 1946).

The respiratory activity of *P. berghei* has been studied only in so far as parasitic activity might encroach on the oxygen and carbon dioxide transport by the blood of the host (Jones, 1951; Jones *et al.*, 1951). The respiration of whole infected blood diluted with phosphate buffer-saline (with or without added glucose) was measured in the Warburg apparatus and the oxygen uptake was observed to be greatly increased when red cells were heavily parasitized as well as in the chronic stage of infection when a large number of reticulocytes were present. It was found that this enhanced respiratory rate was independent of added glucose and that it was almost completely inhibited by the addition of cyanide, indicating that metallo-protein enzymes were probably involved in the process. The authors concluded that the oxygen used by parasitized whole blood in *P. berghei* infections in the albino rat is too small to influence the host directly. In a later paper (Jones *et al.*, 1953), the oxidations occurring in reticulocytes were studied in order to assist projected investigations of the metabolism of *P. berghei* which develops in these cells. Reticulocytes were obtained from young rats which had been subjected to eight to ten daily injections of phenylhydrazine and consequently showed a high reticulocytosis. It was found that the respiration of reticulocytes was much greater than that of mature erythrocytes, a fact well established by other workers (Ponder, 1948), and was independent of added glucose. Results from the use of enzyme inhibitors (particularly monofluoroacetate, which caused suppression of oxygen uptake and an accumulation of citrate) strongly suggested that carbohydrate is being metabolized by the reticulocytes and that a Kreb's cycle is operative in the oxidation of pyruvate. The authors point out that it

may, therefore, be difficult to obtain clear evidence of pyruvate oxidation by *P. berghei* in its natural environment.

Experiments in this laboratory with rat reticulocytes have confirmed the above findings. By making use of a floatation technique, similar to that described by Ferrebee and Geiman (1946) for the concentration of *P. vivax* infected cells, but using egg albumin instead of crystalline bovine albumin (a method also employed by Rao *et al.*, 1951, for concentrating red cells containing *P. gallinaceum*), we have been able to prepare concentrates of rat reticulocytes infected with *P. berghei* and contaminated by only a few uninfected blood cells. A comparison of the respiratory activities of normal and parasitized reticulocytes has revealed no qualitative difference in metabolism, the respiratory quotients and response to enzyme inhibitors being identical in each case. However, although the respiratory rate of unit numbers of reticulocytes and parasitized reticulocytes was found to vary considerably from experiment to experiment (as may be expected with cell populations of such variable composition), the oxygen uptake of the infected cells was significantly greater than that of normal reticulocytes. This difference may be attributed directly to the respiration of the parasite or to an acceleration of the respiratory rate of the host cell due to its presence. When these results are viewed in the light of what is known of the respiration of other species of Plasmodia, however, it seems that at least part of the enhanced respiration can be directly attributed to the enzymatic activity of *P. berghei*. As the respiratory rate of these parasitized cells is independent of added glucose, the parasite may be oxidizing an endogenous substrate. It is more likely, however, that it is using the unidentified reserve of the reticulocyte for this purpose, as Lillie (1947) reported that a carbohydrate reserve is not found in other Plasmodia. We can confirm that the respiration of both normal and infected reticulocytes is inhibited markedly by cyanide, suggesting that *P. berghei* probably contains metallo-protein respiratory enzymes—perhaps a cytochrome system.

CARBOHYDRATE METABOLISM.

Early workers (Bass and Johns, 1912; Johns, 1930; Hegner and McDougall, 1926) have shown the importance of glucose for the survival of the malaria parasite both *in vitro* and *in vivo*. Subsequent studies of the carbohydrate metabolism of Plasmodia, which have been more extensive than those concerned with other aspects of plasmodial physiology, have shown that the pathway of glucose breakdown is essentially similar to that occurring in mammalian tissues (Evans, 1946). Energy for the synthetic activities of the rapidly growing parasite is thus provided by the phosphorylative degradation of glucose to lactic acid, some of which is then oxidized in a stepwise manner. Little information about the carbohydrate metabolism of *P. berghei* has appeared in the literature as yet. Mercado (1952) has studied the *in vivo* glucose content of the blood of infected white rats which was found to fall as the parasitæmia increased. The glucose content of packed infected blood cells was slightly higher than that of normal cells, while the serum glucose concentration decreased markedly, as the infection developed, especially in highly parasitized blood. In a later paper (Mercado and von Brand, 1954) the results of an investigation of the glycogen reserve and glycogen synthesizing power of the liver of rats infected with *P. berghei* were reported. A fall in liver glycogen

in infected rats occurred, and was shown to result from a metabolic disturbance of the host due to the presence of the parasite, not as a result of a decreased food consumption by the infected rats. Similar changes have been shown to occur in animals infected with some other species of malaria (Christophers and Fulton, 1938; Marvin and Rigdon, 1945) and it appears to be generally accepted that the hypoglycæmia and drop in the glycogen reserve of the liver in terminal stages of malaria are due to deficient liver function rather than to actual glucose consumption by the parasite. No work appears to have been reported on the utilization of sugar by *P. berghei*. Our own *in vitro* experiments have shown that the pH of infected blood, diluted with buffered saline and shaken for four hours with glucose, falls markedly. Concentration of the medium and partition chromatography of the acidic end products of metabolism which accumulate under the conditions used, have revealed the presence of formic, acetic, succinic, lactic, pyruvic, α-oxo-glutaric, and small amounts of several other acids yet to be identified. Although similar acids were found to be produced by reticulocytes when shaken under the same conditions, quantitative analyses have shown that lactic and pyruvic acids are produced at a far greater rate by a unit number of parasitized reticulocytes than by normal ones. Glucose disappears from the medium simultaneously. The glycolytic rate of the infected reticulocyte was calculated from the results of these experiments to be about five times that of the uninfected reticulocyte. Although the possibility of an enhanced carbohydrate metabolism by the reticulocyte due to its invasion cannot be excluded, these results suggest that *P. berghei*, like other Plasmodia, is capable of utilizing plasma glucose by aerobic fermentation as a source of energy in addition to oxidizing a substrate from within the host cell.

NUTRITION.

In order to present the subject of nutrition of *P. berghei* in the correct perspective, it has been necessary to review briefly some known facts about the nutritional needs of other malarial parasites. In spite of the large number of researches in this field, only a few relevant papers will be cited.

In vitro *nutritional studies of Plasmodia.*—When studying the nutritional needs of parasites, it would appear that satisfactory results can only be obtained by *in vitro* experiment in which the parasite is maintained in conditions permitting growth and multiplication free from the influence of the hosts' metabolism. A reasoned approach to the cultivation of malarial parasites within the erythrocyte was first made by Trager (1941) who used a complex medium to study the conditions affecting survival of *P. lophuræ in vitro*. Later, the same author (Trager, 1943*a*) found that the addition of calcium pantothenate to the medium improved the survival rate as judged by the ability of male gametocytes to ex-flagellate. Successful growth and multiplication of the monkey malaria parasite *P. knowlesi* in infected blood was later reported by Ball and co-workers in a series of papers from 1945 onwards: (Ball *et al.*, 1945; Anfinsen *et al.*, 1946; Geiman *et al.*, 1946; McKee *et al.*, 1946; Ball *et al.*, 1948). Culture and continued subculture of the latter parasite was attained for seven generations and the material proved infective to fresh hosts at the end of that time. The medium employed was a balanced salt solution resembling monkey plasma in composition with addition of vitamins,

purines, pyrimidines and amino acids. The nutritional need for glucose and para-aminobenzoic acid (PAB) in the culture was demonstrated but not for other individual substances. Later McKee and Geiman (1948) showed that the mixture of synthetic *l*-amino acids in the culture of *P. knowlesi* could be substituted by *l*-methionine. Trager (1947*b*) using similar conditions obtained multiplication of *P. lophuræ* in duck red cell suspensions. He recognized, however, that results of *in vitro* experiments in which the malarial parasite is cultivated within the red cell may lead to ambiguities in interpretation due to the metabolic activities of the host cell. He (Trager, 1950) therefore maintained *P. lophuræ* freed from its avian host cell by specific hæmolysis in a complex medium and followed the development of the parasites which lived for a few days only. In continuation of these studies, Trager (1952) found that the life span of *P. lophuræ* in absence of host red cell was further prolonged by the addition of *l*-malate and coenzyme *A* concentrates to the medium previously used. At the end of three days in culture by far the larger proportion of the parasites remained normal.

So far, however, *P. berghei* has not been cultivated *in vitro* and it has been necessary to approach the question of nutrition of this parasite by studying the effect of diet on the host.

In vivo nutritional studies.—In a review on the influence of nutrition on experimental infections, Clark *et al.* (1949) have outlined the main considerations which have to be taken into account in work of this type. According to these authors, the use of defined strains of both host and parasite is likely to improve the quality of the results. Infection of the natural host by the normal route is preferable to any other. When deficiencies are being studied, an adequate and defined basal diet to which the substances for study can be added in the required amounts, are indispensable for correct interpretation. In all cases, where diets are deficient, it is necessary to distinguish malnutrition from undernutrition. Care must be taken to avoid coprophagy. Other variable factors such as age, weight and sex of animals and those necessary for their well being must be controlled. Finally, in order to ensure that the results are statistically significant, a sufficient number of experiments must be performed. Chandler (1953) has defined the ways in which the diet of the host can affect the parasite which it harbours. The diet may contain nutrients essential to the parasite or factors which are rendered useful to it by the metabolism of the host animal. Conversely, substances directly toxic to the parasite or those which can be converted to inhibitory factors may also be present. As the different components of the diet are metabolized by interdependent pathways in the body of the host, it is not easy to prove that any single dietary factor is required by, or inhibits, the parasite directly. Stimulation or depression of the host's defence mechanism involving antibody production or phagocytosis, especially in the young, may be caused by diet. Thus, Cannon (1942) suggested that antibodies are specifically modified globulins whose formation depends on amino acid and protein intake. Earlier Freund (1930) had shown that young animals cannot form antibodies comparable either in quantity or quality to those of adults of the species. The gut flora possesses the power to synthesize certain necessary factors for host and parasite (Miller, 1945) and can thereby influence the course of infection. The gut flora is itself

readily affected by diet (Gall *et al.*, 1948) and the latter can, therefore, indirectly affect parasitic infections.

AVIAN AND SIMIAN MALARIA.

Dietary studies on malarial infections have been carried out only within the last few years. Passmore and Sommerville (1940) found that monkeys infected with different malaria parasites did not show any difference in the course of primary infections when maintained on an adequate diet or on one which was deficient in a number of factors including vitamins A and C and calcium, thus resembling that of the rice-eating poor in India. Trager (1943*b*; 1947*a*) observed that biotin-deficient ducks and chicks had more severe infections with *P. lophuræ* or *P. cathemerium* than control animals. This substance appears to affect the degree of natural susceptibility to malaria infection, but is not required for *in vitro* cultivation. In a series of papers (Seeler and Ott, 1944; Seeler *et al.*, 1944; Seeler and Ott, 1945*a*: 1945*b*) Trager's results with biotin and *P. lophuræ* have been confirmed. In this infection, however, riboflavin deficiency markedly reduced the parasitæmia whereas restriction of food intake had the opposite effect. When chicks were given a protein-deficient diet in which all other nutrients were present, the infection with *P. lophuræ* was more severe in the acute phase than in chicks on adequate diet. However, mortality was higher in chicks on protein-deficient diet. A deficiency of folic acid and other unidentified factors caused an increase in the severity of the infection. Brackett *et al.* (1946) found that blood- but not sporozoite-induced infections of *P. gallinaceum* in the chick are suppressed by deficiency of pantothenic acid and this fact suggested to the authors that pantothenate is necessary for the asexual forms of the parasite, in agreement with the observations of Trager (1943*a*) on *P. lophuræ*. Roos *et al.* (1946) used both chicks and ducks infected with *P. lophuræ* for studying the effect of vitamin deficiencies on the course of infection. Deficiencies were produced in the hosts with respect to nicotinic acid, thiamine, choline and vitamin *A* by omitting these substances from the diet. The duck proved so susceptible to the infection that nutrition deficiencies failed to suppress it. In chicks, however, vitamin *A* deficiency caused milder infections whereas choline, like nicotinic acid, caused the opposite effect and thiamine was without action. Brooke (1945) when studying the effect in birds of diets deficient in protein and vitamins, found that the primary malarial infection was more severe and more deaths resulted in the hosts on a poor diet. Immunity in them was also weak, as indicated by increased relapse rate and greater susceptibility to super-infection. Before describing experiments with *P. berghei*, some similar work (McKee and Geiman, 1948; Geiman and McKee, 1948) of considerable interest will be briefly quoted. These authors showed that fasting was able to control infection with *P. knowlesi* in monkeys, and the degree of infection was correlated with the length of the fasting period. During fasting, administration of methionine or PAB caused an upsurge of infection. Deficiency of vitamin *C* in the animals also reduced *P. knowlesi* infection in *rhesus* monkeys and the parasite numbers increased when the vitamin was given.

RAT MALARIA.

In the earliest paper dealing with *P. berghei* infection and diet, Fabiani and Grellet (1952) reported that a partial or total absence of vitamin *A* did not increase

the intensity of infection in rats but tended to make the acute phase of the infection milder and the period of infection was reduced. During investigations on hæmoglobin metabolism in rats infected with *P. berghei* by blood inoculation, infection was suppressed in animals receiving a diet of cow's or human milk or of a dried preparation containing small amounts of the B vitamins and Ca pantothenate (Maegraith, *et al.*, 1952). The suppressive effect of the milk diet was sometimes complete and no parasites appeared in the peripheral blood. Latent infection was frequently made apparent, however, when the animals were returned to a normal diet. The parasites themselves seemed to be unaffected as they gave rise to normal infection in fresh hosts. Since infection was not suppressed when a normal diet was fed with milk *ad lib.*, it appeared that there was some factor absent from milk which was necessary for the growth of the parasite. The observations of Maegraith *et al.* (1952) were supported by Mackerras (1953) but Rodhain (1953*a*) found that in mice protection by a milk diet was only of a limited nature in *P. berghei* infections, whereas in the case of *P. vinckei*, a closely related parasite, suppression of infections under the same conditions was complete. Galliard *et al.* (1954) in trying to repeat these experiments found that the number of controls which developed a reasonable infection was so small that they could not draw any conclusions from their results. Schneider and Montézin (1953) fed mice on a milk diet in absence of added vitamins and found that the course of infection with *P. berghei* was practically unaltered, but it should be noted that they gave much larger inocula of parasites than did Maegraith and colleagues in their original experiments. In a short paper Fabiani and Orfila (1954) have reported the results of their studies on the mechanism whereby a milk diet exerts its effects on *P. berghei* infections in mice. They found that such a diet gave rise to very irregular infections and they concluded that these results may have been due to interference with blood cell formation, to alteration in host immunity or to an action on the parasite itself. In this laboratory the serum from rats fed on milk or normal diet was diluted with saline and used in measurement of respiratory rates of washed rat cells infected with *P. berghei* in the Warburg apparatus. This type of experiment failed to detect any inhibitory effect on the parasite by the serum from rats on milk diet.

The effect of milk diet on other malarial infections was investigated about this time. Bray and Garnham (1953) found in *P. cynomolgi* infections of monkeys that a milk diet caused incomplete suppression of blood- or sporozoite-induced infections, whereas Jaswant Singh *et al.* (1953) were able to suppress completely infection with a recently isolated strain of *P. knowlesi*. Ramakrishnan *et al.* (1953) noted, however, that blood- or sporozoite-induced infections with *P. gallinaceum* in chicks on a milk diet were considerably more severe than in controls on normal diet. On the other hand, Greenberg *et al.* (1954) found that the same sporozoite-induced infection was suppressed while blood-induced infection was not. Hawking (1953) reported that on adding PAB to milk, the infection was as heavy as in animals on a control diet, and Rodhain (1953*b*) confirmed this observation in three mice infected with *P. vinckei*. Later Hawking (1954) extended his previous observations to infection of monkeys. In a recent paper Galliard *et al.* (1954) have reported that suckling rats and those newly weaned and kept on a milk diet were not protected against infection by *P. berghei* which invariably caused

death. In agreement with other workers, they also found that mice on a milk diet were not protected. In a discussion on this paper Deschiens aptly points out that whereas the adult rabbit is refractory to infection by *P. berghei*, sucklings are susceptible and may die of infection. Recently, Corradetti *et al.* (1954) found that in a series of 62 rats on a milk diet, no protection was afforded against infection by *P. berghei*. Opinion on the question of the efficacy of a milk diet for suppression of *P. berghei* infection in rodents is thus divided in different parts of the world. Fulton (1954) seldom found in blood-transmitted infections that suppression in rats by a milk diet was adequate for a study of the effect of additions to the diet. Both methionine and PAB sometimes prevented this suppression when it occurred. When rats were maintained on an amino acid diet in combination with fats, carbohydrates and certain vitamins, the effect of supplements to the diet could be studied. Judged by the number of parasites in the peripheral blood the most effective agent in producing parasite growth was methionine. Later work in this laboratory has shown that when a protein source deficient in certain amino acids is used in the diet, it is possible to replace all these missing amino acids and obtain normal instead of suppressed infections with *P. berghei* in rats. When amino acids are replaced singly, however, the resultant infection is usually much less intense. This suggests that the need is not for any specific amino acid but rather for a diet in which all essential building blocks are present. If that condition is not observed, the animals invariably lose weight and their nutritional state is then comparable to that in starved animals. However, we have not found that methionine alone will always cause reversion of suppression of *P. berghei* infection in rats under these conditions, although it has been reported to do so in *P. knowlesi* infection of monkeys. The explanation of this discrepancy may lie in the differing physiological needs of the various species of Plasmodia.

Ramakrishnan (1953); Ramakrishnan *et al.* (1953); Ramakrishnan *et al.* (1953*a*:1953*b*); and Ramakrishnan (1954*a*; 1954*b*: 1954*c*:1954*d*) have made numerous studies on the effect of diet on *P. berghei* infections in the rat. In the first of these studies, the course of blood-induced infection on starved albino rats aged two to six months was described. The fasting period varied from five to ten days and was arranged to begin at different times relative to inoculation. In one group, denied food or water, death of all animals occurred within eight days and no parasites were seen in their peripheral blood. In other experiments, infections were found to be more readily suppressed the older the animals. After a period of five days' starvation, during which parasites were not seen, return to normal diet caused their reappearance. If, however, the animals survived for ten days, no parasites were observed when the host was returned to normal diet. The authors found that all animals on milk diet had parasites in the peripheral blood but the intensity of infection was less than in those receiving a balanced diet. There was no appreciable difference in the weight gain of experimental and control animals showing that the animals were not in a state of starvation. In continuation of earlier experiments on starved rats, these animals now received various substances during the course of fasting. It was found that glucose or biotin did not affect parasitæmia, whereas methionine or PAB caused growth of parasites comparable to that in controls and "The growth was appreciably higher in the methionine series". After the effect of

starvation on *P. berghei* had been considered, it appeared to the authors that the results obtained might have been caused by ketosis and the effect of a ketogenic diet was therefore tested. Only five experimental animals were used and the diet of 93 per cent butter, one per cent salt mixture and five per cent glucose was unpalatable so that food consumption by these animals was reduced; a marked loss of weight followed and parasitæmia was lower than in controls. In another series of experiments, rats were given varying quantities of the same adequate diet during the course of *P. berghei* infections so that some suffered partial starvation. The acute infection was markedly affected by the nutrition of the host, being less severe in the poorly nourished rats. When qualitatively different diets, adequate in quantity, were fed to rats the types of diet employed were vegetarian, lacto-vegetarian and meat. With vegetarian diets, rich in carbohydrate, a more severe infection resulted than in the case of a balanced diet. Mortality was low and immunity high to chronic infection. In a diet with a large proportion of meat, the infection was more severe. In the final paper the author summarizes in table form the known facts regarding the action of vitamins in malarial infections. He fed small numbers of animals for some months on a pyridoxine deficient diet and concluded that pyridoxine was essential for *P. berghei* since the average daily counts as well as peak values of parasitæmia were lowest in the deficient animals.

From the foregoing summary of experimental work on nutrition and malaria, it is seen that deficient diets fed to a host animal may either enhance or suppress malarial infection, according to the nature of the deficiency. The results of experiments in which diets deficient in a known factor suppress the infection are of greater importance from the point of view of interpretation in terms of parasite nutrition. In the opposite case it seems more likley that the effect of the deficiency as evidenced by a rise in parasite counts is due to an impairment of the defence mechanism of the host, and is, therefore, outside the scope of this discussion. However, when the infection is suppressed it is highly probable that the deficient factor under study is either directly necessary to the metabolism of the parasite, or that it is involved in the production by the host of a vital factor or environment, such as the red blood cell. However, as previously noted, deficiency may also cause the host to produce abnormal end products of metabolism which are toxic to the parasites or induce a change in the character of the gut flora. As a result of Maegraith's original observations, a considerable amount of evidence on the effect of a milk diet on *P. berghei* infection is now available, and the complex nature of the problem is apparent. Whereas the above author found in his original experiments that suppression of *P. berghei* in rats was almost complete on a milk diet, his later experiments (Maegraith, 1953) at different times in the year with the same diet failed almost completely to show suppression. He attributed this to a difference in the quality of the milk as a result of change in quality of the pasture. An instance of the results of different workers, being flatly contradictory, has been quoted in the case of *P. gallinaceum* in chicks. Findings from other laboratories have been somewhat variable and the importance of such substances as PAB, methionine and fat content of milk, are still *sub judice*. The variation in physiological requirements of malarial species used in such experiments is undoubtedly of the greatest importance, and may give rise to discrepant results. The difference in virulence of strains, age, breed and sex of the animal host and

size of inoculum used by different workers must account in part at least for the variations in results from different laboratories. The relation of a milk diet to malaria is one of considerable importance in the human infection and has been investigated by Miller (1954). Since no data were available previously, African children naturally infected with malaria were placed on alternate diets of milk and normal food, and for part of the time during the feeding of a normal diet yeast, a rich source of B vitamins and PAB, was added. It was found that neither of these diets altered the course of infection or clinical picture in infections with *P. falciparum* or *P. malariæ*, and it seems that a milk diet offers little hope as a therapeutic agent. It is difficult to come to any conclusions regarding milk diet and the nutritional requirements of malaria parasites on account of the complexity and variation in composition of milk and it would appear from our experiments that neither the addition of PAB, methionine nor any other agent is of exclusive importance in reversing the suppression of *P. berghei* infection in rats. Similar views have been expressed by Refaat and Bray (1953) and Maegraith (1953). Greenberg *et al.* (1954) have, however, laid some emphasis on the inhibitory properties of milk fat and the deficiency of trace minerals such as copper in suppressing *P. gallinaceum* infections.

It has been suggested that in living cells PAB functions in the synthesis of methionine and certain purines (Shive and Roberts, 1946; Woods, 1953). The methyl group of methionine is known to be a precursor of formate, which in turn may provide carbon atom 8 in purines. The extent and importance of this conversion is, however, not known (Welch and Heinle, 1951). The ability of PAB and methionine to reverse the suppression of infection in starving monkeys infected with *P. knowlesi* has been mentioned. Their complementary action in cultures of this parasite as well as their effects on *P. berghei* infection in rats on a milk diet tends to confirm this close metabolic relationship and suggests that these substances may also be of importance to *P. berghei*. Another property common to both parasites is their susceptibility to sulphanilamide which has long been known to be antagonized by PAB. Bray and Garnham (1953) observed that *P. cynomolgi* development is adversely affected by milk diet in the immature schizont stage when nuclear division is occurring. The results of Deane (1945) suggest that active synthesis of DNA takes place at this stage of development, and since purines are essential components of DNA, a source of methionine or PAB which may act as precursors of purines is probably essential to the parasite then.

It is known that deficiency of vitamin *A* in the host is accompanied by lowered resistance to certain helminth and bacterial infections. In the case of *P. berghei*, however, as previously mentioned, a less intense infection results in the rat. Rigdon (1946) and Roos *et al.* (1946) on the other hand have reported that vitamin *A* deficiency in the duck does not affect the course of infection with *P. lophuræ*, since this species is too virulent in that host. The latter authors found, however, that in the chick the same parasite causes a milder infection during vitamin *A* deficiency. Although the deficiency is also followed by suppression of *P. berghei* it seems unlikely that it is an essential factor for the parasite. The effect is more probably exerted as a result of some action on the host. The only other vitamin which has been shown to affect *P. berghei* infections adversely, when absent from the diet, is pyridoxine. In the experiments of Ramakrishnan (1954) already

described, one group of rats was fed on a diet free from pyridoxine and another on the same diet to which pyridoxine was added. In the latter, the usual signs associated with pyridoxine deficiency were not completely abolished. The condition of the rats in both groups indicated that their metabolism was upset and food consumption was reduced. The claim that pyridoxine is an essential requirement for *P. berghei* is therefore open to doubt.

CONCLUSIONS.

In spite of the many difficulties involved in the study of *P. berghei* it will be realized from this short review that the discovery of this parasite has provided a fruitful field of investigation for the malariologist and biochemist. Many problems remain as yet unsolved; for instance, practically nothing is known of the nitrogen and lipid metabolism of this parasite or, indeed, of any other Plasmodia.

The limited studies already made have indicated that the carbohydrate metabolism of *P. berghei* follows, at least in broad outline, the same pattern as other malaria parasites. No abnormal components have been discovered in its nucleic acids and the pigment present is hæmatin, as in other species of Plasmodium. The ready availability of the parasite has led to many studies on the influence of diet on infection but these *in vivo* studies are complex in character and difficult to interpret with respect to the nutritional needs of the parasite.

Although the preference of *P. berghei* for young red cells is marked, the same characteristic has been encountered in other malarial species. Nevertheless the marked predilection of *P. berghei* for the reticulocyte, rather than the mature cell, poses a fascinating problem for the biochemist. It has been suggested in the case of *P. vivax* malaria that only reticulocytes are invaded and that maturation of the host cell occurs subsequent to infection. It is not clear whether this happens in *P. berghei* infections or whether the parasitized mature cells which are frequently seen in early stages of infection are invaded in the peripheral blood. In later stages of infection, the number of circulating reticulocytes present increases markedly and they appear to be preferentially invaded to the exclusion of mature red cells. Possibly some factor in the mechanism of invasion is at work or perhaps the parasite invades both mature and immature cells and survives only in the latter. The failure to observe degenerate parasites within mature cells would appear to favour the hypothesis concerning the invasion mechanism. Further biochemical differences between *P. berghei* and other species may also be involved and, as reiterated in the text, *in vitro* culture of the parasite becomes of outstanding importance for the solution of a number of these problems.

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

XXI. Administration of an extract of male sex hormone to orchidectomised and non-orchidectomised albino rats with blood-induced infection and its effect on the course of infection in its different stages.*

BY

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[May 12, 1955.]

It is well-known that young animals are comparatively more susceptible to experimental parasitic infections than the full-grown adults of the same species. As postulated by Culbertson (1941), it would appear possible that resistance in very young animals could be related to the sex-hormones as well as to those elaborated by the other endocrine glands. According to him, young female rats could be rendered significantly resistant to artificially induced infection of *Trypanosoma lewisi* by either giving them subcutaneous injections of oestrin or transplantation of pituitary glands obtained from adult non-immune rats.

Smith (1942), in course of his work, noted that hypophysectomy in experimental animals resulted in a profound involution of the thyroid, the adrenal cortex and the gonads and that their functional activity was greatly reduced giving rise to a diminished resistance to trauma and infection.

Constantin and Shwartzman (1954) have observed that susceptibility of Syrian hamsters to intracerebrally inoculated poliomyelitis virus was enhanced for the first few days after experimental orchidectomy. They also noted that, conversely, the animals exhibited increased resistance to the virus infection as a result of either administration of testo-sterone in high doses or testicular hypertrophy induced by treatment with GTH or chorionic gonadotrophin.

Ramakrishnan, Satya Prakash and Krishnaswami (1951) while studying the influence of sex on the various stages of infection with *P. berghei*, held the view that

*This paper reports a part of work for a thesis proposed to be submitted for a doctorate.

the female rats were able to overcome the primary parasitæmia comparatively earlier than the males. Greenberg, Nadel and Coatney (1953) also supported the above observation by saying that, apart from the genetic constitution of the host, the female mice of the same strain survived longer than the males.

Galliard and Lappierre (1951) observed that, injections of male hormone (sterandryl) did not cause any modification of *P. berghei* infection in white rats. Zuckerman and Yeoli (1954) found that neither orchidectomy nor oophorectomy has any demonstrable effect on the susceptibility of the host to *P. berghei*. Ray and Bose (1954), on the other hand, noted that, in oophorectomised mice and rats infected with *P. berghei* the parasitæmia remained at a considerably lower level than in the controls.

Satya Prakash, Chakrabarti and Ramakrishnan (1955) while studying the effect of orchidectomy on blood-induced infection of *P. berghei*, in albino rats, showed that the average daily parasitæmia was distinctly higher in the orchidectomised animals than in the non-orchidectomised specimens. The present work was carried out with the objects of (*a*) studying if, and to what extent, the course of infection in the orchidectomised animals differed from that in the intact ones, and (*b*) observing the effect of an extract of male sex-hormone* on the infection in both orchidectomised and the intact groups.

MATERIAL AND METHODS.

The strain of *P. berghei*† maintained in the white rats at the Malaria Institute of India was used. The standard dose of inoculation was 1×10^6 parasites per animal injected through the intraperitoneal route. Details regarding experimental inoculation and enumeration of parasites per 10,000 erythrocytes in the daily blood smears were as described by Ramakrishnan, Satya Prakash and Krishnaswami (1953).

Two series of experiments were carried out with 13 rats in Series I and 24 rats in Series II. All the animals were young adults of about the same age (40 weeks) and approximately of the same weight groups (190 grammes). The animals belonging to Series I (13 rats) were divided into two main groups—Group I consisting of seven animals and Group II six animals. Those of Series II (24 rats) were similarly divided into Groups III and IV, each comprising 12 animals.

Animals of Groups I and III (19 rats) were subjected to bilateral orchidectomy under general anæsthesia (nembutol administered intraperitoneally 15 mg./20 gm. body weight). The technique of orchidectomy was as described by Ingle and Griffith (1949). The animals of each of the four groups were further divided into two sub-groups each, for the study of the effect of parenteral administration of testandrone. Thus, Group I was divided into sub-groups 'A' (four animals) and 'B' (three animals); Group II into sub-groups 'C' (three animals) and 'D' (three animals); Group III into sub-groups 'E' (six animals) and 'F' (six animals) and Group IV into sub-groups 'G' (six animals) and 'H' (six animals). These

*Testandrone—G. W. Carnrick Co., Ltd., Newark, N. J., U.S.A.

† Received in 1952 through the courtesy of Brigadier J. S. K. Boyd of the Burroughs Wellcome Laboratories Ltd., London.

sub-divisions were made in order that one sub-group in each group should serve as control.

Each one of the 37 rats was inoculated with 1×10^6 parasites. Those of Series I were inoculated about a week after the experimental orchidectomy and those of Series II three days after. Concurrently with the parasite inoculation, animals of the sub-groups 'A', 'C', 'E' and 'G' were subjected to intraperitoneal administration of testandrone daily. The dosage schedules were as shown in Table I below:—

TABLE I.

Dosage schedule of testandrone.

Series.	Regime I.		Regime II.	
	250 mg. testandrone/50 kg. body weight daily.		25 mg. testandrone/50 kg. body weight daily.	
	Number of sub-group 'A' animals.*	Number of sub-group 'C' animals.	Number of sub-group 'E' animals.*	Number of sub-group 'G' animals.
I.	4	3	...	...
II.	...	...	6	6

*These animals received their daily injections of testandrone in the prescribed dose from three days prior to the parasite inoculation, while the rest received it from the day of inoculation.

RESULTS.

The main criteria of assessment were the daily parasitæmia and the peak parasitæmia of both the experimental and the control animals. But, since in a biological experiment, individual variations are apt to occur frequently, the average figures for the daily and peak parasitæmia for the animals of the respective groups, are shown in Table II below:—

It will be seen from Table II that, of the animals of Group IV (non-orchidectomised rats), those treated with hormone showed a higher average daily parasitæmia than those without it. It was of interest to know, whether this difference was uniform throughout the period of observation or it was applicable to any particular interval during the treatment. An analysis of the average daily parasitæmia on a weekly basis for animals of both groups II and IV was, therefore, made and the result has been given in Table III below. Although the Group II animals, with and without testandrone, did not evince any significant difference in their average daily parasitæmia, they have also been included in the table for completion, as well as for better comparison with those of Group IV.

It is seen from Table III that the average daily parasitæmia in the intact animals of Series I, treated with testandrone, showed a rapid decline from the second week onwards; whereas in the corresponding animals of the series without

TABLE II.

Average figures of the daily and the peak parasitæmia in respect of the experimental animals and the control animals.

Experiment series.	Rat group.	Average daily parasitæmia/10,000 erythrocytes.			Average peak parasitæmia/10,000 erythrocytes.		
		With testandrone 250 mg./50 kg. body weight.	With testandrone 25 mg./50 kg. body weight.	Without testandrone.	With testandrone 250 mg./50 kg. body weight.	With testandrone 25 mg./50 kg. body weight.	Without testandrone.
I.	Group I. (Orchidectomised)	94 (Sub-group 'A')	...	363 (Sub-group 'B')	812 (Sub-group 'A')	...	1,638 (Sub-group 'B')
	Group II. (Non-orchidectomised)	162 (Sub-group 'C')	...	198 (Sub-group 'D')	660 (Sub-group 'C')	...	1,173 (Sub-group 'D')
II.	Group III. (Orchidectomised)	...	1,883 (Sub-group 'E')	2,365 (Sub-group 'F')	...	4,366 (Sub-group 'E')	4,863 (Sub-group 'F')
	Group IV. (Non-orchidectomised)	...	1,275 (Sub-group 'G')	887 (Sub-group 'H')	...	4,019 (Sub-group 'G')	3,860 (Sub-group 'H')

TABLE III.

Analysis of the average daily parasitæmia.

Experiment series.	Rat group.	Average daily parasitæmia/10,000 erythrocytes.			Statistical significance (result of 't' test).
		First week after inoculation.	Second week after inoculation.	Third week after inoculation.	
I.	Group II with testandrone 250 mg./50 kg. body weight (Sub-group 'C').	200	80 (*a*)	0	(*b-a*) is not significant. (p = about 0·4).
	Group II without testandrone (Sub-group 'D').	200	400 (*b*)	0	
II.	Group IV with testandrone 25 mg./50 kg. body weight (Sub-group 'G').	600	2,000	1,000 (*c*)	(*c-d*) is highly significant. (p = about 0·02).
	Group IV without testandrone (Sub-group 'H').	600	2,000	100 (*d*)	

testandrone, the parasitæmia presented a rise, instead of a decline, during the same period although it eventually came down in the third week. In the intact animals of Series II, on the other hand, the average daily parasitæmia in those treated with

the hormone, exhibited the same rise as in those without the hormone in the second week. In the third week, however, the parasitæmia in the animals treated with hormone remained at a much higher level than in those without the hormone, although it had a tendency towards decline. On a careful scrutiny to ascertain as to whether the above disparity in the result could be attributed to any individual variation or not, it was found that such an event did not occur.

DISCUSSION.

Under the limitations of the investigation it would appear that, absence of the sex hormone causes a more severe course of infection in the male rats. Such an evidence is observed from the higher average daily and peak parasitæmia in the orchidectomised rats than in their controls (Sub-groups 'B', 'D', 'F' and 'H' in Table II).

The observed effect of the sex-hormone in the male can obviously be due to its influence either on the parasitic metabolism or on the host immunity mechanism or both. Moreover, if the influence of the sex-hormone be solely on the host-immunity mechanism, it can be either on the innate or on the acquired immunity or both. Table IV similar in principle to the Table III, presenting the comparative weekly variation of average daily parasitæmia in the orchidectomised and intact animals, is given below:—

TABLE IV.

Experiment series.	Rat group without testandrone.	AVERAGE DAILY PARASITÆMIA/10,000 ERYTHROCYTES.		
		First week after inoculation.	Second week after inoculation.	Third week after inoculation.
I.	Group I (orchidectomised) (Sub-group 'B').	300	800	1
	Group II (Intact) (Sub-group 'D').	200	400	0
II.	Group III (orchidectomised) (Sub-group 'F')	600	3,000	4,600
	Group IV (Intact) (Sub-group 'H')	600	2,000	100

N.B.—The figures have been rounded off to the nearest 100.

It is seen from the above Table IV that the average daily parasitæmia in the intact animals is always at a lower level than that in the experimental ones. The difference between the levels appears to be more pronounced from the second week onwards. Such a trend in the difference, especially in the later phases of the course of infection, possibly indicates that the influence of the male sex-hormone is more upon the specific than upon the innate immunity, if at all.

But, it should also be borne in mind that, as much as the master endocrine gland, the pituitary body, controls the functions of the gonads through its

gonadotrophic hormone, the gonads also by virtue of their hormonic dysfunction can create an imbalance in the pituitary, and through the pituitary they can cause a functional disorder in some other endocrine glands of the system. As a result of such a general endocrinal disorder, originating from dysfunction of the gonads, the metabolic pattern of the animal undergoes considerable changes. Such changes in the host-metabolism may be reflected on to the parasite inhabiting the host, thereby resulting in an alteration of the parasitic metabolism also.

The role of the male sex-hormone in the host-parasite relationship in *P. berghei* infection, of whatever nature it may be, seems to be further confirmed by the result of the substitution therapy with testandrone in the orchidectomised animals. The orchidectomised animals (in both the experiments) treated with testandrone, showed a reduced level of the average daily and peak parasitæmia than those without the hormone.

Another observation was that, in the first experiment, the orchidectomised animals treated with testandrone (250 mg. testandrone/50 kg. body weight) showed a lower average daily parasitæmia and a higher peak parasitæmia than the intact ones treated with the hormone; whereas in the second experiment both the above items were higher in the experimental animals of the corresponding group than in the intact controls. According to the expectation based on the observations discussed above, both the average and the peak parasitæmia in the experimental animals, treated with testandrone in high and normal doses, should have been normally higher than those in the corresponding controls, because, in the intact animals the extraneous male hormone should have supplemented the already existing sex-hormone elaborated by their testes. As far as the peak parasitæmia in both the experiments and the average daily parasitæmia in the second experiment are concerned, the results tally with the expectation. The only disparity that is observed, is in the average parasitæmia of the pertaining groups of rats in the first experiment. A significance test ('*t*' test) performed to determine, if the difference between the mean parasitæmia of the two groups ($162 - 94 = 68$) was actually significant or not, showed that it was but a chance occurrence resulting from certain individual variations and may hence be ignored.

In the normal animals, the results of the two series of experiments were very much different from each other. The Group II animals of the Series I treated with the hormone showed an insignificant decline of the average and the peak parasitæmia as compared with the animals of the same group without the hormone. On the other hand, in Series II (Group IV), the result is seen to be reversed. According to Moore (1942), administration of androgens as well as oestrogens to young animals with actively functioning testes, proved injurious to the testes and that the harmful influence was again secondary to the retardation of the function of the pituitary gland. Wright (1948) also stated that, when either oestrogens or androgens were administered for long periods, certain structural and functional changes occurred in the anterior lobe of the pituitary gland resulting in a derangement of the functions of this gland. The secretion of the gonadotrophic hormone was thus retarded in both sexes leading to proportionate degrees of atrophic changes in the ovaries and testes. The non-orchidectomised animals of the Series I, which were treated with testandrone, were given ten times the dose of the hormone more than the corresponding animals of the Series II. The daily administration

of the hormone in normal dose to the animals of the Series II might have caused some amount of damage to their normally functioning testes, instead of totally accelerating their functions. Thus, it may be possible that on account of the depression of the testicular activity by the extraneous hormone, the animals behaved in a relatively more susceptible manner to the parasitic invasion than the intact animals without the hormone. In the Series I, the hormone administered might have somewhat interfered with the functions of the normal testes but, the dose being high, adequate free hormone might have also remained in the system to keep down relatively the invasiveness of the parasite.

SUMMARY.

Bilateral orchidectomy in albino rats resulted in a higher average daily and peak parasitæmia in blood-induced infection of *P. berghei*. Testandrone administration in both heavy and normal doses in orchidectomised animals checked the invasiveness of the parasite considerably. In the intact animals, testandrone in normal dose boosted up the parasitæmia as opposed to testandrone in heavy dose, which comparatively subdued the infestation. The reason might have been a retardation of normal testicular function brought about by the administration of extraneous testandrone in normal dose. Testandrone administered in heavy dose might have exercised a similar effect but apparently due to perhaps some free hormone in the system, the parasitæmia was comparatively depressed.

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OBSERVATIONS ON THE INCIDENCE AND TYPES OF TUBERCULOSIS IN RHESUS MONKEYS ON AUTOPSY STUDIES.

BY

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[May 19, 1955.]

JASWANT SINGH *et al.* (1951) isolated tubercle bacilli from lung, liver and spleen of *M. mulatta*, showing different types of tuberculous lesions. The organisms were found to be of the human type. Subsequently, Nair and Ray (1954) demonstrated a correlation between tuberculin test and lesions in the same species of monkeys. The present report deals with autopsy findings of 95 tuberculous monkeys out of a total of 232 (41 per cent) autopsies. An attempt has also been made to correlate the incidence and types of tuberculous lesions to the period of captivity.

I. INCIDENCE OF TUBERCULOSIS.

The incidence of tuberculosis and the period of captivity are shown in Table I.

TABLE I.

Incidence of tuberculosis, expressed in percentage and based on autopsy findings, in relation to period of captivity of the monkeys.

Number of monkeys.	Period of captivity up to						
	2 weeks.	4 weeks.	1–2 months.	2–3 months.	3–6 months.	6–12 months.	12–24 months.
Autopsied	52	43	50	25	30	17	15
Positive for tuberculous lesions	12	10	21	11	15	13	13
Percentage showing tuberculous lesions ...	23	23	42	44	50	77	80

Twenty-three per cent of the animals, autopsied within one month of their captivity in the animal house, showed evidence of tuberculosis, as against 50 and 77 per cent up to three and six months, respectively, and 80 per cent after 12 months of captivity.

It confirms the general view that longer the monkeys are kept in captivity the higher is the incidence of this disease.

II. GROSS AUTOPSY FINDINGS.

From the postmortem studies made in the 95 monkeys with evidence of tuberculosis, the gross lesions observed are discussed below :—

(*a*) *Respiratory system.*—The lesions in some monkeys were of the nature of a few tiny translucent nodules of the size of a millet or even larger, located in the upper lobe of one or both lungs and at times in other lobes also. These tubercles were sometimes surrounded by areas of consolidation. Very often they were caseous but occasionally they appeared fibrotic or calcified.

In some animals, either the whole lung or some lobes only, showed smooth firm areas which, when cut, revealed extensive irregular caseated areas resembling caseous pneumonic type of infection in human beings.

Lesions associated with broncho-pneumonic type of infection were also met with. The lung in these cases sometimes appeared voluminous with firm masses scattered over its surface. On cutting, they showed creamy nodules standing out with or without any localization. In some specimens, the tubercles appeared in clusters. In others, caseation was seen in the centre of some of the tubercles or in the whole cluster, thus resembling lesions in the humans. At times, few scattered tubercles were found in the base of the lungs.

Sometimes a lobe or whole of a lung appeared dense with firm adhesions to chest wall which probably indicated a somewhat chronic infection. In these cases, the organ was covered with tubercular nodules of various sizes, some caseating and others even liquified. Cicatrizations and/or tubercular cavities were also not uncommon in such cases.

In many monkeys, miliary tubercles were observed on autopsy. In these cases, grey or greyish yellow tubercles were found scattered throughout the lung from the apex to the base. In these cases also the lungs appeared to be voluminous. The tubercles were seen as minute solid rounded bodies about 1 to 2 mm. in diameter or sometimes even more. In some instances, these tubercles were found clustered into groups. At times these clusters were found confined to one particular lobe, either the upper or lower.

The central part of these tubercles invariably showed caseation and even liquefaction. Some amount of congestion was noticed in the intervening areas not covered by tubercles. Grey tubercles occurred also over the whole pleural surface, especially between the lobes and diaphragmatic surface. Mediastinal glands were found affected and in some cases these were found actually bursting into the bronchi and liberating the liquified necrotic material into the lumen.

(*b*) *Cardiovascular system.*—Pericardial effusion and dilatation of the right side of the heart were seen in some of these animals, and on one occasion caseous nodules (miliary type) were seen in the heart itself.

(*c*) *Gastrointestinal tract.*—Intestinal tuberculosis was also common in the post-mortem findings. Minute grey or yellow tubercles, mainly in the submucosa and the mucous membrane, entirely encircled the lumen. These were more numerous in the cæcal region but extended sometimes in equal numbers up to the rectum unlike the disease in the human. Only in a few of the animals, evidence of infection was seen in the small intestine. Surprisingly enough, tubercular ulceration of the intestine was not usually observed. Intestinal tuberculosis occurred either alone or in association with lung infection. Enlargement of the masenteric glands occurred in majority of the cases. Stomach was found always free from tuberculous lesions.

(*d*) *Liver and spleen.*—Affection of liver and spleen was invariably associated with generalized miliary tuberculosis. Infection in the liver generally appeared as discrete caseating nodules of the size of a cut pea or almond, distributed here and there over the surface of the organ. Grey or caseating miliary tubercles affecting the entire organ were also seen in a few cases.

Whenever spleen was found affected it was usually enlarged. Grey or yellow tubercles were seen sometimes crowding the whole organ. Most often they were discrete and few in number. These tubercles usually were of the size of a small pin's head. Sometimes caseating tubercles of the size of a pea were also found in addition to the innumerable minute ones. On section, the organ appeared to be dark red with tubercles projecting slightly above the cut surface.

In one of the animals showing miliary tuberculosis of lungs, liver, spleen and omentum, the pancreas also showed a caseating mass about the size of $1\frac{1}{2} \times 1\frac{1}{2}$ inch.

(*e*) *Genito-urinary.*—Affection of the kidney was rather rare. On two occasions, in acute generalized tuberculosis, a few scattered minute grey tubercles situated on the cortex were seen under the capsule. Urinary bladder and testicles were not found to be affected in any of the autopsies done.

(*f*) *Bones and joints.*—A few animals which were under captivity for over a period of a year, showed ankylosis of the hip joints.

In many instances, particularly those showing broncho-pneumonic or generalized miliary type of lesions, the diagnosis was confirmed by demonstration of tubercle bacilli in the smears.

III. TYPES OF TUBERCULOUS LESIONS.

The types of lesions, as judged from the above gross postmortem findings, are analysed in Table II in relation to the extent of captivity of the monkeys in the animal house.

It is evident that the tuberculous lesion in the majority of the freshly caught monkeys, was in the form of calcified tubercles but during captivity the incidence of active tuberculosis became very obvious. Caseous pneumonic type was prevalent amounting to 20 to 24 per cent of the total tuberculous lesions up to two months

after captivity but thereafter the incidence was on the decrease. Broncho-pneumonic type was not seen in post-mortem done up to two months but thereafter it showed an incidence of 10 to 13 per cent. Generalized miliary tuberculosis was the main type observed in all late deaths. Its incidence which was nil up to one month was nine per cent between one and two months and 50 per cent between 12 to 24 months. During the various periods, 6 to 20 per cent of the tuberculosis cases belonged to the subacute or chronic type, and 8 to 14 per cent showed infection of the intestines. It is seen from this that in almost all recorded deaths due to tuberculosis after six months, severe lesions were observed indicating that these animals are highly susceptible to the disease. This also shows that the immunity of those animals under captivity to the disease is very low and that once they contract the disease they succumb to it within a few months. These findings corroborate those recorded by Habel (1947).

TABLE II.

Types of tuberculous lesions observed during autopsy and their relation to the duration of captivity of the animals.

Type of tuberculous lesions.	PERCENTAGE INCIDENCE OF THE DIFFERENT TYPES IN RELATION TO THE PERIOD OF CAPTIVITY (IN MONTHS):						
	0–½	½–1	1–2	2–3	3–6	6–12	12–24
A few calcified tubercles	66	40	19	...	...	...	...
Early caseating tubercles	14	20	29	35	28	25	23
Caseous pneumonic ...	20	20	24	5	10	...	7
Broncho pneumonic ...	...	...	...	13	12	10	12
Subacute (chronic) tuberculosis associated with adhesions	...	20	5	13	10	6	...
Generalized miliary tuberculosis... ...	...	...	9	24	27	48	50
Intestinal tuberculosis (without lung infection)	...	...	5	2	3	6	4
Intestinal tuberculosis (with lung infection)	...	...	9	8	10	5	4

SUMMARY.

Morbid anatomy of simian tuberculosis, based on autopsies performed in 95 tuberculous monkeys, has been described.

Up to one month of captivity, calcified tubercles formed the major tuberculous lesions whereas after 6 to 24 months these lesions pertained mostly to generalized miliary type. Infections resembling other forms of human tuberculosis

such as early caseating, caseous pneumonic, broncho-pneumonic, subacute or chronic and intestinal, were also common.

The incidence of the disease was found to increase progressively with the extent of stay of these animals under captivity.

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STUDIES ON NURI STRAIN OF *P. KNOWLESI*.

Part VIII. Comparative study on 4-aminoquinolines.

BY

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[July 19, 1955.]

DURING preliminary studies on the effect of various synthetic drugs against *P. knowlesi* originally isolated by Sinton and Mulligan (1932), it was observed that synthetic preparations of the 4-aminoquinoline series like chloroquine and amodiaquine were more effective than mepacrine, proguanil, aphacrine and metachloridine in the rapid clearance of parasites from the peripheral blood (Jaswant Singh, Ray and Nair, 1949). In the subsequent trial, Jaswant Singh *et al.* (1951) reported that chloroquine diphosphate (Winthrop's) and resochin (Bayer's) were both identical in their effect against the same strain of parasite. Since the isolation of the highly virulent strain of *P. knowlesi* Nuri strain (Jaswant Singh, Ray and Nair, 1953; Edison and Davey, 1953), all chemotherapeutic studies have been undertaken against this strain in preference to the old strain more so as the latter had generally lost its virulence. The results of such trials with pyrimethamine, proguanil, quinine, the active metabolite and the precursor (M. 3349) of proguanil have been recorded previously (Nair *et al.*, 1953:1955). In the present report, the relative merits of the different 4-aminoquinoline drugs have been recorded.

METHODS AND MATERIALS.

Monkey.—Seventy-two healthy *M. mulatta mulatta* weighing from 3 to 6 kg. body weight were used for the tests. Batches of two to six infected monkeys were placed on each dosage regime of the corresponding drugs as shown in Tables I-IV.

TABLE I.

Effect of aralen against blood-induced P. knowlesi *(Nuri strain) infection.*

Dosage mg./kg. (base).	Number of monkeys employed.	Ineffective	Deceleration of parasites only.	Class II Effects:					
				Clearance of parasites.			Relapse.		
		Number.	Number.	Number.	Average (hours).	Range (hours).	Number.	Average (days).*	Range (days).*
1·5	2	2	...	...	...	...	...	...	...
1·8	2	2	...	...	...	...	...	...	...
2·0	2	...	1	1	36	...	1	5	...
2·1†	5	...	...	5	136·8	72–168	5	9	6–12
2·2	4	...	...	4	102	72–120	4	7·25	7–8
2·5	3	...	...	2‡	128	96–168	2	8	7–9
3·0	2	...	...	1§	90‖	84–96	1	13	...

* After the cessation of treatment. † Minimal effective dose. ‡ One died due to intercurrent disease.
§ One showed Class III effect. ‖ Including the one that showed Class III effect.

TABLE II.

Effect of avloclor against blood-induced P. knowlesi *(Nuri strain) infection.*

Dosage mg./kg. (base).	Number of monkeys employed.	Ineffective.	Class I effect	Class II effects:					
				Clearance of parasites.			Relapse.		
		Number.	Number.	Number.	Average (hours).	Range (hours).	Number.	Average (days)*	Range (days)*
1·5	2	1	...	1	144	...	1	6	...
1·6	2	1	1	...	...	...	...	...	...
1·7	2	1	...	1	120	...	1	3	...
1·8†	5	...	...	5‡	129·6	120–144	3	6	3–9
2·0	4	...	...	4	129	120–156	4	6	5–7
3·0	2	...	...	2§	78	60–96	1	12	...

* After the cessation of treatment. † Minimal effective dose.
‡ Two died during observation period. § One died during the observation period.

Table III.

Effect of nivaquine against blood-induced P. knowlesi *(Nuri strain) infection.*

Dosage mg./kg (base).	Number of monkeys employed.	Ineffective.	Class I effect.	Class II effect.					
				Clearance of parasites			Relapse		
		Number.	Number.	Number.	Average (hours).	Range (hours).	Number.	Average (days)*	Range (days)*
2·0	2	1	...	1	156	...	1	5	...
2·2	2	...	1	1	120	...	1	4	...
2·3	2	1	...	1	144	...	1	3	...
2·4	3	2	...	1	132	...	1	7	...
2·5†	6	...	...	6‡	104	96–108	4	7·75	6–10
3·0	2	...	...	2	84	72–96	2	18·5	18–19

* After the cessation of treatment. † Minimal effective dose.

‡ Cure in one; another died during observation period.

Table IV.

Effect of amodiaquine against blood-induced P. knowlesi *(Nuri strain) infection.*

Dosage mg./kg. (base).	Number of monkeys employed.	Ineffective.	Class I effect.	Class II effect.					
				Clearance of parasites.			Relapse.		
		Number.	Number.	Number.	Average (hours).	Range (hours).	Number.	Average (days)*	Range (days)*
2·0	2	2	...	...	...	...	...	...	...
2·2	2	...	1	...	96	...	...	...	...
2·3	2	2	...	...	...	...	...	...	...
2·4	3	1	...	2	114	108–120	2	6	5–7
2·5†	5	...	...	5‡	144	96–168	3	6·3	5–7
3·0	2	...	...	2	108	96–120	2	8	8
3·5	2	...	...	2§	96§§	72–120	1	12	...

* After the cessation of treatment. † Died during observation period.

‡ Two died during observation period. § Class III effect in one.

§§ Includes the one in which Class III effect was produced.

Parasite.—Nuri strain of *P. knowlesi* maintained in *M. mulatta mulatta* by successive serial passages was used for infecting the animals. A standard dose of 5×10^6 parasitized erythrocytes per kg. body weight was introduced by the intravenous route throughout. Blood smears were collected daily from these animals both in the morning and evening up to the treatment period and once daily thereafter, and stained with J.S.B. method (Jaswant Singh and Bhattacharjee, 1944). The parasite densities were determined against 10,000 erythrocytes.

Antimalarials.—The drugs tested were (1) aralen (chloroquine diphosphate) of Winthrop Product Co., (2) avloclor (chloroquine diphosphate) of Imperial Chemical Industries, (3) nivaquine (chloroquine sulphate) of May & Baker, and (4) amodiaquine (camoquin) of Parke, Davis & Co. Different dosage schedules ranging from 1·5 to 3·5 mg. of the drug per kg. body weight of the animal were used for the testing. These doses were all in terms of the base of the drug and were administered orally. The first dose of the drug was given when the initial parasite density reached about 0·01 to 0·1 per cent cell infection (this in most of the cases occurred within 36 to 48 hours after inoculation) and subsequently it was repeated once every day in the morning up to a total of seven doses.

Criteria of activity.—This was determined by the ability of the particular dosage of a drug to clear the parasites from the peripheral blood by at least the day following drug administration (Class II) effect of Shannon (Wiselogle, 1946). The minimum dosage of a drug which produced this Class II effect was recorded as the minimal effective dose (M.E.D.).

If after the initial clearance, no recrudescences were observed during the period of observation (four weeks), splenectomy was performed. Continued absence of parasites in the peripheral blood for a subsequent period of four to six weeks characterized the eradication of infection.

RESULTS.

The details of the findings obtained with aralen are recorded in Table I. Dosages less than 2·0 mg. were found ineffective. Disappearance of parasites was obtained within 72 to 168 hours after the commencement of treatment in all cases treated with 2·1 to 3·0 mg. doses. Of these, except one monkey in which the infection was cured with 3·0 mg. dosage, all the rest exhibited only a Class II effect. The minimal effective dose (M.E.D.) is, therefore, taken as 2·1 mg. Relapses associated with the Class II effect occurred within 5 to 13 days after the cessation of treatment.

Avloclor was tried in dosages ranging from 1·5 to 3·0 mg. Up to a dose of 1·7 mg., one out of two monkeys tried in each schedule failed to show any effect on the parasites but all monkeys treated with 1·8 mg. and above showed Class II effect and, therefore, the latter dosage is recorded as the M.E.D. of the drug (Table II). The disappearance of parasites from the peripheral blood at this dosage was effected within 60 to 156 hours, and relapse within three to twelve days after the cessation of treatment.

From Table III it will be seen that though Class II effect could be observed in some monkeys even with dosages ranging from 2·0 to 2·4 mg. of nivaquin, the minimum dosage required to clear parasites from all monkeys before the cessation of treatment was 2·5 mg. which, therefore, represent the M.E.D. of this compound. At this dosage, parasite clearance was attained on an average of 104 hours (range 96 to 108 hours). The initial clearance of parasites was, however, followed in all the cases by relapses which occurred 3 to 19 days after the treatment.

The effect of amodiaquine is given in Table IV. 2·0 to 2·3 mg. dose of this drug was mostly inactive whereas 2·5 mg. and above were effective in clearing parasites from peripheral blood in 72 to 108 hours. All these monkeys relapsed five to twelve days after discontinuation of treatment. The M.E.D. of amodiaquine is, therefore, considered to be 2·5 mg. for Class II effect.

DISCUSSION.

Nair *et al.* (1953) recorded the minimum effective dose of quinine against Nuri Strain of *P. knowlesi* as 30 mg. In the present investigation, it was found that the M.E.D. of aralen, avloclor, nivaquine and amodiaquine was 2·1, 1·8, 2·5, and 2·5 mg., respectively. Thus the quinine equivalent of aralen is 14·3; of avloclor 16·7 and of nivaquine and amodiaquine 12. Broadly speaking the quantitative therapeutic efficacy of all these four drugs did not show a wide margin of variation, at least against this strain of *P. knowlesi*. However, some differences were encountered against *P. cynomolgi*. The quinine equivalent of chloroquine and nivaquine were observed to be 13·3 vis-a-vis avloclor and amodiaquine, the Q.E. of which were noted to be 20 and 5·7, respectively, (Jaswant Singh, Nair and Ray, 1953), whereas against *P. gallinaceum* in chicks, the quinine equivalent of chloroquine and amodiaquine were reported to be 16 and 32 (Jaswant Singh *et al.*, 1952). These have been summarized as per table below.

	P. gallinaceum.	*P. cynomolgi.*	*P. knowlesi.*
Aralen	16	13·3	14·3
Avloclor ...	...	20	16·7
Nivaquine ...	...	13·3	12
Resochin ...	...	13·3	...
Amodiaquine	32	5·7	12

From these it would be evident that although there are wide variations in the quinine equivalent of chloroquine salts and amodiaquine against *P. gallinaceum* in chicks and *P. cynomolgi* in monkeys, the activity of these compounds would appear to be somewhat similar against *P. knowlesi*. Earlier Jaswant Singh, Ray and Misra (1953) had encountered that against *P. falciparum*, parasite clearance occurred more or less during the same period when patients were treated with chloroquine preparations or amodiaquine. From these observations one is inclined to draw inferences that perhaps there could be some correlation between effects of

antimalarials against *P. knowlesi* and *P. falciparum*. If this could be established with all other known antimalarials as well, the present investigation would seem to be of some value as it demonstrates clearly that what is true for *P. knowlesi* is true for *P. falciparum*.

SUMMARY.

Aralen, avloclor, nivaquine and amodiaquine were tested against blood-induced Nuri strain of *P. knowlesi* infection, following the usual standard techniques adopted at the Malaria Institute of India.

The minimal effective dose (M.E.D.) of these drugs was found to be 1·8 mg. for avloclor, 2·1 mg. for aralen, and 2·5 mg. for nivaquine and amodiaquine.

The existence of direct correlation between the effect of drug against *P. knowlesi* and *P. falciparum* is discussed and the importance of carrying out preliminary investigations on the chemotherapeutic value of new drugs against this simian plasmodium is stressed.

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STUDIES ON NURI STRAIN OF *P. KNOWLESI.*

Part IX. Susceptibility to sulphonamide substituted dihydrotriazine, sulphadiazine and mepacrine.

BY

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PROGUANIL and the active metabolite of proguanil belonging to the dihydrotriazine group were previously tested against Nuri strain of *P. knowlesi* (Nair, Ray and Jaswant Singh, 1953; Nair, Bami and Ray, 1955). Recently, a similar dihydrotriazine (1 ; x = SO_2 NH_2) has been claimed to be very promising (Ray, Bose and Basu, 1954). In view of these observations, bio-assay of this compound was undertaken against this strain of plasmodium. For comparison, drugs like sulphadiazine and mepacrine were also assayed. The present report records the findings on the comparative merits of these three compounds.

MATERIALS AND METHODS.

Healthy *M. mulatta mulatta* (*rhesus* monkeys) weighing from 3 to 6 kg. body weight were used for the experiment. The procedure adopted for testing was on the same lines as described by Nair, Ray and Jaswant Singh (1953). The animals were inoculated intravenously with a dose of five million parasitized erythrocytes per kg. body weight from donor monkey showing very heavy infection. Parasite count was made in terms of their number per 10,000 erythrocytes. Smears were collected for this purpose from the animals twice a day (morning and evening) up to the day following the cessation of treatment and thereafter once a day only. Treatment was started at 0·1 to 1 per cent cell infection and the same was continued once a day up to a total of seven doses. All dosages were in terms of the base of the corresponding drug and were given orally.

Death of monkeys during drug administration with high parasitæmia was taken as an indication of absence of plasmodicidal effect. A dosage that was effective in producing a deceleration of parasites and prolonging the life of

monkeys for a minimum period of ten days from the commencement of treatment, was classified as Class I effect of Shannon (Wiselogle, 1946).

The minimum dose of a particular drug that was found necessary to produce parasite clearance by day following last dose of drug administration in all animals of a particular group (five monkeys generally) was taken as the minimum effective dose (Class II effect).

For Class III effect, the criterion was that monkeys which showed Class II effect initially, should subsequently show sterilization of blood-induced infection.

TABLE I.

Effect of sulphonamide substituted dihydrotriazine against blood-induced P. knowlesi *(Nuri strain) infection.*

Dosage mg./kg. base.	Number of monkeys employed.	Ineffective (Number).	Class I effect. (Number).	Class II effect:			
				Clearance of parasites.		Relapse*	
				Number.	Hours.†	Number.	Average (days)†
5	2	2	...	...	...	...	...
10	2	2	...	...	...	...	...
20	2	2	...	...	...	...	...
30	2	2	...	...	...	...	...
40	3	3	...	...	...	...	...
50	3	3	...	...	...	...	...
75	2	...	2	...	...	...	...
100	2	2	...	...	...	...	...
125	2	1	1	...	...	...	...
150	5	1	2‡	2	80 48-120	...	5·5 5-6
175	5	2	1	2§	96	...	...
200	5	...	...	5§§	84 48,48,60,132,132	...	...
300	2	...	...	2 ¶	48	1	1

*Since cessation of treatment.

†Nominator indicates average and denominator range.

‡Disappearance and reappearance of parasites in one during drug administration.

§Infection was cured in one. The other died during observation period.

§§Two died before the completion of drug administration, one two days after cessation of treatment, one four days after cessation of treatment, and in the fifth monkey infection was cured.

¶One died during treatment before the clearance of parasites.

TABLE II.

Effect of sulphadiazine against blood-induced P. knowlesi *(Nuri strain) infection.*

Dosage mg./kg. base.	Number of monkeys employed.	Ineffective (Number).	Class I effect (Number).	Class II effect: Clearance of parasites. Number.	Clearance of parasites. Average (hours).†	Relapse.* Number.	Relapse.* Average (days).†
0·05	2	2	...	...	...	...	...
0·1	3	...	2	...	...	...	...
0·15	2	2	...	...	...	...	...
0·2	4	1	1	2‡	90 84–96	1	4
0·25	6	...	1§	5‡	69·8 48–84	4	7·7 2–13
0·5	6	1	2§	3	104 72–156	3	3·6 2–6
0·6	3	...	1	2	102 69–144	2	3 2–4
0·7	5	...	1§	4	93 60–120	4	5 3·2
0·8*	5	...	...	5‡	72 48–132	3	5 3–8
1·0	5	...	...	5‡	100·8 72–156	2	5·5 4·7

*Since cessation of treatment.
†Nominator indicates average and denominator the range.
‡Class III effect in 1 to 3 monkeys.
§Disappearance and re-appearance of parasites during the period of drug administration.
*M.E.D. (Minimal effective dose).

RESULTS.

Sulphadiazine substituted dihydrotriazine showed no plasmodicidal effect when administered in 5 to 50 mg. dose. Dosages ranging from 75 to 125 mg. appeared inactive in fifty per cent of the monkeys, and in the rest deceleration in parasitæmia was evident. A dose of 150 to 175 mg. produced Class II effect only in four out of ten monkeys. 200 mg. dose produced Class II effect in all the monkeys tried, but of these three died perhaps due to the toxic effect of the drug

either before the completion of drug administration or soon after that. Similar death of monkeys during the treatment period occurred among those treated with 300 mg. also (Table I).

Between 0·05 and 0·15 mg. doses, sulphadiazine was mostly inactive. Dosages ranging from 0·2 to 0·5 mg. gave unpredictable results, as all types of activity was evident in the animals. However, doses of 0·6 and 0·7 mg. did produce Class II effect in all except one monkey in each regime. These two monkeys showed only Class I effect. Out of ten monkeys treated with 0·8 and 1 mg. doses, all showed Class II and 50 per cent of them Class III effect. From these, it could be inferred that the minimal effective dose of sulphadiazine is 0·8 mg. base per kg. body weight of the animals. Details of these findings are given in Table II.

As shown in Table III, mepacrine was tried in 0·3 to 6·0 mg. dose. Class II effect was seen in some of the monkeys treated with 3·0 to 5·0 mg. doses and in all with 6 mg. dose and thus the last dosage schedule mentioned was taken as the M.E.D. of the drug.

TABLE III.

Effect of mepacrine against blood-induced P. knowlesi *(Nuri strain) infection.*

Dosage mg./kg. base.	Number of monkeys employed.	Ineffective (Number).	Class I effect (Number).	Class II effect:			
				Clearance of parasites.		Relapse.*	
				Number.	(Hours).†	Number.	Average (days.)†
0·3	2	2	...	...	...	...	...
3·0	2	1	...	1	132	1	6
4·0	4	2	...	2‡	72	1	5
5·0	3	...	1	2§	60·96	...	...
6·0	5	...	...	5	76·8 / 60–96	4·5	9·6 / 12,14,10,7,5.

*Since cessation of treatment.
†Nominator indicates average and denominator range.
‡Class III effect in one monkey.
§Died during observation period.

DISCUSSION.

It has been found that the minimal effective dose (M.E.D.) of sulphonamide substituted dihydrotriazine, sulphadiazine and mepacrine is 200, 0·8 and 6 mg. respectively. Corresponding dose of quinine (Nair, Ray and Jaswant Singh, 1953) being 30 mg., the quinine equivalent of the three drugs would appear to be 0·15 for sulphonamide substituted dihydrotriazine, 37·5 for sulphadiazine and 5 for mepacrine. Previous studies have indicated that against *P. gallinaceum* or *P. cynomolgi*, mepacrine has a quinine equivalent of 4 (Jaswant Singh, Basu and

Ray, 1952; Ray, Nair *et al.*, 1954). Hence on a quantitative basis this drug has more or less the same effect against all the three different species of parasites.

Quinine equivalent of proguanil was previously recorded as 150 (Nair, Ray and Jaswant Singh, 1953) and of the active metabolite of proguanil 0·86. From these it would appear that proguanil is the most active, out of the drugs under review. The rest, in order of merit, are sulphadiazine, mepacrine, active metabolite of proguanil and the sulphonamide substituted dihydrotriazine, also known as "supazine". The poor plasmodicidal action of supazine is not only a feature against *P. knowlesi* (Nuri strain) but also against *P. gallinaceum* in chicks as is evident from the fact that its quinine equivalent against the latter strain is only 0·125 to 0·25 (Nair, Misra *et al.*, 1955). Further, though proguanil metabolite has shown quinine equivalent of 533 (Nair *et al.*, 1955) against *P. gallinaceum*, the same against *P. knowlesi* has been observed to be only 0·85. From these it would appear that their activities against various plasmodia are not quite consistent and what is more, they are usually inferior to proguanil. From the above findings, it may perhaps be reasonably assumed that the drugs would not be superior to the well-known standard drugs against human trials, which should be undertaken early for final appraisal on the drugs.

Several workers have proved the therapeutic efficacy of sulphonamide group of drugs against *P. knowlesi* in monkeys. Jaswant Singh and Harwant Singh (1939) found that sulphapyridine (M and B 693) in 1 gm. dose, by mouth, effected cure in monkeys infected with *P. knowlesi*. Dikshit and Ganapathi (1940) recorded radical cure of infection when monkeys were treated with 3 gm. of sulphadiazine given over a period of three days. Coggeshell and Marier (1941) found that sulphadiazine was therapeutically effective against the infection in monkeys in doses of 1·5 gm. when given orally for two to three days. Richardson *et al.* (1946) found that this drug was 175 times as active as quinine. The superiority of sulphadiazine over quinine is no doubt well confirmed in the present investigation also but on a quantitative basis it may appear that Nuri strain is approximately 4·7 times less susceptible than the previous strain of *P. knowlesi* to this drug. Perhaps factors such as strain difference as well as variations in the assessment techniques followed in the present investigation, as compared to that adopted by Richardson *et al.* (1946), may contribute to this relative difference.

SUMMARY.

Sulphonamide substituted dihydrotriazine (supazine), sulphadiazine and mepacrine were assayed in 93 *M. mulatta mulatta* (Rhesus monkeys) infected with the trophozoites of the Nuri strain of *P. knowlesi* and the minimal effective dose estimated to be 200, 0·8 and 6·0 mg., respectively.

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EFFECT OF BENZENE HEXACHLORIDE ON FISH LIFE.

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[June 3, 1955.]

SMALL larvivorous fish, like *Ambassis* sp., *Amblypharyngodon* sp., *Aplocheilus* sp., *Barbus ticto*, *Barilius* sp., *Chela* sp., *Trichogaster fasciatus*, *Danio* sp., *Etroplus maculatus*, *Gambusia affinis*, *Macropodus cupanus*, *Rasbora daniconius*, *Therapon jarbua*, etc., are used to control mosquito larvæ along the shallow margins of ponds. In the presence of much aquatic vegetation and floating debris amongst which the mosquito larvæ take shelter and escape the notice of their natural enemies, mosquito control by larvivorous fish has been found to be slow. In such cases, chemical larvicides will necessarily have to be used to achieve quick and thorough control of mosquito breeding. Some of these insecticides may, however, be toxic to fish life, especially when used in high concentrations. Heavy losses of fishes in streams in the Alabama area of the Tennessee River Valley during the summer of 1950 were attributed to insecticides like toxaphene, benzene hexachloride, D.D.T., and aldrin (Warrick, 1953). Field investigations by Mathis and Quarterman (1953), however, revealed that, benzene hexachloride when applied five times a year for three years consecutively at the rate of one pound per acre for the control of mosquito larvæ in small ponds, had no deleterious effect on fish and that the other insecticides like dieldrin, D.D.T., and toxaphene were all harmful to them. Doudoroff *et al.* (1954) who made a comparative study of the toxicity of some organic insecticides to fish, found that a benzene hexachloride-D.D.T. dust containing eight per cent benzene hexachloride, five per cent D.D.T. and 40 per cent sulphur, was less toxic than toxaphene or aldrin. The authors had the opportunity of studying the effect of B.H.C. 50 per cent on fish life during the year 1954 and the results of this study are briefly reported below.

Fifty per cent B.H.C. water dispersible powder containing about 6·5 per cent gamma isomer, is occasionally used for the control of mosquito breeding in paddy

fields, wells, ornamental tanks, cisterns, ponds and pits in urban and semi-urban areas. For culicine control, two ounces of this powder are suspended in one gallon of water and the diluted suspension sprayed at the rate of about two ounces per square yard of breeding surface in cess-pools, drains, etc. For anopheline control in larger collections of water like ponds, tanks, etc., the dosage applied is about 15 to 20 gallons per acre. In the case of ponds, only the margin for a width of about three feet is sprayed with the suspension. The effect of this treatment on fish life in ponds was studied in March 1954, when larvæ of *A. culicifacies*, the local vector, were found breeding in large numbers along the margins of the fish farm ponds at Bhavanisagar.

The fish farm consists of five similar rectangular ponds of 160 × 64 square feet with an average depth of 3·5 feet. The different species of fish, in the various ponds, were as indicated in Table I below.

TABLE I.

Species of fish in various ponds.

Pond number.	Name of fish.	Number present.	Length range (c.m.).
1.	*Osphronemus gorami* (Gourami)	52	10·0 to 22·5
2.	*Carassius carassius* (Golden carp)	576	2·5 to 17·5
	Cyprinus carpio var. *specularis* (Mirror carp) ...	2,229	2·5 to 17·5
3.	*Cyprinus carpio* var. *specularis* (Mirror carp) ...	3,672	2·5 to 10·0
	Carassius carassius (Golden carp)	1,000	2·5 to 5·0
	Tinca tinca (Tench)	4	15·0 to 17·5
4.	*Catla catla*	875	2·5 to 12·5
	Labeo sp.	400	1·9 to 7·5
5.	*Etroplus suratensis* (Pearl spot)	5,721	1·3 to 3·7

Besides the above food fishes, a few larvivorous fish like *Chela* sp. and *Danio* sp. were also present in the ponds. Pond 4 containing *Catla catla* and *Labeo* sp. was at first treated with half the dose of the insecticide (*one ounce per square yard*) usually employed, the remaining ponds being kept untreated for comparison. The spraying was done with a Cuticura Powder tin and was confined to the marginal strip of three feet all along the pond. The concentration of the insecticide worked out to 0·015 p.p.m. (parts per million). All the mosquito larvæ died within 24 hours of spraying, but none of the fish in the pond was affected. The treatment was repeated in Ponds 3 and 5 containing Mirror carp, Golden carp, Tench and Pearl spot, and the above dosage of the insecticide was found harmless to these species of fish also.

A series of laboratory experiments were also carried out to find whether B.H.C. 50 per cent (gamma isomer 6·5 per cent) was toxic to fish life and if so,

what the lethal limits were. The Orange chromide (*Etroplus maculatus*) of lengths varying from 6·0 to 7·5 c.m. were used as the test fish and its behaviour in different concentrations of B.H.C. was studied. The fish died in 51 hours in 36 p.p.m. concentration of the insecticide (2·34 p.p.m. gamma B.H.C.), in 102 hours in 18 p.p.m. (1·17 p.p.m. gamma B.H.C.) and in 118 hours in 12 p.p.m. (0·78 p.p.m. gamma B.H.C.) and was not affected by concentrations of 6 p.p.m. (0·39 p.p.m. gamma B.H.C.) and lower.

It would appear from the above experiments that B.H.C. 50 per cent at the normal dosage, in which it is usually used for the control of mosquito larvæ along the margins of ponds, is not harmful to fish life. However, the insecticide is lethal to fish life in concentrations above 6 p.p.m. and so care should be taken to see that this concentration of the insecticide is not reached, especially while treating very shallow fish ponds.

ACKNOWLEDGEMENT.

The authors thank Dr. H. Subramaniam, Regional Malariologist, Coimbatore, for his help during the experiments, and the Director of Industries and Commerce, Madras State, for his kind permission to publish this paper.

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A PRELIMINARY NOTE ON THE *ANOPHELINI* OF THE RAPTI VALLEY AREA OF THE NEPAL TERAI.

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[August 17, 1955.]

INTRODUCTION.

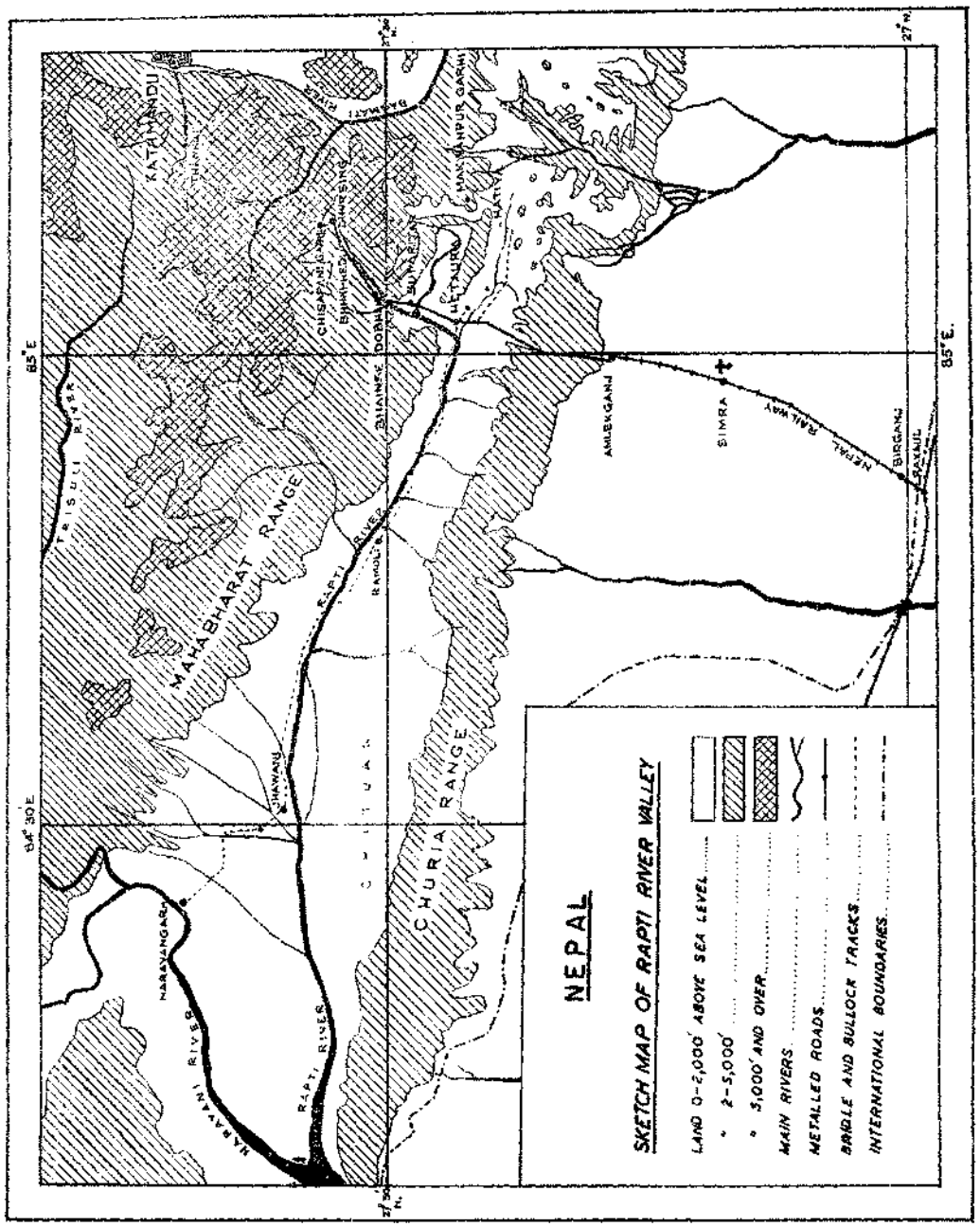
After a brief survey of a section of the Nepal Terai centred on Hetaura, Philips (1925) reported that malaria was hyperendemic in the area and recorded five species of anophelines including *A. fluviatilis*. In the same area, Puri* made a further survey in 1948 which was later extended by Raghavan (1949). Raghavan (1949) collected nine species of anophelines including *A. fluviatilis*, *A. culicifacies* and *A. minimus* and carried out a few dissections without finding any infected insects. In a later paper (1953) he suggested that malaria in this area is probably transmitted by *A. culicifacies* together with one of the *A. fluviatilis*/*minimus* complex.

No account has been published of the mosquito fauna of any other part of Nepal. The present paper is based on a survey carried out as part of the joint World Health Organization—United States Operations Mission to Nepal—Nepal Government Malaria Control Project in the Rapti Valley and Chituan areas of the Nepal Terai between January and May, 1955.

The Rapti River arises in the hills of the Mahabharat Range near Bhimphedi and passes first south-west then south for about 14 miles to Hetaura where it turns north-west then west and follows the Chituan Valley bounded on the south by the hills of the Churia Range and on the north by those of the Mahabharat

*Puri's report was not available at the time of writing this paper.

MAP I.


NEPAL
SKETCH MAP OF RAPTI RIVER VALLEY
LAND 0-2,000' ABOVE SEA LEVEL
" 2-5,000'
" 5,000' AND OVER
MAIN RIVERS
METALLED ROADS
BRIDLE AND BULLOCK TRACKS
INTERNATIONAL BOUNDARIES
KATHMANDU
MAHABHARAT RANGE
CHURIA RANGE
TRISULI RIVER
BHAINSE DOBHAN
HETAURA
MAKWANPUR GARHI
AMLEKGANJ
SIMRA
NEPAL RAILWAY
BIRGANJ
RAXAUL
CHAINPUR
NARAYANGARH
NARAYANI RIVER
RAPTI RIVER
84° 30' E
85° E
85° E.
27° 30' N.
27° N.

Range. About 90 miles west of Hetaura, the Rapti River joins the Narayani River coming from the mountains to the north. The previous surveys extended as far as Narayangarh in this western section and to Hatiya about 10 miles east of Hetaura.

From Bhimphedi (3,800 ft.) the River Rapti drops to about 2,000 ft. at Hetaura and continues at approximately this level for the rest of its course, widening considerably west of Hetaura and flooding much of the broad valley during the monsoons. Most of the enclosing hills consist of sedimentary deposits and the river-bed itself is very stony with numerous sluggish pools and sidewaters in the dry season, especially below 2,300 ft. The valleys east and west of Hetaura are covered by dense foothills jungle with scattered small cultivated areas in which rice is the main crop. The jungle abounds with a large variety of wild game. Here and there, small seepage pools occur in the jungle but most mosquito breeding is associated with the main river-beds and the associated sidestreams and springs feeding them.

No meteorological instruments were available during the course of this survey and no detailed figures are available from other sources. Raghavan (1953) has stated that the total annual rainfall is between 50 and 75 inches, which falls mainly between late June and September. There is a considerable difference between the day and night temperatures but the summer temperatures are probably not over 100°F. or the winter below 50°F.

SURVEY FINDINGS.

A total of 3,398 adult anophelines comprising ten species and one variety were captured in buildings during this survey. Six of the adults reared from the aquatic stages added four more species to the list. A fifteenth species was captured at rest near a small jungle pool. Many more adults were reared from larvæ in addition to many *Culicini* of various species which will be recorded in a subsequent paper. The following species of *Anophelini* were found:—

Species and authors.	Percentage of all adults captured.
Anopheles fluviatilis James, 1902	23·2
A. culicifacies Giles, 1901	22·4
A. minimus Theobald, 1901	2·9
A. maculatus Theobald, 1901 *A. maculatus* var. *willmori* James, 1902 ...	17·6
A. splendidus Koidzumi, 1920	28·6
A. annularis Van der Wulp, 1884 ...	2·3
A. majidi Young and Majid, 1928 ...	0·1
A. subpictus Grassi, 1899	2·6
A. vagus Dönitz, 1902	0·2
A. varuna Iyengar, 1924	1·3

Additional species collected as larvæ:—
A. hyrcanus Pallas, 1771
A. barbirostris Van der Wulp, 1884
A. lindesayi Giles, 1900
A. aconitus Dönitz, 1902

Collected at rest near jungle pool:—
A. aitkeni James, 1903

A. fluviatilis and *A. culicifacies* were only found below 2,300 ft. although it is likely that they are present higher up the valleys in small numbers. They were abundant in the Chituan and Narayangarh areas and in the valley of the Karra River east of Hetaura. *A. minimus* was found only below 2,000 ft. and then too in very small numbers. *A. maculatus* and *A. splendidus* were abundant all over the project area and were the dominant river-bed breeders. Larvæ of these species were also found in isolated shady jungle pools together with those of *A. barbirostris*, *A. hyrcanus*, *A. aconitus* and *A. majidi*. Four male and one female *A. aitkeni* were found resting under small boulders by such pools. *A. annularis*, *A. subpictus*, *A. vagus* and *A. varuna* were found occasionally in buildings in the lower valleys. Larvæ of *A. annularis* were fairly common in irrigation ditches and small pools formed in swampy ground. A few larvæ of *A. fluviatilis* and *A. culicifacies* were found at the edges of small cool streams and irrigation ditches in partial shade. *A. lindesayi* was found breeding in small pools containing filamentous green algæ in the bed of the Rapti River at Dhursing (3,315 ft.) where it was also recorded by Raghavan. Strangely enough this species was seen nowhere else by previous workers or ourselves.

Up to May, *A. culicifacies* and *A. minimus* were relatively commoner in the Chituan and Narayangarh areas than around Hetaura where *A. fluviatilis* predominated. The proportions of *A. fluviatilis* to *A. culicifacies* in the Hetaura area, however, showed a reversal from 3:1 in April to 1:4 by the end of May. South of Hetaura at Amlekanj *A. fluviatilis* was less common but still occurred in small numbers together with *A. culicifacies*. A certain amount of D.D.T. has been applied during the last two or three years in villages on and near the main Bhimphedi-Amlekanj road. It is impossible to say to what degree, if any, this has disturbed the balance of the mosquito fauna.

MALARIA VECTORS.

During March, April and May, 1955 the following mosquitoes were dissected:—

A. fluviatilis	511	*A. splendidus* ...	133
A. culicifacies	378	*A. annularis* ...	3
A. minimus	34	*A. vagus* ...	1
A. maculatus	48	*A. subpictus* ...	9

On April 28, 1955, a single *A. fluviatilis* from the Hetaura area was found with sporozoïtes in the salivary glands [Sporozoïte Rate (S.R.) 0·33 per cent] and in May a second infected specimen was discovered in the same area (S.R. 1·1 per cent). The overall S.R. for the three months was 0·39 per cent. No other infections were detected.

Issaris *et al.* (1953) in the Uttar Pradesh Tarai found a similar S.R. in *A. fluviatilis* in October, March, April and May. This was the dominant species there from October to June with two peaks of population density from October-December and March-May. They found *A. culicifacies* infected in July, August and September at which time that was the dominant species. They also reported that *A. minimus* was too scarce at any time of the year to be of any importance as

a malaria vector there. In view of the similarity of the terrain and our survey findings to date, it seems probable that a similar picture of transmission and seasonal prevalence will emerge in this area as the survey continues.

HOST PREDILECTION.

Mosquito blood meals were collected on filter papers and dispatched to the Malaria Institute of India, Delhi, where precipitin tests were performed on them by Drs. S. P. Ramakrishnan and Satya Prakash to determine the sources of the blood. The following figures were obtained from the small numbers so far examined:—

Species.	Percentage of positive reactions to anti-human serum			
	Sources of specimens (Number examined in brackets)			
	Houses.	Mixed dwellings.	Cattlesheds.	Total.
A. fluviatilis ...	18·7 (16)	21·9 (41)	21·4 (56)	21·2 (113)
A. culicifacies ...	12·5 (16)	4·5 (22)	16·3 (49)	12·7 (87)
A. maculatus ...	12·5 (8)	0 (8)	17·5 (80)	15·6 (96)
A. splendidus ...	18·7 (16)	18·7 (16)	25·4 (71)	22·3 (103)

Issaris *et al.* reported a gross anthropophilic index (A.I.) for *A. fluviatilis* of 41·2 per cent (in 245 bloods examined). The A.I. for insects caught in cattlesheds was 28·4 per cent, out of doors 44·7 per cent and in human dwellings 63·0 per cent. Ramakrishnan and Satya Prakash (1953) have commented on these findings in relation to the possibility of the existence of two biological races. Although the numbers examined so far in our survey are small, it is interesting to note that the overall A.I. of *A. fluviatilis* (21·2 per cent of 113 examined) is lower than that recorded by Issaris *et al.* (1953) and that it is similar in all types of structure. It is tempting but dangerous at this stage to speculate on the extent to which this difference might be due to the irregular application of D.D.T. in parts of this area during the past two years or more. In this respect it is also interesting to note that the following average man-hour densities were obtained in fixed unsprayed catching stations during the months of March and April:—

Species.	Houses.	Mixed dwellings.	Cattlesheds.
A. fluviatilis	7·0	7·9	52·5
A. culicifacies	14·5	15·5	46·5

SUMMARY.

1. A preliminary account is presented of the *Anophelini* of the Rapti Valley area of the Nepal Terai. This is the first account published to date.

2. A sketch map and description of the area are given. No detailed meteorological data were available.

3. 3,398 adult anophelines were captured in buildings between March and May and other examples were taken in the aquatic stages. Fifteen species and one variety were recorded; *A. fluviatilis*, *A. culicifacies*, *A. maculatus* and *A. splendidus* accounting for 91·8 per cent of all adults captured.

4. The proportion of *A. fluviatilis* to *A. culicifacies* changed from 3:1 in March to 1:4 in May. *A. minimus* represented only 2·9 per cent of all adult anophelines captured but was relatively commoner in the Chituan area.

5. Two specimens of *A. fluviatilis* were found infected, one in April and one in May. The Sporozoite Rate was 0·39 per cent of 511 dissected during the three month period.

6. The anthropophilic index of *A. fluviatilis* was 21·2 per cent (of 113 tested) and of *A. culicifacies* 12·7 per cent (of 87 tested).

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A PRELIMINARY NOTE ON THE BEHAVIOUR OF ANOPHELINES IN STRUCTURES TREATED WITH D.D.T. AND B.H.C.

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[August 30, 1955.]

MUIRHEAD Thomson (1947) while working on the control of *A. gambiæ* and *A. melas* first reported the excito-repellent effect of D.D.T. on mosquitoes. He also found that B.H.C. was more effective than D.D.T. in the control of mosquitoes. Bertram (1950) reported similar effect on *A. minimus* in Assam. MacDonald (1950) on the other hand, while reviewing the work of Gilroy in Assam, stated that D.D.T. was more effective against this species. Rajindar Pal and Sharma (1952) observed that both D.D.T. and B.H.C. exerted an excito-repellent action on mosquitoes but that it had no significance in malaria control as the mosquitoes picked up a lethal dose before getting excited and repelled. These authors have also reviewed the work on excito-repellency done in different parts of the world from which it is obvious that there are still conflicting views regarding this manifestation. With a view to investigate this phenomenon as applicable to anopheline species of the sub-montane Malnad tracts of Mysore State, India, studies described in this paper were taken up.

METHODS AND MATERIALS.

The area where experiment was carried out is situated in the Malnad in Hassan District, Mysore State. It is a hyperendemic area and *A. fluviatilis* is the vector. Brookeworth (1953) has published a detailed report of the anopheline fauna of this tract. The species collected during the study were *A. aconitus*,

A. annularis, A. barbirostris, A. culicifacies, A. fluviatilis, A. hyrcanus, A. jamesi, A. jeyporiensis, A. karwari, A. maculatus, A. majidi, A. pallidus, A. splendidus, A. subpictus, A. tessellatus, A. theobaldi, A. turkhudi, A. vagus and *A. varuna.* The most prevalent species was *A. jeyporiensis.*

Two villages, Anemahal and Kollahalli, within a radius of five miles from Saklaspur Town, Hassan District, were selected for observations. D.D.T. residual spraying was being done in the area since the year 1949, leaving the detached cattlesheds unsprayed in the routine operations. For these studies, three cattlesheds, almost contiguous with one another, were chosen in each village. In Anemahal, the sheds were mudwalled with tile roofs, while those at Kollahalli were made of split bamboos with thatched roofs.

The observation period extended from February to May, 1953. Mosquitoes were collected from the sheds before and after spraying with D.D.T. and B.H.C. During the pre-spraying period, the adult mosquitoes were collected by hand, for 30 minutes, at intervals of every four hours (on a twenty-four hour basis) commencing at 18.00 hours (18.00, 22.00, 02.00 hours and so on).

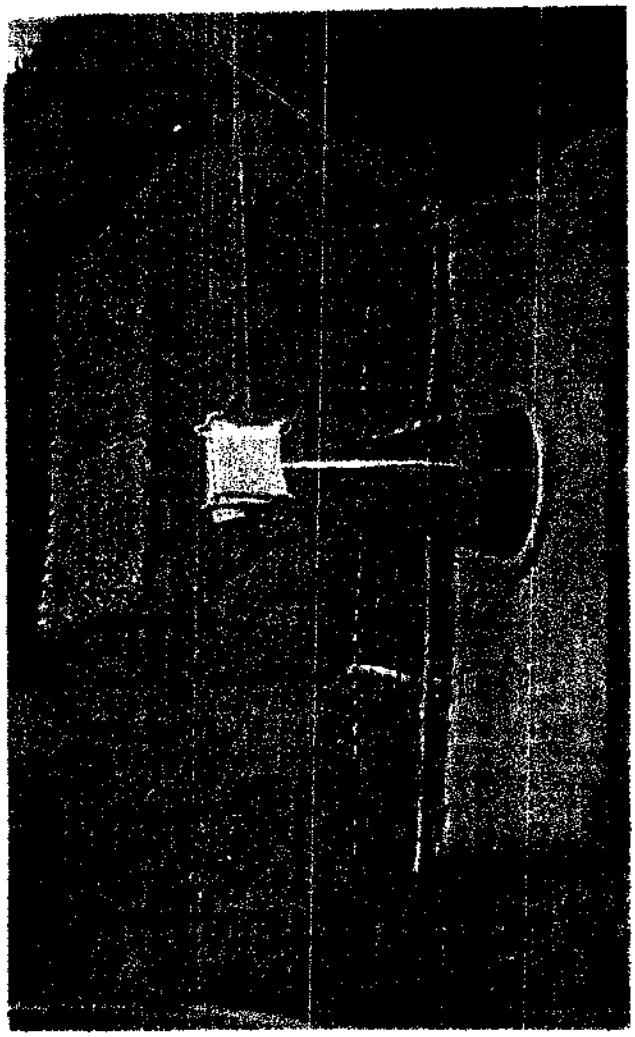
The collections were continued over a period of four weeks. After identification, the abdominal condition of the mosquitoes thus collected was classified and noted on the spot as (*a*) unfed, (*b*) fully fed, (*c*) partially digested, (*d*) fully gravid.

During the second phase of the experiments, in addition to hand collections, window-traps were used to trap the escaping mosquitoes. Before putting up the window-traps, all the openings in the shed, except the eaves, were closed with bamboo thatties. The window-traps were fixed to an opening through which only the light from outside entered the shed. It consisted of a celluloid funnel with the broad end fitting tightly into a plywood ring, and then fixed to a plywood sheet with a circular hole cut into it. This sheet is fixed to the opening in the wall while the narrow end of the funnel led directly into a Barraud cage which was hung outside from a string (Plates I and II).* These cages were changed every four hours during the four weeks period of observation at 22.00, 02.00, 06.00, 10.00, 14.00 and 18.00 hours. Here too the identification and condition of the abdomens of the trapped mosquitoes were noted as with the mosquitoes caught by hand.

After recording observations for eight weeks, the cattlesheds were treated with D.D.T. and B.H.C., and mosquito collections, both by hand and window-traps, were continued as during pre-spray period. In Anemahal Village, cattle-shed 'A' was sprayed with 75 per cent D.D.T. wettable powder at 200 mg. per sq. ft., cattleshed 'C' with B.H.C. P.520 at 22 mg. per sq. ft., while cattleshed 'B' was left unsprayed for comparison. Cattleshed 'A' in Kollahalli was treated with 100 mg. D.D.T. per sq. ft. (water wettable powder) and cattleshed 'C' with 11 mg. B.H.C. (P. 520) and cattleshed 'B' was left unsprayed to serve as comparison. Observations were carried out for four weeks and then discontinued because the mosquito population even in the untreated shed became very low with the onset of summer. In both the villages, cattlesheds were empty during the day time.

* The method of construction of these window-traps has been described in detail by Brook Worth (1953) in *Mosquito News*, **13**, pp. 204–206.

Mudwalled experimental sheds with window-traps in position.



A closeup view of the one-way window-trap, consisting of evenly tapering celluloid cone with circular base fitted with ring inside plywood sheet; exit of cones led directly into Barraud cage.

As these studies were of a preliminary nature, total collection of mosquitoes, 24-hour survival rates of trapped mosquitoes and chemical estimation of D.D.T. deposits, were not carried out. But observations on the trapped mosquitoes indicated that, in the case of the treated sheds, there was heavy mortality (about 80 per cent) within six to eight hours, whereas in the case of the mosquitoes from the untreated sheds a 50 per cent mortality was recorded after 20 hours.

In addition to the study of excito-repellent effect, the observations made before treatment helped to understand the normal behaviour of mosquitoes inside shelters.

The data collected during the pre-spray period are presented in Tables I and II. All species of anophelines collected are pooled together.

TABLE I.

Weekly hand collections and window-trap collections of mosquitoes from six cattlesheds (collections of all the six quarters combined) before spraying.

Week.	ANEMAHAL.			KOLLAHALLI.		
	Cattlesheds.			Cattlesheds.		
	A	B	C	A	B	C
	Hand collections.					
1953						
February 2-9 ...	365	391	274	560	858	280
February 9-16 ...	320	445	230	426	540	221
February 16-23 ...	376	479	186	218	643	212
February 23 to March 2 ...	601	749	265	358	1,006	280
Grand total ...	1,662	2,064	955	1,562	3,047	993
Average per week	415·5	516·0	238·8	390·5	761·8	248·3
	Window-trap collections.					
1953						
March 9-16 ...	830	290	496	72	108	53
March 16-23 ...	824	314	404	33	63	60
March 23-30 ...	495	290	241	25	48	15
April 1-7 ...	406	377	264	14	67	39
Grand total ...	2,555	1,271	1,405	144	286	167
Average per week	638·8	317·8	351·3	36·0	71·5	41·8

TABLE II.

Percentage of mosquitoes, collected by hand and by window-traps, during the six four-hourly collections, analysed according to different abdominal conditions (before spraying.)

Time of collection.	Anemahal.				Kollahalli.			
	a	*b*	*c*	*d*	*a*	*b*	*c*	*d*
Hand collections.								
(*i*) 18·00–18·30	4·2	9·1	69·7	16·8	8·4	9·6	57·2	23·6
(*ii*) 22·00–22·30	1·4	33·9	61·1	3·4	7·7	59·2	31·6	1·4
(*iii*) 02·00–02·30	1·1	17·2	78·7	2·9	1·4	20·2	76·3	1·6
(*iv*) 06·00–06·30	0·9	17·5	78·5	3·1	0·1	10·1	87·3	3·3
(*v*) 10·00–10·30	0·0	0·8	98·2	18·8	0·4	0·0	80·4	19·2
(*vi*) 14·00–14·30	0·5	0·2	79·1	20·1	1·2	0·6	85·6	12·6
Window-trap collections.								
(*i*) 18·00–22·00	0·6	5·2	82·1	12·1	8·9	6·9	6·3	21·6
(*ii*) 22·00–02·00	0·6	4·7	93·9	0·7	3·4	4·5	85·4	6·7
(*iii*) 02·00–06·00	0·3	1·8	97·4	0·5	8·0	4·1	87·8	0·0
(*iv*) 06·00–10·00	0·3	0·0	99·7	0·0	0·8	0·0	99·2	0·0
(*v*) 10·00–14·00	0·0	0·0	2·0	0·0	0·0	0·0	0·0	0·0
(*vi*) 14·00–18·00	0·9	0·0	3·0	0·0	0·0	0·0	0·0	0·0

a = unfed. *b* = freshly fed. *c* = partially digested. *d* = fully gravid.

The data furnished in Table II show that feeding takes place throughout the night but the peak is between 21·00 and 22·30 hours.

The total number of *A. fluviatilis* collected by hand from the two villages, during the period of observation, was 174. In the first collection (18·00-18·30 hours), no freshly-fed specimens were collected; in the second (22·00-22·30 hours) 60 with fresh blood and 13 with partially digested blood were taken; in the third (02·00-02·30 hours) eight specimens with fresh blood and 22 with partially digested blood were recorded, while in the fourth (06·00-06·30 hours) four had fresh blood and 19 had partially digested blood. From these observations, it was evident that the peak feeding time in the case of *A. fluviatilis* in the area was between 21·00 and 22·30 hours (81·0 per cent), though some feeding took place between 02·00 and 06·00 hours also.

The window-trap collections in Anemahal were always higher than those of Kollahalli. This was perhaps due to the different types of experimental sheds prevailing in the two villages. The cattlesheds at Anemahal were ideally suited

for fixing the window-traps while at Kollahalli, in spite of best efforts, there were a number of openings which could not be closed. As a result of this, a large percentage of mosquitoes might have escaped.

The number of *A. fluviatilis* collected in the window-traps was only four from all the six cattlesheds and hence no attempt is made to present the data separately.

The window-trap collections have shown that there is a definite tendency for a large percentage of mosquitoes with partially digested blood to get out of the cattleshed. Whether this is due to the strong outdoor resting habits of the mosquitoes, is yet to be investigated.

The data, obtained after treating the cattlesheds with D.D.T. and B.H.C. with different dosages, are presented in Tables III and IV. These data have been analysed in the same manner as the pre-spray data in Tables I and II. Data regarding the weekly collections are also presented in the form of two charts (Charts 1 and 2).

TABLE III.

Total weekly collections of mosquitoes from six cattlesheds (collections of all six quarters combined). Post-spraying period.

Week.	ANEMAHAL VILLAGE.						KOLLAHALLI VILLAGE.					
	200 mg. D.D.T./sq. ft.		22 mg. B.H.C./sq. ft.		Comparison.		100 mg. D.D.T./sq. ft.		11 mg. B.H.C./sq. ft.		Comparison.	
	Hand collection.	Trap collection.	Hand collection.	Trap collection.	Hand collection.	Trap collection.	Hand collection.	Trap collection.	Hand collection.	Trap collection.	Hand collection.	Trap collection.
Week before spraying ...	614	487	247	285	811	406	195	14	192	39	237	67
Week after spraying												
1	1	206	2	16	44	142	6	23	13	35	285	153
2	1	198	0	102	103	197	1	13	25	18	259	56
3	0	88	0	81	104	116	2	21	54	15	294	40
4	0	163	10	88	115	123	2	21	123	26	172	40

The data presented in Tables III and IV and Charts 1 and 2 show that there was a striking reduction in the number of mosquitoes resting indoors after the application of the residual insecticides. It was also observed that there was a definite drop in mosquito catches even in the untreated (comparison) cattleshed. This is what should be expected because of the close proximity of the treated and untreated cattlesheds. Any way, the reduction was not as remarkable as in sprayed sheds and the number of mosquitoes resting inside was always very much higher than in experimental cattlesheds. However, the number of mosquitoes caught

TABLE IV.

Effect of treating experimental cattlesheds with D.D.T. and B.H.C. on numbers of fed and unfed mosquitoes found resting inside and collected in window-traps.

Weeks.		200 mg. D.D.T./sq. ft.					22 mg. B.H.C./sq. ft.					Comparison.					100 mg. D.D.T./sq. ft.					11 mg. B.H.C./sq. ft.					Comparison.				
		Trap collection.				Hand collection.					Hand collection.					Trap collection.					Hand collection.					Trap collection.				
		a	*b*	*c*	*d*	*c*	*a*	*b*	*c*	*d*	*c*	*a*	*b*	*c*	*d*	*c*	*a*	*b*	*c*	*d*	*c*	*a*	*b*	*c*	*d*	*c*	*a*	*b*	*c*	*d*	*c*
Week before spray.		4	72	387	94	467	1	47	180	19	692	10	147	692	62	692	0	4	4	62	143	12	0	31	4	9	4	5	26	4	130
Weeks after spray 1		1	34	417	11	0	0	1	9	0	107	1	4	111	5	107	0	6	10	4	1	1	0	0	0	0	2	7	15	2	39
	2	2	5	77	4	0	0	0	0	0	39	3	7	96	11	39	1	16	2	3	1	1	2	0	0	0	1	0	0	0	2
	3	0	23	166	20	1	0	0	1	0	62	1	22	164	10	62	0	3	3	2	0	0	1	3	1	0	1	3	8	4	67
	4	1	38	143	24	1	0	1	1	0	24	1	14	126	2	24	4	10	7	2	0	0	3	2	0	0	1	8	7	4	2
		1	72	387	94	467	1	47	180	19	692	10	147	692	62	692	0	4	4	62	143	12	0	31	4	9	4	5	26	4	130

a = unfed. *b* = freshly fed. *c* = partially digested. *d* = fully gravid.

CHART 1

EFFECT OF TREATING EXPERIMENTAL CATTLESHEDS AT ANEMAHAL WITH DDT AND BHC ON NUMBERS OF ANOPHELINES RESTING INSIDE AND COLLECTED IN WINDOW-TRAPS

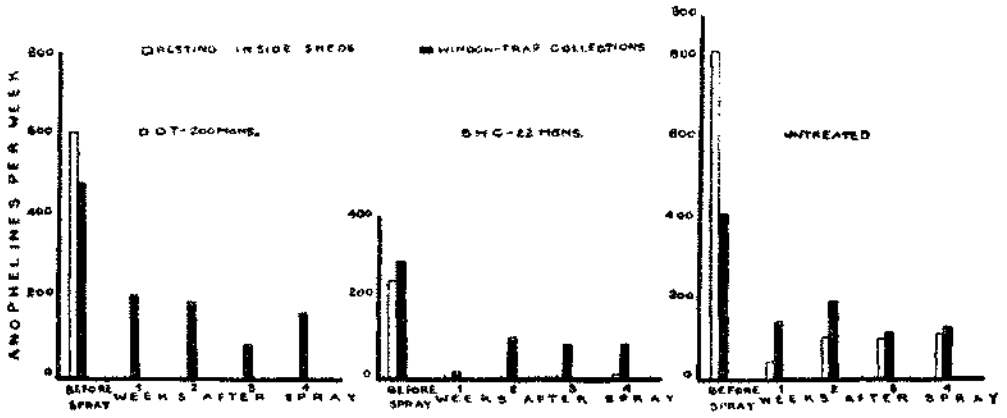
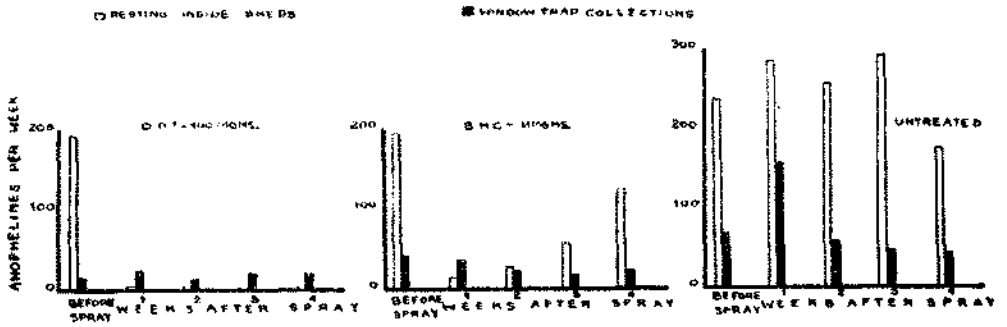


CHART 2

EFFECT OF TREATING EXPERIMENTAL CATTLESHEDS AT HOLLAHALLI WITH DDT AND BHC ON NUMBERS OF ANOPHELINES RESTING INSIDE AND COLLECTED IN WINDOW-TRAPS



in the window-traps in treated cattlesheds was comparatively large and almost all of them were blood-fed females (Table IV). Thus it was evident that active feeding did take place inside all the treated cattlesheds. The peak feeding time was also observed to be the same as in pre-spraying period. The presence of a large number of mosquitoes in window-traps indicated that there was a definite excito-repellent effect. It was also actually observed in a few instances that mosquitoes flew towards the window-trap immediately after coming into contact with the treated surface. For the first two to three days, the mosquitoes, in the window-traps in the D.D.T. treated cattlesheds, exhibited characteristic symptoms of D.D.T. poisoning, namely, lying on their backs with one or two pairs of dislodged legs.

This shows that the mosquitoes did pick up a lethal dose before they became excited. A large proportion of mosquitoes, caught in the window-traps during the first week after spraying in the cattleshed treated with 22 mg. B.H.C., was either dead or in the process of dying, thus proving that B.H.C. was very toxic. Even at a dosage of 11 mg./sq. ft., B.H.C. exhibited a slight excito-repellent effect in the early stages, but not to the same extent as D.D.T. The residual effect of B.H.C. in 11 mg. dosage did not last beyond the fourth week as was evident from the large numbers of mosquitoes resting inside the cattleshed.

In all the treated huts *A. fluviatilis* was virtually absent and only one specimen was caught by hand-catching in the cattleshed treated with 11 mg./sq. ft. B.H.C., while in the untreated (comparison cattleshed) six specimens were caught. The density of *A. fluviatilis* in the window-traps even during prespraying period was very low and as such no definite conclusion can be drawn regarding the excito-repellent effect on this species.

DISCUSSION.

In assessing the data of these studies, it has to be borne in mind that these observations were made during the months February to May and there was a rapid natural decline in the mosquito population from April owing to the onset of the dry season. It is, however, quite evident that there is a definite excito-repellent effect exerted both by D.D.T. and B.H.C. and even in the absence of any figures for survival rate of the trapped mosquitoes, there was enough evidence to show that mosquitoes did pick up a lethal dose before escaping. These findings are in accordance with those of Rajindar Pal and Sharma (1952), Hocking (1947) and Nair (1951). The two different dosages of D.D.T. applied did not show any significant variation and the effect on mosquitoes was more or less similar. But with B.H.C. at 11 mg./sq. ft. dosage, the repellent effect was not so pronounced as in the case of D.D.T.

The loss of residual effect of D.D.T. and B.H.C. with different dosages could not be followed up because the period of observations was limited only to four weeks. But with B.H.C. 11 mg. dosage, it was evident that the residual toxicity did not extend to the fourth week as judged from the large number of mosquitoes found resting inside treated cattleshed.

SUMMARY.

Behaviour of mosquitoes in cattlesheds before and after treatment with D.D.T. and B.H.C. was studied in two villages in Malnad, Hassan District, Mysore State.

D.D.T. appeared to exert an excito-repellent effect on mosquitoes. The repellent effect of B.H.C. was comparatively much less marked and was present only in the earlier stages with the dosage of 11 mg./sq. ft. There was evidence to show that the mosquitoes picked up a lethal dose before escaping.

The normal behaviour of the mosquitoes did not change due to insecticidal treatment of shelters. Active feeding did take place in treated cattlesheds.

ACKNOWLEDGEMENT.

The authors express their grateful thanks to the Director of Public Health, Mysore, for facilities extended and to Dr. B. Ananthaswamy Rao, Deputy Director, Malaria Institute of India, Delhi, for his valuable suggestions and reviewing the manuscript. The authors are obliged to Messrs. Sunder Raman and Rahiman Beig for assistance in the work.

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OBITUARY.

Dr. S. RAJENDRAM.

One of the active promoters of malaria eradication programme in Ceylon, Dr. S. Rajendram, L.R.C.P. & S. (Edin.), L.R.F.P. & S. (Glas.), D.T.M. & H. (Eng.), D.P.H.(Edin.), passed away on May 9, 1955, at the age of 56. His death is deeply mourned not only by his colleagues in Ceylon but also by malariologists all over the world.

Dr. Rajendram joined the Department of Medical and Sanitary Services of Ceylon in 1925. After his studies abroad in 1935 and 1936 and training at the Malaria Institute of India and at the League of Nations malaria course at Singapore, he was absorbed in the Department of Antimalaria Campaigns in 1941 of which he became Superintendent in 1946.

He was a member of the panel of the World Health Organization Expert Committee on Malaria. Based on the experience gained by his studies in India, Italy, Cyprus, Egypt and U.K. on the Rockefeller Foundation and the World Health Organization fellowships in 1947 and 1949, the malaria eradication programme in Ceylon was drawn up and launched in 1950. His achievements in this malaria control project attracted the attention of eminent world malariologists who studied the splendid work carried out by him.

He made many valuable contributions and his articles on 'Malaria in Ceylon' which appeared in the special CEYLON NUMBER of the *Indian Journal of Malariology* (Vol. V, No. 1, March 1951) will remain a lasting monument and a guide to all malariologists.

J. S.

PLATE III.

Brigadier J. A. SINTON, C.I.E., O.B.E., I.M.S., Retd.
Editor: 1929-1935.

EDITORIAL

THE *Indian Journal of Malariology* has successfully completed 25 years of its service to the malaria world. Starting as *Records of the Malaria Survey of India*, its nomenclature was changed to *Journal of the Malaria Institute of India* in 1938 and finally switched over to *Indian Journal of Malariology* in 1947.

So far 22 volumes, each consisting of four issues, have appeared. It is a quarterly publication but in 1932-1933 and during the war years (1941-1946), when there was paucity of material for publication, one volume was completed in two years. For the same reason, two issues were combined into one, four times during the entire period. The total number of printed pages run to 11,506, average 523 pages per volume. During this period, 805 articles have appeared, average 37 per volume (Table I).

TABLE I.

Number of articles and pages published.

Volume.	Year.	Number of articles.	Number of pages.
Records of the Malaria Survey of India.			
1	1929–30	33	771
2	1931	23	664
3	1932–33	39	856
4	1934	28	426
5	1935	32	528
6	1936	33	656
7	1937	21	272
Journal of the Malaria Institute of India.			
1	1938	39	480
2	1939	36	463
3	1940	44	609
4	1941–42	43	642
5	1943–44	37	477
6	1945–46	42	513
Indian Journal of Malariology.			
1	1947	40	547
2	1948	19	327
3	1949	35	426
4	1950	41	556
5	1951	38	694
6	1952	41	590
7	1953	50	392
8	1954	50	395
9	1955	41	402
Total		805	11,506
Average per volume		37	523

Articles published are not only accounts of work done by the staff of the Malaria Institute of India (formerly Malaria Survey of India) and other Indian workers, but the journal has enjoyed the confidence of eminent research workers in foreign countries as well and includes as many as 58 contributions from them as per following details:—

India		...	746
Africa	9		
Belgium	1		
Burma	1		
Ceylon	12		
Formosa	6		
France	3		
Holland	1		
Indonesia	4		
Ireland	1		
Italy	2		
Malaya	1		
Nepal	1		
Pakistan	2		
Palestine	1		
Switzerland	1		
U.K.	8		
U.S.A.	4		58
	58		804

So far, 28 abstracts of articles of local importance (the original MSS. having been kept in the library of the Malaria Institute of India for reference by workers who wish to consult them) and nine obituaries of eminent workers who left a wide gap among the malariologists of the world, have appeared in the journal. Review of important books has only recently been started and so far only four reviews have appeared.

The total number of authors who have contributed to the journal from time to time is 365. The largest number of pages contributed by a single author so far is 1,057 in 43 articles by Major-General Sir Gordon Covell (Director, Malaria Institute of India from 1936 to 1947) and the largest number of contributions by a single author is 83 comprising 759 pages by Lt.-Colonel Jaswant Singh (Director, Malaria Institute of India from 1947 to date).

The subjects covered include malaria in all its aspects (human, avian, simian and recently rodent—physiology of malaria parasite, pathology, chemotherapy, immunology, epidemiology and control); studies on mosquitoes, both Culex and Anopheles (distribution, vectorial capacity, relation to malaria, destruction by the use of insecticides, resistance to insecticides); blackwater fever; kala azar; filariasis; etc.

The Director, Malaria Institute of India, has been the ex-officio editor of the journal. Brigadier J. A. Sinton remained the editor from 1929 to 1935 and

PLATE IV.

Major-General Sir GORDON COVELL, C.I.E., K.B.E., D.M.S., Retd.
Editor: 1936-1946.

was assisted at times by Lt.-Colonel H. W. Mulligan during 1935. *Major-General* Sir Gordon Covell took up the editorship in 1936 and continued till 1946. He was assisted by Lt.-Colonel M. K. Afridi up to 1939 and by Lt.-Colonel Jaswant Singh from 1941 onwards. Lt.-Colonel Jaswant Singh became the editor in 1947 and is still continuing.

The journal is financed by the Indian Council of Medical Research and besides money realized from sales, advertisements, etc., receives an annual grant of Rs. 12,500 per year for its publication only.

It has a world-wide circulation and has a special place among the malaria publications. There are at present 70 foreign and 98 Indian subscribers. Besides 114 copies issued in exchange, 112 copies are distributed free to eminent workers and institutions both in India and abroad. A large number of journals of international repute are received in exchange of this journal, which considerably augment the resources of the library at the Malaria Institute of India.

With the formation of Malaria Survey of India in 1927, it was felt necessary to dig out the old reports and articles relating to malaria all over the country. Some of these were included in provincial publications and many were still in the MS. form. In the course of a three-month tour throughout India, Sinton carried out a systematic search and compiled a bibliography of malaria in India which includes references of 2,200 papers with journal index, authors index, subject index and geographical index. This monumental work formed the first number of the *Records of the Malaria Survey of India* in 1929. In the second number, many valuable memoranda on malaria which had appeared during previous years but were no longer available for general use, were reprinted.

There have been outstanding contributions by workers in India, *viz.*, malaria in Sind by Sinton, Covell, and Baily; treatment with cinchona alkaloids and what malaria costs India nationally, socially and economically by Sinton; contributions on anopheline fauna of India and their distribution by Christophers, Barraud, Covell, Puri, Viswanathan, Ramachandra Rao, Mohan, Senior White, Iyengar, Muirhead-Thomson, Pal and others; monkey malaria and extensive studies on synthetic antimalarial drugs by Sinton, Mulligan, Wats, Jaswant Singh, Ray, Nair and others; studies on insecticidal sprays by Sinton, Wats, Paul Russell, Knipe, Puri, Viswanathan, Rao, Senior White, Pal, Sharma and others; malaria and its engineering aspects by Jaswant Singh, Henderson and others; studies on *Plasmodium berghei* by Ramakrishnan, Satya Prakash, Krishnaswami and colleagues; progress of antimalaria operations in Delhi by Covell, Afridi and Jaswant Singh; in Bombay by Viswanathan, Ramachandra Rao and colleagues; in Mysore by Sweet, Rao and others; on B. N. Railway by Senior White and co-workers. The study of malaria and its vectors in Borneo by McArthur and Colless; studies on the bionomics of *A. aquasalis* and its relation to malaria in British West Indies by Senior White; malaria in Formosa by Watson and colleagues, are some of the other notable contributions. Besides, special issues have been published from time to time on Paludrine, Insecticides, Pyrimethamine, Malaria in Ceylon, and a written symposium on *Plasmodium berghei* in which workers from all over the world participated.

The journal has been one of the most important organs of the East (perhaps the only one of its kind) for making known the trend of malaria research and control. It has tried to help in the fulfilment of two of the main functions of the Malaria Institute of India: (1) " To publish scientific results, useful guides, bulletins, etc.", and (2) " To keep alive interest in malaria study and prevention and to see that such interest, wherever present, is nursed and assisted ". If India has launched upon the most gigantic programme of malaria control, it is because the Malaria Institute of India has maintained a continuous propagation of ideas on malaria prevalence and its effects on the economy of the country, and in this the *Indian Journal of Malariology* has played a very prominent part. It has kept up its standard through many hazards it had to face during the war years.

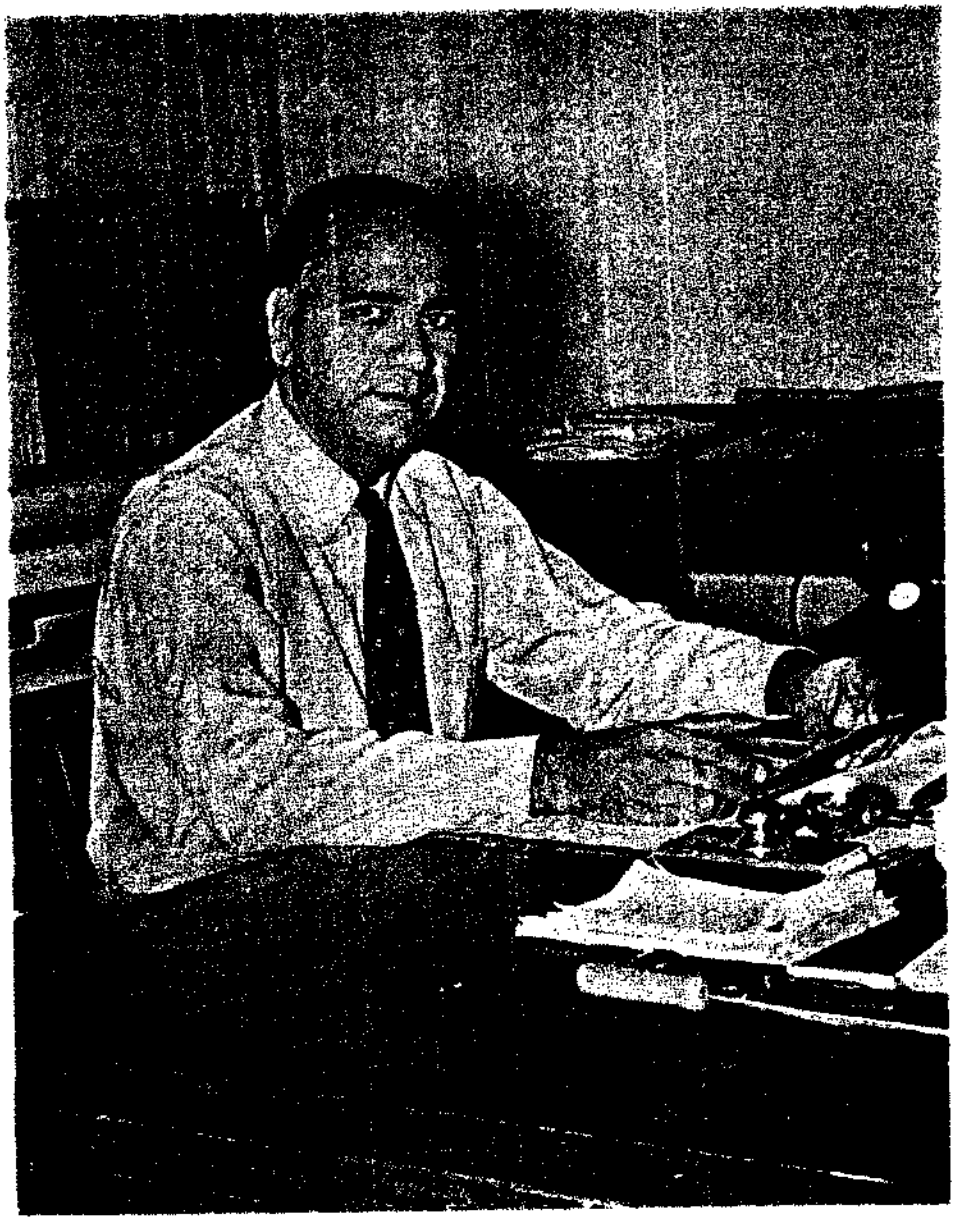
The Jubilee Issue gives useful important reviews of the past researches and studies by eminent workers, and suggestions for the future on different aspects of the subject. It also depicts the present position of malaria control and research and paves a way for future programmes. It is a useful guide and a source of inspiration for those entering the field.

Recently the activities of the Malaria Institute have expanded to include some of the other communicable diseases, and the Malaria Advisory Committee of the Indian Council of Medical Research has also been redesignated as " Malaria and other Arthropod-borne Diseases Sub-Committee " of the Communicable Diseases Advisory Committee. Whether the *Indian Journal of Malariology* should also widen its scope and welcome in its fold valuable knowledge about other arthropod-borne diseases and thus change its name once again, is a problem which will have to be considered in the near future.

This period of 25 years has been of great interest not only for contributors and readers but also for those connected with editing of this publication. Its future will no doubt be watched with interest.

The editor solicits the cooperation and patronage of contributors, institutions engaged in research and control of arthropod-borne diseases and also of those who are interested in emancipating the world of the misery caused by many preventible diseases.

PLATE V



Lt.-Colonel JASWANT SINGH,
Asstt. Editor: 1944-1946,
Editor: 1947 to date.

Indian Journal of Malariology, **9**, 4, *December 1955*.

SOME LACUNÆ IN OUR KNOWLEDGE OF THE MALARIA PARASITE.

BY

J. A. SINTON, M.D., D.SC.

(Formerly Director of the Malaria Survey of India.)

[August 30, 1955.]

It is now seventy-five years since Laveran first described the malaria parasite. During this period it has been given more study than any other cause of protozoal infection in either man or other animals. In spite of this there still remain many serious gaps in our knowledge of the plasmodium.

When the Indian Research Fund Association placed me on special duty in 1921 to do malarial research, my first impression was that little was left to be discovered about this protozoon. As time went on and the subject was studied in greater detail, it soon became apparent that very many problems still remained unsolved. Each new problem was recorded as it arose. Careful notes were afterwards maintained about any references, information or ideas which might help in the elucidation of the difficulties. In this way, a very large amount of data was collected up to the outbreak of the Second World War. Several of the problems were solved, but many still remain untouched or have been left in an unsatisfactory position.

In a note of this nature, it is impossible to give all the hundreds of references collected, so only a few of the more relevant ones are appended. There are doubtless many which have been missed by me during the war years and afterwards. Such omissions are regretted. Some of them may show that the lacunæ are not so great as have been indicated in this article.

Nowadays problems in malaria, apparently purely scientific, seem to have fallen into a secondary position as compared with the more immediate practical ones of insecticides and synthetic anti-malarial drugs. While most of the questions raised are controversial, it is hoped that their ventilation will result in a clearer understanding of the problems discussed, and may stimulate renewed research into these aspects of malariology.

DO ALL THE HUMAN MALARIA PARASITES BELONG TO ONE GENUS?

As discussed by Christophers and Sinton (1938), Grassi and Feletti in 1890 divided the malaria parasites into two genera—first, *Hæmamœba* to include the quartan and benign tertian parasites, and second, *Laverania* to include those parasites which produced crescentic gametocytes. This classification was later rejected by Schaudinn (1902), who, from comparison with the Coccidia, did not consider that mere differences in the form of the gametocytes was sufficient to justify the erection of two separate genera. He grouped all these parasites in the genus *Plasmodium* Marchiafava. This ruling has been followed by most workers from that time.

Since the discovery of the exo-erythrocytic cycle of schizogony in malaria parasites, this problem needs reconsideration. The latest classification of this type of schizogony, as given by the World Health Organization's Drafting Committee on "Malaria Terminology" (Covell, Russell and Swellengrebel, 1953), shows that of the human species of *Plasmodium* s.s. as quite different from that of *Laverania*.

Now that the exoerythrocytic cycles of schizogony has been found to differ greatly in these two groups, does not this suggest that the classification given by Grassi and Feletti is justified? Should the genus *Laverania* be officially recognized as the correct name for such parasites as *Plasmodium falciparum* [vel *L. malariæ* (Laveran)].

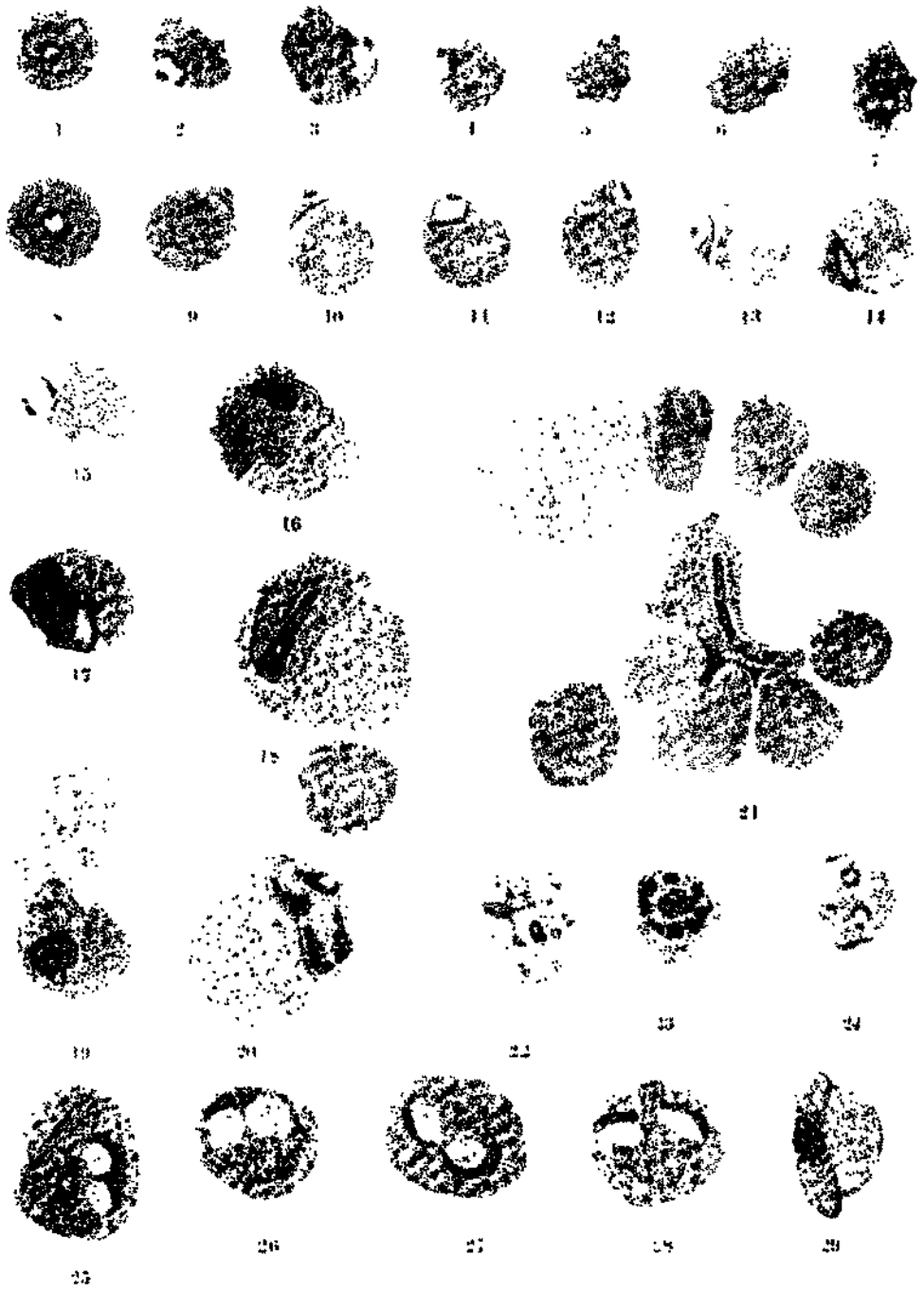
IS THE MALARIA PARASITE INSIDE OR OUTSIDE THE ERYTHROCYTE?

Laveran in his original description of the malaria parasites believed these to be extraglobular. Most of the other older writers like Marchiafava and Bignami (1894) asserted that they were within the red blood cells. Mannaberg (1894), however, thought that the *young* rings of the æstivo-autumnal parasites were outside the erythrocyte.

Maurer (1902), in his paper on the stippling of red cells infected with plasmodia, came to the conclusion that the large and small ring forms of the pernicious parasite remained extracellular while they were in the peripheral blood. At the stage of development, however, when they disappeared into the internal organs, they entered the red cells.

The description given by Schaudinn (1902) of the entry of the sporozoite and the merozoite into the erythrocyte seemed to settle their intracellular position definitely. Rowley-Lawson (1914;1918) again raised the question and produced evidence which cast doubt on the above finding.

The question was re-opened by Sinton (1922). He pointed out that, on account of the very thin flattened shape of the erythrocyte, it was impossible by microscopical examination to ascertain whether the parasite lay in or on the cell (Plate VI, Fig. 1 and 8). He subjected the infested cells to hypertonic and hypotonic conditions to change their shape from a flat to a globular form. In *falciparum* infections this caused a great increase in the number of accolé forms



FIGS. 1 and 8. Erythrocytes with ring forms of *P. falciparum*.
FIGS. 2-7. Accolé forms of *P. falciparum* produced by hypertonic conditions.
FIGS. 9-15. Accolé forms produced by hypotonic conditions.
FIGS. 16-17. Extracellular forms of *P. vivax* produced by hypotonic conditions.
FIGS. 18-20. Extracellular forms of *P. vivax* from moist chamber preparations.
FIG. 21. *P. vivax* migrating from its host cell under moist chamber conditions.
FIG. 22. "*P. lenue*" with Maurer's dots in parasitized erythrocyte.
FIG. 23. Mature schizont of "*P. lenue*".
FIG. 24. Erythrocyte infested with multiple rings of *P. falciparum*.
FIGS. 25-28. Early stages of division of *P. ini* schizont.
FIG. 29. Gametocyte of *P. falciparum* with red cell "bib".

(Plate VI, Fig. 2-7; 9-15). While in *vivax* infections hypotonic conditions caused many of the parasites to appear as if attached to the outside of the cell (Plate VI, Fig. 16 and 17). He concluded that " results have been obtained which I am unable to explain satisfactorily except on the hypothesis that the sub-tertian and the benign tertian malarial parasites are, for the most of their asexual cycle in the human host, attached to the outer surfaces of the red blood cells ". " These results are published in the hope that renewed interest will be stimulated in this problem and that further investigations will be made which will settle definitely the disputed point of the relative positions of the malarial parasite and the red blood corpuscle ".

In his experiments, Sinton (1922) noted the extracellular position assumed by many of the parasites in thin films from *vivax* malaria, when kept in a moist chamber. Not only do some of the parasites still attached to red cells appear to be extracellular (Plate VI, Fig. 16-20), but some of them are found at a distance from the apparenlty unbroken Schuffner-dotted host cells (Plate VI, Fig. 21). This phenomenon must have been noted by many workers while studying the exflagellation of *P. vivax* in a moist chamber.*

Thomson and Woodcock (1922) thought that the majority of *vivax* parasites were intracellular, but that some completed their cycle extracellularly. The parasites depicted by them in their Plate LXIV, Figs. 38-42, are very similar to those produced by hypotonic conditions. It is possible that slow drying in a moist atmosphere before fixation may have been the cause of these appearances.

Schuffner and de Graaf (1937) also noted that quartan parasites freed themselves from their host cells in moist preparations.

Sections of infected blood were made by Sinton (1922) and these appeared to support the extracellular theory. Ratcliffe (1927;1928), however, did a much larger series of sectional experiments and concluded that the parasites were intracellular. A critical study of the latter work shows that his sections were 2μ thick. As the thickness of the erythrocyte is about 2·5μ at the periphery† and only 1·5μ at the centre, red cells lying on the flat in sections would give the same appearance as these cells in thin films, i.e., intracellular. It must also be remembered that blood cells placed in a fluid fixative usually become distorted—globular, cupshaped or irregular. So in sections any parasites lying in such cupped cells would appear to be intracellular.‡

In the case of crescents (Plate VI, Fig. 29), it is difficult to understand why the outline of the red cell should appear as a curved line and not as a straight one, *if* the cell was being stretched by an *internal* expanding body—the parasite. The position of the crescent has been examined by Stephens (1924).

*Shute and Maryon (1955), while this paper was being prepared, have called attention to these " free " parasites in moist films, and consider that this " suggests that the parasite is on and not in the red cell ".

†In the abstract of Sinton's paper in the Tropical Diseases Bulletin, the reviewer makes great play of the fact that the measurement μ was printed as " mu ". The author was not responsible for this printer's error, which was not in the original manuscript.

‡Since the above was written, Fulton and Flewitt (1955) have used the electron microscope on sections 0·02-0·05 μ thick of red cells parasitized with *P. berghei* and *P. knowlesi*. They conclude that " all the evidence so far obtained by the methods employed indicates that the two parasites studied lie within the red cell ".

Erythrocytes carrying multiple infections, even as many as six, are not rare in severe malignant tertian infections (Plate VI, Fig. 24). On the other hand, it is very rare to find even double infections with mature parasites of this species apart from any question of triple or quadruple ones. What happens to the other parasites? Are they swept off the outside of the host cell and perish, or do they then acquire fresh host cells as suggested by Rowley-Lawson (1914;1918)? It may be, of course, that so heavily burdened erythrocytes never emerge again into the peripheral blood from the internal organs, so escaping detection.

While the extracellular position of the parasites may help to explain some problems, yet it is difficult to understand the position of mature parasites in double infections such as were mistaken by Schaudinn (1902) for parthenogenesis, except on the assumption that the parasites are held in position by a globular wall (*vide* Plate LXIV, Figs. 44-47 of Thomson and Woodcock, 1922). Tobb (1930) also depicts erythroblasts harbouring parasites, and in these cells the parasites appear to have displaced the nuclei. This might, of course, have been a mechanical displacement such as caused the distortion of the red cells in infections with *P. ovale* (Sinton, 1955).

Even as late as 1953, the Drafting Committee on " Malarial Terminology " appointed by the World Health Organization (Covell, Russell and Swellengrebel, 1953) are not agreed in an authoritative pronouncement on the matter. They state that " in this report the word ' in ' has been used, but the possibility that the above (extracellular) theory is correct has not been overlooked ".

From the evidence available it is seen that the relation of the plasmodium to its host cell at various stages of its development, still remains doubtful. A more exact knowledge of the physical structure of normal and parasitized erythrocytes would help to explain the various observations mentioned above. A renewed study of the migration of *P. vivax* should be made under fresh conditions in a moist chamber on a warm stage or in a hot room.

HAVE WE A PROPER PICTURE OF THE CYTOLOGICAL STRUCTURE OF THE MALARIA PARASITE?

The earliest work on the structure of the malaria parasite was carried out in fresh preparations. Since the advent of the Romanowsky stains half a century ago, few other methods of examination of malarial blood have been used.

These stains produce a beautiful picture of the plasmodia. They form the most reliable method of diagnosis. Unfortunately, this method of dry fixation and staining gives very varied results. It is not, therefore, recognized by most protozoologists as producing a reliable picture of the minuter and more complicated features of the cytological structure of most protozoa*. So much use has been made of this method of staining that the more reliable cytological method of wet fixation and iron-hæmotoxylin coloration (Sinton and Mulligan, 1930) has been neglected. We have none of the very careful descriptions of the plasmodia as have been given by Wenyon and by Dobell in the study of the intestinal amœbæ.

*As Dobell once said—" The day is now past when one can go into the field with a bottle of Giemsa and hope to rival Schaudinn." This was also emphasized by an Editorial in the *Lancet* (1926).

Until this is done there will still remain many of the doubtful questions such as those detailed below. It is suggested that, on account of its slow growth and the presence of all stages of its schizogonic cycle in the peripheral blood, *P. inui* would form a most suitable subject for a study of this nature.

WHAT IS THE NATURE OF THE VACUOLE SEEN IN THE RING FORMS OF THE MALARIA PARASITES?

Grassi and Feletti (1890) describe the vacuole as a large clear bladder-like nucleus with a delicate, often invisible, nuclear membrane and a nucleolus (the chromatin). This interpretation of the structure as a vesicular nucleus was followed by such older workers as Marchiafava and Bignami, Romanowsky and Mannaberg until the studies of Schaudinn (1902). The last worker, however, considered that the vacuole was formed inside the ring of protoplasm to give the young parasites a large absorptive surface for nutriment. So great was the authority of Schaudinn that the idea that the vacuole was nutritive and that the chromatin alone was the true nucleus, has largely held sway ever since. Even the Drafting Committee on "Malarial Terminology" appointed by the World Health Organisation has refrained from committing itself on the subject of the "vacuole". It states—"The nature (i.e., whether nuclear or nutritive) of the 'vacuole' seen in the ring forms is still uncertain, so that provisionally the best term appears to be vacuole" (Covell, Russell and Swellengrebel, 1953).*

Not all modern malariologists appear to be in doubt as to the nature of the vacuole. For example, Le Dantec (1924) and many other workers describe the nucleus as vesicular with a karyosome; Boyd (1935) speaks of the nucleus of *P. vivax* as round or oval with a limiting membrane, within which is a karyosome formed of one or an agglomeration of many chromatin granules; and Thomson (1932) stated that the nuclear structure of a *falciparum* gametocyte is a definite vesicular one.

While there is litte doubt that the 'vacuole' with its chromatin mass is a definite vesicular nucleus, further work with more precise cytological methods is needed to confirm this. A few trials made on *P. vivax* with the iron-hæmotoxylin method of Sinton and Mulligan (1930), gave results which made the vacuole resemble very closely the limax type of vesicular nucleus seen with some intestinal amœbæ.

The absence of a vacuole is usually given as one of the diagnostic characters of the early stages of gametocyte growth. A careful study of these forms shows that the vacuole is still there but that the chromatin is evenly scattered through it, so obscuring its usual empty bladder-like appearance.

The chromatin has a very close connection with the vacuole. In *P. inui* with its slow rate of growth this is very well seen at all stages (*vide* Sinton, 1934, Plates III and IV).

*Ewing in 1898 and later Rowley-Lawson considered the vacuole as a small portion of the erythrocyte surrounded by two pseudopodia coalescing at their ends.

WHAT ARE THE CYTOLOGICAL CHANGES WHICH OCCUR WHEN THE MALARIA PARASITE SEGMENTS?

Few workers appear to have studied the details of the schizogony of plasmodia using well-recognized cytological methods. Schaudinn (1902) described a sort of primitive mitosis in the asexual forms of *P. vivax*. In the same parasite, Ivanic (1937) described a form of promitosis going on to true mitosis in the later stages of schizogonous division. More recently Wolcott (1954) has studied the nuclear structure and division of *P. vivax*. He says that he never found the vesicular nucleus and karyosome reported by Ivanic (1937). However, he describes a mitotic stage in the cycle of schizogony with two chromosomes and a well marked achromatic spindle. He used wet films stained with Giemsa, so his observations require confirmation by more precise cytological methods.

Here again the slow rate of growth of *P. inui* and the abundance of all stages in the peripheral blood, should make this species an admirable one for the study of nuclear division. The early stages of chromatin division and their relation to the vacuole are shown in Plate VI, Fig. 25-28. At this stage of schizogony the findings suggest simple biniary fission of a vesicular nucleus.

IS MULTIPLE INFESTATION OF ERYTHROCYTES IN MALIGNANT TERTIAN INFECTIONS DUE TO PREMATURE DIVISION OF YOUNG SCHIZONTS?

In very severe infection of *falciparum* malaria, it is not uncommon to find some red cells infested with many parasites, even as many as six per cell (Plate VI, Fig. 24).

Some workers suggest that this is due to biniary fission of the young schizonts and not to the attack of the cell by several merozoites (Alessandrini, 1933).

Schuffner and de Graaf (1937) have discussed this hypothesis and come to the conclusion that this multiplicity of infestation was due to the greater attraction afforded by certain erythrocytes to the young parasites. Hingst (1934;1938), however, attributes such multiple infections as due to amitotic division of young schizonts already infesting the cell rather than to multiple invasion of single merozoites.

DOES THE METHOD OF PREPARATION CHANGE THE MORPHOLOGICAL CHARACTERS OF THE PARASITE?

Sinton (1955) has discussed the causation of ' banded ' forms in infections with *P. malariæ* and *P. inui*. He has produced reasons to support the view that these are not found *in vivo*, but are distortions produced by the method of preparation of the thin film. They give indications of the physical condition of the parasite at the time that the film was made. They are valuable aids in diagnosis. Similarly with *P. tenue* the characteristic forms seen, appear to be produced in a similar manner (Callanan, 1926). They indicate a special physical state of the plasmodium at the time. This shows a tertian periodicity.

WHAT IS THE NATURE OF THE CHANGES IN THE ERYTHROCYTES INFESTED BY MALARIA PARASITES?

WHAT CAUSES THE CHANGES IN THE SIZE AND SHAPE OF INFESTED ERYTHROCYTES?

The enlargement of the infested red cell in *vivax* infections is well known. On the other hand, the æstivo-autumnal parasite in its later stages is found more commonly with a smaller cell, which may show marked colour changes when examined in the fresh. We have still to discover the different causations of these changes.

The oval and fimbriated erythrocytes, which are seen in thin films of the blood at some stages of infections with both *P. ovale* and *P. knowlesi*, are often of great diagnostic importance. Sinton (1955) has discussed the probable causation of these forms. He thinks they do not occur *in vivo* but are produced mechanically during the preparation of thin films which dry very quickly; they indicate a special physical character of the cells.

WHAT IS THE CAUSE OF THE DIFFERENT TYPES OF " STIPPLING " SEEN IN ERYTHROCYTES INFESTED BY DIFFERENT SPECIES OF PLASMODIUM?

Schuffner (1899) was the first to describe these changes. He considered that the Schuffner's dots of *vivax* infections were due to degenerate particles in the stroma of the infested red cells.

These changes were examined in some detail by Maurer (1901;1902), who gave the same reason as Schuffner for the stippling seen in *vivax* infections. In the case of the " red-coloured dots, little rings and lines " with the *Laverania* species (Maurer's dots), he concludes that these were due to " changes or losses of substance on the upper surface of the erythrocytes, which are the result of the attacks made by the parasite in order to adhere to its host cell and to obtain nourishment ". The numbers are said to increase with the size of the parasite and are not stationary as with *vivax* stippling.

Billet (1913) seems to have had the same ideas but Chatton (1917) does not agree. The latter author thinks in both types of infection all these markings are of mechanical origin, and are impressions left by the pseudopodia of the parasites upon the thin periplastic pellicle of the erythrocytes. He has traced the ends of the pseudopodia to some of these spots, which he believes are either a secretion of the parasite or a little of its own substance. The number of the dots is thought to be a function of the amœboid activity of the parasite species.

The exact nature of these markings is still in doubt. In addition to the usually recognized forms of stippling, are the curious filamentous bodies described by Blanchard and Langeron (1913) in parasitized cells in monkey malaria. These they believed to be nuclear in origin. The same kind of filamentous bodies and a dark red deposit around segmenting forms is reported and discussed by Sinton and Mulligan (1933*b*), Sinton (1934), and Mulligan (1935) in all three species of oriental simian plasmodia. Malamos (1934) found a capsule with *P. vivax*, *P. ovale* and *P. knowlesi*. Garnham (1931) and Thomson (1933) investigated curious bodies seen in red cells with immature gametocytes of *P. falciparum*.

Stippling and similar changes have been seen in infections with most kinds of malaria infection.* The demonstration of these markings depends very largely upon the type and intensity of the stain used. The use of alkaline distilled water, intensive staining or Shute's technique is needed in some cases to demonstrate the stippling, etc. In the method of staining thick films recommended by Christophers, Sinton and Covell (1936) the Schuffner stippling is very conspicuous and the spots of Maurer are much less so. This suggests that they may not have a similar origin.

In the same category of changes comes the " capsule " which is sometimes so conspicuous around deeply stained crescents. This is discussed by Garnham (1931;1933) and Thomson and Robertson (1932). Warasi (1932) thinks that the parasite is covered by two membranes, first the periplast of the red cell and second the periplast of the parasite. Between these two is the hæmoglobin, and as the parasite grows, this disappears and the two membranes fuse together to form a capsule. Apart from this, Mannaberg (1894) states that in fresh preparations a perfectly recognisable double capsule is seen.

Many of the appearances seen support the idea of Schuffner (1899) that the origin of Schuffner's dots is different from Maurer's ones. The latter look as if they were produced directly by the parasite and were not merely due to degenerative changes in the erythrocyte.

ARE MORE THAN ONE SPECIES OF PARASITE INCLUDED UNDER THE NAME OF " *PLASMODIUM FALCIPARUM*?

Even from the earliest days of the separation of the crescent-forming parasites from the quartan and tertian ones, there has been doubt as to how many species of parasite were included in the former category. These were first divided by the supposed periodicity of their febrile paroxysms. Later morphological differences were described as well as variations in their febrile reactions, in the effects of treatment, and in their susceptibilities to infecting the same insect host.

WHAT IS THE DURATION OF THE ERYTHROCYTIC CYCLE OF SCHIZOGONY OF THE ÆSTIVO-AUTUMNAL MALARIA PARASITES?

The earlier workers on the malaria parasites, such as Marchiafava, Bignami and Grassi considered that there were two kinds of æstivo-autumnal parasites—a tertian one and a quotidian. Mannaberg (1894) divided them into a tertian and two quotidians. Even as late as 1914, Manson following the last worker grouped them as (*a*) Subtertian due to *Laverania malariæ* (syn. *P. falciparum*), (*b*) Pigmented quotidian due to *P. præcox*, and (*c*) unpigmented quotidian due to *L. immaculata*.

The fever curve in these infections is very often remittent, not intermittent, so that they may appear to have a quotidian periodicity. In recent years all these forms have been grouped as " malignant *tertian* ", and the absence of tertian febrile periodicity explained as due to the schizogony of all the parasites not being simultaneous but distributed over several hours. Why such an abnormal schizogony

*The dots seen in quartan malaria are often called Ziemann's but Seyfarth (1924) mentions them as Brug's.

should occur in this species of malaria parasite and not in the other three infecting man, seems curious, unless this is another difference possessed by the genus *Laverania* as compared with the genus *Plasmodium*.

Even although the term 'malignant tertian' is now used by most English writers, there still seems to be great doubt as to the duration of the cycle of erythrocytic schizogony. Various authors give this as 24-28 hours, 36-40 hours and 36-48 hours. The reason for this uncertainty is that a large proportion of the schizogonic cycle of this group, occurs in the internal organs, so it cannot be followed with the same ease as those of the quartan and tertian parasites can be.

The prolongation of the febrile paroxysm in these supposedly tertian infections could be accounted for by a double infection, such as gives rise to quotidian periodicity in some *vivax* infections. It is possible, however, that the older workers were correct, and that a true quotidian species occurs as in the case of *P. knowlesi* in simian malaria. Indeed, Craig (1909;1921) has reported such a parasite, which he named *P. falciparum quotidianum*. Row (1917) also describes and pictures the parasites seen in a culture of 'quotidian malaria' (*Laverania præcox*).

One needs more careful study along the lines used by Sinton (1934) and Mulligan (1935) to determine the duration of the cycles in the species of monkey malaria. Sinton (1922) using this method showed that *P. tenue* had a definite tertian periodicity. Cultural methods might also be useful. The strains used would be more easily studied under conditions of malaria therapy. It is suggested that possibly strains from the North-West of India should be compared with those from the Eastern parts, and African ones with Italian.

From this it is seen that the question still remains unsolved as to whether all 'malignant tertian' parasites have a true tertian periodicity, i.e., are only one species.

CAN SEVERAL SPECIES OF ÆSTIVO-AUTUMNAL MALARIA PARASITES BE SEPARATED BY THEIR MORPHOLOGICAL CHARACTERS?

While the older writers separated their different species of the *Laverania* group mainly upon clinical features, they also distinguished them on morphological grounds (Marchiafava and Bignami, 1894; Mannaberg, 1894). As these descriptions were reported mainly from fresh specimens, they made differential diagnosis difficult.

The sinking of all these crescent-forming plasmodia in one species—*P. falciparum* (*P. præcox*; *P. immaculatum*)—seemed to settle the question, but in more recent years many workers have doubted the unity of this species. For example, Ross (1911) was still inclined to believe that there are two or three species in this group; O'Gorman-Lalor (1913) reported the unusual morphology of such parasites in a blackwater-fever area in India; Marchoux (1922) points out differences between African, Italian and Macedonian forms; Schuffner and Hylkema (1922) reported what they thought was a special form during an epidemic at Belwan in Sumatra; many other workers are also doubtful.

The American worker, Craig (1909;1921) describes what he considers to be a new variety of *P. falciparum* which he has named *P. falciparum quotidianum.*

Grave doubt has been cast by many workers on the unicity of the Italian and the African forms of *P. falciparum.* Ziemann (1915) thought that the form found in the Cameroons, West Africa, was so different from the Italian form that he named it *P. perniciosum*, and has since described it in comparison with other forms of *P. falciparum* (Ziemann, 1938).

James and Kauntze (1930) considered that the malignant tertian parasite seen by them in East Africa differed from the classical description of *P. falciparum.* Many Italian workers noted differences between the Italian parasite and some found in Abyssinia. The latter parasite has been named by Giovannola (1938) as *P. falciparum æthiopicum.*

A curious type of *P. falciparum* was found by Marzinowsky (1916) in South Russia, which showed segmenting forms in the peripheral blood. This he named *P. caucasicum.**

The species most in dispute has been *P. tenue*, described by Stephens (1914) from specimens received from the old Central Provinces in India, and later from West Africa (Stephens, 1915).

Sinton (1922) carefully examined five cases from Nagpur Jail, and was convinced that this was a separate species of malignant tertian parasite. Since then other data have been collected to support this conclusion.

In the Indian sub-continent there seem to be at least two morphological species of *Laverania*—(*i*) the ordinary classical form, only seen with small rings in the peripheral blood (except in pernicious cases), occurring commonly in the north-western parts of the country, and (*ii*) the large-ringed form (small rings may sometimes be detected in the very early stages) common in the eastern and southern parts of the country. It is the latter parasite which shows a "tenue" stage.† Every year blood films taken from cases in the latter parts of India, were given to the post-graduate students in our Annual Malaria Class. Students from the northwestern areas almost invariably diagnosed these initially as *P. vivax*, because they were quite unaccustomed to seeing such large ring forms in malignant tertian malaria. In my experience of some thousands of infections contracted in the old Punjab and the North-West Frontier Provinces, 'tenue' forms are of extreme rarity there, while they are relatively common in those from some of the eastern parts of this sub-continent (Sinton, 1927).

Apart from the 'tenue' stage with its tertian periodicity, the parasite is seen for a longer time in the peripheral blood, even as late as 36th hour of its cycle, and the rings are much larger than the classical *P falciparum.* The pigment sometimes

* In the winter of 1934-35 some slides were sent me from Gilgit in Kashmir showing parasites resembling those described by Marzinowsky (1916). Unfortunately the specimens were mislaid before a full investigation could be made.

† It is almost certain that the 'tenue' form is not a true morphological characteristic of this parasite *in vivo.* It seems to be a distortion, similar to 'banded' forms of *P. malariæ*, caused by the method of preparation (Sinton, 1955). This morphology is an indication of a special physical state of the parasite at one stage of its development which makes it liable to this distortion. A similar stage has not been found in the cycle of *P. falciparum* s.s., possibly because this species retires from the peripheral blood at an earlier point in its cycle.

seen in the largest rings is finer, more scattered and lighter in colour than the coarser dark brown or jet-black masses seen in *P. falciparum* at a similar stage. The golden-yellow colour of this pigment was well seen in the only mature schizont (Plate VI, Fig. 23) observed in Sinton's cases, and which contained eight merozoites (*cf.* Marchoux, 1926). The Maurer's dots seen in the classical *P. falciparum* are usually described as irregular in shape and few in number, and as often being difficult to demonstrate. In *P. tenue* they stain more easily, and are more numerous, rounded, linear and coccoid in shape (Plate VI, Fig. 22).

These forms have been reported by workers in many other tropical countries, for example Hucks and Bowden (1924) and Darling (1925) in the United States, Perekropoff (1917) in Russia, Sergent *et al.* (1913) and Vialatte (1922) in North Africa, and Jerace (1932) in Italy, among a host of others. I have received beautiful slides of this form sent by Dr. G. Fraser from Assam and Dr. K. L. Rustomjee from Ceylon. Russell (1928) gives a careful review of the literature up to that date.

Is the small-ring form that of areas of seasonal and epidemic malaria, while the large-ring form is that of hyperendemic and blackwater fever areas? It has been argued that the morphological differences are due to variations in immunity, or in the chronicity of the infections found in those two different areas. Sinton and Mulligan (1933*a*;1933*b*) were unable in the cases of *P. knowlesi* and *P. cynomolgi* to find that such conditions caused any morphological changes in the character of these parasites.

In spite of the evidence produced, the latest authoritative statement says—"*P. tenue* Stephens, 1914, is no longer accepted as a distinct species" (Covell *et al.*, 1953).

If *P. tenue* be accepted as a true species, what is its proper specific name? The parasite is probably the same as one of those originally described as either *P. præcox* or *P. immaculatum*. As pointed out by Christophers and Sinton (1938) both these names are invalid as having first been used to designate avian parasites. Similarly, the specific name *tenue* is also invalid, because a few months before Stephens (1914) used it, a parasite of the Peking nightingale had been named *Hæmamœba tenuis* by Laveran and Marullax (1914). Ziemann (1915), in his discussion of the human parasites, names it in his description of the figures given on his plate—"*Plasmodium khartunense* (From Khartoum) Synonym *Plasmodium tenue* Stephens".

ARE THERE CLINICALLY DIFFERENT 'STRAINS' OF THE ÆSTIVO-AUTUMNAL PARASITE?

Apart from the question of the periodicity of the febrile paroxysms, other distinct clinical differences have been reported between 'malignant tertian' infections in various parts of the world.

When James (1932) compared the Rome strain of *P. falciparum* with an Indian one, he found that it needed almost ten times the dosage of quinine to control the former infection, which was, however, easily affected by atebrin. In the Panama, very heavy doses of quinine, as compared with European practice, are required to reduce pyrexia. Fairley (1946) has reported that almost double

the normal dosage of atebrin was necessary to suppress some *falciparum* infections in the Aitepe-Wewak area of New Guinea. Other workers in describing their new species have noted clinical differences.

O'Gorman-Lalor (1913) and Hassal-Wright (1920) drew attention to unusual forms of the parasite found in blackwater-fever areas of India. Sinton (1927) remarked upon the similarity of the recorded distribution of *P. tenue* and the same syndrome. Cort (1929) noted a special form of subtertian parasite in association with hæmoglobinuria, and Ziemann (1938) also thought that there was a relationship between *P. perniciosum* and this disease. James and Kauntze (1930), in drawing attention to the differences between the East African parasite and the text-book descriptions, conclude that " the subject certainly merits careful enquiry in Kenya and Uganda, particularly in districts where blackwater fever occurs ".

FAILURE TO INFECT SOME ANOPHELINES WITH FOREIGN STRAINS OF *P. FALCIPARUM.*

James, Nicol and Shute (1932) found great difficulty in getting any infections in *A. maculipennis* var. *atroparvus* with Indian strains of *P. falciparum* as compared with the Rome one, although clinically the latter was more pathogenic to man. This was confirmed by Shute (1940) with other strains of tropical origin.

Raffaele and Lega (1937) remark upon the difficulty of infecting *A. maculipennis* with their new variety, *P. falciparum aethiopicum.* Similar results with both *P. falciparum* and *P. vivax* have been recorded in America.

As we do not know what are the factors which govern the susceptibility of mosquitoes to any malarial infection, it is not possible to decide whether these failures are due to something in the insect or something in the parasite, either of a strain or a specific character.

WHAT IS THE FATE OF THE SPOROZOITES IN THE INSECT HOST?

Ross (1905) in describing his original discovery said—" I saw the thread-like bodies, although apparently without motion themselves, were soon scattered by the insect's circulation all through its body ". This passive distribution was also noted by Grassi and Schaudinn. Muhlens (1921; 1931) showed that the sporozoites could be found in the muscles and even in the legs and in the appendages of the head.

The concentration of these forms in the salivary glands is great, but it is uncertain whether this is due to chemiotaxis or purely a matter of chance (Schaudinn ,1902). It is easy to understand how the sporozoites in the salivary glands are got rid of in the acts of biting, but the fate of the others is still uncertain.

In the early stages of the infection, at least, if a piece of a segment of a leg is severed from an infective living insect and the fluid expressed from it, this is found to contain large numbers of sporozoites. What is the ultimate fate of these? Are they infective? Do they form a reservoir from which the supply in the glands is replenished as these become depleted by biting? How long can they be detected and are the insects still infective after they disappear from the appendages?

Schuffner, Korteweg and Swellengrebel (1929) found that in the autumn the bites of one or two mosquitoes failed to cause malaria in the usual incubation period, but infection appeared after eight to nine months. The impression formed by me while working in the Malaria Laboratory at Horton was that, with the Roumanian strain of *P. vivax*, these prolonged incubations occurred more often when the sporozoites had been present for several weeks in the insect host. Were these latent infections caused by sporozoites which had been a long time in other parts of the insect's body? Did this give rise to senility of these parasites or was it merely a smaller dose of infection that was injected?

WHY ARE SOME SPECIES OF ANOPHELINES SUSCEPTIBLE TO HUMAN MALARIAL INFECTIONS WHILE OTHERS ARE NOT?

It is well known that the gametocytes of all the human species of *Plasmodium* will become gametes and conjugate to form zygotes, when taken into the stomach of even culicine mosquitoes. These zygotes never develop further in culicines and only in certain species of anophelines. As mentioned above the zygotes of some strains of parasite may behave in a refractory manner in anophelines which are normally susceptible to such infection of other strains.

Why this occurs has never been determined. Is it due to some factor in the insect or some in the parasite? The best researches on the subject have been those of Huff (1934), but even these have failed to give a satisfactory explanation.

MISCELLANEOUS QUESTIONS.

(*i*) WHAT IS THE MALARIA 'TOXIN' AND WHAT IS THE CAUSE OF THE MALARIA PAROXYSM?

Is there no true malaria toxin? Is the paroxysm due to a condition of anaphylactoid shock caused by the discharge into the blood stream of particles of pigment, remains of parasitized blood cells, and pieces of parasite at the moment of schizogony (Sinton *et al.*, 1928)?

(*ii*) CAN DIFFERENT IMMUNOLOGICAL STRAINS OF PARASITE BE DIFFERENTIATED BY DERMAL OR BY SEROLOGICAL TESTS?

Sinton and Mulligan (1932) showed that an antigen prepared from an almost pure suspension of *P. knowlesi* produced a marked dermal reaction with animals infected with a similar parasite. Could this method be used to differentiate the different strains of this parasite?

Before it became possible to isolate large quantities of parasite substance (Sinton and Mulligan, 1932), many experiments were made to perfect a complement-fixation test. Could a better antigen now be produced which would give a differential diagnosis, between the species or even the strains of monkey malaria?

Several precipitin tests have been tried for malarial infection. The results in these appear to depend merely upon their effects upon the increased globulin content of the blood, and as such are poor diagnostically.

(*iii*) WHAT IS THE NATURE OF THE ANTIBODIES IN MALARIA?

From a study of malarial immunity it is evident that two factors are involved—an anti-parasitic one and an anti-toxic one (Sinton, 1939). The former acts by a destruction of the parasites and the latter by neutralizing their effects.

Is the anti-parasitic one a lysin which renders the parasites more readily destroyed by the macrophages, or is it an opsonin which causes the macrophages to attack the parasites with greater avidity?

While we have no information as to the nature of the anti-toxic one, we can only consider its results.

Does it pass through the placenta and so cause a passive immunity in the fœtus, or does the latter produce its own antibodies under the stimulation of malarial 'toxin' entering its circulation from the blood of its mother (Sinton, 1939*a*)? Can acquired maternal immunity be transmitted through her milk?

(*iv*) WHAT IS THE ORIGIN OF THE GAMETOCYTES?

Are these derived from special exoerythrocytic merozoites or from the blood ones? Probably at least from the latter, as they occur after blood inoculations.

At what stage of their development do these forms take on their sexual character, or are they so *ab initio*? Do they have an intracellular position as compared with asexual forms, and if so would this determine their fate? Does the environment in which they develop have any effect upon their fate, *i.e.*, do the special conditions of the spleen and bone marrow have any action in determining their development into sexual forms?

(*v*) WHAT SPECIES OF PLASMODIUM OCCUR IN THE LOWER MONKEYS OF AFRICA?

Sinton and Mulligan (1932*a*: 1933), Mulligan (1935) and Sinton (1934) made a careful study of the malaria parasites of the lower monkeys of the Old World.

Those from Asian countries were found to be infected with at least three distinct species—(*a*) *P. knowlesi* Sinton and Mulligan, 1932, having a 24-hour cycle of schizogony in the erythrocytes: (*b*) *P. cynomolgi* Mayer, 1907, with a 48-hour cycle, and (*c*) *P. inui* Halberstadter and Prowazek, 1907, with a 72-hour cycle. Among the lower African monkeys, *P. gonderi* Sinton and Mulligan, 1932 (vel. *P. kochi* Gonder and Berenberg-Gossler, 1908) and *P. kochi* Laveran, 1899, with varieties were described. The latter parasite has since been found not to be a *Plasmodium* but to belong to the genus *Hepatocystis*.

Except for the careful studies made by Gonder and Berenberg-Gossler (1908), Berenberg-Gossler (1909) and Gonder and Rodenwaldt (1910), the plasmodial parasites of the lower African monkeys seem to have had little attention. Is *P. gonderi* identical with *P. cynomolgi*? How many African species are there?

SUMMARY.

It is pointed out that there are many unsolved problems in our knowledge of malaria parasites and these are discussed.

1. Should genus *Laverania* be again separated from the genus *Plasmodium*?
2. Is the malaria parasite inside or outside the host erythrocyte?
3. Have we a proper picture of the cytological structure of the malaria parasite?
   - (*a*) What is the nature of the 'vacuole'?
   - (*b*) What cytological changes occur when the parasite segments?
   - (*c*) Are multiple infections of the red cells due to simple biniary fission and not to invasion by multiple parasites?
   - (*d*) Is the morphological appearance of the parasite in thin films influenced by the method of preparation?
4. What is the nature of the changes in parasitized erythrocytes?
5. Does the present species '*P. falciparum*' contain more than one species?
   - (*a*) What is the duration of the cycle of schizogony?
   - (*b*) Can several species be separated by their morphology?
   - (*c*) Do the infections caused by these species vary clinically?
   - (*d*) Do they vary in their ability to infect anophelines?
6. What is the fate of the sporozoites in the insect host?
7. Why are some species of anophelines susceptible to malarial infection while others are not?
8. Miscellaneous questions.
   - (*a*) Is there a malaria 'toxin'?
   - (*b*) Can different immunological strains be separated by laboratory tests?
   - (*c*) What is the nature of the 'antibodies' in malaria?
   - (*d*) What is the origin of the gametocytes?
   - (*e*) What species of *Plasmodium* occur in the lower monkeys of Africa?

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SOME EVOLUTIONARY POSSIBILITIES IN THE HISTORY OF THE MALARIA PARASITES.

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THE problem of the evolution of the malaria parasites is, in the nature of things, one which can never be wholly solved, but its very difficulty makes it the more challenging to the medical men and to the biologist. It is a question which has puzzled malariologists almost ever since the causative organisms of malaria were discovered, some 75 years ago, and perhaps we are even now not much nearer a solution. Yet so much new light has been shed on what may be called the natural history of malaria in the past decade or two, that it is worthwhile taking a new look at the problem.

Involved, of course, are not only the parasites themselves, but their hosts, both vertebrate and invertebrate. Comparative morphology, always a mainstay when biological relationships are being considered, may shed light on the degree of kinship of existing species of *Plasmodium*, or of larger groups. But it is likely to tell us little of what we might call the third dimension of the problem, or time. And the ancestral relationships are those in which are we most interested. From what did the malaria parasites evolve? What may have been their earlier hosts? What light, if any, can be thrown on the evolutionary development of the complex life cycle of this genus of parasites?

The best evidence for the evolutionary history of any living thing is undoubtedly that afforded by fossils, but fossil malaria parasites are, of course, non-existent, as indeed are fossil remains of most other parasitic organisms. Yet fossil Foraminifera are known from Cambrian times (some 500 million years ago), and Radiolaria may be even older, having been reported from rocks of pre-Cambrian age. (Chapman, 1902; Pirsson and Schuchert, 1924; Glaessner, 1945). Both groups of Protozoa are free-living, and both are almost exclusively marine, and their antiquity is, therefore, only of interest here because it shows the great age of the phylum, but it suggests that the parasitic protozoa may also have had a very long evolutionary history.

Indeed, it may safely be assumed that there were protozoa of at least the two orders just mentioned long before the oldest of these fossils were laid down, for the morphology of many of the latter is much like that of contemporary forms, and it is, therefore, probable that their life-histories were also similar. Since the life-cycles of the relatively few of these organisms which have been thoroughly studied are complex, including the production of gametes and fertilization, evolution must have already gone a very long way even in pre-Cambrian times. It seems likely that life-cycles as complex as those exhibited by such parasites as the malaria plasmodia were even then commonplace.

Additional evidence that this was probably the case is furnished by the occurrence of fossil algæ in rocks of this age. Algæ are closely related to the plant-like flagellates (Phytomastigida), and in many cases have similar life-cycles, also involving the production of gametes. Fossil Dinoflagellates are also known (Glaessner, 1945) though they date only from Cretaceous times, perhaps 140,000,000 years ago. Ciliophora and Sporozoa are unknown in the fossil state, although since some of the former have skeletons or tests it is not impossible that such remains may eventually be found.

Of course, one must suppose that free-living organisms antedated those of parasitic habit, and (except possibly the viruses) that parasitic organisms had free-living ancestors. Yet parasitism must be a very ancient method of making a living. Sandon (1932) remarked " The animal kingdom presumably had its origin when some unicellular organisms, previously accustomed to nourishing themselves after the manner of plants, began to eat the bodies (either living or dead) of their neighbours ". Some parasitic protozoa are perhaps, even now, not far removed from that stage, since they closely resemble free-living species. But the Sporozoa, all of which are parasites, resemble no free-living protozoa known to day, and as the great protozoologist, Gary N. Calkins, used to say in his lectures many years ago, they represent a group the members of which are so different that it is often impossible even to suppose any mutual relationship. Thus,however ancient some of the free-living protozoa may be, we have no direct evidence bearing on the antiquity of the Sporozoa. We have, therefore, to fall back on similarities in life-cycles, occasional similarities in morphology, physiological peculiarities as indicated by host-parasite relationships (and sometimes more definitely shown by cultural requirements), and the indirect evidence which knowledge of the evolutionary history of their hosts may afford.

The malaria parasites are classified as Hæmosporidia, subclass of the Telosporidia. This subclass contains two families which are certainly very closely related: the Hæmoproteidæ and the Plasmodiidæ, to the second of which belong the malaria parasites. Both families are large as far as the number of species is concerned, but small in the number of genera contained. There are at least 50 recognized species of *Plasmodium*, the only genus of the Plasmodiidæ, and probably many more species of *Leucocytozoon* and *Haemoproteus* (the two genera comprising the Hæmoproteidæ).

The second order in the class Hæmosporidia contains only the single family Babesiidæ, together with two genera of doubtful status: *Dactylosoma* and *Toxoplasma**.

* According to the classification of Hall (1953).

The Babesias are parasites of red cells, and superficially resemble the malaria plasmodia, but, although they are incompletely known, the two orders do not appear to be closely related. Both vectors and life-cycles differ considerably.

It has long been thought that the malaria parasites may have originated from the coccidia. Mesnil (1899) seems to have been the first to point this out, and the view was supported by Schaudinn (1899), and later by Reichenow (1912). Wenyon (1926) spoke of the affinities existing between coccidia and the hæmosporidia, from the first of which the second group " may be supposed to have evolved ".

The evidence for the belief that the malaria plasmodia (and presumably also their close relatives, the genera *Hæmoproteus* and *Leucocytozoon*) arose from the coccidia, lies in the close similarity of their life-cycles. All of these organisms are typically cell parasites, and all exhibit sexual stages. The coccidia are generally parasites of the intestinal epithelium, in the cells of which they undergo a number of asexual generations, culminating in the production of gametes. After fertilization, the zygote develops into a resistant oöcyst, containing sporocysts which in turn contain sporozoites. These constitute the infective stages, and initiate a new infection after ingestion by a fresh host. There is no vector (but sometimes an alternation of hosts), and hence resistant stages are generally necessary for survival without the host.

The life-cycles of the malaria parasites are obviously very similar to this pattern, the chief differences being the addition of a second host which, in all species where the life-histories have been completely studied, is a mosquito, and the lack of a resistant oöcyst stage. The fact that the parasites reside in the erythrocytes of the vertebrate host, rather than in the intestinal epithelium, seems of much less significance than it did before the exoerythrocytic stages of the cycle were discovered two decades ago.

The chief controversy revolves around the question of whether the ancestral organisms from which the Plasmodiidæ evolved were parasites of invertebrates, and, therefore presumably of biting flies (such as mosquitoes, hippoboscids, *Simulium*, and others), or of the vertebrates. And if it is conceded that this family may first have been parasites of vertebrates, were their hosts in the beginning perhaps reptiles, since it was from the latter class that both birds and mammals evolved?

These problems are unlikely ever to be solved with certainty. But it is worth pointing out that the coccidia are typically parasites of vertebrates, although numerous exceptions occur and perhaps the disparity in number of species occurring in hosts with and without backbones would not seem so great if these parasites were better known. It seems also to be true that the majority of coccidia of invertebrates do not occur in insect hosts.

The fact that most coccidia are parasites of the gut suggests that they were originally acquired as the result of ingesting food or water contaminated with them. Of course, the forms from which they evolved must once have been free-living, although it is at least conceivable that they may have parasitized invertebrates before becoming adapted to life in veretbrate hosts, and that the latter were first infected by ingesting the former. In any case, it seems probable that these ancestors of the coccidia of vertebrates had already developed a highly complex life-cycle,

just as those protozoa we know as fossils must have done. Doubtless cysts, the production of which often climaxes a period of asexual reproduction and may also involve a sexual cycle, were the infective stage. Certainly a good deal of adaptation must have been required for life within a living host, but protozoan cysts have often been observed to survive passage through the intestine and some free-living species may even persist there for a limited time.

Huff (1938; 1945) has urged the point of view that the malaria plasmodia and their close relatives were at first parasites of insects, and became secondarily adapted to life in vertebrates after their insect hosts developed the blood-sucking habit. The fact that these parasites do not seem to be pathogenic to their vectors favours this theory, and yet, as Ball (1943) has rather convincingly maintained, there are too many exceptions to the rule that the degree of pathogenicity is inversely related to the duration of association between host and parasite to make this type of evidence more than doubtfully suggestive.

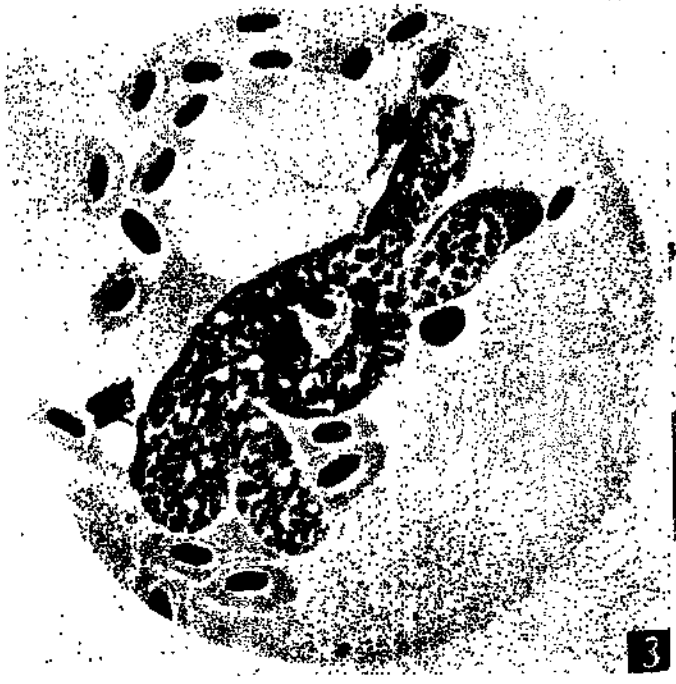
Huff also emphasizes the fact that the life-cycles of the Hæmoproteidæ and Plasmodiidæ are more similar as far as the insect host is concerned than for the vertebrate host. This is true (although we still know little or nothing of the life histories of many species), but it is worth pointing out that the newer knowledge of the vertebrate portions of these life-cycles makes them seem ever more alike.

The known insect hosts of the Hæmoproteidæ and Plasmodiidæ include hippoboscid flies (*Hæmoproteus*), *Simulium* (*Leucocytozoon*), and mosquitoes (*Plasmodium*). The first of these vector groups is only distantly related to the last two, and it may (as Huff concedes) suggest—despite the undoubted similarity of the insect portions of the life-cycles of all three genera of blood parasites—that this portion of the cycle developed later than that in the vertebrate host.

But, in any case, the insect-vertebrate type of life-cycle characteristic of this group of hæmatozoa could not have evolved before the ancestors of the present vectors adopted their blood-sucking habit. That this dietary requirement may not be very ancient is perhaps indicated by the fact that it is, even today, restricted to females. As for the antiquity of mosquitoes, sufficiently like those of today to be recognizable as such, they are known from Eocene times, some 60 million years ago. A fossil form, believed to be a male mosquito (note the plume-like antennæ), found by the writer in the famous Oligocene shales of Florissant, Colorado, is shown in Plate VII, Fig. 1. Mosquitoes are, of course, older than this, and doubtless this is also true of other blood-sucking Diptera. Diptera as a group appeared as early as the middle Jurassic, but they were at first restricted to midges and cranefly-like forms (Carpenter, 1953). The oldest known fossil insects (notably the extinct Palæodictyoptera, and cockroaches) were extant in the later Carboniferous period, which is believed to have been about 250 million years ago. It is worth noting that insect evolution had gone a long way even then, and it is very likely that insects were already well supplied with protozoan and other parasites.

The classes of vertebrates to which the known natural hosts of the malaria plasmodia belong include, as perhaps we should expect, all those of terrestrial habitat.* But susceptible host species are not evenly distributed among these classes,

*Malaria parasites have also been reported from the bull frog by Fantham, Porter and Richardson, (1942), but nothing else is known about them.



FIGS. 1. A fossil mosquito (male) from the famous Oligocene shales of Florissant, Colorado. Note the typical and conspicuous brushy antennae. × 12

2. An exoerythrocytic schizont in a brain capillary of an orange-crowned warbler infected with *Plasmodium hexamerium*. × 1500

3. A large schizont in a lung capillary of a *Haemoproteus* (sp.)-infected song sparrow. × 1000

4. A schizont of a *Leucocytozoon* (sp.)-infected purple grackle. Note the vacuoles in this parasite and also in that shown in Figure 3. Such vacuoles are rather typical, and are also seen in the exoerythrocytic stages of at least some species of avian malaria parasites (e.g., *Plasmodium relictum* var. *matutinum*.) × 1000

nor are such species relatively numerous. Most of the known reptilian host species of plasmodia are lizards, and the majority of avian hosts are passerines. There is much more variety among the malaria-susceptible species of mammals.

The striking parallelism between the reptilian and avian species of *Plasmodium* has often been noted. Together these species constitute almost two-thirds of the recognized species of the genus. Unfortunately very little is yet known about the life-cycles of the malaria parasites of reptiles, but the resemblances in morphology of the avian and reptilian types point to a close relationship. It may be that such similarities are to be explained as due to parallel evolution, but it seems more likely to be the result of relatively slow evolutionary change on the part of the parasites since the original separation of avian and reptilian stocks, even though bird and lizard lines of descent must have diverged at a very early period.

The relatively small number of malaria-susceptible species among the mammals, and their spotty taxonomic distribution (antelopes, water buffalo, rodents, primates, bats, to mention the more important) seems to suggest that malaria among them was a kind of after-thought on the part of nature. Perhaps the greater pathogenicity of some of the mammalian species of *Plasmodium* points to the same conclusion.

Another fact which may support the belief that malaria was originally a disease of reptiles, and then also of birds, is the very common occurrence of the two closely related genera* of blood protozoa, *Hæmoproteus* and *Leucocytozoon*, among birds and, in the case of the former, among reptiles also. As with the avian malarias, infections of *Hæmoproteus* and *Leucocytozoon* seem usually to affect the host very little, a fact which seems to indicate a very long period of host-parasite association.

Still another fact which is in accord with the possible earlier occurrence of malaria in reptiles and birds is their greater antiquity. Except for the monotremes, mammals apparently were virtually non-existent before the Tertiary, whereas birds go back to the Jurassic and reptiles to the later Carboniferous periods, (Romer, 1941). Thus both the two last groups probably antedated in origin the Diptera, and, especially, the blood-sucking types now serving as vectors of malarial and malaria-related parasites. (But it must be remembered that the vectors of the great majority of species of *Plasmodium* are still unknown, and there is no certainty that all are mosquitoes. It is also true that the vectors of most species of *Hæmoproteus* and *Leucocytozoon* still remain to be discovered).

The occurrence among the coccidia of genera such as *Shellackia* and *Lankesterella* (family Lankesterellidæ), in which a life-cycle not unlike that of the Hæmosporidia occurs, has often been regarded as supporting the theory that parasites of this group originated in vertebrate rather than invertebrate hosts. Perhaps this is best regarded as simply the result of parallel evolution, but it certainly indicates that the original hosts of parasites such as these need not have been insects, for here the cycle involves in the one case (*Shellackia*) lizards and mites,

*There are also certain hæmatozoa of mammals which differ so little from the true malaria parasites that they were for a long time put into the genus *Plasmodium*. Such are *Hepatocystis* (*Plasmodium*) *kochi* of monkeys and certain similar parasites of bats. What is known of their life history suggests that they may perhaps be regarded as the mammalian analogues of *Hæmoproteus* of birds and reptiles. (Hawking and Hunt, 1947; Garnham, 1948-54; Manwell, 1946).

and in the other frogs and leeches. The nature of the cells parasitized in the vertebrate hosts also certainly suggests that the coccidian ancestors of the Hæmosporidia may have developed along similar lines, for *Shellackia* multiplies in the intestinal epithelium of lizards, the sporozoites later entering erythrocytes, and *Lankesterella* reproduces in the endothelium of the capillaries of the frog, with subsequent sporozoite invasion of the red cells. The tissue stages may be regarded as corresponding to the reproductive stages of *Hæmoproteus* and *Leucocytozoon* in the vertebrate, and to the exoerythrocytic forms in malaria.

In the light of the occurrence of life-cycles such as these among certain of the coccidia, it is rather tempting to think of *Hæmoproteus* as perhaps the oldest of the three closely related genera, *Hæmoproteus*, *Leucocytozoon*, and *Plasmodium*. In this genus asexual stages do not occur in the blood, and it is found in both reptilian and avian hosts. Possibly the very highly specialized nature of the known vectors (hippoboscids), presumably indicating a long period of evolution on their part, points to alike conclusion.

Leucocytozoon may have originated later, and still seems to be confined to birds. That the biological relationships of all three genera are still close, however, is indicated by the ability of certain stages of each to live in erythrocytes,* and by the occurrence of asexual multiplication in tissue cells of one kind or another—often with striking similarities in morphology—(Plate VII, Figs, 2, 3, 4). Since all types of blood cells (except lymphocytes) are believed to have a common origin, differences in the blood cell type parasitized may not be very significant. Nor does it seem that it would have been a very long step from reproduction in tissue cells of some type in the vertebrate to reproduction in cells of the blood itself, as in the case of malaria. If some gametocytes or sporozoites also spilled over into blood cells from their hypothetical ancestral habitat in the intestinal epithelium, the way would be open for the evolution of an insect-vertebrate cycle.

Little can be said as to the possible or probable mutual relationships of the different species of *Plasmodium*. The life-cycles of all, as far as is known, are similar, and follow a pattern similar to that in the genus *Hæmoproteus* and *Leucocytozoon*. It might be thought that those avian and reptilian species of *Plasmodium* which most closely resemble one another arose the one from the other. This may, of course, have been the case, but parallel evolution may also have occurred. It is possible to divide species of the genus according to whether the gametocytes are elongate, or round or irregular, and according to the nature of exoerythrocytic schizogony. If the latter is done, the types are *Plasmodium gallinaceum* and *P. elongatum*. But *Plasmodium mexicanum* of reptiles exhibits both kinds of exoerythrocytic multiplication (Thompson and Huff, 1944). On the whole, although such characteristics afford convenient bases for classification, so little is yet known about the cycles of most species of *Plasmodium* (and this is equally true of species of *Hæmoproteus* and *Leucocytozoon*) that it does not now seem worthwhile to try to work out the detailed relationships of any of these parasites.

*Fallis *et al.* (1951) and Cook (1954) have shown that *Leucocytozoon simondi* may invade erythrocytes; the writer has also observed this in other species occasionally.

SUMMARY.

The problem of the evolution of the malaria parasites and their close relatives among the Hæmosporidia is considered, and it is suggested that they may well have arisen from coccidia of vertebrates rather than from those of insects, as has been more commonly supposed. This possibility is consistent with, or is supported by, various facts of palæontology and of host-parasite relationships. But too little is known about the life-cycles and physiology of many species of the Hæmosporidia to give anything like a final answer to the problem of their evolution.

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ABSENCE OF CROSS-IMMUNITY BETWEEN *PLASMODIUM CYNOMOLGI* AND *PLASMODIUM GONDERI*.

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THREE species of *vivax* like malaria parasites are known to occur in lower monkeys: *Plasmodium cynomolgi* (Mayer, 1907) in oriental macaques (chiefly from Malaya), *P. gonderi* (Rodhain and van den Berghe, 1936) in African mangabeys (chiefly from Belgian Congo) and *P. simium* (Fonseca, 1951) in Brazilian spider monkeys. All three species are much alike: they exhibit a tertian periodicity in the blood, cause enlargement of the erythrocyte accompanied by Schüffner's stippling, and morphologically resemble *P. vivax* in the asexual and sexual stages. When Sinton and Mulligan (1933) tried to disentangle the muddled systematics of monkey malaria, they remarked that *P. inui* var. *cynomolgi* and *P. inui* var. *gonderi* (as they were then called), would very possibly prove to be identical, and they were unable to find any differential characters between the two parasites. Rodhain and van den Berghe (1936) re-described *P. gonderi*, pointing out that its cycle in the blood occupied 48 hours and that it could, therefore, hardly be called a subspecies of the quartan *P. inui*. Mulligan (1935) described *P. cynomolgi* in considerable detail, and from this description and from our own observations, it is possible to specify a few characters by which this species may sometimes be differentiated from *P. gonderi* (Table III). Little is known about *P. simium* and a strain has never been isolated; from the descriptions given by Fonseca (1951), this South American representative of simian benign tertian malaria parasite has no features distinguishing it from other members of the group.

Strains of malaria parasites behave differently in their immunity reactions. Avian species as a whole have a wide spectrum of immunity, the larger species producing fairly strong cross-immunity (e.g., between *P. relictum* and *P. cathemerium*—Redmond, 1939), and the smaller also (e.g., between *P. rouxi* and *P. hexamerium*—Draper, 1953). The human parasites on the other hand show no cross-immunity between species, and although strains of *P. ovale* are antigenically similar (Jeffery

et al., 1955), so-called strains of *P. falciparum* and *P. vivax* are so different (immunologically and in other ways) that they sometimes receive sub-specific names. Rodhain (1954) has recently used the absence of cross-immunity between *P. berghei* and *P. vinckei* as confirmation that they represent distinct species.

We felt that it would be useful to discover if cross-immunity tests would give a clue to the specific status of *P. cynomolgi* and *P. gonderi*. We possessed a number of monkeys highly immune to the former, and thus had a good opportunity of carrying out the requisite experiments.

IMMUNIZATION OF MONKEYS.

Four *Macaca mulatta* (Numbers 81, 99, 101, 102) were immunized against *P. cynomolgi** by blood and sporozoite infections as shown in Table I.

TABLE I.

Immunization of monkeys against P. cynomolgi.

Monkey Number	Date of first infection with *P. cynomolgi*	Mode of first infection	Superinfection with *P. cynomolgi*		
			Date of super-infection	Mode of super-infection	Subsequent course of infection
81	May 29, 1951	Sporozoites	14/5/53	Blood	*
			24/8/53	Blood	‡
			28/7/54	Sporozoites	‡
			2/2/55	Blood	†
99	April 25, 1952	Sporozoites	14/5/53	Blood	†
			24/8/53	Blood	†
			28/7/54	Sporozoites	‡
			2/2/55	Blood	‡
			25/2/55	Sporozoites	†
101	July 23, 1952	Blood	14/5/53	Blood	†
			24/8/53	Blood	†
			28/7/54	Sporozoites	†
			2/2/55	Blood	‡
102	July 23, 1952	Blood	14/5/53	Blood	†
			24/8/53	Blood	‡
			28/7/54	Sporozoites	‡
			2/2/55	Blood	†

* Shortened infection. † Transitory infection. ‡ No infection visible.

It will be noted that when blood containing *P. cynomolgi* is inoculated into monkeys immune to this species, usually no visible parasitæmia follows; if parasites appear, they are very scanty and persist for a few days only. Sporozoite infections are no more effective.

*Strain originally came from the Malaria Institute of India and came to us via the Rockefeller Foundation, New York.

RESULTS OF INOCULATING *PLASMODIUM GONDERI** INTO MONKEYS IMMUNIZED AGAINST *PLASMODIUM CYNOMOLGI.*

Fifteen mls. of blood were withdrawn from a rhesus monkey which had a chronic infection of *P. gonderi.* The blood was citrated and was divided into five equal portions, each containing about 250,000 parasites. These were inoculated into four immune monkeys (Table I) and into one control (M. 152). The course of the ensuing infections was studied in daily blood films, and the density of parasitæmia is shown in Table II. Unfortunately, the control monkey died after 11 days, but the normal course of this infection in *M. mulatta* is sufficiently well known for us to be able to predict what would have happened in the control monkey had it lived, that is to say—a chronic infection would have developed and persisted for years.

TABLE II.

Course of parasitæmia of P. gonderi *in* cynomolgi-*immune monkeys. Number of parasites per 10,000 erythrocytes.*

Days after infection	Monkey number.				
	Control 152	81	99	101	102
1	−ve	−ve	−ve	−ve	−ve
2	+ve	+ve	−ve	−ve	−ve
3	+ve	+ve	+ve	+ve	−ve
4	+ve	+ve	+ve	+ve	−ve
5	6	1	1	−ve	−ve
6	8	6	+ve	−ve	−ve
7	18	7	8	+ve	+ve
8	45	43	15	1	+ve
9	116	87	37	2	1
10	274	327	100	8	4
11	Died	444	71	24	10
12		121	73	68	20
13		41	31	56	124
14		19	12	34	146
15		20	12	12	182
16		16	13	9	105
17		36	11	18	35
18		43	7	21	5
19		77	2	25	1
20					
21		60	4	23	8
22		47	3	9	8
23		33	2	6	13
24		14	1	6	35
25		16	3	15	28
26		17	1	25	52
27					
28		14	+ve	31	24
29		11	+ve	23	34
30		17	+ve	36	23
31		51	+ve	11	38

*Strain obtained from the School of Tropical Medicine, Antwerp.

Each immune monkey showed a density of parasitæmia of the same order as that of the control, with many minor fluctuations. All the animals became anæmic, and Monkey 81 became quite ill, its blood containing numerous normoblasts and other abnormal elements.

Each infection was examined critically to determine if it were really *P. gonderi* and not a recrudescence of *P. cynomolgi* provoked by the injection of foreign blood. The minor morphological and clinical differences were observed in each case, the time of rupture of schizonts was approximately 3 p.m. (G.M.T.) on alternate days, instead of 3 a.m. when schizogony of *P. cynomolgi* occurs, and the behaviour of the parasite in *Anopheles maculipennis* var. *atroparvus* was typical of *P. gonderi*. The infection in mosquitoes thus developed in the normal way up to the ripe öocysts stage (about 50 per cent of a batch of 120 becoming infected after feeding on M. 81), then the oöcysts ruptured and rapid lysis of most sporozoites occurred. The salivary glands became only lightly infected (50 per cent). *P. cynomolgi* develops readily in this species of mosquito and the salivary glands remain packed with sporozoites for weeks. These three tests suffice to show that the new infections were due to *P. gonderi* and not to *P. cynomolgi*.

This experiment indicates that monkeys immune to *P. cynomolgi* are susceptible to *P. gonderi*.

RESULTS OF INOCULATING *PLASMODIUM CYNOMOLGI* INTO A MONKEY CURED OF A CHRONIC INFECTION OF *PLASMODIUM GONDERI*.

Rhesus monkeys never become immune to *P. gonderi* in the way that they do to *P. cynomolgi*: instead a chronic infection persists for years, often at quite a high level of parasitæmia. It was, therefore, impossible to plan an experiment on the same lines as that described in the preceding section, and a monkey with such a chronic infection was used.

A monkey (M. 129), which had suffered from a blood-inoculated *P. gonderi* infection for two years, was cured by the administration of 300 mg. of quinine bihydrochloride. A few days later it was infected with *P. cynomolgi* by exposure to mosquitoes heavily infected with this parasite. On the ninth day, rings were found in the blood, and an infection typical of *P. cynomolgi* followed, terminated by spontaneous recovery. *Anopheles maculipennis* were fed at a suitable time on this monkey and the resultant infection (80 per cent) was typical of *P. cynomolgi*: sporozoites appearing and persisting in large numbers in the salivary glands.

This experiment again demonstrated the absence of cross-immunity between the two species.

DISCUSSION.

The only final criterion for differentiating species of parasites is morphology; perhaps the next best is geographical separation in a different host. Alterations of behaviour, such as inability to complete development in the invertebrate host, or different response to drugs, probably indicate the beginning of a species change, but are generally regarded as too intangible for taxonomic use. The existence of

cross-immunity between two organisms must mean the possession of a common antigen; its absence the lack of one, indicating a fundamental difference which may be specific in character. The absence of cross-immunity alone would have little diagnostic value, but when associated with other evidence might provide useful confirmation of the status of a parasite. Our experiments suggest that there is no cross-immunity between *P. cynomolgi* and *P. gonderi* in rhesus monkeys, confirming that they are two distinct species. Table III presents the characters which may be used to differentiate these two species.

TABLE III.

Differential characters of Plasmodium cynomolgi *and* Plasmodium gonderi.

Character	*P. cynomolgi*	*P. gonderi*
Origin	East Indies	West Africa
Host (Vertebrate : nature)	*Macaca irus*	*Cercocebus* spp.
Host (Insect : laboratory)	*A. maculipennis*-excellent	*A. maculipennis*-poor
Course of infection in rhesus	Self-limiting	High chronic parasitæmia
Time of rupture of schizonts	Early morning	Late afternoon
Cross-immunity	Nil	Nil
Effect on erythrocyte	Distinct enlargement	Slight enlargement
"Double chromatin dots"	10 per cent in young rings	Rare or absent
Numer of merozoites	15·4	12

Both species have a 48 hour blood cycle, produce Schüffner's dots, show double invasion of red blood cells, exhibit indistinguishable gametocytes and have sporozoites of equal length (11·5 μ dried).

SUMMARY.

1. Monkeys immunized against *P. cynomolgi* are susceptible to *P. gonderi.*

2. A monkey cured of a long-standing infection of *P. gonderi* proved to be susceptible to *P. cynomolgi.*

3. This absence of cross-immunity plus other evidence indicates that the two species are distinct from each other, though both belong to the "benign tertian malaria" group.

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A NEW APPROACH TO THE EPIDEMIOLOGY OF MALARIA.

BY

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OUR knowledge of the epidemiology of malaria owes more to workers in India than in any other country of the world. It was first advanced by Dempster (1848) who established the general circumstances in which malaria occurred, the means by which it could be accurately measured, and some of the means by which it could be prevented. The transmission of the disease by mosquitoes, the differing vectorial capacity of anopheline species, the causation and nature of epidemics, and the nature of hyperendemic malaria were all described in India as the highlights of a mass of work which has no comparison elsewhere. Before 1929 this was distributed in a number of journals but since then has been for the most part collected in one series which is worthy of the work which it records.

The author owes much to the example of workers on malaria in India, to the experience he has had there, and also to the opportunity to contrast malaria in India with that elsewhere. The contrast has, however, often only complicated understanding. Types of malaria which in description seemed similar became markedly dissimilar when examined closely. Hyperendemic malaria in India was not the same as hyperendemic malaria elsewhere. The explanation of regional epidemics was completely satisfying in India but failed to explain why they did not occur in places with apparently similar surroundings in other countries. An attempt has been made to resolve some of these difficulties through the process of mathematical analysis of transmission of malaria along lines originally prepared by Ronald Ross (1916). The outcome has been a series of papers (Macdonald, 1950*a*:*b*; 1952*a*:*b*; 1953:1955) which, though essentially simple in themselves, are partly written in a language unfamiliar to most malariologists, of mathematics. The object of the present paper is to translate much of this working, attempting to expose the methods of work used and show how the products of mathematical analysis can be used to elaborate understanding of the normal epidemiology of malaria in the field.

FACTORS INVOLVED.

The state of parasitæmia is an intermittent one varying in length with characteristics of the parasite and the immunity of the individual concerned. Proper account can be taken of these variations but it is convenient to consider happenings in a totally non-immune person, in whom it has been shown that *Plasmodium falciparum* infection may typically be patent on about 200 to 250 days, whilst gametocytes may be present on about 80 days following a single infection. On each of these days the patient may be bitten by vector mosquitoes, let us say in illustration by about 10, and if the infection is established it proceeds through oöcyst development to the production of sporozoites. The time taken for the production of sporozoites is a function of temperature. Figure 1 shows the approximate speeds of development of *P. falciparum* and *P. vivax* in the mosquito to the stage of production of sporozoites. In ordinary tropical climates one might say that the normal time of development of *P. falciparum* is 12 days, though this may be much prolonged in cooler weather. The proportion of mosquitoes which survive sufficiently long for this development depends on the mortality to which they are exposed. Figure 2 shows how this varies with mortality. Circumstances in which the daily mortality is ten per cent—a not unreasonable figure—may be used in illustration, in which case about 30 per cent will survive for this period.

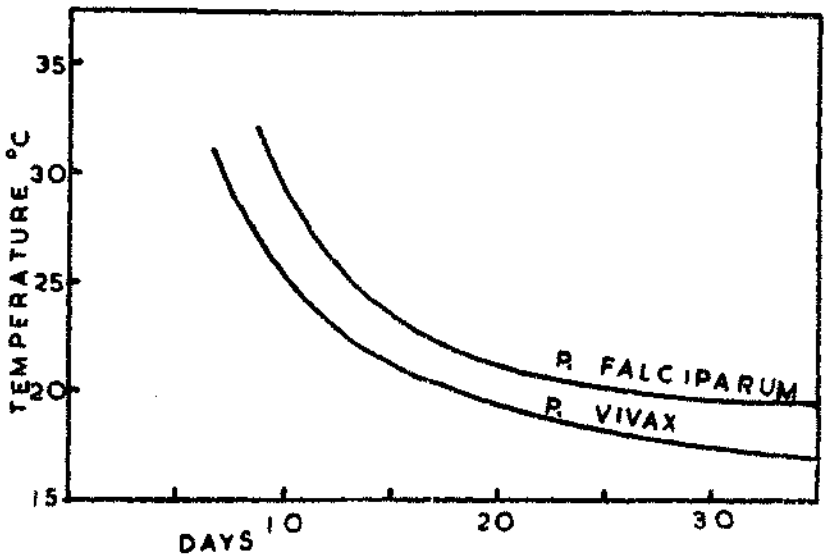

FIG. 1. The approximate time of the extrinsic cycle in relation to temperature.

Next comes the question of how long the mosquito may be expected to survive to bite other people should it have lived long enough for the development of sporozoites. Again it depends on the mortality to which the mosquitoes are exposed and is illustrated in Figure 3. In the example chosen with a 10 per cent mortality the expectation of such life would be about ten days. The number of

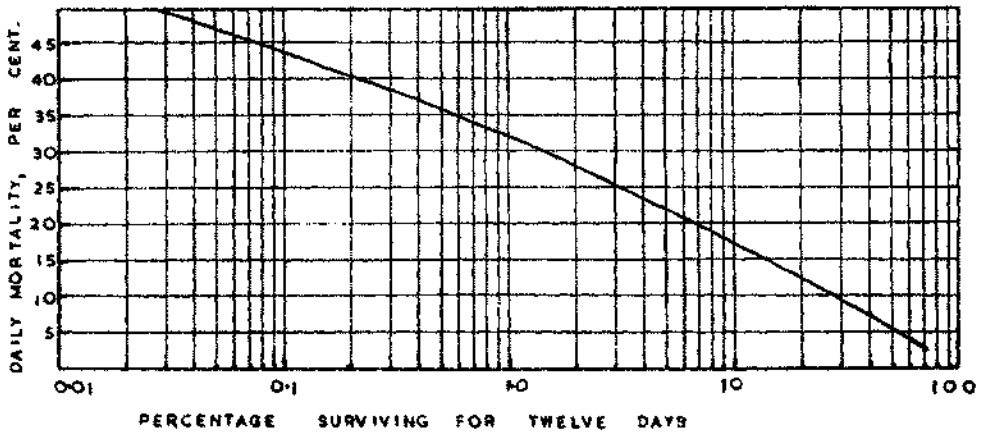

Fig. 2. The effect of mosquito mortality on survival through the extrinsic cycle.

people whom the mosquito will bite during that period depends on its biting habit; under tropical conditions the majority of anophelines take a blood meal once in every two days, but their habit of selection of man or other animal varies very greatly. In some, for instance *A. gambiæ*, the meal is almost invariably on man; in others, the meal is rarely on man if other foods are available. In the example used for illustration it may be taken that it feeds normally on man and once in every two days, that is to say that it might be expected to take five such feeds after the development of sporozoites in the glands. The success of the infection which it thus transfers turns on the infectivity of the sporozoite and susceptibility of the individual, but assuming success, infection is followed by an incubation period which can be taken as ten days, and in the case of *falciparum* infections by a further ten days before gametocytes appear and the case becomes infective to mosquitoes.

CONCEPTS.

In the example chosen the individual was bitten on each of 80 nights by ten mosquitoes of which 30 per cent survived for the development of sporozoites, and each of which took five subsequent feeds. There is a potential infection of as many as 1,200 people from the original. This introduces the idea of a reproduction rate, the number of secondary cases which potentially could be infected from a primary one, and the example chosen is not an extreme one. This reproduction rate is at the core of all epidemiology. The figure just given—1,200—is a gross rate; obviously this rate of multiplication could not go on unchecked for ever and it is worth examining what are the forms of brake on multiplication which eventually restrain it. In the case of insect-borne diseases there are two brakes, one is the existence of previous infections in the individuals who receive infective bites, so that the subsequent bites are non-effective and do not actually produce new cases of the disease. The other brake is the occurrence of superinfection in the mosquito, which may have a previous infection so that a second infection does

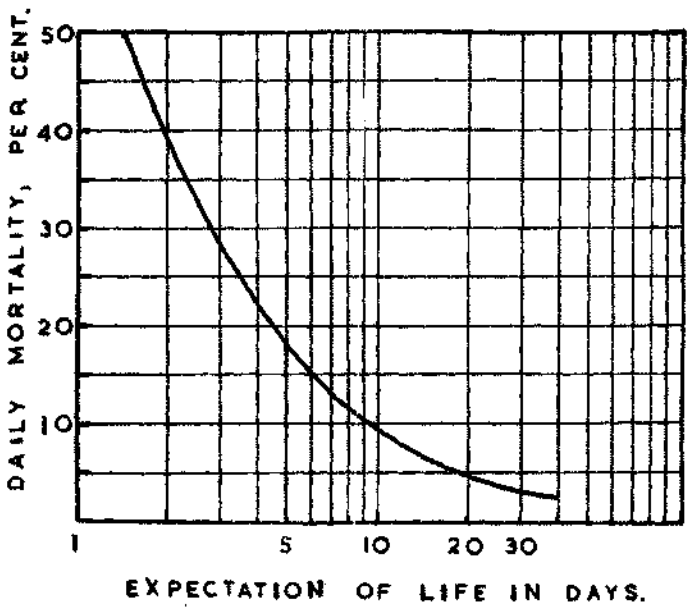

FIG. 3. The effect of mortality on expectation of life.

not materially increase its infectivity. These factors together reduce the gross reproduction rate to a net one which may be much lower. Should this fall below one, successive generations of cases will be smaller than their predecessors and the disease will disappear; should it be greater than one, successive generations will increase and the disease will mount in the population. Obviously the objective of all control is to keep the reproduction rate below one so that successive generations decrease in size and the disease disappears. Since in the last resort when it is in process of disappearing, the gross and net reproduction rates are the same it can be said from the start that the objective is to reduce the gross reproduction rate below one.

The idea of a critical level is also introduced here. It is not necessary to eliminate transmission completely in order to get disappearance of the disease but only to reduce it below some significant level, after which the disease will decrease indefinitely. This is a most important concept in general epidemiology and particularly in that of malaria, and explains the occurrence in some parts of the world of anophelism without malaria, the transmission being at such a low rate that the disease automatically extinguishes itself.

INTERACTION OF FACTORS.

Fundamental epidemiology considers this cycle of transmission in various stages. The sporozoite rate depends on the number of gametocyte carriers in the population, on the period of the extrinsic cycle and on the mortality rate of the mosquitoes in a manner illustrated in Figure 4. The mortality is here put as a survival

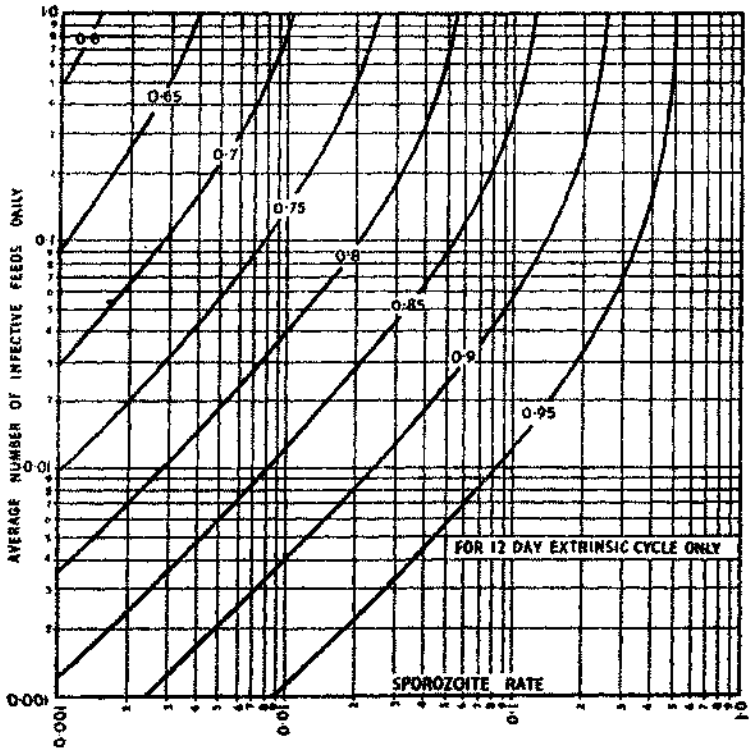

Fig. 4.—A series of graphs showing the sporozoite rate corresponding to given frequencies of infective feeds. Each graph refers to a different probability of survival which is shown on the line. The frequency of infective feeds is the product of the frequency of biting man and the infective gametocyte rate.

rate, 0·95 meaning that 95 per cent survive through one day. The graphs have been developed as a result of theoretical working which has been well checked in the field and shown to be substantially correct. In the most malarious conditions of Africa the sporozoite rate is usually of the order of ten per cent which represents a survival rate of about 0·95, that is to say a five per cent daily mortality, a 12-day extrinsic cycle, and about a one per cent chance of a mosquito biting an actually infective person on any particular day, for in these conditions in Africa the prevalence of infective people is reduced by the occurrence of immunity. In parts of India where malaria is transmitted by *A. culicifacies* very much lower sporozoite rates of the order of 0·1 per cent are often recorded. In an extensive series of surveys made by Russell and his co-workers in Madras it was 0·064 per cent; this was attributable to a 22·5 per cent daily mortality of the mosquito which took only, on the average, 1 out of 40 of its feeds on man, an extrinsic cycle for *P. vivax* of about nine days, and a gametocyte rate in the population of about 13 per cent.

Having analysed the relationship of the sporozoite rate to the gametocyte rate the next point is to examine the parasite rates which would be given by a constant inoculation rate. The series of graphs in Figure 5 shows how the parasite rate in infants might be expected to mount with passing time and increasing probability of infection according to the inoculation rate to which they are exposed. In each case the curve mounts to a plateau. Where the inoculation rate is lower than the recovery rate this plateau is below the level of 100 per cent infections. In other cases the parasite rate rises rapidly to 100 per cent and subsequently remains at that level, the majority of individuals actually suffering from two or more concurrent infections. This theoretical graph can be and has been checked against similar curves observed among infants in nature. The curves fit with extraordinary accuracy in a large number of cases, which establishes the general veracity of their shape and derivation. There are, however, a number of cases in hyperendemic parts of Africa where the inoculation rate derived from this form of working is very much less than that derived by entomologists examining the numbers of mosquitoes and their sporozoite rates, and it is thought that the explanation lies largely in the very low grade infections of the mosquito, consequent on low gametocyte counts in the population which is highly immunized.

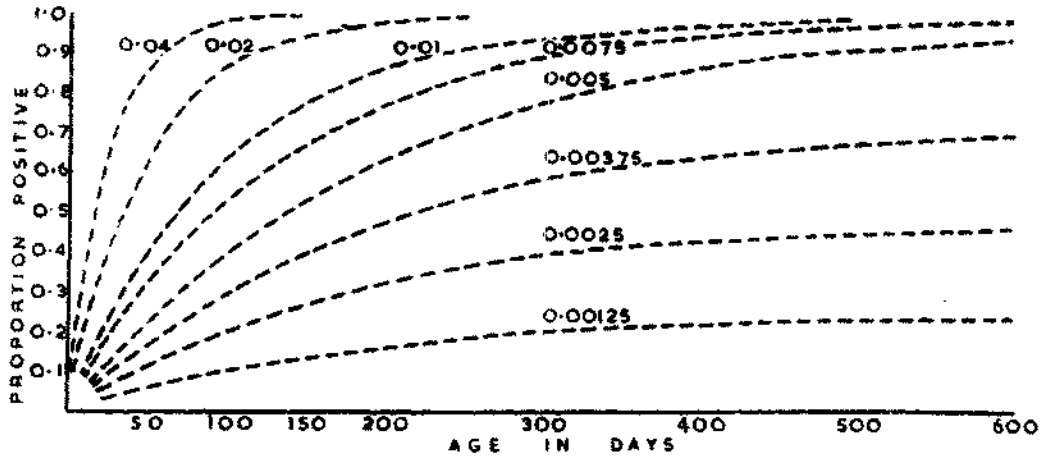

Fig. 5.—Theoretical infection rates by ages corresponding to various inoculation rates (h). The limiting value for all values of h exceeding 0·005 is 1·0.

In nature the parasite and sporozoite rates are dependent on each other. An increase in the parasite rate automatically increases the sporozoite rate, which in turn increases the parasite rate, and so on *ad infinitum* until the brakes on multiplication, the occurrence of superinfection in man and the mosquito, bring increase to an end. The next process in analysis is to examine the form of happenings when both the parasite rate and the sporozoite rate are allowed to vary in a manner dependent upon each other. Assuming for the sake of illustration that there has been some mild malaria in a community and thus a reservoir from which the disease can arise, and that transmission is abruptly increased, analysis produces the form of epidemic curve which would result. It has a curious form

starting with an original abrupt step, followed by a pause, and after a lag by a second more rounded curve.

Epidemics in nature are almost always mixed ones of *P. vivax* and *P. falciparum*, and the fact that gametocytes appear earlier in the case of *P. vivax* alters the timing so that in reality there are two separate epidemics which together form a combined curve like the upper one in Figure 6. This may be compared with actual happenings in nature. In every one of the local Ceylon epidemics of 1934/35,

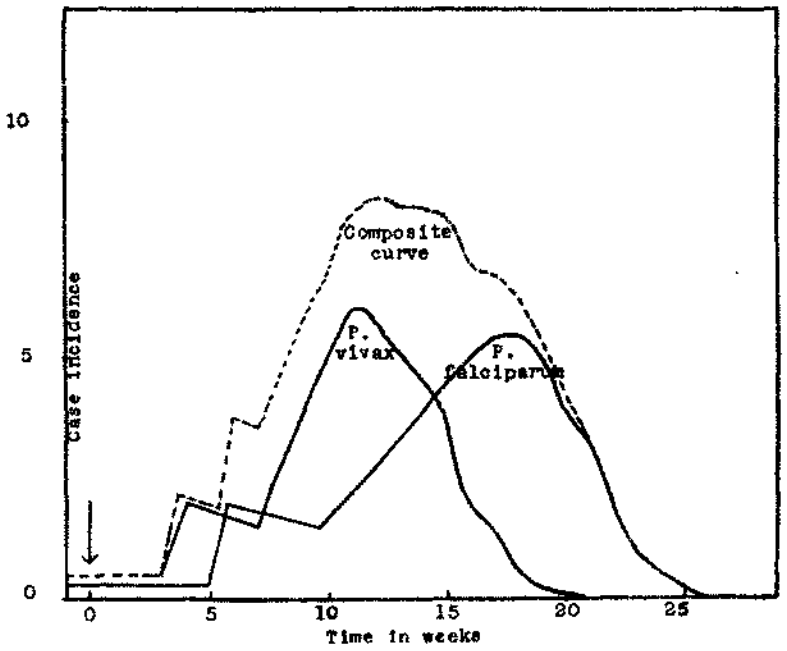

FIG. 6. Two synthetic epidemic curves built on identical data except for differences in incubation interval and extrinsic development, corresponding to those of *P. vivax* and *P. falciparum*.

and in most subsequent ones, one can trace the same general form. All natural epidemics do in fact consist of two separate curves as has been shown, and the occurrence of a very abrupt start, the preliminary appearance of *P. vivax* and the subsequent appearance of *P. falciparum* with a high mortality are accepted features. With this verification one can fit theoretical curves to the actual curves of Ceylon and from this process of fitting find the order of events, the amount of multiplication of mosquitoes necessary to produce such a catastrophe.

Epidemic curves reflect the admission of new cases but there are two factors: new cases and the parasite rate. The latter mounts from the start of the epidemic to achieve a plateau, which may be 100 per cent of the population or less. In analysis it is represented by a theoretical formula which cannot be directly checked in nature, because when this steady stage is reached immunity sets in, alters the value of one of the factors involved—the duration of the disease—and invalidates

direct comparison. The expression might, therefore, remain one of theoretical academic interest except that it can be used in subsequent examination of the stability of equilibrium, a very important concept which may be illustrated by a mechanical analogy: no engine however well governed runs perpetually at the same speed, some increase of the load on it produces some slight decrease in speed though this may later be adjusted by the governing mechanism. Now imagine two engines one of which is fitted with a reasonably efficient governor which maintains it running at roughly the same speed whatever the load that may be put upon it, and another which has an extremely inefficient governor so that with variation of the load or the amount of fuel supplied the speed shows great divergences from the normal. The speed of the first is fairly stable and of the second unstable. Exactly such a mechanism happens in any insect-borne disease according to the efficiency of the two brakes mentioned earlier. The brake of cancellation of infections falling on already infected people is, of course, always the same. The brake to multiplication applied by the probability that infections would fall on mosquitoes which are already infected varies very greatly with the characteristics of the mosquito and the probability of its biting more than once. Theory suggests that the greater the probability of the mosquito biting man in the form of its biting habit, choice of man and length of life in which to bite, the greater will be the overlap of infections in the mosquito and the more efficient the governing mechanism, resulting in a much more stable epidemiological condition with less tendency to vary from the mean. It is in exactly this characteristic of degree of variation about the mean that natural conditions differ so markedly from each other. In some places malaria repeats itself annually with astonishing regularity, and in parts of the perennially warm tropics the disease shows very little variation over several years. However, in other places and notably in northern India, parts of Pakistan and in Ceylon, the variations from year to year are extreme. In some there is a periodic variation with a cycle of about eight years, but apart from this, good and bad years follow each other in an irregular manner. Examination of the vectors of malaria shows that these two types do in fact depend on the longevity and the biting habit of the mosquito as theory had indicated they would.

EPIDEMIOLOGICAL TYPES.

By this route the concept of two quite different types of malaria, stable and unstable, is introduced. The first is caused by transmission by a mosquito which feeds often on man and has good prospects of life. The critical level is extremely low and in consequence anophelism without malaria is extremely rare. The disease shows little tendency to fluctuate from its normal, and epidemics amongst the indigenous inhabitants are rare. In consequence of the regular transmission of the disease immunity is fairly readily established amongst the local population, adults often show a firm resistance to it, and the disease is very difficult to control by anti-larval measures and relatively difficult by imagicidal measures. On the other hand there is unstable malaria caused by transmission by a short-lived vector not feeding normally on man; the critical level is relatively high; anophelism without malaria is common; the disease is extremely fluctuant in character varying very greatly from year to year, sometimes with dramatic epidemics of extreme severity

as those in Ceylon and the Punjab. The irregularity of transmission and the occurrence of consecutive years when it happens only on a small scale, results in a poor stimulus to the develpment of a firm immunity in a population.

These processes can show what is happening in epidemiology and why it is happening, why malaria is so vastly different in, for instance, central Africa where it is of the extreme stable type, and in Ceylon and the plains of India where it is of the extremely unstable type; one can in fact map out in the world zones where the epidemiological types differ. In northern Europe, for instance, malaria was relatively unstable, and is in fact in process of natural control by deviation of anophelines from man, whereas in southern Europe it is of the extremely stable type and remained unchanged until the development of imagicidal control in recent years.

CONTROL.

The object of all control is to reduce the reproduction rate below one. Without suggesting that the rate should be accurately calculated in every region, one should at least form a concept of the nature of the reproduction rates and the degree of reduction necessary to establish control of the disease. Theoretical examination can show the changes in the density of anophelines, in their daily mortality, or in their habit of feeding on man which would be necessary to eliminate the disease. It is the function of the epidemiologist to find as accurately as possible the value of the factors listed in the first section and particularly the biting habit and longevity of the local vector; from them to determine the actual amount of transmission and the potential amount should the entire population be non-immune, in the form of the basic reproduction rate; from these, estimates must be made of the changes in conditions necessary for control. It is then for the executive to determine how these changes can best be produced, though in most cases it will be by the use of residual insecticides. The determination of the actual mortality achieved by the use of particular insecticides in the field, using experimental trap huts, when related to estimates of the mortality necessary can, undoubtedly, avoid wastage.

Though the immediate attack should usually be through insecticides, their great value should not distract attention too much from the utility of other methods. The most significant feature in the epidemiology of malaria is the fact of its apparently natural disappearance from vast areas of the globe. These include the greater part of northern Europe, the greater part of the United States and parts of Canada which were once intensely malarious, and lesser zones within the tropics. These "spontaneous" regressions have occurred in places where the local mosquito is readily deviated from man to cattle, and it seems probable that the regression follows some slight change in agricultural pattern which has led to the more ready availability of animal food. There are large areas in India where even before the application of the modern methods of control, malaria was absent despite the presence of some vector mosquitoes, and there are other considerable areas where the disease has shown itself to be evanescent, coming and going in an irregular way following minor changes in the environment. This indicates that over very large tracts indeed the basic reproduction rate was naturally near its critical level

of one, and the amount of change needed to bring it permanently below that level would be small. Any deviation of the mosquito from man to animal must act in this direction and the influence of such deviation is very marked. The most interesting epidemiological study in India therefore lies in those areas of anophelism without malaria, and should concern itself with the precise reasons why malaria is absent, and the levels of mosquito prevalence, longevity and anthropophilism which permit the co-existence of man and mosquito without the transmission of the disease. With the knowledge gained in such studies the present great malaria control programme could rationally go on to one of elimination throughout India, to be succeeded by a programme of agricultural development which would take account of the protective value of cattle as an insurance against re-introduction of the disease, just as the present agricultural pattern of northern Europe and northern America is an insurance in those areas.

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SOME PROBLEMS ON CHEMOTHERAPY OF MALARIA.

BY

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WITH the advent of modern synthetic antimalarials, treatment of acute attacks of malaria nowadays presents few difficulties. The most powerful of the series (by oral route), as judged by the speed of action in relieving clinical symptoms as well as by the clearance of asexual parasites from the peripheral circulation, are the drugs of 4-aminoquinoline series. Even in extremely severe cases, parenteral administration of chloroquine preparations has been found to be as effective as quinine though some consider that the former is somewhat better. However, that would need detailed comparative studies against all the available strains of plasmodia before a final conclusion can be drawn.

As a blood schizonticide, neither proguanil nor pyrimethamine is as rapidly active as the 4-aminoquinolines. Besides their comparatively slower action, reports have also appeared from various quarters, particularly in India, indicating that occassionally some cases, particularly of *P. falciparum*, do not respond to the treatment (Jaswnt Singh *et al.*, 1952, Chakravarty and Chaudhuri, 1953; Srivastava *et al.*, 1953). Consequently, their field for therapeutic use becomes narrow. Although satisfactory reports on mepacrine have been presented by many workers, even as a single dose (Chaudhuri, 1954), it is well known that compared to the 4-aminoquinolines, its speed of action is somewhat slower, and that it is likely to produce untoward side symptoms like toxic psychosis, even though in a very small percentage of cases. In repeated doses quinine acts almost as fast as mepacrine.

Thus the choice of antimalarials for treatment of acute attacks of malaria naturally falls on the 4-aminoquinolines administered orally as a single dose of 0·6 gm. (adult) in partially immune population, if the condition is not too serious. Under emergency conditions and in cases where the patient is unconscious or if there is incessant nausea and vomiting, parenteral administration of chloroquine or quinine hydrochloride or dihydrochloride would obviously be called for.

Thus in the discovery of compounds like the 4-aminoquinolines a stage has now reached that perhaps further development of a more potent and rapidly effective remedy for clinical cure may not be necessary in view of rapid reduction of the incidence of malaria on account of large scale control measures.

Prima facie it would appear that the main problem in the treatment of malaria has practically been solved. But on deeper consideration, it becomes evident that there are still many lacunæ in our knowledge of the subject. For example, it is not clear why certain drugs like quinine, mepacrine and the 4-aminoquinolines which are such powerful blood schizonticides, yet have no effect on the sporozoites or the tissue forms (primary or secondary) of the plasmodia. Again drugs of the 8-aminoquinolines which are tissue schizonticides, particularly effective against the secondary exoerythrocytic forms, have poor action against the asexual erythrocytic forms, specially in respect of *P. falciparum.* Similarly, explanations may have to be sought as to why drugs like proguanil, pyrimethamine or 8-aminoquinolines are effective against the pre-erythrocytic forms of *P. falciparum* but not against those of *P. vivax,* and totally ineffective against the sporozoites of all plasmodia. Further, it is also common experience that some strain of a plasmodium is highly susceptible to a particular drug while others are comparatively refractory and need much larger doses. It is also not clearly known as to why should plasmodia develop resistance to certain particular groups of compounds like proguanil or pyrimethamine, and not against drugs like quinine, mepacrine or the 4-aminoquinolines.

Factors like the phases of plasmodium life cycle; somewhat different pattern in the life cycle of *P. vivax* and *P. malariæ* as against *P. falciparum* in the human host; the metabolic processes of plasmodia in the tissues like the liver and also in the blood; the chemical constitution of antimalarials and their effect on plasmodium metabolism; metabolites of antimalarials; pharmacological considerations like break-down products; absorption and distribution of the drug in the tissues of the host thereby aiding its penetration; and many other problems of similar nature have to be carefully considered.

As to the life cycle of malaria parasites, most of the lacunæ in our knowledge have been filled since the discovery of the tissue phase. Although, it is the general opinon that secondary exo-erythrocytic forms develop in *P. vivax* and *P. malariæ,* there is reason to believe that such forms are absent in *P. falciparum.* As to why such stages are not developed in the last named plasmodium, raises a fundamental issue. Obviously the behaviouristic pattern of this parasite in the liver is different. Further, it is well known that certain antimalarial drugs like proguanil, pyrimethamine and the 8-aminoquinolines are effective against the primary tissue phase of this plasmodium but not against the others. Since the rate of absorption, distribution, and tissue concentration of the same drug could not be fundamentally different in the same host, it is difficult to understand as to why there should be such a marked difference in the reaction of plasmodia to these compounds. Naturally, therefore, this leads one to speculate that perhaps the metabolic processes of the pre-erythrocytic forms of *P. falciparum* are different from those in respect of *P. vivax* and *P. malariæ.* Again it would appear that perhaps similar differences exist in the metabolic processes in the same plasmodium, like *P. vivax* at the primary and secondary exoerythrocytic forms, because 8-aminoquinolines, though active

against the latter, are ineffective against the former even though some workers have demonstrated prolongation of the prepatent period (James, Nicol and Shute, 1931). According to Huff and Coulston (1944) cytologically there is a great deal of similarity between sporozoites and the pre-erythrocytic forms which are considered to be an extension of the sporogony phase of the plasmodium, yet drugs which are effective against the tissue phase are totally ineffective against the sporozoites, in spite of the somewhat similar cytological character. Since it is believed that antimalarials act by interfering with the enzyme system, there is reason to believe that the metabolism of the two stages, sporozoites and pre-erythrocytic forms are different. This variation perhaps depends on the particular environment where they are usually lodged like the salivary glands or the liver.

There is also the possibility of differences in the metabolic processes of tissue forms and erythrocytic forms; firstly, because powerful blood schizonticidal drugs like quinine, 4-aminoquinolines, etc., are ineffective against the tissue forms of the same plasmodium and secondly powerful tissue schizonticidal drugs, like 8-aminoquinolines, are poor blood schizonticides. Again it may be noted that most of the existing antimalarials, which are powerful blood schizonticides, are poor gametocytocidal drugs. On the other hand, 8-aminoquinolines which have powerful gametocytocidal action (also tissue schizonticides), are poor blood schizonticides. Could there be differences in the metabolic processes even in the sexual and asexual forms of the same plasmodium? Also, could the metabolic pattern of gametocides and tissue forms of the same plasmodium have some degree of similarity?

As to the metabolic processes of malaria parasites and drug action, the present day knowledge is limited. Although quinine has been known for centuries to have specific action against malaria parasite, it is surprising to note that as yet so little is known as to how it acts. This is true for other antimalarials as well. The earliest workers like Christophers and Fulton (1938) demonstrated that quinine, mepacrine and pamaquin inhibit the oxygen consumption by the parasites. Mepacrine has been shown to interfere with the respiration of malaria parasites but quinine much less so. Silverman *et al.* (1944) believe that quinine affects the carbohydrate metabolism in *P. gallinaceum* by inhibition of the oxidation of pyruvate. Proguanil is supposed to act by interference with the poryphyrin metabolism of the parasite as suggested by Curd and Rose (1946), while the other view was that the activity of proguanil and pyrimethamine was due to antagonism with pteroglutamic acid. Marshall *et al.* (1942) reported that P. aminobenzoic acid (PABA) inhibits the action of sulphanilamide against *P. lophuræ*. This has been found also true for exo-erythrocytic forms of *P. gallinaceum* in tissue culture (Tonkin, 1946). According to Hellerman *et al.* (1946), atabrin and quinoline bases are enzyme inhibitors. These are likely to combine with certain acidic groups of the enzymes. If such groups combine with co-enzymes, a competition is set up between co-enzyme and the inhibitor. Work and Work (1948) believe that this inhibition may not be specific to one enzyme or class of enzymes. According to them " the danger of misinterpreting the action of an inhibitor, as a result of testing on an insufficient number of enzymes, is well illustrated in the case of the antimalarial drugs ". Findlay (1951) observes that quinine, mepacrine and pamaquin might interfere with a number of reactions essential for the metabolism of malaria parasites.

According to him, " there is still uncertainty as to how far any such inhibition is responsible for antimalarial action ". In the same way, there is hardly any indication as to the mode of action of proguanil.

As to the correlation between chemical constitution and antimalarial drug, Magidson *et al.* (as quoted by Findlay, 1951) suggested that different parts of the molecule of a drug, like mepacrine, have different functions and that basic side-chain is primarily of pharmacological importance, controlling absorption and distribution of the drug in the host and aiding its penetration into the parasite, while the substituted acridine or quinoline nucleus is responsible for the plasmodicidal action. But by experience, and from studies on newer antimalarial drugs, it has been observed that it is difficult to establish such correlation. Findlay (1951) observes that " to attempt to correlate chemical constitution and antimalarial action has so far proved an impossible task ".

It is also not clearly understood whether a compound exerts its action through the parent or its metabolites. It is known that quinine is degraded in the system to a 2-hydroxy derivatives. But this has been found to be inferior to quinine (Marshall and Rogers, 1948). Spectrographic study of mepacrine does not indicate acridines as the possible metabolic products. During their studies on pamaquin, Josephson, Taylor *et al.* (1951) had observed that concentrates obtained from blood, tissues and droppings of the chickens treated with pamaquin, possessed antimalarial activity *in vitro* equivalent to 16 times that of the parent compound. This activity could not be accounted for on the basis of pamaquin present in these concentrates. But subsequent *in vivo* studies on one of the metabolites isolated by (Josephson, Greenbergh *et al.*, 1951) did not show any antimalarial activity (Schmidt, 1951). Elderfield and Smith (1953) were also unable to establish any definite metabolite of pentaquin. Similarly ultraviolet irradiation of 4-amino-7-chloroquine derivatives and study of their breakdown products, did not reveal any new product (Price *et al.*, 1948).

Hawking and Perry (1948) suggested that proguanil itself is not active but it is converted to an active metabolite. Carrington *et al.* (1951) and Crowther and Levi (1953) were able to isolate an active metabolite of proguanil as well as an inactive compound originally isolated by Crounse (1951). The active metabolite is a dihydro triziane derivative but according to Schmidt *et al.* (1952), the parent compound was found to be two to four times more active than the metabolite. Subsequently, however, a large number of triazine compounds have been synthesized and some of them have shown high degree of activity against *P. gallinaceum* in chicks.

In view of the points raised above it is apparent that the existing knowledge on the problem of chemotherapy in malaria is still limited. At the moment our primary objective is to develop a suitable compound which in non-toxic doses is highly and equally effective against all the phases of the plasmodial life cycle, or at least against the asexual erythrocytic and tissue forms. In other words, it should be a powerful blood schizonticide as well as tissue schizonticide. If this proves effective against the gametocytes and sporozoites as well, it would be an added advantage. This is particularly necessary in case of *P. vivax* infection for though it is quite easy to effect a clinical cure with the usual antimalarials, its radical cure continues to be a baffling problem. No doubt some of the newer 8-aminoquinolines

possess curative action but there are always the toxic hazards encountered, though in small number of cases.

In order to achieve this objective, it would be essential to understand clearly the metabolic processes of plasmodia at all the phases, particularly the tissue and blood forms. Once the basic requirements of the plasmodia are well understood, it should not be an insurmountable task to find a suitable drug which would be antagonistic to one or more nutrients essential for the parasitic growth. There is, therefore, an urgent need for intensive studies on these problems which will require the resources and skill of a team of expert biologists, biochemists, and chemists alike, and not the wishful enthusiasm of a single individual.

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ANOPHELES RESISTANCE IN RELATION TO MALARIA CONTROL PROGRAMME PLANNING.

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INTRODUCTION.

THE great expansion in malaria control activities which has taken place within the past ten years with the development of D.D.T. and related residual insecticides has created many new problems and increased the importance of many old ones. In addition to perplexing problems related to the development of effective and adequate malaria control organizations, is the problem of resistance to residual insecticides by anopheline vectors.

The extent to which these issues can be resolved naturally varies with the existing situation in each country, including financial resources, the availability of suitably trained professional personnel, ways of thinking, and competing exigencies. In this connection, the author is reminded of his experience of 25 years ago in a State-wide programme of malaria control in one of the States of the United States of America, in which the situation can be summed up by the following bare facts: Area served—58,000 square miles. Population served—three million. Number of malaria cases—50,000 to 100,000. Number of professional personnel assigned to malaria control—one. Paradoxically, it was not until malaria had largely ceased to be an important socio-economic burden that funds became available for the development of adequate malaria control organizations in this and other malarious States of the United States.

Under such circumstances, it was futile to propose new plans for the existing malaria control "organization" to carry out, which invariably required additional trained personnel. Today, throughout the world, malaria control programmes fortunately are staffed somewhat more adequately, but major shortages of key personnel are still all too common. Such shortages understandably impede the adoption of "ideal" plans based on theoretical concepts, some of which may be involved in this article.

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ANOPHELES RESISTANCE.

In considering any matter of insect resistance, two governing natural laws should be recalled to mind. One is that the development of resistance is a product of selection pressure, its duration, and the genetic capacity of the species to develop resistance. The other is that, barring eradication, any species inevitably will develop resistance of one type or another, provided the degree and duration of selection pressure in combination are sufficient to threaten species survival.

When one surveys the results of ten years of global application of the chlorinated residual insecticides for agricultural, public health, and pest control purposes, the comparative lack of confirmed resistance development in the field among the anophelines is little short of amazing. This is especially true of many of the more anthropophilic anopheline species which, in some areas, have been bombarded for eight years or longer in specific localities with D.D.T. residual house spray. Malaria has been eradicated in extensive subnational areas where the biotic potential of the disease was high, with and without vector eradication, and has been controlled in other far more extensive areas of high biotic potential. Malaria eradication also has been realized in other extensive areas where the disease was not so firmly intrenched. In a few special cases where the vector had not achieved a status of long, indigenous adaptation, species eradication was even achieved as a partly unexpected dividend from malaria control operations. Yet, so far, only four of about 50 significant anopheline vector species are considered to have acquired physiologic resistance in local or more extensive areas. These are *Anopheles sundaicus* in Indonesia; *A. maculipennis*, *A. sacharovi* and *A. superpictus* in Greece; and *A. sacharovi* again in Lebanon (Pampana, 1954; Garrett-Jones, 1954; and Crandall, 1954:1955). With the exception of Lebanon, physiologic resistance development has occurred in the presence of adulticidal treatments *cum* larviciding, although these selection pressures were not necessarily applied continuously and concurrently. Even in Lebanon, the possibility of larval exposure incidental to agricultural insecticiding cannot be ruled out. Earlier reports of resistance in *Anopheles quadrimaculatus* in the T.V.A. area of the United States of America have not been substantiated (Hawkins and Hall, 1954). The species is under larvicidal treatment in this area, but such treatment is restricted to federally-owned properties bordered by private lands.

Behaviouristic resistance as a developmental factor cannot be as readily evaluated, nor, as pointed out by others, is it necessarily adverse to man's interest. This term is intended to apply both to anophelines which do not normally enter or rest in buildings capable of being sprayed, and to those possessing hyper-irritability to D.D.T. or other chlorinated hydrocarbons. It is at times difficult to determine whether observed behaviouristic resistance was a pre-existing characteristic of the dominant population group, of an important biotype fraction which has not increased in prevalence, or whether the resistant biotype has become more prevalent as a result of the selection process. An example of behaviouristic resistance which has impeded malaria control is provided by *A. sergenti* and *A. superpictus* in the Jordan Valley (Farid, 1954). One which has not prevented malaria control is that of *A. albimanus* in Panama (Trapido, 1952:1954).

Compared with the house fly, this relatively favourable state of affairs reflects the lower genetic capacity of the genus *Anopheles* to develop resistance, supplemented in some cases by fewer generations per year. The exposure of a smaller fraction of the species population to insecticides is an added possibility. The latter factor highlights the well-established principle that residual house spray in malaria control is a highly selective tool which is intended to intercept only that fraction of the species population which is infective to man.

Yet, the importance of anopheline resistance should not be minimized. It is already a problem of major importance in some of the countries or local areas in which it has appeared (especially in Greece), and more instances of serious resistance are almost certain to occur. Slight resistance may exist at present in many places where it is not recognized. Because the potential significance of *Anopheles* resistance outweighs its present status, the primary issue is still prevention of spread of anopheline resistance, rather than contending with existing resistance problems.

Efforts which are being made, or might be made, to solve the resistance problem may be divided into two categories: (1) Laboratory studies, and (2) Field operations.

LABORATORY STUDIES.

These consist of: (1) Basic physiologic, biochemical, and genetic studies on the mechanism of resistance in arthropods, particularly anophelines, and the genetic processes by which physiologic and behaviouristic resistances are developed, and (2) the development of new insecticides, formulations, and techniques of application. The apparent purpose of the basic studies is to permit making more intelligent searches for new insecticides and synergists, but the theoretical implications of such research extend further.

In combination, the present main hopes of such studies are in the following directions, with the proviso that any insecticide which satisfies one or more of these objectives must also be relatively safe to man in application and in use, and must be economical in cost:

A. Development of an even more effective residual insecticide which will facilitate eradication of the anthropophilic biotypes of indigenous anopheline species, preferably solely by the routine application of residual house spray techniques. This is based on the hypothesis that the eradication of indigenous vectorial species of long standing is generally infeasible due to the existence of highly zoophilic biotypes, but that biotype eradication is feasible where species eradication is not. Fortunately, the more anthropophilic biotypes tend to be endophilic, but this is not always the case. According to Holstein (1954), the paucidentate race of *A. gambiæ*, which has marked anthropophilic tendencies, with human precipitin test results of 70 to 90 per cent, tends to be exophilic in resting place with respect to human dwelling places, while the multidentate zoophilic race tends to be endophilic. This hypothesis, accordingly, may not apply in exceptional cases.

B. The development of new insecticides with differing periods of residual effectiveness, but which in other respects would have substantially equal merits.

By selecting for each area an insecticide having an effective residual life equal to, but no greater than, the length of the seasonal period of disease transmission, selection pressure during the remainder of the year could be avoided. Such insecticides should have high effectiveness with an abrupt termination point. The merits of this objective are sharply limited by the facts that (1) a high proportion of the number of annual generations of vectorial species in the more malarious areas occurs within the malaria transmission season, (2) variation in length of effective life can be achieved with existing insecticides to a considerable extent by choosing between available insecticides and by selection of dosage rate, and (3) it is not operationally feasible for residual spray crews to apply insecticide to all houses at the optimum moment—several months may be required for a spray crew to cover its territory with a single application.

C. The discovery and development of new insecticides, formulations, and techniques of application by both haphazard and systematic methods. The development of very high resistances and cross-resistances to many of the newer insecticides by arthropods of agricultural or public health importance has generated what appears to be a perpetual race between man and insect in insecticide development. Although centered mainly on agricultural insect problems, the control of the house fly and various culicine mosquito species also provides important motivation in the quest for new insecticides. In a mosquito abatement district in the State of California in the United States, for example, the entire list of chlorinated hydrocarbon insecticides was thrown into the discard three years ago, due to mounting resistance by *Culex tarsalis* and *Ædes nigromaculis* (Geib, 1955). Since then, a number of organic phosphorus compounds have been resorted to on this larviciding programme.

Such further new developmental work possesses somewhat less direct significance in malaria control, in view of the slow rate of resistance development by anophelines to residual adulticidal treatments, the apparent absence of total spectrum cross-resistance in them, and the present availability of one or more acceptable alternative residual insecticides in the chlorinated hydrocarbon group. But there is always the possibility that a new insecticide may evolve which is more effective or more economical, and yet safe for extensive indoor application.

FIELD OPERATIONS.

The foregoing laboratory studies have significant potential value in malaria control and eradication, and should be pursued. But it is of great importance that the management of malaria control operations in the interest of avoiding resistance not be neglected in any particular. In a large scale programme even minor operational changes may be difficult or slow to execute, and may temporarily impede operational efficiency and the accomplishment of other urgent objectives. Programme decentralization, although desirable in many respects, may complicate the acceptance and adoption of such changes. The achievement of effective malaria control under adverse operational conditions often is an exacting and difficult task at best. In spite of such obstacles, consideration of the resistance problem in programme planning and execution is essential.

Differences undoubtedly exist in the genetic capacities of different *Anopheles* species to develop resistance, and in the actual resistance of different species, biotypes and even individuals. However, the main variables within this genus in the selection pressure—duration—genetic capacity—equation are considered to be selection pressure and its duration. Certainly this is the case when the house fly is used as a plane of reference. Were it not so early, extremely high resistance would have appeared many years ago in those of the more anthropophilic species which also had genetic potentialities materially higher than the mean level for the genus.

While the degree of selection pressure exerted is very importantly affected by behaviour of the adult anopheline, it is otherwise a controllable variable. Duration of pressure is totally controllable aside from the inherent characteristics of the insecticide employed. Even these characteristics can be modified by varying application rates and practices.

The present main trend of thought in certain malariological circles is to achieve the eradication of malaria as rapidly as possible, and in as large areas as possible, in order to permit the interruption or termination of residual house spraying. Avoidance of resistance is only one element in the total motivation and justification for this concept, but it is a carefully considered factor.

It may be postulated that the optimal condition favouring *Anopheles* resistance development which would be encountered in the field is that of moderately high selection pressure applied perpetually. The degree of pressure would be as high as possible without eradicating the biotype, species, or parasite. In theory, departure in either direction from this optimal selection pressure should reduce the rate of resistance development or totally avoid it.

It is generally considered that even very low selection pressures continued long enough will result in some degree of resistance development. No doubt this is true of isolated populations, but it is unlikely to have significance in the case of many of the more zoophilic anopheline vectors due to dilution by unexposed populations. *Anopheles albimanus* is a case in point. This species enters houses at dusk or after dark, dispersing within the night or at dawn to concealed outdoor daytime resting places. Precipitin tests for human blood in this species are commonly below five per cent. Assuming the figure of five per cent to roughly represent the percentage of the population which becomes exposed to D.D.T. residual spray within houses in obtaining a single blood meal, that the percentage of survivors from this exposure is not over 20 per cent (or one per cent of the total population), and that this one per cent is then diluted 95-fold by zoophilic feeders in the perpetuation of the local species population, it is apparent that the degree of selection pressure would be negligible. While this example is deliberately oversimplified, it illustrates the principle of population dilution in combination with low selection pressure on the local species population.

However, avoidance of *Anopheles* resistance is not an end unto itself, nor is it an overruling consideration in most areas at the present time. For this reason, overall objectives are best served by increasing the intensity of selection pressure as a calculated risk in order to achieve more rapid malaria eradication and to terminate residual spraying.

Malaria control and malaria eradication have much in common, but differ in important particulars from a programme design standpoint. Both concepts demand in principle a high order of operational competency in the malaria control organization in the interests of economy and achievement of objective. Nation-wide malaria control and eradication programmes are large, complex enterprises which, in order to be ideal, require the skills of the three basic professional interests—medical, engineering, and entomological—and the utmost in teamwork.

The inherent competency and aptitude of the engineer is in the management of labour forces and of logistical functions—responsibility for residual spray crew operations, and for the supply pipeline. That of the entomologist is basically in observation of the anopheline vector and in programme guidance based on these observations, but is not necessarily limited to these functions. That of the medical profession is basically the epidemiological observation of the disease in man and, traditionally in many areas, over-all command of programme.

Globally, programmes for malaria control *per se* may be considered as having conformed to two main patterns. The more common programme has followed the concept of prompt, effective suppression in project areas where operations were carried on. The other pattern, dictated by limitations of supplies and equipment in relation to magnitude of problem, has been aimed at controlling the greatest number of cases of malaria with available resources. By use of relatively high D.D.T. dosage rates, coupled with an optimal frequency of application, malaria transmission in the first pattern might, for example, be reduced 80 per cent in the first year. In the second pattern, the same quantity of D.D.T. might be spread over twice as many houses with the expectation of reducing malaria transmission by only 60 per cent, but among twice as many people. Although the objective of the second pattern is praiseworthy, and was no doubt fully justified a number of years before the threat of resistance became apparent, careful re-examination is warranted if such programmes still exist.

As with species eradication, malaria eradication is directly dependent upon eliminating the last surviving reproduction source, in this case the gametocyte carrier, from each "useful unit" area. This is the major point of difference between malaria control and malaria eradication. In consequence, malaria eradication, in principle, demands a higher order of programme effectiveness, as previously mentioned. It also demands the regrouping and extension of project areas along different lines than with malaria control programmes. Generally, malaria control and malaria eradication operations should be initiated in areas of highest malaria endemicity. With malaria control, this would be in conformance with the principle of controlling the greastest number of cases at the lowest cost; with malaria eradication, it would be to lower malaria rates to parity with adjoining less malarious areas. Having achieved this parity, however, the project area in a malaria eradication programme should be expanded as rapidly as possible toward inclusion of the total malarious territory comprising the trading area. Since such territories generally conform to transportation routes, they are likely to be irregular in shape. Expansion into areas of low endemicity within such a territory would have priority over initiating work in a new, more malarious, but remote area.

The absolute prevention of reintroduction of malaria into a clean area is, of course, not to be expected until national, regional, or even hemispheric and global eradication has been achieved in all areas from which travellers may originate. The goal is rather to reduce the frequency of reintroduction to such a low level that the prospects of restoring large scale malaria transmission and the burden of malaria surveillance are minimized.

In the interest of reducing the hazard of resistance development, as well as economy of effort, many short cuts in finally terminating residual spraying in an area may be postulated. Certainly, continuation of full-scale spraying in a district of, say, a million people, until several years after the termination of malaria transmission in the last remote village, is absurd. How far and how rapidly a programme can proceed in curtailing spray operations, or in substituting selective chemotherapeutic measures in the mopping-up stage, is directly contingent on the adequacy of malariologic services. There is almost certain to be an acute shortage in the quantity of such service which might ideally be desired in every country where malaria is a primary public health problem. Thus, rather than creating a problem of technological unemployment among malariologists, D.D.T. potentially has created a far greater need than ever before existed.

In the interest of avoiding resistance, there are certain other measures which warrant serious consideration before the tapering off period; two of them, in fact, ideally should be carried on not only continuously throughout the programme, but should antedate residual house spraying.

The first measure is the determination of prevailing resistance baselines for each vector species at judiciously selected observation posts and recurrent testing of the resistance level. At least two published objective tests have been developed for this purpose (Expert Committee on Malaria, World Health Organization, 1954, and Fay *et al.*, 1953). Such testing is underway, but should be far more extensive. When resistance is detected on operating projects by less sensitive methods, it is often too late. Diagnosis is never a substitute for cure, but is an essential prerequisite.

The second measure is the careful restriction of use of chlorinated hydrocarbon insecticides for anopheline larviciding. The circumstances under which the World Health Organization Expert Committee on Malaria has indicated such larviciding should be practised, do not preclude the development of resistance, but do tend to limit its occurrence to restricted geographical areas.

Instances in which larviciding for malaria control should be the method of choice over residual house spraying on economic grounds consist mainly of urban areas or small breeding surfaces in isolated communities such as oases. Larviciding on biological grounds also has been advocated for the control of important exophilic vectors, as is repored to be the case with *A. superpictus* and *A. sergenti* in the Jordan Valley (Farid, 1954). Justification for larviciding *cum* residual house spraying would be more often encountered in suburban areas where the joint control of anophelines and certain other mosquito species are involved, but also can be visualized in exceptional situations where malaria is primarily transmitted by an endophilic species and, secondarily, by an exophilic species. In the latter case, differences in breeding habitat and adult behaviour might result in the selective exposure of each species.

The importance of avoiding use of the same chlorinated hydrocarbon insecticide against the immature and adult stages of the same anopheline species at the same time and place has been stressed by others. Under such circumstances, use of two chlorinated hydrocarbon insecticides which are not closely related has been suggested. However, the extremely high resistances (up to 1300 fold) (Geib, 1955) to a variety of chlorinated hydrocarbon insecticides which have developed in the United States of America among species as *Culex quinquefasciatus*, *C. tarsalis*, *Aedes taeniorhynchus*, *A. sollicitans*, and *A. nigromaculis*, where larvicides are intensively and extensively employed, provides striking evidence of the extremely high degree of selection pressure exerted by this method of control. With this in mind, one might say that as long as the patient took cyanide (*i.e.*, larviciding) it matters little whether he also took some other relatively innocuous poison (*i.e.* adulticiding) at the same time. It also poses the question whether the intensive use of chlorinated hydrocarbon larvicides on an extensive scale is ever warranted for malaria control, even with exophilic vectors. Except where a high degree of sexual isolation exists, it may be postulated that resistance developing in a localized area will be rapidly dissipated by dilution upon interruption of selection pressure, but such development over a large area gives cause for much concern.

The third measure is to pay attention to control of cause as well as control of effect. Malaria eradication is one method of control of cause. Another is the elimination of anopheline breeding places and prevention of the establishment of new breeding places by man. The need for keeping in mind the more traditional methods of malaria control and of avoiding excessive preoccupation with the newer forms of insecticidal work has been pointed out by others (World Health Organization Expert Committee on Malaria, 1954). Yet, it is generally acknowledged that many of these measures are not susceptible of either near-term adoption or of widespread adoption over the vast rural areas of the world which form the stronghold of malaria.

However, in many countries, the predominance of man-made over natural vector breeding places throughout large areas, is apparent. This is particularly the case in India and many other tropical countries with their vast numbers of roadside borrowpits and of brick pits, and their extensive irrigated acreages. The elimination of existing breeding places in these categories would be a gigantic undertaking, and even the prevention of new ones is no easy matter, especially where changes in cultural practices are required.

On the other hand, corrective practices in new undertakings which in themselves yield direct economic benefit (e.g., water conservation in certain irrigation projects), or can be performed at nominal cost incidental to construction (*e.g.*, connecting shallow hillside borrowpits), are the most economical of all malaria control practices. The important consideration which has been given to these problems by some malariologists in India is noteworthy.

The fourth measure is further experimentation with each vector species in the field with respect to dosage rate and frequency of application of residual house sprays. Early field trials of this nature were made in a number of areas in the 1940s. However, they were focused on the interruption of malaria transmission as the main criterion rather than in combination with evaluating selection pressure.

In countries where they are not already underway, the carrying out of further limited experimental studies in conjunction with local operating projects might well be undertaken in order to jointly evaluate malaria reduction and resistance development. Would it not be desirable to deliberately attempt to induce resistance in each important vector in the smallest feasible area, in order to establish a safety factor for general programme operations or to disprove the possibility of resistance development in a particular species under the conditions of such a programme? The length of time needed to carry this through might, of course, be an important weakness.

Would it not be desirable similarly to carry on other limited experimental studies in conjunction with local operating projects which would employ different dosage rates and frequencies of treatment than those conventionally used? Intensification of selection pressure on the general programme in lieu of its reduction might well be in order if the margin of safety is ample and malaria eradication could be expedited by so doing. On the other hand, present pressures in some cases may be greater than necessary to obtain a prevailing, satisfactory interruption of malaria transmission, with lessening of pressure indicated. These variables are also interrelated with those of vector species or biotype, cultural practices, housing, quality of D.D.T. wettable powder, and quality of labour force and supervision. In consequence, such experiments must be standardized as much as possible and carefully evaluated. No doubt, experiments along these general lines are being undertaken in some areas, but are they being undertaken even to the minimum extent necessary in all indicated countries?

SUMMARY.

The problem and possible solution of *Anopheles* resistance threats are considered from the standpoints of laboratory research and field operations. The development of resistance is a product of selection pressure, its duration, and the genetic capacity of the species to develop resistance. Any species inevitably will develop resistance of one type or another, provided the degree and duration of selection pressure in combination are sufficient to threaten species survival. The slowness of anophelines to develop resistance to insecticides is commented on.

Three end purposes of laboratory studies concerned with *Anopheles* resistance are described. Adjustment of operating practices in the field is advocated in the interest of avoiding resistance problems. Principles underlying the variation of selection pressure and duration of pressure in field practices and programme design are discussed. Brief mention is made of the role of certain traditional methods of malaria control and of the malaria control organization.

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SPOROGONY CYCLE OF MALARIA PARASITES IN RESISTANT AND NON-RESISTANT STRAINS OF MOSQUITOES AFTER EXPOSURE TO D.D.T.

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INTRODUCTION.

In a previous communication it was reported that in *A. fluviatilis* and *A. stephensi* (type) a sublethal contact with D.D.T. did not inhibit the development of eggs whether the females were exposed to the insecticide before or after blood feed or as half gravids (Mohan, 1955). There was close correlation between fertilization and maturation of ovaries irrespective of the exposure to D.D.T. Fertilized females of both the species showed a significantly higher percentage of mature ovaries than the unfertilized females. It was also observed that exposure to D.D.T. affected a fair number of gravid *A. fluviatilis* and *A. stephensi* in that the eggs were laid at random although suitable water for oviposition was available. The present studies were undertaken to find out the effect, if any, of lethal and sublethal contact with D.D.T. on malaria parasites in resistant and non-resistant strains of mosquitoes, respectively. The effect on *P. gallinaceum* of D.D.T. orally fed to infected fowls as measured by sporozoite infection in *Ædes ægypti* was also investigated.

MATERIALS.

The host and parasite species used were a D.D.T.-resistant and a non-resistant strain of *C. fatigans* for *P. relictum* in local sparrows, a D.D.T.-resistant and a non-resistant strain of *A. fluviatilis* for human malaria and only a non-resistant strain of *Ædes ægypti* for *P. gallinaceum* in domestic fowls. All these strains of mosquitoes were furnished by the laboratory colonies.

D.D.T.-resistant strains of *C. fatigans* and *A. fluviatilis*.—While a fuller account of the development of the resistant strains of *C. fatigans* and *A. fluviatilis* will be published in due course, a few relevant details concerning each species are given below.

C. fatigans.—Both the resistant and non-resistant strains of *C. fatigans* originated from the same single raft of eggs laid by a wild-caught female. Signs of resistance* to D.D.T. first became apparent in the fourteenth generation as a result of the exposure of the adults, males and females, to sublethal doses of D.D.T. at each successive generation. In the subsequent generations, there was some progressive increase in resistance which had reached a high level at the time of conducting the experiments with mosquitoes of the forty-third and forty-fourth generations. A brief resume of some of the salient points about D.D.T.-resistant *C. fatigans* may be relevant at this juncture.

While the non-resistant strain of *C. fatigans* evidenced 100 per cent mortality on being exposed to 200 mg. of D.D.T. per sq. ft. for one hour, the resistant strain showed little or no mortality on being subjected to similar treatment under identical conditions. Adults of the latter strain were resistant not only to mortality but also to the paralysing effect of D.D.T. The resistant strain showed striking variations in its resistance to D.D.T. With the dosage remaining the same, some individuals in a given batch of females of the same generation, died after six hours of exposure, others after 12 hours, while still a few others required even more than 18 hours of exposure to die.

A. fluviatilis.—Similar efforts were made to raise a D.D.T.-resistant strain of *A. fluviatilis*. Feeble resistance† was first evidenced in the twenty-ninth generation. There was slight increase in the subsequent generations but the level of resistance still remained low in mosquitoes of the forty-seventh generation which were used in the experiments. In this susceptible species, resistance was very late in appearance and equally very slow in building up.

DEVELOPMENT OF SPOROGONY CYCLE OF *P. RELICTUM* IN RESISTANT AND NON-RESISTANT STRAINS OF *C. FATIGANS* AFTER EXPOSURE TO D.D.T.

In all, four experiments were carried out. Resistant *C. fatigans* used in the first experiment were of the forty-third generation and in the subsequent three experiments of the forty-fourth generation.

Experiment 1.—Females of the resistant strain of *C. fatigans* and of the non-resistant strain were kept in separate cages and given an opportunity to feed on the same gametocyte-carrying sparrow, the latter during the first half of the night and the former during the second half. Mosquitoes of each strain which had become fully engorged were, in part, given two separate exposures to 200 mg. of D.D.T. per sq. ft. for 40 minutes. The first exposure was given within 12 hours and the second on the third day of the infecting blood meal. In other words,

* Indian Council of Medical Research Technical Report of the Scientific Advisory Board for the year 1952, New Delhi, p. 12.

† Indian Council of Medical Research Technical Report of the Scientific Advisory Board for the year 1954, New Delhi, p. 7.

the effect of these exposures was timed to coincide with the early stages of development of the parasite in the mosquito host. Some blood-fed females of each strain which were not exposed to D.D.T. were kept for comparison. There were thus four groups of mosquitoes, two of the resistant and two of the non-resistant strain, each including exposed and unexposed (comparison) insects. They were kept in separate cages under identical conditions and fed on ten per cent glucose solution until dissection. Mortality resulting from two separate exposures to D.D.T. was practically nil among the resistant mosquitoes whereas it was heavy in the non-resistant strain. In spite of a much higher proportion of the latter exposed to D.D.T., only a few were left alive for dissection. All the groups of mosquitoes were dissected on the same day particularly for the determination of sporozoite infection of the salivary glands after the normal incubation period.

Experiment 2.—Each group of the resistant and non-resistant strain was given four exposures, the first immediately after infecting blood meal during the night and the subsequent three on alternate days. The dosage of D.D.T. was 200 mg. per sq. ft. in all the cases and the period of contact was 40 minutes for each strain in the first exposure but in the subsequent exposures it was increased to one hour in the case of the resistant strain and decreased to 20 minutes in the non-resistant strain so as to get a fair number of the mosquitoes for dissection after the incubation period of the parasite in the mosquito.

Experiment 3.—Resistant females which had fed on a naturally infected sparrow were exposed to D.D.T. as above for one hour once daily for the first four days. Some mosquitoes of the same stock which had fed on the same sparrow were not exposed to D.D.T. but kept for comparison.

Experiment 4.—In this experiment 300 resistant *C. fatigans* females were first exposed to 200 mg. of D.D.T. per sq. ft. for as long as six hours and then, on release, immediately given an opportunity to feed on an infected sparrow, during the night. The following morning 24 were 'up' including 14 fully engorged females. All these engorged specimens remained alive until dissection.

Data concerning the foregoing experiments are set out separately in Table I.

The results of comparative infections indicate that mere acquisition of resistance in *C. fatigans* was not accompanied by any concomitant change in its susceptibility to infection with *P. relictum*. Both the resistant and non-resistant strains were about equally infected up to the sporozoite stage.

The effect of exposure to D.D.T., if any, on the development of sporogony cycle was not reflected in resistant or in non-resistant strains of *C. fatigans* as measured by the results of comparative infections. Sporogony cycle was completed in a normal manner in resistant *C. fatigans* in which the mechanism responsible for aborting or counteracting the lethal action of the toxicant was activated by one sublethal and three lethal exposures to D.D.T. on alternate days which covered the entire incubation period or by four lethal exposures once daily during the early stages of the parasite development. Likewise, the results were similar in non-resistant *C. fatigans* after exposure to sublethal doses of D.D.T.

There was no modification of the host-parasite relationship when resistant *C. fatigans* were subjected to a lethal contact with D.D.T. for as long as six hours

preceding infective blood feed. The presence of absorbed D.D.T. in the surviving specimens did not seem to operate against the conjugating gametes or oökinetes that were being then formed in the stomach of the mosquitoes. In other words, acquisition of infection was not inhibited.

TABLE I.

Infection with P. relictum *in D.D.T. resistant and non-resistant strains of* C. fatigans *exposed to D.D.T.*

Experiment number	Exposed or not	*C. fatigans*									
		Resistant strain					Non-resistant strain				
		Number dissected.	Gut positive.	Glands positive.	Total positive.	Per cent total positive.	Number dissected.	Gut positive.	Glands positive.	Total positive.	Per cent total positive.
1	Exposed	13	—	7	7	54	2	—	1	1	50
	Unexposed (comparison)	11	—	6	6	55	7	1	3	4	57
2	Exposed	21	—	8	8	38	11	—	5	5	45
	Unexposed (comparison)	21	2	9	9	43	14	—	6	6	43
3	Exposed	51	5	25	30	59	—	—	—	—	—
	Unexposed (comparison)	33	1	18	19	58	—	—	—	—	—
4	Exposed	14	3	5	6	43	—	—	—	—	—

Details:—

Experiment 1. Two exposures of 40 minutes each, the first within 12 hours and the second on third day of the infective blood meal in both.

Experiment 2. First exposure of 40 minutes immediately after infective blood meal followed by three separate exposures, once every other day, of one hour each for resistant *C. fatigans* and of 20 minutes each for non-resistant *C. fatigans*.

Experiment 3. Four exposures of one hour each once daily, commencing from the first day of infective blood meal.

Experiment 4. A single prolonged exposure of six hours preceding infective blood meal.

Note: 1. Resistant *C. fatigans* were of Generation 43 in Experiment 1 and of Generation 44 in Experiments 2 to 4.

2. Dosage of D.D.T. was 200 mg. in acetone per sq. ft. in all exposures.

3. Usually only salivary glands were dissected for sporozoite infection. In a few cases, guts were also examined, particularly if glands were found negative.

From the foregoing observations it seems reasonable to conclude that sporogony cycle of *P. relictum* is completed in a normal manner in resistant and non-resistant strains of *C. fatigans* subjected to lethal and sublethal exposures to D.D.T., respectively.

DEVELOPMENT OF SPOROGONY CYCLE OF HUMAN MALARIA IN RESISTANT AND NON-RESISTANT STRAINS OF *A. FLUVIATILIS.*

Experiment 1.—Resistant and non-resistant *A. fluviatilis* were caged separately in screened bamboo rings and applied to a gametocyte carrier of *P. vivax.* All the mosquitoes could not be fed on the donor who on account of tender age (three years) would not tolerate mosquito bites. The blood-fed mosquitoes being small in number, were not exposed to D.D.T.

Experiment 2.—One lot of resistant and another of non-resistant strain were fed simultaneously on a crescent carrier.* Here, again, the mosquitoes were not exposed to D.D.T. in the hope of utilizing them for a neuro-syphilis case under the treatment of the Medical Officer, Government Hospital, Mettupalaiyam, South India. This, however, did not materialize. The results of dissection of both the experiments are shown in Table II.

TABLE II.

Infections with human malaria in D.D.T.-resistant strains of A. fluviatilis.

Experiment number	Species of Plasmodium	*A. fluviatilis*									
		Resistant strain					Non-resistant strain				
		Number dissected	Gut positive	Glands positive	Total positive	Per cent total positive	Number dissected	Gut positive	Glands positive	Total positive	Per cent total positive
1	*P. vivax*	49	2	2	3	6	...	...	...	...	...
		*20	0	0	0	0	*14	0	0	0	0
2	*P. falciparum*	52	4	18	21	40	75	...	39	39	52

Note: 1. In both the experiments mosquitoes were not exposed to D.D.T.
*2. Partially fed.

It would appear that both the resistant and non-resistant strains of *A. fluviatilis* were almost equally susceptible to infection with human malaria.

DEVELOPMENT OF SPOROGONY CYCLE OF *P. GALLINACEUM* IN NON-RESISTANT STRAIN OF *ÆDES ÆGYPTI* AFTER EXPOSURE TO D.D.T.

Experiment 1.—In this experiment, mosquitoes were divided into three groups. Females in group (*a*) were first fed on an infected fowl and then exposed to 25 mg. of D.D.T. for five minutes. Out of 300 engorged females, 227 (or 76 per cent)

* Thanks are due to Dr. A. Kanagaraj, Medical Officer, Government Hospital, Mettupalaiyam, South India, for according permission to feed mosquitoes on this patient.

were found to have died the following day. In group (*b*) females were first exposed to D.D.T. as in group (*a*) and then given a chance to feed on the same infected fowl. Very large numbers of mosquitoes were used because only a small proportion of the excited females could pierce the skin and take a blood meal. The third group (*c*) which was not exposed to D.D.T. was kept for comparison.

Experiment 2.—In this experiment, *Ædes ægypti* females were exposed to D.D.T. (25 mg. per sq. ft.) as gravids for three minutes. The following morning mortality among these mosquitoes was found to be 83 per cent (166 out of 200 females).

The results of dissection of mosquitoes of the different groups of Experiments 1 and 2 are detailed separately in Table III.

TABLE III.

Infection with P. gallinaceum *in non-resistant strain of* Ædes ægypti *exposed to sublethal doses of D.D.T. (25 mg. per sq. ft.) for five minutes.*

Experiment Number	Conditions of experiment	Number dissected	Gut positive	Glands positive	Total Positive	Per cent total positive
1*a*.	First given infective blood meal and then exposed to D.D.T.	36	—	19	19	53
b.	First exposed to D.D.T. and then given infective blood meal	19	—	10	10	53
c.	Comparison (unexposed)	21	—	10	11	52
*2 *a*.	Exposed to D.D.T. as gravids	24	19	14	19	79
b.	Comparison (unexposed)	26	16	10	16	62

*Period of exposure, only three minutes.

It will be seen that a sublethal exposure to D.D.T. of *Ædes ægypti* preceding or succeeding infective blood meal or in a stage of gravidity was found to have no effect on the development of *P. gallinaceum* inside the mosquito host. The index of infection was practically the same in all the groups of *Ædes ægypti* whether exposed to D.D.T. or not.

Experiment 3.—This experiment was started with *Ædes ægypti* most of which had recently become infected with sporozoites of *P. gallinaceum* in the salivary glands. About 75 of these females were exposed to 25 mg. of D.D.T. per sq. ft. for five minutes.

As soon as the exposure was over, the mosquitoes were given an opportunity to feed on a normal fowl (G. 79) enclosed suitably inside the cage. The mosquitoes, with developing symptoms of D.D.T. poisoning, were unable to penetrate the skin for a blood feed until after an hour when one female had become engorged. This was dissected immediately and it showed a moderate sporozoite infection of the salivary glands. Again, one hour later, the same fowl was bitten by three

mosquitoes in the course of 25 minutes, of which two were found infective. At this point feeding was discontinued. The normal fowl (G. 79) was thus inoculated with sporozoites by bites of three known infective specimens which succeeded to obtain a blood meal after exposure to D.D.T.

Another group of infected mosquitoes from the same stock was exposed to D.D.T. as the above group and released into a separate cage. A new normal fowl (G. 80) was brought into contact with these irritated mosquitoes by holding it against a side of the bobbinet cage from outside. It was bitten by two proved infective mosquitoes, one after 50 minutes and another after one hour of the exposure.

Both the normal fowls (G. 79 and G. 80), which were inoculated with sporozoites by bite of the infective mosquitoes after sublethal exposure to D.D.T., developed patent infection which ended fatally in one and in a spontaneous recovery of another. The results demonstrate that a sublethal contact with D.D.T. of infective mosquitoes did not render the sporozoites non-infective to normal mosquitoes.

The marked change in the biting potential of *Ædes ægypti* after exposure to D.D.T. is noteworthy. The same specimens of *Ædes ægypti* which would have immediately crowded and fed on the fowl in a few minutes, were practically unable to do so after exposure to D.D.T.

Experiment 4.—Mulligan *et al.* (1940) have reported that sporozoite agglutination in high dilutions of malarial serum is a specific reaction. This test was made use of to find out if sporozoites in salivary glands of *Ædes ægypti* had undergone any change after exposure to 25 mg. of D.D.T. for ten minutes. Sporozoites from heavily infected specimens with self amputated legs and almost at death point, were dissected in different dilutions of homologous chronic serum. The results suggest that sporozoites in highly paralyzed *Ædes ægypti* were not impaired antigenically.

Inoculation of sporozoites from a highly paralyzed specimen into a normal fowl (G. 81) caused patent infection after an incubation period of eight days, showing thereby that the sporozoites were infective. The parasites reached their peak on the fourth day and disappeared from peripheral circulation on the seventh day.

VIABILITY OF *P. GALLINACEUM* IN INFECTED FOWLS POISONED WITH ORAL ADMINISTRATION OF D.D.T.

Having demonstrated that the development of malaria parasites in resistant and non-resistant strains of mosquitoes was not affected in any way by exposure to D.D.T., it was thought desirable to ascertain whether D.D.T. orally fed to fowls, would inhibit the multiplication of parasites and/or render the gametocytes non-infective to mosqiutoes. The idea came from the work of Wilson (1948) who estimated biologically the effects of B.H.C. and D.D.T. in the bovine blood by feeding arthropods on treated animals. The two experiments carried out are described below.

Experiment 1.—One normal fowl was inoculated with the blood of a fowl harbouring chronic infection. When infection first became patent, the fowl was given D.D.T. in olive oil by mouth at the rate of 100 mg. per kilogramme of the body-weight for three days in succession. On the fourth day, the fowl began to develop early symptoms of D.D.T. poisoning and its blood picture revealed a number of male and female gemetocytes. On the same night when the fowl was in convulsions and prostration, it was placed bodily inside a cage containing large numbers of *Ædes egypti* which became engorged in a short space of time. Soon after blood feed, most of the mosquitoes began to show toxic symptoms. In some specimens, the symptoms deepened and they fell down in the cage. After about 24 hours, more than half the females were dead. A few females which survived to complete the extrinsic incubation period, were dissected for finding out oöcysts and sporozoite infection. All the 12 specimens were found infected with both oöcysts and sporozoites. These sporozoites were normal in appearance in fresh and stained preparations. One normal fowl which was bitten by two infective specimens developed infection on the ninth day and died of malaria seven days later.

It would appear from the foregoing that in a normal fowl poisoned with D.D.T., the parasites multiplied and produced viable gametocytes which on being ingested by mosquitoes resulted in the production of sporozoites which, in turn, were proved to be infective to a normal fowl.

Experiment 2.—A normal fowl was inoculated with blood from the fowl with chronic infection. Before infection became patent, the fowl was given D.D.T. in olive oil as in the above experiment on three consecutive days. For some unknown reason, the fowl showed no signs of illness on the fourth day as expected, but died on the twentieth day of D.D.T. poisoning. The parasites which appeared in the peripheral blood after a prepatent period of six days, multiplied and when the density of gametocytes was adequate, the fowl was exposed to the bites of *Aedes egypti*. The engorged females were slow in developing sysmptoms of D.D.T. poisoning. After about four hours, most of the mosquitoes were unquestionably irritated in varying degrees. The surviving females showed 85 per cent infection of salivary glands with sporozoites (17 out of 20 dissected).

DISCUSSION.

It is well known that certain species of mosquitoes have developed resistance to D.D.T., particularly in areas where D.D.T. has been used extensively and intensively in massive doses. This insecticide resistance may be slight so that mosquitoes are still killed by the insecticide or may have reached a stage where D.D.T. is of little or no practical value in mosquito control. The importance of the development of resistance, particularly in a vector species, would seem to depend largely on whether the ability of a resistant strain to withstand the doses of D.D.T. which would kill a non-resistant strain of the same species, would also operate adversely on malaria parasites in an infected mosquito. In discussing the possible effects of residual insecticides on the interruption of malaria transmission, Gabaldon (1953) observes: "If physiological resistance appears, its importance may depend on the fact that the sorbed insecticide may injure or not

the malaria parasite in any of its stages in the mosquito, a fact unknown at the present time". The same author again records "Nobody knows what happens to oöcysts or to sporozoites inside slightly intoxicated mosquitoes ".

On the basis of data on hand, it is evident that mere acquisition of D.D.T. resistance which was high in *C. fatigans* and low in *A. fluviatilis*, did not result in any concomitant change of the vectorial capacity of these two species. Resistant and non-resistant *C. fatigans*, which originated from the same parent, were almost equally infected with *P. relictum*. Similarly, resistant and non-resistant strains of *A. fluviatilis* showed no difference in their susceptibility to infection with human malaria.

It is also evident that activation of the mechanism of resistance in resistant *C. fatigans* by lethal contact and in non-resistant *C. fatigans* by sublethal contact with D.D.T., produced little or no effect on the development of sporogony cycle. Both the groups were infected with *P. relictum* to about the same extent as were the comparison groups.

It will be further observed that sporogony cycle was completed in a normal manner in resistant *C. fatigans* which were exposed to lethal doses preceding infective blood meal or thereafter during the early or entire extrinsic incubation period.

Experiments with *P. gallinaceum* indicate that a sublethal exposure to D.D.T. had no effect on the parasite in any stage in *Ædes ægypti* and that when D.D.T. was fed orally to fowls, the parasites multiplied and gametocytes remained viable.

Finally, it would also appear that if contact with D.D.T. resulted in any change, physical or chemical, in the body of an infected mosquito, the malaria parasites were not apparently influenced by it in their natural environments.

It is not known whether D.D.T. absorbed by mosquitoes found its way to the parasites in an unchanged condition or in the form of some less toxic metabolite. It is again problematical whether malaria parasites are subject to the lethal action of D.D.T. or whether they behave like plankton organisms which are not killed by this insecticide (Bishop, 1947).

SUMMARY AND CONCLUSIONS.

1. The acquisition of D.D.T. resistance by *C. fatigans per se* had no apparent effect on its vectorial efficiency. Both the resistant and non-resistant strains originating from the same female were about equally infected with *P. relictum* up to the sporozoite stage. There was no change in the host-parasite relationship when the mechanism of resistance was stimulated by exposing the resistant strain to lethal doses of D.D.T. preceding infective blood meal or thereafter during the early or entire extrinsic incubation period. Sporogony cycle was completed in a normal manner in resistant and non-resistant strains, irrespective of the exposure to D.D.T.

2. Resistant and non-resistant strains of *A. fluviatilis* were almost equally susceptible to infection with human malaria. Resistance in *A. fluviatilis* was, of course, low but it was clear-cut.

3. A sublethal exposure to D.D.T. of *Ædes ægypti*, preceding or succeeding infective blood meal or in a stage of gravidity, was found to have had no effect on the acquisition of infection with *P. gallinaceum* or its subsequent maturation to sporozoites. Exposure of the infective females did not render the sporozoites non-infective to normal fowls.

4. Oral administration of D.D.T. to fowls infected with *P. gallinaceum* did not inhibit the multiplication of the parasite, nor did it have any apparent effect on the viability of gametocytes.

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POLICY IN RELATION TO MALARIA CONTROL.

BY

S. R. CHRISTOPHERS.

(August 1, 1955.)

How important is policy in measures directed to the control of malaria in a country? By policy is meant all that goes to the thinking out of what should be done. It includes what it is desired to do, what it is possible to do and how this last can best be done. It is sometimes not very clearly thought out, often based on imperfect information and with the best intentions it may not be the best that could be adopted. One thing it very commonly lacks, *viz.*, adequate provision to show what results have been achieved. It is, therefore, worthwhile to give careful thought to what it is hoped to do, how it can best be done and how if it is done it can really be known to be done.

What it is hoped to do may be the elimination of malaria as a human disease. To have any relation to reality such hope must be more specific in time and place. There are two words in use in the fight against malaria, *viz.*, control and eradication: those whose expectations are aimed high prefer eradication, those less optimistic use the word control. As in many human affairs a great deal depends on the particular circumstances and what is reasonable to hope in some circumstances would be unreasonable in others. Whatever the objective, however, whether control or eradication, much the same techniques must be used. Malaria is almost unique as a disease in the great varieties of ways in which it may be attacked and the number of techniques that can be used in doing so. The important point that policy has to decide upon is which of these techniques to use. It may be useful to indicate briefly what these techniques are.

Early control measures, following upon Ross's discovery of the mosquito transmission of malaria, were almost entirely based on action taken against the breeding places of Anopheles. The only important rivals to such measures were communal quinine prophylaxis and screening. Discovery of paris green as a more efficient and easily handled larvicide and the work of Malcolm Watson in Malaya exploiting the use of drainage and other methods of larval control, together with the conception of species sanitation, appeared to show that control through antilarval action was possible and that where it could be carried out it was the method of choice as being the most fundamental. Other measures such as the

trapping and spraying or fumigation of adults, use of communal quinine and protection took their place as measures to be used where antilarval work was unsuitable or as supplementary measures of securing success. So successful in certain cases had such work been shown that in place of merely reducing Anopheles there came the ideal, where circumstances allowed, of eradicating once and for all a vector species. The conditions necessary for this method were that the area must not be too great, though it was not necessarily restricted to an island, and considerable areas were treated on this basis.

Some operations of this kind have been highly successful, *e.g.*, *Anopheles gambiæ*, which appeared to have invaded Brazil from Africa and there had occasioned serious epidemics of malaria, as a result of a determined and systematic attack launched by the Rockefeller Foundation in association with the Brazil Government under Doctors Soper and Bruce Wilson, was completely eradicated from North East Brazil and up to the present (Russell, 1955) Brazil has remained free from this species. Another successful anti-*gambiæ* eradication operation has been carried out in Upper Egypt (invaded area 4,270 sq. miles and population some three million). In Sardinia, at a total cost of 12 million dollars and with the assistance of some thousands of scouts, an attempt was made to eradicate the vector species. But though malaria was eradicated the operations were not completely successful in eliminating the indigenous species of Anopheles. In Cyprus, an island with endemic malaria (spleen rate about 25 per cent), there was an example of the use of the new insecticide D.D.T. as a larvicide. Here also an attempt to eliminate the indigenous Anopheles was unsuccessful, though as a result malaria was much reduced.

In the first world war antilarval control, with in addition quinine prophylaxis where larval control was impractical or required supplementing, was still the recognized major control measure. Extensive operations in Palestine, Mesopotamia and other areas based on such measures were, however, but moderately successful. Nevertheless much was learnt regarding protection of troops that was to bear fruit in the second world war.

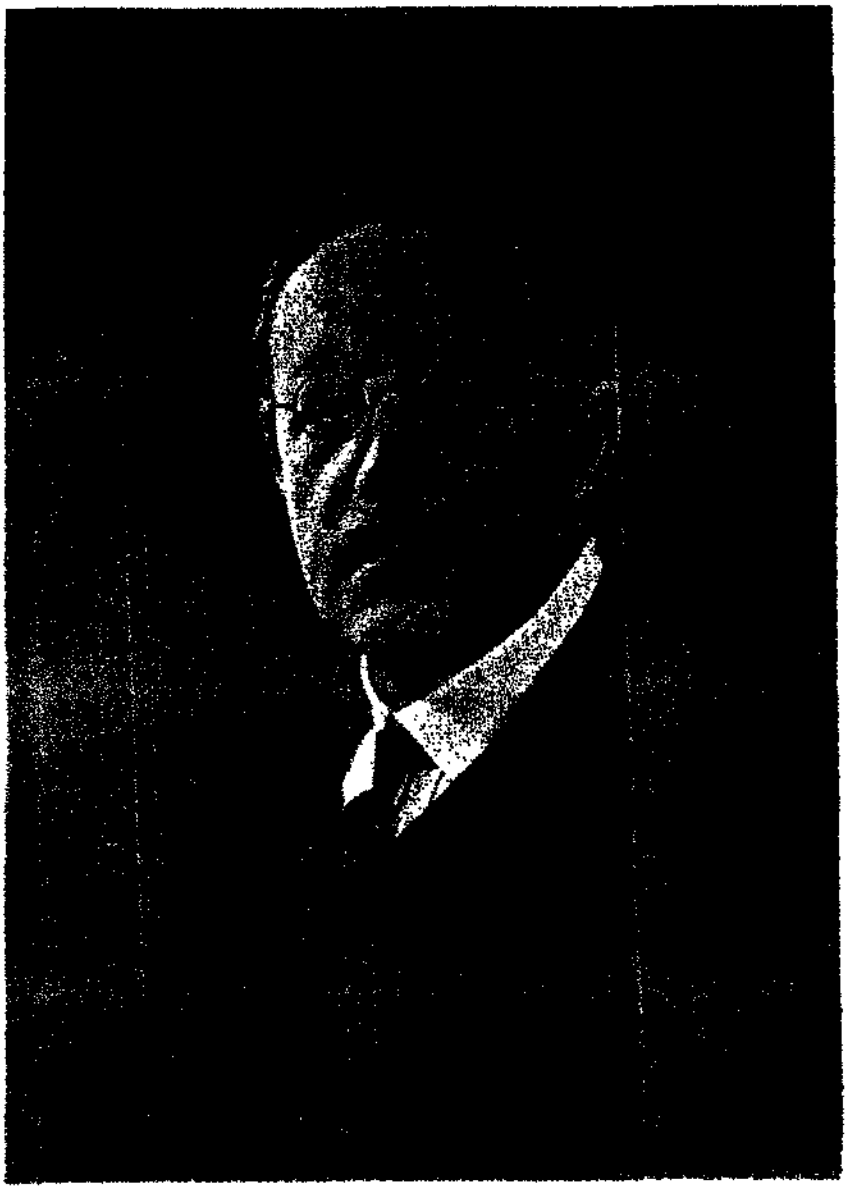
In the period between the two wars, two things revolutionized ideas regarding control. These were the discovery of new more and more effective synthetic antimalarial drugs and the results from the new insecticide D.D.T. used in residual spraying. Drug prophylaxis through mepacrine, now as a result of the previous war pushed as a prophylactic measure with the full and effective support of the military commands, was practically the answer to malaria control in troops. The new and powerful insecticide D.D.T., used first as a larvicide and eventually along with a number of related compounds in residual spraying, has been so dramatically successful that many have considered it the final answer as to how malaria can be fought.

With this brief resume of the present position with respect to various forms of techniques used against malaria, we may usefully examine some considerations that affect policy. It will be most convenient to do so chiefly in relation to experience in India.

AREA AND POPULATION.

In Table I, are given the area, population and persons per *square mile* in a number of tropical malarious countries.

PLATE VIII.



Br.-Colonel Sir RICKARD CHRISTOPHERS, [illegible]. Retd.
In charge, Central Malaria Bureau, Kasauli, 1910-1916 and 1918-1924.

TABLE I.*

Area, population and persons per square mile in a number of tropical malarious countries.

Country	Area in square miles	Population	Persons per square mile
An English county (Cambridge)	492	177,000	360
Trinidad	1,863	678,000	356
Cyprus	3,570	505,000	140
Sardinia	9,300	1,220,000	131
British Guiana	83,000	450,000	5
Venezuela	352,000	3,323,000	15
,, (treated area)	69,000	2,431,000	35
Bolivia	415,000	3,788,000	9
Gold Coast	24,000	2,223,000	92
Nigeria	373,000	31,200,000	83
Belgian Congo	910,000	12,115,000	13
Tropical Africa as a whole (From 15° N. to the level of Beira)†	6,000,000	60,000,000	10
Pakistan	364,700	75,840,000	207
India	1,143,000	356,179,000	312

* Data, except for tropical Africa as a whole, from Whittaker's Almanac, 1955.

† Area for tropical Africa has been measured from map and given approximately. The figure for population is probably an under estimate.

Such a list serves to show the relative scale of operations that would be required for these countries and emphasizes the special cases of India and some tropical African countries.

ECONOMIC STATUS.

There will almost certainly be a great difference in what a prosperous and perhaps largely europeanised country can do as compared with a relatively poor country much of the population of which may be living in rural or even jungly conditions. Between two such, the whole question of malaria control differs enormously and, except as experiment for some particular purpose, what one might almost term smash and grab operations are not usually the most suitable in the latter circumstances.

VITAL STATISTICS.

One point that it is very necessary to know in a country where the question of action against malaria arises is how much malaria there actually is. Normally it is assumed that this will be shown by the vital statistics, as also whether any steps that have been taken have produced the desired result. Unfortunately, where the malaria problem is most urgent, vital statistics of any value commonly do not exist. India is fortunate in this respect since, especially in some areas, registration of deaths with age, village, etc., is well kept and if studied with a knowledge of the method of collection may be relied upon up to a certain extent. The cause of death is another thing and cannot be expected to be accurate. In general, vital statistics are of little use except in very special cases in determination of malarial incidence. Hospital records in such countries often represent only a fraction of the real number of cases of a disease. Medical men may be, and usually are, so small in proportion to the population in rural and jungly areas that any attempt to arrive at an estimate of the effect of operations on a large scale through medical diagnosis would be liable to grave doubt. To do so from films sent in to headquarters might be extremely misleading. Unless a good system of returns of death is in existence or there is a sufficiency of effective dispensaries any real estimate of the amount of malaria would require expert investigation.

DISTRIBUTION OF MALARIA.

It is not usually that a country is uniformly malarious, or even that there are not extensive areas where malaria is not a serious problem. In India there are some considerable areas that come under the designation of " healthy ". In such areas malaria is often restricted to small foci of infection where conditions happen to be favourable to the disease. Other tracts may be hyperendemic, *i.e.*, malaria is no longer an occasional disease, but one in which infection in childhood is universal, or almost so, and adults are largely immune. Even the children, though infected, often show little evidence of being ill and in true hyperendemicity as seen in some primitive tribes it may be difficult to show that there is an increased child mortality. On these accounts the urgency or even the desirability of taking action under such conditions has been considered by some authorities to be doubtful. It is a matter of opinion with little factual evidence to go on. Such areas may be quite extensive and have been mapped and much studied in India. In other forms of high incidence communities may be in a miserable state of perpetual illness from malaria and in need of urgent relief. These may be the conditions in communities colonizing new areas and perhaps labour connected with important industries or projects. Without some system of ascertaining what areas and communities are in need of action to be taken, wide-scale institution of some particular method of control is not a policy to be recommended. Much attention in India has been given to field studies in the villages in different parts of the country. In tropical Africa similar enquiries have been made, *e.g.*, by Bagster Wilson, but there must be large areas about the malaria in which little is known.

EPIDEMIC MALARIA.

One form of malaria, that termed seasonal epidemic, or when very severe fulminant malaria, is especially important. Its characters are sudden onset with

almost universal infection and high death rate. Such epidemics in India occur only at intervals of a decade or so, between which times it is characteristic that malaria is of relatively low incidence. It has been shown by Gill who has made a very thorough research into the nature and origins of such epidemics in India and Ceylon to be a phenomenon related to a normal low malaria incidence with decreased immunity followed in certain years by unusual and still not very well understood conditions favourable to malaria. In the year 1908, a great epidemic of this kind swept through north west India in the course of which in some three months tens of thousands of people died, the mortality in some areas reaching the fantastic figure of 400 per mille, whilst the area covered almost simultaneously was an area nearly as large as England and Wales. A careful study following upon the epidemic showed that its most characteristic feature was widespread flooding from the rivers. Since 1908, the attention of engineers has been especially directed to this danger of flooding and appropriate measures on a large scale to mitigate such effects have been taken. Whether on this account or not, these epidemics which usually occurred about every eight years as far back as records go have never since occurred on a like scale. Study of relatively small affected areas has shown that the measures which can be adopted are mainly organized arrangements for treatment and free drug distribution. Areas liable to such epidemic effects have been mapped and it has even to some extent, through studies of the spleen rate and other factors, been possible to foretell likely affected areas.

STAFF.

For operations on any scale trained staff is a serious problem. This must necessarily include, besides labour and operators for whatever method is adopted, a sufficiency of trained malariologists to direct and supervise operations. During the last war the sudden call for personnel to equip antimalarial units operating in the various countries in the Middle East was largely met from the Malaria Institute of India, this being about the only large organization where training was, and had been, systematically carried out. In the years of the war, the Institute supplied for this purpose 555 trained malaria officers, 426 trained personnel and 87 engineers that had received malaria training.

ORGANIZATION.

It will be clear from this brief account that organization and research are a very essential part of anti-malarial work. Without them measures must be taken blindly and proof that anything has been achieved may leave much to be desired. Whilst all-out attacks can be made on malaria where large funds are available and circumstances favourable, it would seem these are not most suited to undeveloped areas or countries, such as many are in the tropics, where malaria assumes a variety of forms and degrees of intensity. What seems to be more needed in this case, especially where the country at issue is large, is some permanent organization where investigation and control can work hand in hand, its staff able to cover all the necessary fields of expert knowledge, familiar with field conditions, expert in malaria techniques, with access to government and supported on a scale proportionate to the task committed to them. In India in the course of the last

thirty years or more there has been developed a centre of malaria research and control on a scale and with an output of results that can safely be said to be second to none other in the world, *viz.*, that now named the Malaria Institute of India.

Only very brief mention can here be given of the history and work of this Institution. A very instructive and complete account, however, will be found given by Covell in two communications, the first dated 1938 in Vol. 1 of the *Journal of the Malaria Institute of India* giving the history and work of the Malaria Survey of India (1927-1937) and the second dated 1947 in Vol. I of the *Indian Journal of Malariology* giving the history and work of the Malaria Institute of India up to that date. Up to 1909, there was in India no organized plan for malaria research or control. In that year, however, proposals were made for a malaria organization for India by Lieut.-Colonel J. T. W. Leslie, Sanitary Commissioner with the Government of India, whose far-sighted policy was also responsible for the building up of the Bacteriological Department of the Government of India in which was combined the running of the large Central and Provincial Laboratories and much else. The scheme included a General Committee (with the Minister as President) with a delegate from each Province and Provincial Committees to obtain information and supervise local enquiries. A part of the scheme was the Central Malaria Bureau (1910-1916 and 1919-1927). At the Bureau were instituted a reference library on malaria, collections which later formed the basis for work on the mosquitoes of India, and other activities aimed at advancement of malaria work throughout India and Burma. The Bureau maintained close connection with researches and surveys carried out by the Provincial Malaria Officers many of whose names have been then and later familiar in the literature. It also held an annual class of instruction for medical officers taking up malaria work in which both field work and necessary laboratory techniques were undertaken. Unfortunately the first world war necessitated the calling up of most of the Provincial Malaria Officers for duty in different theatres of war and the organization was for a time in abeyance. Proposals, however, in 1924 for a renewed organization led in 1927 to the formation under the Indian Research Fund of the Malaria Survey of India with Lieut.-Col. (now Brigadier) J. A. Sinton as Director. At first located at Amritsar, a city severely affected by the 1908 epidemic, it was later moved to Karnal where operations were in progress, but later at the director's suggestion to the present headquarters in Delhi. In 1938, at a meeting of the Indian Research Workers Association, the Government of India agreed to finance the now greatly enlarged organization, which under the name of the Malaria Institute of India continued work under the directorship of Col. (now Major-General Sir Gordon) Covell, followed on his retirement in 1947 by Lieut.-Col. Jaswant Singh. Throughout these years innumerable surveys have been made of different areas in India, control measures carried out and in almost every field of malaria control practical measures and techniques perfected. Some idea of the extent and variety of the work carried out may be gathered from the list of papers published in the Journal of the Institute. In recent years a great deal of study and experimentation has been given to residual spraying and its effects on which many papers will be found in the Journal. Should residual spraying be found to give all that it promises one may safely leave its full exploitation in control of malaria to the present director and his expert staff. That there

is no lack of initiative is shown by the recent formation of an associated Society with its own Bulletin, *viz.*, the National Society of India for Malaria and other Mosquito Borne Diseases, of which Lieut.-Col. Jawsant Singh is the President and the objects of which include work on filariasis in India which causes much sickness and is a mosquito-borne disease.

Quite recently I have seen the announcement that a Malaria Institute has been established under the directorship of Dr. D. Bagster Wilson in tropical East Africa, the East African Malaria Unit of the East Africa High Commission having become the East African Institute of Malaria and Vector-Borne Diseases. Such a step has very much to recommend it.

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DEVELOPMENTS IN MALARIA CONTROL METHODS DURING THE PAST FORTY YEARS.

BY

SIR GORDON COVELL, M.D., D.P.H.

(July 4, 1955.)

A PERIOD of 40 years has been selected for this review because it corresponds approximately with the writer's own experience of malaria and its control. The developments which have evolved during this time in the methods used to combat the disease may be considered under the headings of (*a*) antimosquito measures and (*b*) chemotherapy.

ANTIMOSQUITO MEASURES.

During the first half of the period the measures adopted were directed almost exclusively towards the destruction of the aquatic stages of the mosquito, the egg, larva and pupa, usually referred to as antilarval measures. Some of the earlier campaigns, such as those undertaken in Malaya, Ismailia, Cuba and the Panama Canal Zone, were very successful. They were costly, but in each case important financial considerations were at stake, and the expenditure was amply repaid by the improvement in health which resulted, particularly among the labour forces employed.

In India, the prosecution of antilarval measures received a serious setback from the comparative failure of the campaign undertaken at Main Mir, later known as Lahore Cantonment. The extreme flatness of the land in this area made drainage operations difficult, and though mosqutio breeding within the cantonment and in the immediate vicinity was controlled to a great extent, these measures were largely vitiated by the infiltration of *Anopheles culicifacies*, the chief mosquito vector, from outside the protected area. The campaign was further discredited when the great regional malaria epidemic of 1908, one of the most severe ever experienced, swept over the Punjab. Main Mir lay within the epidemic area, and many cases occurred among the troops stationed in the cantonment and their families.

During the next 25 years, antilarval measures were practised with some success in various urban areas, in tea, coffee and rubber plantations and in connection with large scale engineering projects. Following Bentley's survey of 1909-1911, a Special Malaria Department was created for Bombay City and a

number of antilarval measures were put in force, directed chiefly towards the prevention of mosquito breeding in wells and cisterns, the favourite breeding places in Bombay of the vector, *A. stephensi*. As a result, the malaria rate was greatly diminished, so much so that the local authorities decided that the measures could be relaxed. Accordingly in 1918 the Malaria Department was disbanded; but the meagre staff remaining proved entirely inadequate to cope with the situation. Covers and trap-doors of wells and cisterns were removed or allowed to get into disrepair and numbers of wells which had been sealed were re-opened. The incidence of malaria began to increase and in 1922 the heads of 40 commercial houses sent in a petition to the Corporation drawing attention to the prevalence of the disease in the city and to its harmful effects on commerce. In 1923, the Malaria Department was reconstituted, but much of the work previously accomplished had to be re-done: in 1924, the disease was again very prevalent in Bombay and there were a number of cases among the crews of ships berthed in the docks. A Central Malaria Committee was appointed and the staff increased, but malaria continued to be a serious problem and it was decided to hold another survey of the whole island. This survey, which lasted six months and involved the examination of more than 30,000 children, was carried out by the writer in 1928. The recommendations then put forward, which were directed solely towards the prevention of mosquito breeding, were implemented with excellent results.

In Assam, antilarval work was carried out in a number of tea estates under the auspices of the Ross Institute, notably by Ramsay in and around Labac. An interesting development here was the growing of dense vegetation over ditches, streams and swamps to prevent mosquito breeding. This measure was very successful for some years, but in the course of time the bushes used for shading began to invade the tea, necessitating the employment of extra staff to keep it cut back. Eventually in many instances estates reverted to open drains and oiling for the control of mosquito breeding. A similar course of events occurred in Malaya, where extensive subsoil draining was installed in rubber estates. Here again the measure was at first successful, but eventually the roots of trees grew down in between the tiles in search of water and choked the channels, so that an open space 1½ times the height of the trees had to be left on each side of the pipes. Since a rubber tree may grow to a height of 60 feet or more, a large proportion of the plantation was thus put out of action. In many estates the subsoil pipes were pulled up and substituted by open drains and oiling.

Mention may be made of two engineering projects in India whose construction was made possible solely by the rigid application of antilarval measures, namely the Sarda Canal Headworks Project, in what was then known as the United Provinces, and the building of the Raipur-Vizianagram branch of the Bengal-Nagpur Railway. The construction of the latter project was held up for 40 years owing to the prevalence of intense malaria, the incidence of the disease among the survey parties being so high that the first three were unable to complete their task, while the fourth succeeded only when every engineering post in the party was duplicated. The construction of the railway was finally made possible by the work of the late Major Senior White, Malariologist to the railway, who instituted rigid antilarval measures in and around the camps in which the labourers were concentrated.

During this period, the chief methods employed for the control of mosquito breeding were drainage and the application of various kinds of oil to water collections. A notable advance was the introduction as a larvicide of paris green, following the demonstration of its value in the field by Barbar and Hayne in 1921. From this period until the advent of D.D.T., paris green was used very extensively in large-scale antimalaria campaigns throughout the world. It was the principal method employed in the Brazilian campaign of 1939-40, which resulted in the eradication of *A. gambiæ* from that country.

The experience gained during the earlier antimalaria campaign in India and elsewhere showed that the disease could probably be controlled anywhere by antilarval measures, *provided that sufficient funds were available for the purpose.* Under urban and industrial conditions, where the population at risk was concentrated in a limited area and where its potential output was economically productive, it could usually be demonstrated that the cost of malaria to the community was considerably greater than that of effective control, even by the measures then available. It was the control of *rural* malaria that for so many years proved an insoluble problem to the health authorities of tropical and subtropical countries. Here the population, instead of being concentrated in compact areas, is scattered over wide tracts of country. Villages often consist of isolated groups of houses dispersed over several square miles of country which usually harbours innumerable breeding places for malaria-carrying mosquitoes. Malaria was, indeed, controlled in rural areas in certain instances as a demonstration project, but only at a cost far greater than any sum which the local authorities could afford. Under such circumstances the only way of ameliorating the condition was to provide treatment for the sick, a palliative rather than a control measure.

This was the position as late as 1936, when the publication of the remarkable results achieved in South African villages by the spray-killing of adult mosquitoes with pyrethrum insecticides at a moderate cost encouraged the hope that here at last was a weapon which might prove practicable for the control of malaria in rural India. Just at that time a comprehensive scheme of malaria control was being put into operation in Delhi urban area, which covers about 75 square miles of country. The quarters occupied by certain selected communities of Government employees located in particularly malarious sections of this area were regularly sprayed throughout the malaria season. The results were remarkably good, and in one set of quarters the malaria rate was reduced to 1·4 per cent, a figure of 45 per cent being recorded in adjacent quarters which were left unsprayed. The method was immediately recommended for use throughout India for personnel such as police, railway, forest or other government employees and labour forces in estates, mills and other industrial enterprises. It was at first thought that its usefulness would be limited to such conditions, but in 1937 it was tried as an experimental measure in two villages on the outskirts of Delhi and was subsequently extended to a number of others further afield, with encouraging results. Further experimental work on these lines was carried out by the Malaria Investigations Unit of the Rockefeller Foundation International Health Division in certain villages in southern India during the period 1938-41. The first large-scale routine application of this measure in Indian villages was, however, carried out in Mysore State.

When the Bombay State Malaria Organization was created in 1942, the spray-killing of adult mosquitoes with pyrethrum insecticide became an important feature of the control programme in Kanara District. Early results in this area were somewhat disappointing, but the adoption of a programme of spaced spraying based on a series of patient researches was followed by a striking reduction in the malaria rate. In September 1945, a supply of two tons of D.D.T. which had been imported into India for military purposes was made available to the Bombay Malaria Organization for field trials. From then onwards the spray-killing of mosquitoes with residual insecticides became the main feature of the campaign, which was progressively extended to cover the whole of the malarious parts of the State.

In the same year a large-scale project was launched in Venezuela with the object of eradicating malaria from the entire country by D.D.T. residual spraying, and similar campaign was inaugurated in British Guiana. Since then, D.D.T. spraying projects have been carried out in Brazil, Argentina, Bolivia, Peru, Ecuador, Columbia and French and Dutch Guiana. In the United States a joint programme for eradicating malaria was also launched in 1945, with the result that the disease has now practically ceased to exist in that country. Large-scale campaigns are in progress in Italy (including Sicily and Sardinia), Cyprus, Greece, Mauritius, Ceylon, Thailand, the Philippines, Borneo, Iraq and Iran, as well as in India and Pakistan. In all these projects spraying with residual insecticides has been the chief and in many cases the sole, antimalaria measure employed.

It is interesting to recall that the systematic destruction of adult mosquitoes was practised in the Panama Canal Zone as early as 1908, the first to use it being W. R. Procter, a sanitary inspector. It was done by negro labourers armed with chloroform tubes and acetylene lamps. Mosquitoes were also caught in wire gauze traps, placed over the windows. These measures met with considerable success in reducing the malaria rate. The destruction of hibernating adult mosquitoes by hand-catching was also practised in Holland from 1920 onwards, and in 1926 pyrethrum sprays were introduced for this purpose. It was this work in Holland, largely inspired by the insistence of S. P. James on the importance of destroying the infected mosquito and thus interrupting transmission, which formed the basis for the successful campaign in South Africa alluded to above.

CHEMOTHERAPY.

During the first decade of the period under review the only drugs available for the prophylaxis and treatment of malaria were the cinchona alkaloids, of which the most commonly used was quinine. It was customary to give the drug over an extended period, beginning with 20 to 30 grains daily for seven to ten days; this was usually followed by a similar dose twice or thrice weekly for several weeks or even months. During the first world war, quinine was given to the troops in Macedonia in enormous doses, up to 100 grains daily, and was continued for varying periods after the subsidence of symptoms with the object of diminishing the likelihood of relapse. One of the regimens adopted consisted of 45 grains daily for 30 days, a total of 1,350 grains. Another, known as a " sterilizing course ", consisted of 30 grains

of quinine orally plus 30 grains intramuscularly daily for 12 days; 60 grains orally for two days; and 20 grains orally for the following two weeks; a total of 1,180 grains.

The numerous investigations conducted between the two world wars, notably those sponsored by the League of Nations Malaria Commission, together with experience gained during the treatment of neurosyphilis with malaria therapy, resulted in the general adoption of comparatively short courses of treatment for malaria. It was established that any dosage of quinine exceeding 30 grains daily not only fails to affect the course of the disease, but is also detrimental to the health of the patient; and that to extend the course beyond seven days has no effect on the relapse rate.

The evolution of the synthetic remedies now so largely used in the treatment and prophylaxis of malaria makes an interesting story. Quinine is an effective drug for terminating the clinical attack in most malarial infections, and it is doubtful if any of the synthetic drugs now in use would have been developed had not the Germans been deprived of all sources of quinine during the first world war. It was the necessity for finding an effective substitute for quinine which inspired the researches which led to the synthesis first of plasmochin (pamaquin) and later of atebrin (mepacrine).

Up to the time when this work was planned, all attempts to synthesize quinine had failed; but Guttmann and Ehrlich, many years earlier, had discovered that methylene blue stains, and, therefore, presumably penetrates, the malaria parasite and had observed some abatement of clinical symptoms in patients suffering from *vivax* infection to whom the dye had been administered. With these experiments in mind, a team of German scientists embarked on a line of research which was destined to have far-reaching consequences. They introduced a basic side chain into the formula of methylene blue, and found that one of the resulting compounds had considerable activity against bird malaria. It seemed likely that the activity of the quinoline nucleus present in quinine might also be enhanced by the introduction of a similar basic side chain. This line of approach culminated in the synthesis of pamaquin, the first synthetic quinoline compound to exhibit effective action against human malaria parasites.

Pamaquin was found to have a powerful destructive action on the gametocytes of *Plasmodium falciparum*, a property not possessed by any of the cinchona alkaloids; another quite unexpected development was the demonstration in India by Sinton and Bird that when used in conjunction with quinine it effected a marked reduction in the relapse rate of *vivax* malaria. In certain respects, however, pamaquin proved unsuitable as a therapeutic agent, chiefly by reason of its relatively high toxicity and the fact that it has little action on the asexual erythrocytic forms of *P. falciparum*. The Germans, therefore, embarked on further studies; they attached the basic side chain which had been evolved for pamaquin to other heterocyclic nuclei, and finally, in 1930, produced the acridine compound mepacrine.

Mepacrine proved to have a powerful destructive action on the asexual erythrocytic forms of all species of human malaria parasite. It possesses all the antimalarial properties of quinine, and against some strains of *P. falciparum* it is considerably more active. It has, however, the disadvantage of turning the skin yellow and of producing in certain individuals undesirable side-effects.

After preliminary tests on patients undergoing malaria therapy first in Germany and later in England, pamaquin and mepacrine were subjected to an extensive series of field trials under the auspices of the League of Nations. These tests afforded clear and decisive proof of the high suppressive action of mepacrine in all forms of human malaria, though some uncertainty remained as to the possible harmful effects of long-continued administration. When Indonesia fell to the Japanese in 1942, thus cutting off the supply of quinine to the Allies, steps were immediately taken to manufacture mepacrine in large quantities in both Great Britain and in the United States. Mepacrine prophylaxis was rigidly enforced among the Allied troops operating in the South-west Pacific and South-east Asia Commands. This measure resulted in effective control of malaria in both areas and played an important part in achieving final victory.

The German programme of research on synthetic antimalarials had not ceased with the production of mepacrine. They continued their investigations with the object of producing a drug with the same antimalarial properties but without its disadvantages. Removal of the methoxy-bearing ring from the mepacrine molecule gave rise to resochin (chloroquine), a member of the 4-aminoquinoline group of compounds. Preliminary tests of this drug on a small series of patients in a mental hospital in Germany were interpreted as indicating a considerable degree of toxicity, and further research was undertaken to counteract this supposed defect.

The second world war broke out while this work was still in progress and as field tests of the 4-aminoquinolines were as yet incomplete, the Germans adopted mepacrine as the standard antimalarial drug for their troops. After the occupation of France supplies of chloroquine and of an allied compound, sontoquine, were made available to the French authorities for tests in North Africa; when this area was occupied by the Allies, stocks of these fell into the hands of the Americans, who were already engaged in a gigantic research programme in which more than 14,000 compounds were eventually tested for antimalarial activity.

The Americans found that the early German tests of chloroquine had created an exaggerated picture of its toxicity; they also found it superior to sontoquine as an antimalarial drug. It had the advantage over mepacrine of not causing discoloration of the skin; it was found to be in some respects more active than the latter drug and less likely to produce unpleasant side-effects. It was not used to any great extent during the second world war, but was soon afterwards adopted as the standard antimalarial drug for the United States Army. Two other 4-aminoquinolines, amodiaquine (camoquin) and hydroxychloroquine (plaquenil), have similar properties and are claimed to be equally effective.

During the latter half of the second world war, British chemists, adopting a new line of approach, produced a biguanide compound, proguanil (paludrine) which proved to have remarkable antimalarial properties. This drug acts on the pre-erythrocytic forms of *P. falciparum*, and is, therefore, a causal prophylactic of infection due to this species of parasite; it is a good suppressive against all forms of malaria; it inhibits the late sporogonic forms of the parasite, so that mosquitoes feeding on a gametocyte carrier receiving therapeutic doses of the drug do not become infective; it has a lower toxicity than any other antimalarial drug known; and it can be produced at very low cost. Its action on the asexual erythrocytic

forms of the malaria parasite is not sufficiently rapid to render it suitable for treatment of the clinical attack, and its principal use is in prophylaxis. Proguanil has been used very extensively for this purpose among troops and civilian populations in Malaya and other parts of the Commonwealth during the post-war years with excellent results.

More recently another antimalarial drug of importance has been placed on the market. This is pyrimethamine (Daraprim), a member of the diaminopyridine group. It was synthesized in the United States, though many of the biological tests have been conducted in England. In most respects its action closely resembles that of proguanil, and this is not surprising, since its chemical structure is not unlike that of an active metabolite produced in the body by the latter drug. Like proguanil, it is too slow in action for therapeutic use, and its chief value is likely to be in prophylaxis. It is active in very small doses and can be produced at relatively low cost.

Proguanil and pyrimethamine share one grave disadvantage, namely, a tendency to provoke resistance in parasites subjected to prolonged contact with either drug in sub-therapeutic dosage. Cross-resistance between the two drugs has also been demonstrated. It is not possible to forecast at this stage how far this phenomenon will affect their future use. Fortunately no such tendency has yet been observed in respect of either chloroquine, amodiaquine, hydroxychloroquine, mepacrine or quinine.

Another important new antimalarial drug developed in the United States is primaquine, one of the 8-aminoquinoline series. The researches which led to its production were inspired by the urgent need for preventing relapses of *vivax* malaria in trooops returning from Korea. It is claimed that the maximum tolerated dose of primaquine is twice as high as that of pamaquin, and that it is four times as active as the latter drug in the radical cure of *vivax* malaria. Since its adoption as a routine method of treatment, the relapse rate among the United States personnel returning from Korea has fallen to less than one per cent.

COMMENT.

No student of malariology can fail to be impressed by the profound influence exerted by the exigencies of war on the development of malaria control measures during the past 40 years.

The greatest advance in the conduct of antimosquito measures during this period was the introduction of D.D.T. This compound had been synthesized as early as 1874, but its insecticidal properties were not discovered until 1939, when Swiss chemists were searching for a chemical which would destroy clothes moths. D.D.T. was first used on a large scale in the early years of the second world war against the Colorado beetle, which threatened the Swiss potato crop at a time when military considerations had made the preservation of all foodstuffs of the utmost importance. The need for a synthetic insecticide has been intensified by the shortage of pyrethrum, the bulk of which was then grown in Dalmatia and Japan, and in 1942 D.D.T. was made available to the military authorities in Great Britain and in the United States. It was used with great effect for the prevention of typhus during the Italian campaign and later in the war it was employed on a

large scale by the military authorities for the destruction of mosquitoes and other insect vectors of disease. Although D.D.T. was already in existence when the war broke out, the researches which demonstrated its possibilities as an agent for malaria control were inspired directly by military considerations.

Wartime conditions had an even greater influence on the development of synthetic antimalarial drugs than on that of residual insecticides. As noted above, it was the fact that they had been deprived of the sources of quinine during the first world war which inspired the German researches which culminated in the production of pamaquin and mepacrine. Chloroquine was evolved as a further development of the same line of research and its outstanding properties as an antimalarial drug were demonstrated by the Americans in the course of their wartime research programme. The investigations which led to the production of proguanil were begun because the Allies in their turn were cut off from sources of quinine when the Japanese occupied Indonesia. Finally, as already noted, primaquine was produced in an attempt to evolve a safe substitute for pamaquin for the treatment of personnel returning from the Korean battlefront.

Thus the wars which have devastated the world during the past 40 years have had at least one beneficial effect, in that they have stimulated the development of synthetic insecticides and drugs which bid fair to rob malaria of most of its terrors and perhaps even to lead to its eventual eradication from the globe.

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THE NATIONAL MALARIA CONTROL PROGRAMME OF INDIA.

A Review.

BY

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(September 12, 1955.)

INTRODUCTION.

AWARENESS of the enormity of the problem of malaria, both from the public health and the socio-economic points of view, has existed in India through several decades. Indeed, it had been repeatedly demonstrated for over two decades in different States that it was feasible to control and prevent malaria efficiently and urban malaria even economically. The social and economic betterment of communities in whom the malaria was controlled, was only too obvious. Between 1946 and 1953, several States had carried out large-scale successful antimalaria programmes with indoor residual spray of D.D.T. in the rural areas. At the close of the financial year 1952-1953, about 30 million people out of an estimated 200 million exposed to the risk of malaria, were being protected from the disease at an annual cost of 15 million rupees (Jaswant Singh, 1953).

The need for and the feasibility of a national programme for malaria control had been visualized as early as 1946 when the Health Survey and Development Committee recommended a nation-wide effort. It was, however, in 1952 that a plan was formulated as a part of the health development programme of the first Five Year Plan for the country. The Indo-American aid made it possible to launch this first national health drive.

THE PLAN.

Extensive knowledge of malaria and methods of its control under widely differing epidemiological conditions involving several vectors, was a great help in the formulation of the Plan. Although based on certain assumptions dependent on the data available, it was dynamic, capable of modifications as and when

necessary. The plan is technically sound and has had necessarily to be an experiment in so far as its administration, organization and execution are concerned. While it is true that the experience of a few nation-wide malaria control programmes elsewhere in the world was available, for sheer magnitude, the variety of technical problems and the number of vectors involved, the National Malaria Control Programme of India remains to date unique and the biggest in the world. It is proposed to review this programme in retrospect and prospect after three years of execution.

Based on the assumption that 200 million people of the country's population are exposed to the risk of malaria (with an estimated annual morbidity of 75 million and mortality of 0·8 million), the plan was drawn with a defined objective to protect the entire population at risk from further infection. The emphasis of the plan would in retrospect appear to be mainly rural malaria control.

The method of malaria control provided was to intercept the transmission of malaria by the application of residual insecticides in the dwellings (75 per cent water dispersible D.D.T. applied once or twice and rarely three times a year, depending on local conditions, using a total dosage of 200 mg. of technical D.D.T. per sq. ft. of wall surface annually). For over-all calculation, an average house was reckoned to have one thousand sq. ft. of indoor surface to be sprayed. Each house was reckoned to have a population of five.

The plan envisaged an operational phase of three years and a maintenance phase thereafter. The financial implication of the plan during the years of the operational phase was estimated at Rs. 1,505 lakh. The major part of this, namely, Rs. 772·7 lakh, was to be American aid in kind (transport, equipment, D.D.T.), while the Government of India was to contribute Rs. 227·3 lakh for the expansion of Malaria Institute of India and the provision of antimalaria drugs, locally produced insecticides and customs duty on material coming from abroad. The United Nations International Children Emergency Fund gave Rs. 15 lakh worth D.D.T. The State Governments' contribution was estimated to Rs. 490 lakh to cover the salaries of the staff employed and running expenses.

The plan provided for the protection of 125 millions only out of the 200 millions estimated to be at risk. The implementation of the programme was to be phased to set up the required organization in the different States for the protection of 75 millions in the first year of the plan and the full 125 millions during the second and third years. Thereafter, it was expected that the tempo of the control operations would be scaled down to the maintenance phase. It will be seen later that some changes from the original planning had to be made, even within the first three years.

RESPONSIBILITIES.

Direction, co-ordination, over-all supervision, training of staff, procurement and supply of equipment, transport and insecticides and final assessment were allocated as central responsibilities to be discharged by the Malaria Institute of India. The States were to be responsible for the recruitment of staff, execution of the programme, immediate supervision and concurrent assessment of results.

In accordance with the Constitution, Public Health being a State responsibility, it was necessary to circulate the plan and invite the concurrence of the States to participate in the national effort. Each unit of the National Malaria Control Programme was designed to protect a population of one million and the States were requested to indicate the total number of units required to cover all the malarious areas in each, and the number they could support. It was fully realized that the organization for control of malaria was not equally developed in all the States and it was, therefore, one of the main objectives during the operational phase to strengthen the malaria organizations in the States wherever required, and to initiate them where none was present with a view to developing an efficient organization as quickly as possible.

IMPLEMENTATION.

Saturday, December 13, 1952, will be remembered as a landmark in the history of malaria control in India when the relevant operational agreement was signed in New Delhi, between India and the United States of America. That agreement made it possible to equip and provide insecticides, transport and equipment for 75 units in 1953-1954, each unit protecting one million population. It was found necessary, however, by a second agreement in the same year, to provide for 15 additional units, bringing the total for the first year to 90. This addition was made possible by the free grant of 400 tons of D.D.T. by the United Nations International Children Emergency Fund. In March, 1954, as a result of review of State requirements, another agreement was signed for 35 more units, which again was increased by another 11 units, briniging the total to 136 to protect 136 million people in the operational phase. This number has been increased to 162 by allocating additional 26 units during 1955-1956.

It would thus be seen that out of an original estimate of population at risk, namely 200 million, there still remain 38 millions requiring protection.

Despite the fact that all details of planning and logistics of supplies, etc., were given due consideration, there were some inevitable birth pains. Perhaps due to the fact that everything, except personnel, had to be procured from different sources in distant America and shipped to this country, together with the fact that the ports were not the ultimate destination of the commodities imported and the bottle-necks of rail and roadways had to be overcome, it took some considerable time for the transport, equipment and supplies to be distributed to the ultimate destinations where they were going to be used. It is a tribute to the zeal and willingness of all concerned that there was at no time any avoidable delay.

Some Government policy decisions and procedural formalities resulted in the supply of a few items of transport and equipment not suitable for use under the varied field conditions in the country. The trucks, for example, were found to be too large for being manipulated underneath overhead railway bridges, etc. The power sprayers were found to be unusable due to their huge size. These difficulties were freely discussed at a conference of field workers and were got over by local initiative and rectified in subsequent procurement.

The insecticide formulation (75 per cent water dispersible powder) received from America was from different manufacturers in that country. In addition, the

periods of storage and transit for various consignments were different from one another. When the first complaint was received about the difficulty of obtaining a good water suspension out of a batch of insecticide received in the country, it became necesary to inspect and test every consignment of insecticide received at the different ports before distributing them to the various States. Simple devices for testing of the insecticide formulations, as well as for rendering them suitable where found to be wanting in the required specifications, were quickly developed with the full cooperation of the U.S. Technical Cooperation Mission to India.

BOTTLE-NECKS.

There was initial delay in the recruitment of staff both in the Centre and in the States on account of procedural formalities. The latter have also been responsible for long delays in procurement of supplies. It is time that necessary action was taken to see that such bottle-necks are not allowed to interfere with the scheduled implementation of an emergency programme like the National Malaria Control Programme.

ACHIEVEMENTS.

The training of Malaria Medical Officers and ancillary personnel required, was provided in the plan, and the staff and facilities in the Malaria Institute of India were suitably expanded to meet this obligation. Seventy-five medical officers, six entomologists, 402 malaria inspectors, 14 engineers and 21 laboratory technicians have been trained from 1953 onwards at the Malaria Institute of India. The latter is fully equipped to meet all the future training requirements of the personnel.

To ensure uniformity of methods of control, assessment and accounting, it was quickly realized that an operational guide was necessary. This was prepared with due regard to the divergent conditions of endemicity, vectorial behaviour, population density, transmission period, communications and a host of other factors. The document was deliberately called a " Guide " with the full knowledge that modifications would be necessary from time to time and place to place, to avoid harmful regimentation and provide full scope for individual initiative. A second edition is already in the press.

A detailed assessment of results is being done but the over-all picture reveals progress. The results, however, are not, and could not in their nature be expected to be uniform everywhere. The quality and extent of control established with the inauguration of the National Malaria Control Programme has varied from State to State, being more intensive in the States where malaria control operations had been well established prior to the national programme, and correspondingly less in the States where an organization had to be built up with the inauguration of the national plan. A few States which had a good nucleus of malaria organization prior to the plan, have, however, not advanced to the degree and extent possible during the plan. One among several reasons for this state of affairs appears to be a conflict in ideology and policies pursued by State Governments with reference to participation on voluntary basis of local people in developmental

plans in general. Such voluntary participation in a malaria control programme has some obvious limitations.

To achieve successful control in the entire country (or even a State) it is essential to spray all the houses in malarious areas for the requisite number of times within as short a period as possible. The time interval between sprayings as well as the spraying periods are governed to a great extent, among other factors, by the periods of the year when malaria transmission actually occurs. Experience has shown that neither of the above conditions can be effectively satisfied by utilizing voluntary labour which is not amenable to any kind of control.

At the time of inception of the plan, there was no agency to manufacture standard spraying equipment in the country and all such equipment had, therefore, to be imported. In pursuance of a general policy local industry was stimulated to interest themselves in the manufacture of this equipment. Full use was made of a specialist in the line, made available to the Institute by The Rockefeller Foundation to draw up a set of standard specifications based on the World Health Organization general specifications for sprayers, to suit local conditions. The industry in the country has in the course of two years been able to produce standard equipment in sufficient number to avoid the necessity of importing this item altogether. Besides, the easy availability of much needed spares has helped considerably in the smooth working of the programme.

The production of insecticides locally during the plan period to make the country as nearly self-sufficient as possible, by mobilizing both public and private sectors in the country, has progressed satisfactorily. One D.D.T. plant established in Delhi as a joint project by U.N.I.C.E.F./W.H.O./Government of India has gone into production with an annual capacity of 700 tons and a second plant is proposed to be set up in the south with a capacity of 1,400 tons. Steps have already been taken to double the capacity of the Delhi plant. The private sector is producing B.H.C. and it is expected that an equivalent of nearly 1,500 tons of D.D.T. would be available.

ANALYSIS OF RESULTS.

The data received from the units in the monthly reports for the year 1953-1954 and 1954-1955 are under compilation and analysis. Appendices I, II and III give (1) the population protected and D.D.T. consumed, (2) the malaria indices, and (3) the morbidity figures.

Appendix I is prepared on the basis of the largest number of houses sprayed in any one round of spraying during the transmission period and assuming the number of people per house as five. The recommended dosage of D.D.T. application is 200 mg. per square foot in two applications of 100 mg. each. On the basis of the assumed average square surface of one house as 1,000 ft. sq., it should be possible to protect about eight persons per lb. of D.D.T. This average of the number of persons protected per pound of D.D.T. is liable to vary if (1) the average square surface of a house is more or less than 1,000 sq. ft., (2) if all the houses are not given a 200 mg. dosage during the transmission season each year and/or if the number of persons per house is more or less than five per house as assumed. It would, therefore, be necessary to make allowances for these variations when

interpreting the data presented in the table. It would be seen that against a minimum of 3·8 persons protected per lb. in Coorg, the maximum is 21·9 in Manipur in 1953-54 and corresponding figures for 1954-55 are 3·4 and 13·3 in Coorg and Manipur respectively.

Appendix II indicates a general trend towards lowering of the malariometric indices in most of the States. There are, however, a few States like Madhya Bharat, Tripura and Uttar Pradesh, where there is a reverse trend. There is also a suggestion that some units in the States of Bombay, Mysore, and Coorg, have reached the end-point of malaria transmission.

Appendix III presents comparative morbidity rates. As indicated therein, the difference in the number of malaria cases reported from the dispensaries in the unit areas for the two years is multiplied by ten to arrive at the total reduction in the number of malaria cases in the areas controlled in each State. This estimate is based on the data presented by Sinton (1939).

It will be seen that there has been a fall of nearly 19·4 million malaria cases in 1954-1955 as compared to 1953-1954. It would appear that on the basis of three days sickness per case and a daily wage of Rs. 2/-, the amount of money saved would be nearly 11·64 crores of rupees, very nearly the sum expected to be spent on the programme for the three year period.

The data presented in Appendices II and III indicate only general trends. Statistical analysis will be attempted later when complete reports have been received and compiled.

COSTS.

	1953–54 Rs.	1954–55 Rs.	1955–56 Rs.
1. Expenditure by the Centre on equipment and material for the various units, customs duty and expansion of the Malaria Institute of India	2,12,60,592	3,01,49,113	3,09,48,000*
2. Expenditure by the States	1,13,48,147	1,24,68,362	1,99,01,000 (budget)
Total ...	3,26,08,739	4,26,17,475	5,08,49,000
	Total Rs. 1,260·75 lakhs.		

*Revised budget as anticipated.

APPRAISAL OF THE SITUATION.

The appraisal of the progress of the plan has necessarily to be against the background of the magnitude and the variety of problems involved. The working plan has, therefore, necessarily to be dynamic, and no rigidity either in planning or execution is indicated. Practical difficulties, some of which have been enumerated above, have been encountered and most of them have been surmounted. It may now be appropriate to review the original objectives, targets and organization,

particularly for assessment of results and future policy in respect of some of the States not coming up to expected standard.

At the time when the N.M.C.P. was planned, the factor of anopheline vectors developing resistance to insecticides had not assumed the importance it has within the last year or two. Insect resistance to insecticide was certainly known but observations at that time appeared to indicate that the danger of resistance in anophelines was not so imminent as in the case of other insects which had a lower degree of natural susceptibility to insecticides. But recent studies indicate that the danger is real. The time taken by anophelines to develop resistance requires to be determined. It would probably vary from species to species and a number of factors like dosage of insecticide, and its deterioration, etc., will affect it. In the circumstances, if malaria is ' controlled ' within the time (actually ' eradicated ' would be more appropriate) in all contiguous areas simultaneously, then and then only would it be possible to interrupt insecticide spraying, and thus possibly avoid the risk of development of insect resistance to the insecticide in use. Thus it is seen that the time factor has assumed considerable importance. Once the resistance phenomenon is allowed to develop, the problem of rural malaria control with insecticides synthesised with chlorine, may become impracticable. It is because of this factor that we have to re-examine our original objectives and targets and modify them where necessary to suit present day concepts.

The objective of the plan as defined originally was to provide protection from malaria to 125 out of 200 million estimated to be at risk. The scope of the plan was extended to cover 162 million people though only about 750 lakh (75 million) people were actually protected up to the end of March 1955. In selecting this population out of the 200 million, preference was to be given to those living in hyperendemic areas, the potential food growing areas, and for those living in community project areas within them. The concept of eradication will involve the inclusion in the programme of all the areas even those of very low endemicity; and the States which have left out areas with spleen rates less than ten per cent in the original planning, will have to reconsider their requirements.

As indicated already in the original plan, the operational phase was limited to three years. Experience of workers in this country, corroborated by the experience elsewhere (World Health Organization, 1954), has made it necessary to extend it by an additional period of two years, so as to continue the full control operations for a period of at least five years. The point to consider now would appear to be whether this additional period of two years, followed by the necessary maintenance period, would be sufficient to achieve malaria eradication? If eradication is to be the goal, then ensurement of its achievement everywhere or at least in large contiguous areas, is of paramount importance. This will require a very careful and continuous vigilance during the maintenance period after the end point is reached and will largely depend on the calibre and application of the vigilance staff in the units.

The indices to determine the achievement of eradication have necessarily to be more sensitive than the ones we adopt at present to assess malaria control. They will in all probability consume infinitely more time and involve greater expenditure in being determined. One important essential factor in this connection will be a thorough search for every new case of malaria. On this will depend the rest of the epidemiological verification, investigation and measures to

eradicate the focus. It will require intensive study to evolve quickly a suitable organization for spotting new cases and their microscopical verification.

It has been indicated that each unit of the National Malaria Control Programme was designed to afford protection to one million population. This has been found to be feasible only under certain conditions of population density, communications, etc. There are, however, areas where population is scarce and communications scanty, in which circumstances the units are unable to cover much more than nearly half a million. It may, therefore, be a more accurate overall estimate to place the target at 800,000 per unit on an average.

The main emphasis of the National Malaria Control Programme was on malaria control in the rural areas. With large rural areas under control, and the conception of eradication having come in the fore-front, the problem of urban malaria has taken on a new urgency. In the light of experience and repeated demonstration in this country and elsewhere, urban malaria control should, if feasible, be based largely on permanent engineering measures. Such measures are not only more economical than the measures suited to control malaria in the rural areas but also take care of general mosquito nuisance. Where there are other mosquito-borne diseases, indeed, the permanent engineering measures are the ones of choice in their prevention, the second choice being given to recurrent anti-larval measures. To the administrators and the public, the National Malaria Control Programme would appear to have come to mean the spraying of D.D.T. and nothing else. It would seem appropriate at this juncture to emphasize the need for planning and execution of permanent engineering measures and subsidiary anti-larval measures for mosquito control in urban areas.

SUPERVISION.

It has been pointed out that the pace at which control has been established has been greater in some States than in others. It has also been indicated that such progress has not been uniform even in some of the States that had considerable experience and staff for malaria control prior to the advent of the plan. In the States where there was little or no organization to control malaria prior to the plan, perhaps it was to be expected that the progress would be slow. In view of the above, it would appear necessary now to take stock of the situation and plan for greater supervision where necessary. In this connection, establishing regional centres and utilizing the highly trained and experienced personnel, both at the Centre and the States, to staff such centres, seems essential to achieve the objectives in full during the second Five Year Plan.

RESEARCH.

It has been said that a unique contribution to science by America lies in making research a career and a normal avocation. The realization that research is not something that is mystic, and should be pursued in exclusive laboratories by only a few who are removed from conditions in the field, does not yet seem to have pervaded this country, at least not widely enough among practical malariologists. The problems to be solved are many; it is the field malariologist that comes across

the problem at first hand. It is he that most requires a solution to each problem in order that his work can progress along scientific lines. Many of the problems of the highest importance can only be solved by work in the field and would be difficult to tackle in the laboratory.

A number of research projects covering the different aspects of the epidemiology and control are in progress. The vectors of malaria in some areas like Tripura, Manipur, Vindhya Pradesh, Madhya Bharat, have been incriminated. The insecticidal properties of the newer synthetic insecticides and the best material as also the most economical dosages of application, are under continuous study. The normal susceptibility to D.D.T. of the different malaria vector species and their present status after different periods of exposure to D.D.T., are currently under detailed investigation. A technique for working with lower pressures in the spraying equipment has been developed.

Perhaps the single most important problem of the day is insect resistance to insecticide. The study of the problem requires planning, definition of objectives and priorities—a tremendous amount of work. It requires no prophet to say that postponement of this phase of activity will be fatal to the cause of malaria control. It will not be any exaggeration either, to say that all available hands trained in research in the field of malariology will have to be mobilized immediately to tackle this problem if results and possibility of their practical application are to be exploited before it is too late.

PUBLICITY.

It is a sad reflection that the progress of health activities in the country, not to mention the National Malaria Control Programme in particular, did not receive adequate publicity in the feature articles commemorating eighth year of freedom. The reason for this must be sought. Is it because that the National Malaria Control Programme is not sufficiently known to the people and the administrators? And yet in other contexts it has been stated times out of number that the National Malaria Control Programme is one of the very few health programmes under the National Plan that has made good progress and one of the very few projects in the entire plan whose benefits have been within the reach of an average householder. The results achieved by the National Malaria Control Programme thus far are concrete and not imaginary or theoretical. It would appear that the people and the administrators have not yet felt the tremendous activity with regard to prevention of malaria that is in progress in the country on a scale unprecedented anywhere in the world. The necessity to get the people and the administrators to feel the progress and take a live interest, seems only obvious. Publicity like everything else has to be planned and organized to be effective.

PUBLIC PARTICIPATION.

It is a sufficiently recognized principle that success in any large undertaking requires participation of all concerned in the activity. The more active the public participation the greater the success of the undertaking. This principle has been emphasized in the second Five Year Plan. What then is the practical feasibility of

public participation in the National Malaria Control Programme? It would appear to lie in the field of active cooperation with the duties of those who are trained, employed and paid to carry out certain functions for protecting the public from malaria. Such cooperation can only be obtained by education of the people in the rationale of the activities.

A technique to carry the people all the way with the progress is to organize malaria committees comprising representative members who will from time to time meet to take stock of the situation, progress, difficulties and obstacles and take steps to overcome them. Such committees formed at all levels like the panchayat, municipal, district, State, regional and national levels, would help to keep the plan alive and bring home to the people the full realization of the possibilities of a nation free from such a crippling disease as malaria.

Appendix I.

Number of houses sprayed, population protected and the number protected per lb. of D.D.T.

State.	1953–54				1954–55			
	Number of houses sprayed at least once.	D.D.T. consumed. (lbs.)	Population protected.	Number of people protected per lb. of D.D.T.	Number of houses sprayed at least once.	D.D.T. consumed (lbs.)	Population protected	Number of people protected per lb. of D.D.T.
Ajmer ...	42,481	38,826	212,405	5·4	67,080	65,610	335,400	5·1
Bhopal ...	88,574	21,867·5	442,870	20·2	183,074	103,065	915,370	8·8
Bihar ...	255,210	87,049	1,276,050	14·6	1,592,587	77,892	7,962,935	10·2
Bombay ...	3,252,999	1,638,660	16,264,995	9·9	3,486,399	1,676,542	17,431,995	10·3
Coorg ...	39,479	51,428	197,395	3·8	38,801	57,015	194,005	3·4
Delhi Urban	63,354	38,752	316,770	8·1	105,657	53,322	528,285	9·9
Delhi Rural	56,120	73,118	280,600	3·8	70,673	99,071	353,365	3·5
Himachal Pradesh	31,417	19,899	157,085	7·8	160,507	79,133	802,535	5·0
Kutch ...	43,328	24,341	216,640	8·9	69,387	34,022	346,935	10·1
Madhya Bharat	235,738	196,077	1,178,690	6·0	273,446	283,364	1,367,230	4·8
Madhya Pradesh	1,487,745	451,821	7,438,725	16·4	1,558,922	683,655	7,794,810	11·4
Manipur ...	3,461	790	17,305	21·9	66,502	24,950	332,510	13·3
Mysore ...	687,432	307,096	3,437,160	9·3	635,629	602,375	3,178,140	5·2
Orissa ...	310,967	203,895	1,534,835	7·6	1,052,272	461,313	5,261,360	11·4
Punjab ...	214,074	208,342	1,070,370	5·1	544,342	610,307	2,721,710	4·4
P.E.P.S.U. ...	159,157	77,958	795,785	10·2	409,956	256,992	2,049,780	7·9
Tripura ...	27,428	7,398	137,140	18·5	120,877	49,923	604,385	12·1
Uttar Pradesh	155,584	48,413	777,920	16·0	296,724	239,793	1,915,865	7·9
West Bengal ...	2,718,430	1,118,167	13,592,150	12·1	3,181,989	1,481,459	15,909,945	10·7

Appendix II.

Spleen rate, parasite rate and infant parasite rate in different States in India.

State.	1953–54			1954–55		
	Spleen rate (per cent)	Parasite rate (per cent)	Infant parasite rate (per cent)	Spleen rate (per cent)	Parasite rate (per cent)	Infant parasite rate (per cent)
Andhra	24·4/27·5	3·3/7·2	1·0/7·3	19·9/29·6	7·4/9·7	5·6/7·9
Bihar	11·9/59·6	9·5/16·8	...	11·2/39·6	0/21·3	0·0/13·3
Bombay	0·0/20·7	0·0/9·7	0·0/8·8	0·0/14·9	0·0/10·0	0·0/13·7
Coorg	0·02	0	0	0·02	0	0
Delhi } Urban ...	0·18	0	0	0·06	0	0
Delhi } Rural ...	1·30	0·1	0	0·80	0	0
Hyderabad ...	3·0/21·0	0·6/30·6	0	4·0/8·5	0·6/7·2	0/1·4
Kutch	6·4	13·4	...	4·1	5·8	...
Madhya Bharat ...	7·8/26·6	14·7/23·0	...	14·5/25·6	9·9/15·7	...
Madhya Pradesh ...	6·6/64·9	...	0/28·7	6·4/44·9	0/32·4	0/17·8
Manipur	23·2	1·5	...	17·7	1·0	0·8
Mysore	0/12·2	0/15·0	0/5·1	1·2/13·4	0·5/15·9	0/0·3
Orissa	29·9/41·5	1·6/1·9	3·8/33·3	24·7/27·3	0·3/1·7	0
Punjab	7/14·8	...	0/0·6	3·3/9·0	0/17·3	0/0·8
P.E.P.S.U.	7·5/7·6	0·2/2·1	0	0·5/2·5	0	0
Saurashtra	22·1	0·6	0	11·1	0·5	0·9
Travancore-Cochin ...	19·2	7·1	0	10·6	1·4	0
Tripura	55·8	17·1	14·2	61·1	21·6	12·5
Uttar Pradesh ...	5/24·9	0·6/15·5	0/14·2	11·4/28·3	1·5/10·1	0/11·4
Vindhya Pradesh ...	18·7/27·8	...	...	7·9/16·4	...	...
West Bengal ...	5·3/64·1	...	...	2·7/53·0	...	...

Note.—For purposes of conciseness, the spleen, parasite and infant parasite rates are presented as ranges in each State. The numerator in each instance represents the lowest rate and the denominator the highest amongst the different units in operation in each State.

APPENDIX III.

State-wise reduction in malaria cases from the year 1953-54 to 1954-55.

State.	Malaria cases reported from hospitals and dispensaries:			On the assumption that one out of 10 cases reported to a dispensary, the total figure of fall in cases arrived at by multiplying the figures in Col. 4 by 10.
	1953-54	1954-55	Reduction from 1953-54 to 1954-55.	
1	2	3	4	5
1. Bhopal ...	1,44,205	1,00,774	43,430	4,34,300
2. Bihar	11,07,819	7,59,410	3,48,409	34,84,090
3. Bombay ...	2,53,006	1,95,229	57,777	5,77,770
4. Coorg ...	3,439	3,210	229	2,290
5. Delhi	9,172	7,035	2,137	21,370
6. Himachal Pradesh ...	1,01,110	84,838	19,272	1,92,720
7. Hyderabad ...	1,04,776	56,740	48,036	4,80,360
8. Kutch	18,202	12,094	6,108	61,080
9. Madhya Bharat ...	1,10,638	90,782	19,856	1,98,560
10. Madhya Pradesh ...	5,88,716	3,32,678	2,56,038	25,60,380
11. Madras	1,56,099	1,30,454	25,645	2,56,450
12. Manipur ...	65,260	55,813	9,447	94,470
13. Mysore ...	1,93,530	1,45,053	48,477	4,84,770
14. Orissa	14,234	7,192	7,042	70,420
15. P.E.P.S.U. ...	1,03,330	61,505	41,825	4,18,250
16. Punjab	5,08,636	3,80,496	1,28,140	12,81,400
17. Saurashtra ...	47,597	33,381	14,216	1,42,160
18. Travancore-Cochin ...	55,013	51,478	3,534	35,340
19. Tripura	1,27,339	90,877	36,462	3,64,620
20. Uttar Pradesh ...	6,03,644	5,55,882	47,762	4,77,620
21. Vindhya Pradesh ...	94,314	64,741	29,563	2,95,630
22. West Bengal ...	16,18,924	8,76,955	7,41,969	74,19,690
Total ...	63,29,001	40,93,627	19,35,374	1,93,53,740

Notes.—The Director of Public Health, Andhra, has reported that 3,507 deaths occurred from malaria in 1954 as compared to 3,526 deaths in 1953.

The Civil Surgeon, Ajmer, has stated that there was about 40 per cent fall in the number of malaria cases from 1953-54 to 1954-55.

National Malaria Control Programme did not operate in the States of Assam, Jammu and Kashmir, Rajasthan and Coalfields during 1953-55.

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MALARIA CONTROL IN THE PUNJAB (INDIA) WITH SPECIAL REFERENCE TO THE NATIONAL MALARIA CONTROL PROGRAMME.

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THE Punjab is a border State at the extreme north-west corner of India. After the partition of this Province in 1947, the State has been greatly truncated and instead of 27 districts, it consists at present only of 13 districts. Except for the entire Kangra District and a small portion of Simla which are mountainous, the rest of the State is a great alluvial plain which stretches from the foot of the Himalayas to the Rajputana desert. In other respects the country is featureless with the exception of mighty rivers which traverse the plains and are flooded almost every year after heavy monsoon rainfall. The affected country-side is inundated extensively resulting in the loss of lives, property and outbreaks of malaria and other epidemic diseases.

The soil of the Punjab is for the most part a fine silt deposited by the rivers whose action has virtually formed the land. It is peculiarly impervious to water in most places and this, added to the level character of the country, results in large surface collections of water in the event of floods temporarily submerging extensive tracts of the State.

Between the rivers of the Punjab are strips of country known as the '*Doabs*'. These are situated on comparatively high level but they are not entirely safe from being adversely affected by heavy rainfall. On the whole, the soil is very suitable for production of chief agricultural crops namely, cotton, wheat, sugarcane, millets, gram, rice, etc. There is an extensive network of canals throughout the State which greatly help in the production of superior agricultural crops. Other modes of irrigation are by wells in the plains and '*Kuhls*' in the hill tracts.

According to the 1951 census, the total population of the Punjab has been estimated at 12,641,205 spread over a land area measuring 37,378 square miles. The distribution of the population in 13 districts of the State is given below:—

TABLE I.

Distribution of rural and urban population in the Punjab (India).

District.	Total population.	Rural population.	Urban population.
1. Hissar	1,045,645	877,945	167,700
2. Rohtak	1,122,046	970,987	151,059
3. Gurgaon	967,664	827,511	140,153
4. Karnal	1,079,379	876,067	203,312
5. Ambala	943,734	695,784	247,950
6. Simla	46,150	...	46,150
7. Kangra	936,042	893,592	42,450
8. Hoshiarpur	1,091,986	986,962	105,024
9. Jullundur	1,055,600	761,202	294,398
10. Ludhiana	808,105	602,218	205,887
11. Ferozepore	1,326,520	1,101,438	225,082
12. Amritsar	1,367,040	958,533	408,507
13. Gurdaspur	851,294	688,034	163,260
Total ...	12,641,205	10,240,273	2,400,932

As is usual in India, the rural population greatly exceeds the urban, being nearly eighty per cent of the total. There are only three cities namely Amritsar, Jullundur and Ludhiana with a population exceeding 100,000. The housing conditions are more or less similar in the rural areas of the State. The villages consist of solid mud built houses, lying compactly around the agricultural lands. In the hill tracts, however, a village is conventional aggregate of small hamlets known as '*Tikas*' which are remotely scattered according to the situation of the agricultural land of the villagers. The houses in the hills are usually smaller as compared to those in the plains.

The partition of the Punjab during August 1947 involved considerable exchange of populations between the East and West Punjabs—the latter having become part of Pakistan. This exchange had a tremendous influence in introducing malarial conditions in some of the hitherto non-malarious tracts of the East Punjab where the anopheline potential was high. On the other hand, certain highly malarious tracts such as the Bet area of Ludhiana District, which was populated predominantly by Muslims, had shown substantial lowering of malarial incidence after this mass exodus of infected Muslim population.

The normal climate of the Punjab is characterized by extremes of cold and hot weather. The cold weather gradually gives way to the hot weather at the end

of March, from which time it gets steadily hotter, and the relative humidity gets steadily less and less till the middle of June when great extremes of heat and dryness are normally experienced. The monsoon breaks suddenly usually about the third week in June, with a consequent increase in the relative humidity and decrease in the maximum temperature. The minimum temperature at night remains practically unchanged at about 80°F. Periodical rain falls throughout the monsoon during July and August, gradually diminishing in September, when the relative humidity again decreases. Gradually the winter is ushered about the middle of November. It has been observed (MacDonald and Majid, 1931) that the monsoon is the sole apparent climate controlling factor in the spread of malaria, the amount of rainfall influencing the number of breeding places, and the associated increase in humidity, which lasts just as long and no longer than the monsoon, making it possible for the anophelines to live a long time and consequently to be capable of transmitting malaria.

INCIDENCE OF MALARIA.

Nearly seven million out of a total population of nearly 12·6 million in the Punjab live in rural malarious tracts. This disease is chiefly prevalent in the plains of the Punjab but in the hill-tracts at an altitude varying from 2,000 to 4,000 feet above sea-level, it has been reported to be very active. Extensive areas in Kangra District are known to be highly malarious and in certain parts conditions of malarial hyperendemicity have also been recorded.

Malaria is endemic almost throughout the Punjab and manifests itself chiefly in the plains from August to November. In the Kangra hill-tracts, the malaria transmission commences a month earlier owing to comparatively more favourable conditions of temperature and humidity.

In the plains of the Punjab, a very large number of endemic tracts exist which although may not exhibit epidemic conditions for years, are in some ways so predisposed to them that on a certain minimum of rainfall they will become the seat of epidemic malaria. Such tracts are located mostly near the rivers. In addition, there are other areas which are not obviously riverain but nevertheless behave in the same way. Such tracts usually receive much of the drainage from the district in which they are situated. The foot hills throughout the State, more particularly near the Siwaliks in Hoshiarpur District, develop epidemic conditions in this manner. Such tracts not only easily become affected but exhibit a high degree of malarial intensity.

As a measure of the endemicity of malaria the spleen and parasite indices have been studied during the course of malaria surveys conducted in different parts of the State in the recent years.

Before the National Malaria Control Programme was introduced in the Punjab, the spleen rates amongst children under ten years of age in some of the important endemic areas of the State were as under:—

District.	Locality.	Spleen rate (Per cent.)	
Kangra	Jowali ...	70·1	
	Kulu ...	33·1	
	Jowalamukhi ...	22·9	
	Dera Gopipur ...	58·0	
	Kangra ...	12·9	
	Shahpur ...	24·3	
	Nurpur ...	43·0	
	Indaura ...	66·0	
Gurdaspur	Behrampur ...	51·1	
	Norat Jaimal Singh	28·1	
	Sri Gobindpur	27·5	
	Derababa Nanak	25·0	
Amritsar	Patti ...	70·1	
	Ram Das ...	27·9	
	Taran Taran ...	24·5	
	Ajnala ...	21·6	
	Lopoke ...	29·8	
	Majitha ...	21·6	
	Chabal ...	24·2	
	Tung Bala ...	58·0	Suburban area.
	Amritsar ...	40·5	Suburban area.
Ferozepore	Guru Harsahai ...	89·3	
	Zira ...	49·3	
	Dharamkot ...	22·1	
Ludhiana	Sidhwan Bet ...	47·6	
	Machiwara ...	21·1	
Hoshiarpur	Santokhgarh ...	31·1	
	Gagret ...	34·5	
	Amb ...	25·6	
	Garh Shanker ...	22·9	
	Hajipur ...	26·6	

District.	Locality.	Spleen rate (per cent).
Karnal	Indri ...	38·0
	Thanesar ...	26·9
	Bajida ...	31·7
	Khojgipur ...	34·3
Rohtak	Gohana	23·8
	Juan ...	22·9
	Mundlana ...	23·8
Gurgaon	Dhauj ...	52·8
	Sohna ...	35·3
	Punahana ...	59·2
	Hassanpur ...	15·2
Ambala	Panchkoola ...	29·6
	Chamkaur ...	15·4

It is evident that conditions of high endemicity and hyperendemicity prevailed in the Punjab before the National Malaria Control Programme was introduced in 1953.

Three species of malarial parasite, namely, *P. vivax*, *P. falciparum* and *P. malariæ* have been detected in the blood films examined from various parts of the State. The last-named species is, however, very much restricted in its distribution.

At intervals of years, wide-spread epidemics of malaria are liable to occur in this State. The disastrous epidemic of malaria which broke out during 1908 in the Punjab, was of such a type where, in the space of three months, it occasioned over 300,000 deaths amongst a population of approximately 20,000,000. Even before this, malarial epidemics of marked intensity had been experienced in 1868-69, 1877-78, 1895-96 and in 1899-1900 (Gill, 1928). Subsequent to the year 1909 there have been several epidemics of malaria but the outstanding outbreaks were recorded in the years 1917 and 1923, 1942 and 1950. Such epidemics of malaria usually develop during years of excessive monsoon rainfall associated with overflow of rivers following a series of years in which the rainfall has been in defect, and the greater the number of years which have elapsed since the last epidemic year, the more likely is an epidemic to occur.

As a result of the study of distribution of these malaria epidemics during the long series of years, it has been found that the districts of the Punjab vary in their liability to experience visitations of epidemic malaria. For instance, they do not occur in any part of the Himalayas, although Kangra Valley is one of the most intensely malarious tracts. The precise location of such wide-spread epidemics of

malaria is more or less restricted to the low-lying plains which are liable to inundation.

Amongst the chief factors which complicate malaria problem in the Punjab may be included (1) flooding of the rivers and the associated storm-water channels after excessive monsoon rainfall, (2) canal irrigation, (3) uncontrolled flow of storm water from hill sides into the adjoining low-lying plains after heavy rains, (4) extensive swamps in low-lying areas which receive local drainage, as in Gurdaspur District and (5) the impounded water as a result of the construction of protective embankments in certain districts.

At least nine districts in the Punjab, namely, Gurdaspur, Amritsar, Ludhiana, Jullundur, Hoshiarpur, Ferozepore, Karnal, Rohtak and Gurgaon are liable to inundations to a greater or lesser extent almost every year following excessive monsoon rainfall when the rivers Ravi, Beas, Sutlej and Yamuna are in spate. Owing to the impervious nature of the soil, the flood water is retained for a sufficiently long period to create malariogenic conditions in such tracts.

It is true that malaria has followed canal irrigation in the Punjab by raising the level of subsoil water and created swampy conditions in the adjoining areas. Canal irrigation has become gravely prejudicial to health in areas where it has been improperly carried out. Most of the districts in the Punjab, which are traversed by the great canal system, have become greatly menaced by malaria.

In the hill tracts, the high incidence of malaria is usually associated with paddy cultivation which depends on the water supply from '*Kuhls*' arising from hill-streams.

MALARIA FORECAST.

The Punjab perhaps claims a unique position inasmuch as it is only in this State that attempts have been made to develop and bring into administrative use a method for forecasting epidemics of malaria. This method, as elaborated by Gill (1928) and Yacob and Satya Swaroop (1944), utilizes information pertaining to rainfall, enlargement of spleen amongst school children, economic conditions, and the variability of malarial incidence in individual localities recorded in previous years.

This forecast, which is issued every year in the beginning of September, has proved of considerable value in past years in indicating areas in which more than ordinary prevalence of malaria has in fact subsequently occurred. A malaria forecast of this type is certainly invaluable to a State which is every year exposed to malaria epidemics owing to the vagaries of monsoon rainfall.

ANOPHELINE FAUNA.

There are at least thirteen species of anopheline mosquitoes found in the East Punjab. These are listed below:—

A. culicifacies	*A. maculatus*
A. fluviatilis	*A. annularis*
A. stephensi	*A. pallidus*

A. lindesayi
A. pulcherrimus
A. splendidus
A. hyrcanus
A. subpictus.
A. barbirostris
A. gigas simlensis

Of these anophelines, *A. culicifacies* is the accepted chief vector of malaria in the Punjab. This is evident from the following results of dissections, carried out by different workers from time to time:—

Observer.	Species dissected.	Number dissected.	Gut infected.	Gland infected.
Perry (1940)	*A. culicifacies*	100	...	...
Gill and Singh (1917-20) ...	—do.—	295	...	...
Chowdhury (1930)	—do.—	152	15	...
Hicks and Majid (1931-36) ...	—do.—	8,815	20	32
Mehta (1940-42)	—do.—	455	10	6
Gill (1925)	*A. stephensi*	155	3	3
Chowdhury (1930)	—do.—	3	...	...
Hicks and Majid (1931-36) ...	—do.—	254	...	10
Mehta (1940)	*A. stephensi*	42	...	...
Chowdhury (1930)	*A. fluviatilis*	24	2	...
Hicks and Majid (1931-36) ...	—do.—	381	...	10
Mehta (1940)	—do.—	146	...	...
Mehta (1940)	*A. maculatus*	21	...	...
Mehta (1940)	*A. pallidus*	103	...	...
Chowdhury (1930)	—do.—	7	...	...
Chowdhury (1930)	*A. splendidus*	26	...	...
Hicks and Majid (1931-36) ...	—do.—	98	...	...
Mehta (1940)	—do.—	21	...	...
Stephens and Christophers (1902)	*A. subpictus*	496	...	...
Gill (1917)	—do.—	102	...	...
Hicks and Majid (1931-36) ...	—do.—	159	...	...
Hicks and Majid (1931-36) ...	*A. annularis*	206	...	...
Mehta (1940)	—do.—	195	...	...

From the above data it would be evident that *Anopheles stephensi* and *A. fluviatilis* have also been incriminated in the carriage of malaria. According to

Covell (1927), sporozoites were detected in the salivary glands of *A. willmori* James by Adie (1911) in the Kangra Valley. The precise rôle of this species in the transmission of malaria is being studied.

The dissections of *A. culicifacies* during different months of the year have indicated that the transmission of malaria is active chiefly from August to October. In view of the fact that the transmission is so restricted, the measures aimed at the destruction of anopheline vectors have to be regulated accordingly.

PREVIOUS HISTORY OF ANTIMALARIA WORK IN THE PUNJAB.

The first serious attempt to study malaria problem in the Punjab relates to a report of a committee assembled to enquire into the salubrity of the area near Karnal watered by the Western Jamuna Canal (Dempster *et al.*, 1847).

In the year 1908, the Punjab was visited by a regional malaria epidemic of marked severity in consequence of which an Imperial Malaria Conference was held at Simla during October 1909 and this led to the creation of the Punjab Malaria Bureau. The functions of this institution were confined solely to the study of malaria in this Province. Sometime later Christophers (1911) wrote a comprehensive memoir entitled '*Malaria in the Punjab*' which embodied a scientific exposition of the intricate malaria problems of the State. These findings laid the foundation for further investigations on malaria (Perry, 1911; and Gill, 1914: 1915:1917:1920:1921:1923:1924:1928).

During the subsequent reorganization of the Public Health Department, Punjab, in 1923, the scope of the Malaria Bureau was enlarged so as to include the investigation and control of other epidemic and endemic diseases also. This resulted in weakening the efforts towards the study of malaria. In the year 1939, a field epidemiological unit was set up which was responsible for conducting malaria investigations and instituting antimalaria measures which consisted chiefly of the destruction of anophelines by pyrethrum sprays and administration of antimalaria drugs to the sick. In addition limited antilarval measures were also applied.

At the recommendations of the Bhore Committee (1946), a malaria organization was set up for the State with its headquarters at Lahore but before it could establish itself, the Punjab was faced in 1947 with partition based on political considerations.

Prior to the year 1950, antimalaria measures were applied in the Punjab in a very restricted manner by the provision of a small antimalaria unit in each district and consequently feverish endeavours had to be made whenever serious outbreaks of malaria were reported. Such recurring measures proved very expensive and helped in providing relief only for the time being.

In the year 1950, the antimalaria policy was revised under the guidance of Lt.-Colonel Jaswant Singh, Director, Malaria Institute of India. Accordingly, the existing State Malaria Organization was expanded with its headquarters at Karnal. This organization includes malaria and entomological laboratories where specialized investigations on malaria, biological assaying of insecticides and other cognate problems are conducted. In addition, two antimalaria demonstration

units were set up for combating rural malaria in Karnal and Gurgaon Districts and another malaria control project unit was detailed to demonstrate if malaria could be controlled in a badly affected urban tract like the city of Amritsar. During the period from the year 1950 to 1952, the number of demonstration teams was raised to five and these amply showed that under proper technical direction and control, a substantial lowering of malarial incidence could be achieved by the indoor application of insecticides such as D.D.T. and B.H.C.

NATIONAL MALARIA CONTROL PROGRAMME.

In the year 1952, the total population protected against malaria by the State Malaria Organization was nearly four lacs (0·4 million) which involved an expenditure of nearly Rupees 2,61,000. This inadequacy of the protection afforded against malaria was realized by the Punjab Government early in 1953 when a bold and outstanding decision was taken to participate in the National Malaria Control Programme initiated by the Government of India under the Indo-American Point-IV Agreement. During the year 1953-54, the Punjab Government earmarked a sum of Rs. 7,47,700 towards this programme and the Government of India's contribution was nearly Rs. 11,38,004. This assistance was in the shape of grants of D.D.T., antimalarials, motor transports, and essential spraying equipment provided through the Technical Cooperation Administration. The expenditure on the operational costs was borne by the Punjab Government. In this manner, four malaria control units were created for operation in areas worst affected by malaria in Gurgaon, Karnal, Rohtak, Ambala, Ludhiana, Ferozepore, Gurdaspur, Amritsar and Kangra Districts.

Intensive antimalaria operations consisted chiefly of indoor spraying of village houses with five per cent D.D.T. watery suspension at a dosage of 100 mg. per square foot twice during the malaria season. These operations were commenced during July 1953, in nine out of the 13 districts of the State. In the remaining districts, routine antimalaria measures were applied by the normal anti-epidemic staff on similar lines under the District Medical Officers of Health.

Each malaria control unit consisted of one Medical Malaria Officer (Gazetted), eight Malaria Inspectors, 23 Sanitary Supervisors, 120 Sanitary Beldars (field workers), five Motor-drivers, five Cleaners and one Mechanic. In addition, adequate personnel had been provided for the office of each unit at Gurgaon, Karnal, Gurdaspur, and Ferozepore. The mobility of the four Malaria Control Units was ensured by the provision of four trucks and one jeep for each unit.

The programme of work was framed in advance taking into consideration the requirements of the areas to be served. In this manner, a total of 8,03,056 rooms in 2,526 villages were sprayed with D.D.T. to afford protection against malaria. In addition, antimalaria drugs were administered by these units to 10,142 malaria cases. By the application of these measures nearly 14,00,000 population was directly protected against malaria.

These operations were also extended to deal with community projects of Faridabad, Sonepat, Nilokheri, Jagadhri and Batala, where 2,00,999 persons were

protected against malaria. The community projects, as conceived by the Government of India, have been designed to promote the pre-requisite for additional productivity, such as can cater for all the basic elements of rural life.

Assessment of the first year's work under the National Malaria Control Programme in the Punjab indicated that although the target in regard to the total population to be protected could not be reached, still there had been a dramatic effect on the life of the people served by this project. A reduction of 50,000 malaria cases was recorded in the recognized hospitals and dispensaries of areas in the State served by the National Malaria Control Programme. In addition, there was a striking reduction in the spleen and parasite rates amongst the children. Similarly, there was a conspicuous lowering of the population density of mosquitoes.

The outstanding evil influence of malaria is directed towards the great loss of man power in industry and agriculture. The economic gain to the Punjab, as it affected the ' Grow More Food ' campaign in the year 1953, has been estimated for Karnal and Ferozepore Districts. It has been revealed that there had been an increase of 1,30,562 acres in the area cultivated in three tehsils namely, Karnal, Panipat and Kaithal during the period from July to December 1953, as compared to the preceding year. Similarly, it has been estimated that there has been an increase of 11,327 acres in the area under paddy cultivation alone in three tehsils mentioned above which equals, by a modest official estimate of Rs. 126 per acre, to a sum of Rs. 14,27,202. This increase in the area under paddy cultivation has been reported only in the case of three tehsils in which intensive antimalaria measures have been carried out during 1953. On the other hand, in Thanesar Tehsil of Karnal District which could not be included in the National Malaria Control Programme there has been a marked reduction in the area under paddy cultivation. Similarly there has been an increase in the area under paddy cultivation in Ferozepore District to the extent of 4,834 acres in 1953 as compared to the previous year which at a modest estimate equals Rs. 6,03,084. Similar data is being collected from other districts also and it is expected that the net economic gain to the Punjab would definitely repay to a large extent the expenditure on malaria control programme (Rs. 7,47,700) incurred by the Punjab Government during 1953.

Encouraged by the results obtained, the Punjab Government extended their antimalaria programme so as to cover the entire State during the year 1954. For this purpose, seven malaria control units, including the existing four units, were raised at an expenditure of nearly Rupees 10·8 lacs (1·08 million). Once again the Government of India, through the Technical Cooperation Mission, agreed to provide the required quantities of D.D.T., motor transports, essential spraying equipment, etc., at an approximate cost of nearly Rupees 8·5 lacs (0.85 million).

Seven malaria control units were accordingly raised, equipped and trained by June 1954, when intensive antimalaria operations were commenced throughout the State. The disposition of these seven units, total population to be protected and areas to be covered are given below:—

Units.	Area to be covered. (sq. miles).	Population to be protected.	
1. **Gurgaon Unit.**			
Gurgaon District	1,776·3	7,16,089	10,00,000
Rohtak District	590·0	2,83,911	
2. **Karnal Unit.**			
Rohtak District	826·6	3,77,916	10,00,000
Karnal District	1,726·0	6,22,084	
3. **Ambala Unit.**			
Karnal District	542·0	1,88,030	
Ambala District	960·0	4,24,481	10,00,000
Hoshiarpur District	785·0	3,87,489	
4. **Jullundur Unit.**			
Ludhiana District	688·3	3,85,690	
Jullundur District	380·1	3,01,002	10,00,000
Hoshiarpur District	558·0	2,71,361	
Amritsar District	89·0	41,947	
5. **Kangra Unit.**			
Kangra District	3,020	5,64,060	10,00,000
Gurdaspur District (Pathankot and Gurdaspur Tehsils) ...	712	4,35,940	
6. **Gurdasper Unit.**			
Gurdaspur District	569·0	3,24,060	10,01,118
Amritsar District	971·0	6,77,058	
7. **Ferozepore Unit.**			
Amritsar District	28·0	13,037	
Ferozepore District	2,580·06	7,31,963	10,00,000
Hissar District	1,306·0	2,55,000	
Total ...	18,070·26	70,01,118	

These units conducted malaria surveys in hitherto uninvestigated areas of the State during April and May 1955 and intensive antimalaria operations were commenced during the following month. The technique employed in regard to the functioning of the malaria control units was in conformity with the instructions contained in the '*Operations Guide*' issued by the Malaria Institute of India. In

this manner 33,42,333 persons living in 5,868 villages were protected against malaria. In this connection 20,24,400 rooms were sprayed with five per cent D.D.T. watery suspension at a dosage of 100 mg. per square ft. once or twice during the malaria season depending on the requirements of the areas served. In addition to this measure, resochin was administered to the sick in the villages.

Assessment of the antimalaria work carried out so far has revealed an unprecedented reduction in the number of malaria cases in the Punjab during the year 1954 as compared to the previous years. This is evident from the data reproduced below:—

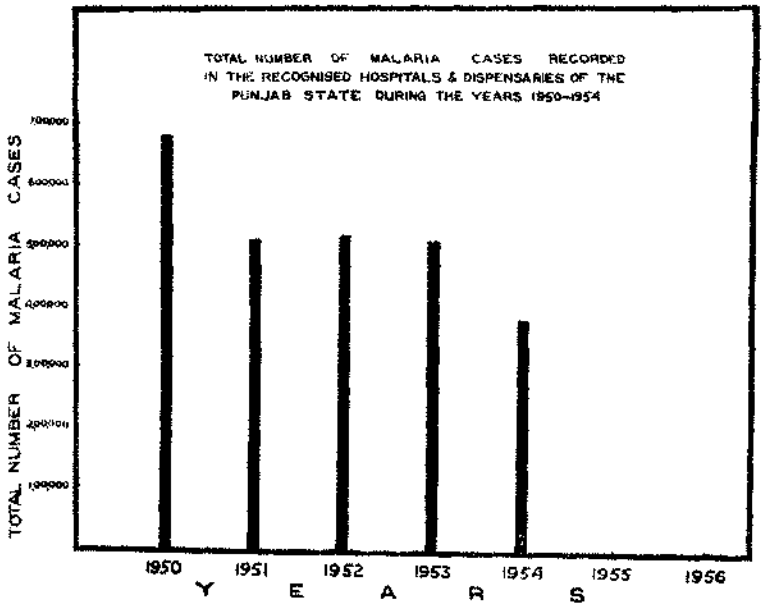
Year	Number of malaria cases recorded in the hospitals and dispensaries of the State.
1950 ...	6,80,059
1951 ...	5,11,775
1952 ...	5,16,777
1953 ...	5,08,636
1954 ...	3,80,496

It will be observed that there has been a reduction of nearly 3,00,000 malaria cases during the year 1954, as compared to 1950 when residual insecticides had not been brought into use for malaria control on a very large scale throughout the State (Chart 1). This reported reduction pertains to the records in recognized Government dispensaries and hospitals. Considering that a very large number of the affected population do not visit such centres of medical relief, it is expected that the total reduction in the number of malaria cases throughout the State during the whole year must be very great. Similarly reductions in the (*i*) spleen rates of children, (*ii*) parasite rates, and (*iii*) number of mosquitoes have been observed (Appendix I).

The transmission of malaria has been considerably reduced as indicated by pronounced lowering of infant parasite rates in all the areas served by the National Malaria Control Programme in the Punjab.

From the foregoing account, it is evident that there has been a reduction of nearly 3,00,000 malaria cases during the year 1954 as compared to 1950 when residual insecticides had not been brought into use for malaria control on a very large scale throughout the State. A case of malaria usually incapacitates a person for at least three to six days of his wage earning capacity. At the lowest daily rate now provided by the various Governments, this involves a loss of Rs. 12 per head on account of a single attack of malaria. It is, therefore, evident that nearly Rs. 36,00,000 were saved in terms of wage earning capacity of 3,00,000 persons protected from attacks of malaria during the second year of the National Malaria Control Programme in the Punjab State.

CHART 1.



A substantial reduction in the number of fever deaths has also been reported in the Punjab during the year 1954. The relevant data are given below:—

Year.		Total number of fever deaths.
1950	...	2,10,961
1951	...	1,58,871
1952	...	1,63,140
1953	...	1,84,858
1954	...	1,25,005

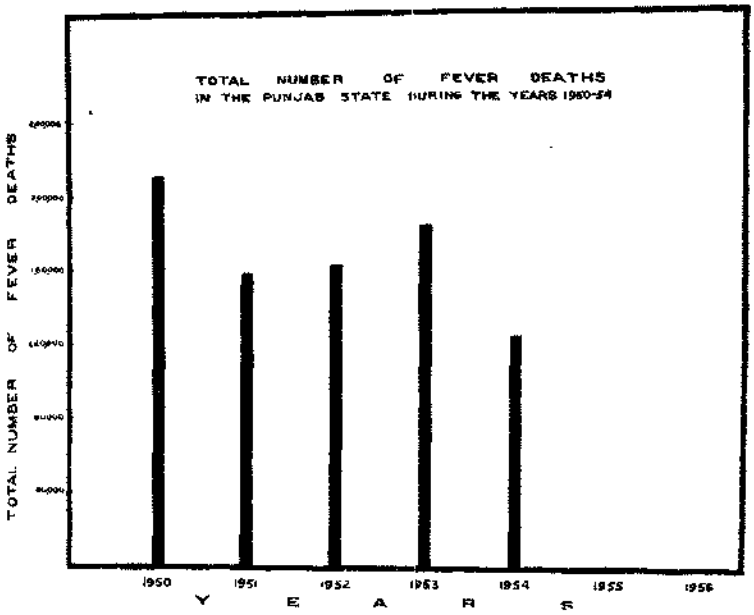
It will thus be observed that there had been a reduction of nearly 85,956 fever deaths in 1954 as compared to the year 1950 (Chart 2).

It is emphasized that apart from other advantages on account of the saving of 85,956 lives, there had been a net saving of Rs. 42,97,800 which would have been spent on unprofitable funeral expenses.

COLLATERAL BENEFITS.

Amongst the collateral benefits, as a result of intensive antimalaria operations in the Punjab, may be mentioned a substantial reduction in the population of flies, fleas, etc., which are usually abundant in the villages. There is, however, no yard-stick by which this could be measured except that three is considerable public appreciation in this field as manifested by the growing demand for D.D.T. spray even in areas where malarial incidence is extremely low.

CHART 2.



In the Punjab, complete control against plague was effected in the year 1950 by the use of residual insecticides chiefly in the indoor rat harbourages. This control has been effectively maintained up till now chiefly by indoor D.D.T. spraying which has been carried out under the National Malaria Control Programme.

The available data also indicate that there has been substantial reduction in the number of total deaths due to diarrhœa and dysentery in Amritsar District since the year 1951 when indoor spraying with D.D.T. and B.H.C. was carried out on a large scale for protecting the population against malaria. The relevant data are reproduced below :—

Year.		Total deaths due to diarrhœa and dysentery.	
1942	...	973	
1943	...	933	
1944	...	915	
1945	...	647	
1946	...	871	
1947	...	1,141	
1948	...	714	
1949	...	619	
1950	...	806	
1951	...	489	Mass indoor spraying with residual insecticides.
1952	...	441	
1953	...	453	

CONCLUSIONS AND SUMMARY.

Encouraging results have been achieved during the first two years of the working of the National Malaria Control Programme in the Punjab State.

Besides the lowering of malarial incidence there has been considerable saving of human lives. It has also been possible to improve the economic condition of the people by antimalaria operations. A healthy and productive population is the basis of national economic progress which malaria definitely inhibits. The National Malaria Control Programme was introduced at a very critical stage in the political life of the Punjab State which had been badly hit by partition during the year 1947. The alleviation of human suffering by intensive antimalaria operations has to a very large extent been responsible for the upgrading of the economic status of the people.

Judging from the success attained and the progress maintained so far, it is expected that the incidence of malaria would be driven to a very low point during the next few years as a result of the National Malaria Control Programme. Having stopped malaria transmission, efforts will be concentrated on 'strategic control' in order that malaria may not reappear.

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APPENDIX I.

Spleen rates amongst children in the Punjab before the National Malaria Control Programme and in November, 1954.

District.	Locality.	Spleen rate before N.M.C.P. (per cent).	Spleen rate in November, 1954 (per cent).
Kangra	Nurpur ...	43·0	5·6
	Shahpur ...	24·3	16·2
	Jowali	70·1	16·8
	Dera Gopipur	58·0	6·9
	Jowalamukhi ...	22·9	11·4
	Kulu	33·1	12·0
	Indaura ...	66·6	14·8
Gurdaspur	Narot Jaimal Singh	28·1	10·8
	Behrampur ...	51·1	1·0
	Dera Baba Nanak	25·0	2·5
	Siri Gobindpur ...	27·5	4·8
Amritsar	Patti	70·1	1·3
	Ram Das ...	27·9	2·9
	Taran Taran ...	24·5	7·5
	Ajnala ...	21·6	4·1
	Lopoke ...	29·8	18·1
	Majitha ...	21·6	2·9
	Chabal ...	24·2	3·3
	Amritsar (suburban)	40·5	5·3
Ferozepore	Guru Har Sahai ...	89·3	3·0
	Zira	49·3	14·2
	Dharamkot ...	22·1	5·2
Ludhiana	Sidhwan Bet ...	47·6	2·0
	Machiwara ...	21·1	7·1
Hoshiarpur	Santokh Garh ...	31·1	5·3
	Garh Shanker ...	22·9	4·6
	Hajipur ...	26·0	21·3
Karnal	Indri	38·0	4·3
	Thanesar ...	26·9	1·9
	Khojgipur ...	34·3	4·3
Rohtak	Gohana ...	23·8	4·6
	Juan	22·9	0·0
Gurgaon	Dhauj ...	52·8	12·9
	Sohna ...	35·3	6·4
	Hassanpur ...	45·2	13·7
	Punahana ...	59·2	9·4

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THE CHANGING ASPECT OF HAND-OPERATED EQUIPMENT IN MALARIA CONTROL.

BY

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(July 28, 1955.)

PUBLICATION of the Silver Jubilee issue of the *Indian Journal of Malariology* presents an appropriate time to review some of the important changes made during the past half-century in hand-operated malaria control equipment, as well as some of the reasons for the modifications and improvements.

The stages of development might be loosely divided into three periods. The first of these periods began a number of years before recognition of the vector of malaria which occurred in the late 1890's. The second period began about 1930 when attempts to control the disease through spreading of larvicides were expanded; this was a time when concentrated efforts in comparatively small-scale control programmes were the practice. The third period began about 1940, when control of mosquito larvæ was replaced by control of adults in their natural resting habitats.

Hand-operated equipment of one kind or another was used throughout the three periods, in limited quantity during the first period, but in increasing volume as programme requirements increased. It may be of interest to note some of the alterations which came about with changing techniques in control measures.

Even before the *Anopheles* mosquito was identified as the transmission agent of malaria, "oil" was spread on water surfaces from time to time to control mosquito breeding. As early as 1892, practical application techniques for spreading oil were developed by Howard (Ginsburg and Rudolfs, 1941). Thereafter oil was the only larvicide extensively used until 1918 when Thibault experimented with pyrethrum powders in dust form (Barber, 1941*a*). Despite the development of pyrethrum powders and other products for malaria control, however, oil in one form or another, sometimes combined with other toxicants such as paris green or D.D.T., has always remained one of the principal larviciding agents.

A very great change in larviciding techniques came about in 1921 when Barber and Hayne demonstrated the practical use of paris green diluted with an

inert material such as road dust (Barber, 1941*b*). The use of this mixture became standard procedure and remained so until 1936 when Barber suggested a modification—paris green diluted with oil (kerosene) in the presence of egg albumen (Barber, 1941*c*), instead of road dust. When D.D.T. came into prominence, it was used in solution with oil (naphtha) to improve the quality of the larvicide (Russell *et al.*, 1946). These, then, were some of the popular materials developed to control mosquito larvæ. To distribute these materials, hand equipment of one type or another was used.

Pyrethrins, the toxicant elements in pyrethrum, were first used to destroy adult mosquitoes late in the 1930's (Thornton, 1935). The toxicant was dissolved in kerosene and applied as a space spray. The efficiency of the method was proved in India early in the following decade (Russell *et al.*, 1943), and for a time this spray solution was widely used in spite of its high cost and its lack of residual killing properties.

Pyrethrum space sprays do not compare, though, in overall residual effectiveness, with the newer toxicants represented by chlorinated hydrocarbons, commonly referred to as the D.D.T. group. These sprays, properly applied to the resting places of adult mosquitoes, have brought about radically beneficial results in malaria control throughout the world.

Any agent for mosquito control must be applied. Whether the agent is oil, paris green, pyrethrum, or D.D.T., it must be distributed by some type of equipment to the resting place of the larvæ or the adult. For a long time the development of such equipment was left mostly to chance. Certain types, for instance knapsack sprayers, were taken over from other fields with little attempt to modify them in relation to the new problem. In fact, no active effort was made to develop hand equipment for malaria control until pyrethrum came into use as a space spray. Since then more and more attention has been paid to this problem. The movement has gained impetus in recent years due to leadership of the World Health Organization Expert Committee on Insecticides. Through its efforts, standardization of hand-operated equipment has rapidly advanced to the point where spray equipment manufacturers everywhere actively seek the Committee's advice.

In the early days of larviciding, when oil was first used, it was apparently applied with any tool at hand. Sometimes the oil was poured directly on the water surface from a container, and spread only because of its repellency toward water. No effort at economy was made, and no spreading agent was knowingly employed. Sprinkling containers of the garden type were frequently used, perhaps the first equipment employed to dispense oil on water as a mosquito control measure. Once malaria transmission had been linked to the mosquito, knapsack sprayers such as those used in agriculture were taken over and used without modification. Throughout practically the entire larviciding era, only slight improvement which could be directly attributed to malaria control influences, was made in this apparatus. Even today, wherever the knapsack sprayer is still used it remains the same heavy, cumbersome oiler. No real attempt has ever been made to improve the quality of discharge from the nozzle tip in order to secure better spreading of the larvicide. Actually, little thought was given during the early years to the improvement of larviciding by use of oil; today, because of

the efficiency of other control methods, so little larviciding is carried on that specialized equipment for this means of control is hardly considered necessary.

The knapsack sprayer may be symbolic of all hand-larviciding oil equipment. It was pressed into service, performed a useful function without undergoing extensive alteration, and has been almost entirely replaced by techniques better suited to the solution of the problem.

When Barber and Hayne developed the technique of destroying *Anopheles* larvæ by spreading paris green on water surfaces, they recognized the need for using a very small quantity of paris green per unit of water surface. Consequently a way to dilute paris green had to be devised; and it was necessary to find some inert material with which the toxicant could be mixed. Ordinary road dust became the popular diluent.

It was quite a task to prepare this diluent. First the dust had to be collected, and in malarious areas it was not unusual to see gangs of men along a well-travelled highway assiduously collecting road dust. Then the dust had to be screened; an efficient hand-operated device was constructed for the purpose. This device was probably one of the first pieces of equipment designed primarily for use in malaria control; another was the mixing drum for combining the dust with the toxicant. Such equipment is now usually found only in museums featuring discarded control methods.

After a proper larviciding mixture of paris green and dust had been prepared, it still had to be distributed. A common early method was to put the mixture in a bucket and throw it by hand onto the water surface. Hand bellows, to which a small hopper containing the mixture was attached, were also used. A pipe carried the mixture from the hopper to the tip of the bellows, where ejection under low air pressure took place whenever the bellows was operated. This outfit had advantages over hand distribution; but the low capacity of the hopper, as well as other drawbacks of a mechanical nature, weighed against great popularity of the apparatus.

Probably the most popular equipment for the distribution of road dust was a type of knapsack duster, held on the operator's back with straps. It consisted of a hopper with a capacity of about one cubic foot of mixture, and a bellows arrangement, activated by a hand lever, which pumped air under low pressure and fair volume into a small compartment of the duster. This compartment was automatically refilled with dust. Air pressure agitated the dust. The dust-air mixture escaped through a distribution hose and was deposited on water in fairly uniform quantity. Although many modifications of the apparatus were developed, the principle of distribution remained practically unchanged. Distributors of this type continued in use in diminishing numbers as long as the road dust-paris green technique survived, or until about 1937-38.

Beginning about 1930, demand slowly grew for improved equipment, particularly for hand-operated dust distributors with rotary blowers. As with the knapsack larviciding machine, these rotary blowers were borrowed from agriculture and were not efficiently designed. The difficulties experienced in their use were many indeed. Because most of them were badly assembled, they leaked dust through every seam. In addition, little attention had been paid to " balance ", with the result that prolonged use tired the operator excessively. Air

passages were not streamlined; this resulted in low-grade dust distribution. Gears and cranks were crudely made. Air vents and accompanying fans were not constructed to render maximum air delivery with a minimum of effort. One example of such equipment was a square box referred to by the makers as a venturi. It was used as a mixing chamber for air and dust. This box, in no sense a venturi, frequently became clogged and caused endless trouble. Furthermore, as the operator walked forward to dust a water surface, he had to turn the crank counter-clockwise, in order for the apparatus to function at all!

Considerable improvement was made in this type of distributor during the following six or eight years, until the rotary duster was no longer extensively used for malaria control. Possibly due to the need for a better product for malaria operations twenty years ago, this machine is much more substantial and dependable today and is still extensively used in agriculture.

A very important revolution in equipment was brought about after 1936 by the introduction of Barber's wet method of paris green distribution. This method soon eliminated road dust as a diluent. It was no longer necessary to collect, screen, and store vast quantities of dust; nor was it necessary to mix and distribute tons of powder. Instead, an operator carried with him sufficient kerosene-paris green mixture to last one day. Water was always available to dilute the mixture to proper proportions.

This system of larviciding required completely different equipment, a type best operated by developing air pressure in the mixture container. The mixture was ejected through a primitive type of nozzle in the form of a spray which readily spread a thin film over the water surface. The apparatus was the forerunner of the hand-compression sprayer so extensively used in residual spray operations during recent years. These original hand-compression sprayers had mechanical and structural weaknesses, but they were the most efficient tools used for malaria control through the kerosene-paris green control period.

Other types of equipment were put into service during the late 1930's and early 1940's. These were, in fact, the real pioneer years for designing equipment adapted to malaria control. Most equipment proved inadequate for the proper distribution of insecticides, however, until about 1940. Since then equipment has been designed more and more to meet this specific problem.

One type of equipment worthy of note for larvicide application was the automatic paris green distributor, a machine originally designed in the Philippines and later perfected in India, which distributed proper quantities of paris green continuously over irrigation canals (Knipe and Russell, 1942). Current in the canals activated the distributor, and the same current carried a charcoal-paris green mixture downstream with it, doing a remarkably efficient job of larvæ destruction *en route*. However, popularity of the apparatus waned when the kerosene-paris green mixture became popular, even though the equipment could readily be adapted to distribute this material.

Prior to 1940, malariologists frequently demanded some means of successfully attacking adult mosquitoes. Pyrethrum used as a space spray was the first practical answer to this demand. It was tried out experimentally in South Africa and in northern India in the late 1930's (Covell *et al.*, 1938). Beginning in 1939, an

active field programme in which pyrethrum was used as a space spray, was instituted in southern India. Ultimately, this programme proved beyond doubt the effectiveness of the product as a space spray (Thornton, 1935). So-called "flit guns" were used originally with fair success (Russell and Knipe, 1939). However, these cheap sprayers were not well made, did not sufficiently atomize the spray, were small in size, and were inadequately designed. In order to overcome these defects, certain modifications were made: an adequate nozzle tip which assured good atomization was constructed; the continuous discharge type was perfected; and a model hand sprayer called the "Cobra", which markedly increased overall efficiency, was developed (Knipe and Sitapathy, 1942). Concurrently the Malaria Institute Sprayer Hand (M. I. S. H. pattern) was evolved in Delhi. It possessed all the qualities of good atomiser. It was widely used in civil organizations and by the army in all contonments and in forward areas during war years.

Many other models of sprayers were investigated and developed in this project in southern India. One of these dispersed space sprays through the use of "dry ice"—solidified carbon dioxide—as the propellent (Knipe and Sitapathy, 1942). This apparatus did away with all labour of hand pumping. Another was the so-called "one-man" petrol-powered unit (Knipe and Sitapathy, 1942). This unit, which weighed only 28 pounds and was entirely self-contained, was quite efficient. Still another model tried successfully was the prepressurized unit—the prototype of the present-day hand-compression sprayer. This was pressurized, through a Shrader valve, to 100 pounds per square inch from one of several different sources of air pressure (Knipe and Sitapathy, 1942). A modified Cobra sprayer unit carried the insecticide, which was ejected and atomized by air pressure stored in the hand-compression sprayer tank.

At this time the need for some sort of regulator to control pressure on the nozzle tip was recognized, and a lightweight commercial regulator was found which served the purpose (Knipe and Sitapathy, 1942). It was placed on practically all sprayers using pyrethrum, except hand sprayers of the Cobra type. This type needed no such attachment because it developed only one-half atmosphere pressure (Knipe and Sitapathy, 1942). This regulator was the forerunner of the present-day pressure regulator now advocated for use in one form or another on practically all types of residual spray equipment, whether power driven or hand activated.

Pyrethrum sprays, although they were exceedingly popular and exerted highly lethal effects on mosquitoes without exhibiting any injurious properties toward higher animals, did not have significant residual property. To be effective they had to be used repeatedly and at close intervals. Furthermore, the cost was quite high. Consequently, when sprays possessing residual properties came in the market, the popularity of pyrethrum quickly receded.

Residual spray applications posed new equipment problems but not so many as one might expect. The groundwork in sprayer development had been well laid during the period when pyrethrum sprays were popular. Although the action of the new spray was different, the principles of application remained much the same. The result has been the development of two types of hand-operated sprayers which have almost entirely replaced all others. These are the hand-compression sprayers, used practically throughout the world, and the stirrup-pump sprayer, widely used in India but not to any extent in other countries.

An excellent residue of toxicants may be applied with either type of sprayer. Although the sprayers differ considerably in action, the same amount of insecticide is distributed since distribution is made through the same type of liquid discharge line by each type. Features of this line which have undergone intensive investigation include the following: a bayonet type of attachment (for the hand-compression sprayer); a polyvinyl chloride (a plastic), lightweight, highly abrasive resistant hose; a cut off valve which does not leak; a sturdy lance; an automatic non-drip valve in the nozzle; and a carefully designed stainless steel nozzle tip which accurately determines the desirable flat spray pattern as well as the rate and angle of discharge.

Since the hand-compression sprayer is the type universally used, it has been subject to the greatest improvement. In contrast to its prototype, which was, at best, a cheaply designed agricultural tool with many defects, the present-day hand-compression sprayer is a first-class tool for residual spray application and meets practically every requirement demanded of it. This equipment has long since surpassed its predecessor, the agricultural sprayer, in quality. Features include an all-stainless steel tank construction, welded seams which ensure against leakage, an adequate filler hole which may be semiautomatic in action, a Shrader valve for prepressurizing, adequate safety features, plastic leak-proof gaskets, a pressure regulator specially designed for the equipment, and a bayonet connection (quickly detachable) for the discharge line.

These improvements have been made through the cooperation of individuals working on malaria control projects who have consulted with interested equipment manufacturers in several countries. Much of the coordination of details in the programme of development has been carried on by the World Health Organization Committee previously mentioned. This Committee meets from time to time to discuss equipment and to formulate specifications of a general nature which seem to lend themselves to acceptance on a world-wide basis.

Hand equipment used in the field of malaria control has indeed passed through an interesting period of development. The early prototypes, borrowed from any available source, served a purpose. They were not always suited to the problem, but successful modifications were usually made. Then specialized models, such as the automatic paris green distributor, began to appear. Later, types adaptable to pyrethrum distribution were developed and gradually improved, with the result that when residual sprays appeared, operators in the malaria control field were fairly well-acquainted with the types of equipment required. Models have now been perfected to the point where a malaria officer knows he has dependable equipment with which to carry on his control programme. In fact, spray equipment for malaria control is now of such excellence that technical features are being copied for use in other insect pest control programmes.

The advances made thus far do not mean that the perfect apparatus has been designed. Improvements will continue to be made. Everyone would like to escape the drudgery of pumping or pressurizing, a problem that may be solved in the near future. Better pressure regulator control mechanisms will be designed. Longer lasting nozzle tips which produce even more uniform insecticide distribution will become available.

In other words, spray equipment, like most modern equipment, has gone through several periods of development and change. Today's equipment is far superior to yesterday's. The end is still not in sight.

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ON THE POSSIBILITY OF *A. SUNDAICUS* ERADICATION IN INDIA.

BY

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(July 15, 1955.)

THAT it is possible to eradicate vector anophelines from particular areas under certain conditions, and thus to eliminate permanently all chances of malaria transmission there, has been amply borne out by recent work. The eradication measures which have so far been carried out successfully may be broadly classified into three groups as follows:—

(*a*) Measures dealing with imported vectors;

(*b*) Measures carried out in limited areas having natural barriers; and

(*c*) Measures where eradication is obtained, not by deliberate action, but rather unexpectedly as a bye-product of indoor residual spraying with insecticides.

The first concerted attempt at eradication, which falls in the first category mentioned above and which was attended with conspicuous success, was made in Brazil by Soper and Wilson (1943) against *A. gambiæ* and the next attempt was also made against the same species by Shousha (1948) in Egypt with similar results. These measures involved antilarval as well as anti-adult operations, though it was recognized that the former constituted the primary means of attack with the latter only as supplemental measures wherever necessary. In both cases, paris green was used against the larvæ and pyrethrum against the adults. Soper and Wilson (1943), however, cautiously observed that their success was due to the fact that the vector was imported into Brazil less than ten years previously and did not yet gain a firm foothold in the country and that *A. gambiæ*, having selective breeding habits, was more vulnerable to attack than other species which are capable of breeding in a large variety of waters. They doubted whether eradication would ever be possible with autochthonous vector species.

After the advent of D.D.T., when it became possible to contemplate the elimination of malaria on a global scale, eradication was tried in Cyprus and Sardinia, two small islands in the Mediterranean Sea. Whereas complete success was obtained in Cyprus (Aziz, 1948), the vector of Sardinia has not yet been wholly

eradicated, though it exists in only very small numbers and malaria is brought completely under control (Logan, 1953). In these cases also, anti-adult measures were supplemented by antilarval measures.

On the other hand, eradication as a bye-product of indoor residual spraying with D.D.T. has been observed by several workers in various parts of the world. Gabaldon (1949) and Giglioli (1951) have reported the eradication of *A. darlingi* from Venezuela and British Guiana, respectively. Dowling (1951) and Hamon and Dufour (1952) have succeeded in eradicating *A. funestus* from Mauritius and Reunion. *A. sacharovi* has been eliminated from certain parts of Italy (Missiroli, Mosna and Allessandrini, 1948). *A. minimus*, a notorious vector of the Oriental Region, has been virtually eliminated from the sprayed areas of Thailand (Dy, 1954). It should, however, be noted that all these cases have been observed on the border-line areas of the geographical distribution of the species concerned (Gabaldon, 1953).

It, therefore, appears that eradication is possible where the vector is imported into a new region, where the area concerned is limited and bound by natural barriers against re-infestation or where the vector is occupying the marginal zones of the area of its distribution. The question now to be discussed is whether *A. sundaicus* satisfies any or all of these conditions and, if so, to what extent.

A. sundaicus is a species of the Malayan sub-region of zoological distribution and is not properly a member of the Indian anopheline fauna. The only locality in India where it is known to exist with certainty for a long time is the Sunderbans in the deltaic tracts of the rivers Ganges and Brahmaputra (Christophers, 1933) where physical features and ecological conditions are similar to those in the Malayan region. However, by 1910 it was discovered that the range of its distribution was extended up to Matla and Port Canning, about 30 miles from Calcutta (Gravely, 1912). In 1930, the mosquito made a further push inland up to Budge-Budge, only 16 miles from Calcutta (Iyengar, 1931) and, within another five years, it occupied practically the whole of the 24 Parganas District, including the salt lakes of Calcutta and even extended westward up to about 20 miles from that city. Severe malaria outbreaks occurred in all these areas soon after they were invaded by *sundaicus*.

Another area where *sundaicus* is known to exist is around the Chilka Lake in Orissa, though for how long it has been there cannot be definitely stated. When Hunter (1872) toured the area extensively to prepare a comprehensive report on Orissa to the Government, there was no malaria around the Lake, though Puri Town towards the north and Ganjam Town towards the south of the Lake, were observed to be malarious. However, after a lapse of 40 years, Fry (1912) found an intense degree of malaria infection in the same area, with child spleen rates indicating hyperendemic conditions along the Lake shore. Sarathy (1932) observed similar conditions twenty years later, showing that there was little change in the situation during the intervening period. Though the time of invasion of Puri is not certain, there is some evidence of when the invasion of Ganjam took place. Ganjam is situated about five miles to the south of the Lake. This was a flourishing port and the local headquarters of the East India Company, with a population of 35,000, till 1815, when a violent epidemic of malaria occurred. The

town was then abandoned by the Company and soon became a ruined and insignificant place. If we assume, as perhaps we must, that the malaria in Puri and Ganjam found by Hunter (1872) was due to the presence of *sundaicus*, it was about the year 1815 that *sundaicus* first invaded this area (Senior White and Venkat Rao, 1946). Though the mosquito was present in these two isolated places, it did not extend into the Chilka Lake as long as the Lake had direct access to the sea and its water was too saline for the mosquito to thrive. The subsequent choking up of the outlet and the resultant freshening of the water and the abundant growth of fresh water flora there might, in later years, have facilitated the rapid extension of the mosquito into this area (Venkat Rao, 1949).

The next extension, which was directed southwards, appears to have taken place during or about the year 1930, when violent epidemic exacerbations were reported from Chatrapur Town (five miles south of Ganjam) and from a somewhat extensive area further down the coast known as the " Uddanam ", including Naupada, a salt manufacturing centre. Both these areas were previously healthy, having been used as sea-side health resorts for a number of years. While, as in the case of Puri (Panigrahi, 1942), Chatrapur suffered only from periodical epidemic outbreaks, highly endemic conditions were soon established in the Uddanam area, which became notorious for what came to be known locally as " coastal malaria ".

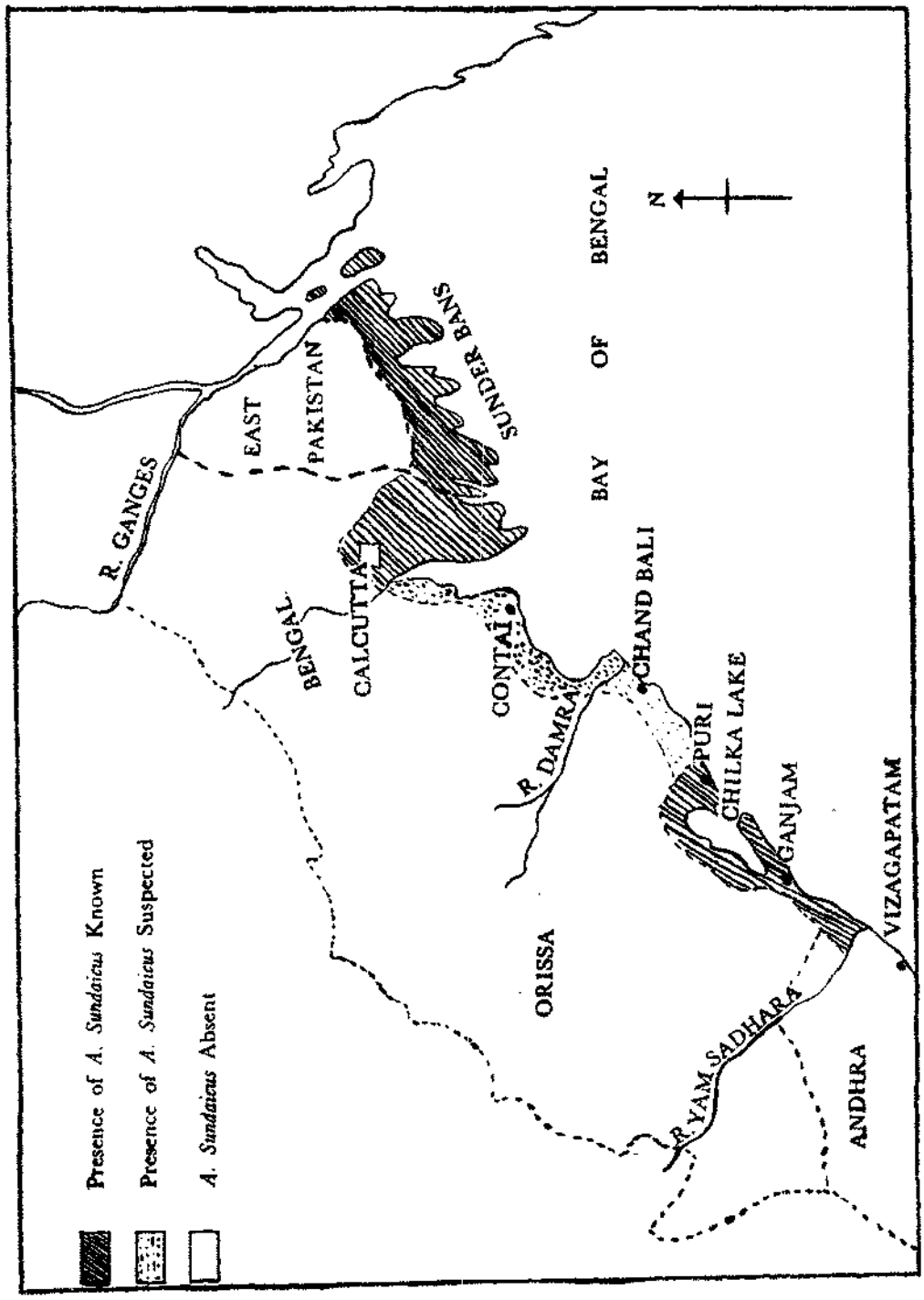
Curiously enough, the latest infiltration to occur was not directed southward along the coast as might have been expected but extended inland up to about twenty miles from the sea, though the worst affected localities were situated within ten miles of the coast. This happened during the years 1942 and 1943 (Senior White *et al.*, 1947).

Thus, the areas where *sundaicus* is now known to be present are Lower Bengal, Chilka Lake area and the coastal tracts of Ganjam and North Vizagapatnam districts (Map 1).

The mechanism of extension of this mosquito into Lower Bengal has been described by some authors. After a careful investigation of the means of transport available in Bengal, Sen (1941), observed that, though railway trains were also partly involved, it was the small " country " boats plying the various localities between Sunderbans and Calcutta that were really responsible for the establishment of the mosquito in Calcutta, and that particularly active dispersal occurred during the years 1933 and 1934. This might possibly have been connected with the increased forest fellings followed by rice cultivation near Port Canning (Senior White, 1937).

The first invasion of Puri and Ganjam in the early years of the last century might have been facilitated likewise by small sea-going vessels plying between Bengal and Orissa. At that time railways did not exist in India.

It is, however, obvious that the mere availability of railway and boat transport does not by itself result in the expansion of the mosquito into new areas. A number of railway trains run daily between the Chilka Lake and other *sundaicus*-infested areas further south and the town of Vizagapatam (since renamed Visakhapatnam), and regular coastal shipping services are also available but the mosquito has not yet extended into the latter area, though suitable breeding conditions are also present.



MAP 1.
North-East India.

The invasion of Chatrapur Town, which is only five miles south of Ganjam, occurred over a hundred years after Ganjam was itself invaded, while the area immediately south of Chatrapur remained free from *sundaicus* for at least ten years more. Probably, it is not availability of means of transport but a cataclysmic disturbance like a sudden and unprecedented increase in the population of the species that is responsible for bursts of colonization. Deforestation and extended cultivation near Port Canning referred to above might have resulted in augmentation of breeding places and, therefore, in the increase of *sundaicus* population in that area. During 1942, there was an immense increase in the prevalence of *sundaicus* in the Chilka area, pushing up the density in houses to over one hundred per man-hour (Venkat Rao *et al.*, 1942). As Senior White (1948) has observed, passive migration may extend the species into new areas but it is likely to die out there, as it did in the areas west of Calcutta.

It is thus shown that, except in the Sunderbans, *sundaicus* is an imported mosquito everywhere else. Except in the isolated localities of Puri and Ganjam where the mosquito appears to have been introduced over a hundred years ago, the importation into the other areas occurred in more recent times, the last burst occurring only about twelve years ago.

Regarding the second postulate, *viz.*, limitation by natural barriers, it is clear that *sundaicus* has a strong predilection for coastal conditions and does not exist far away from coastal and brackish water areas. In Bengal, it has never been found more than six miles from tidal waters (Senior White and Venkat Rao, 1946). Throughout the Chilka area, if isolated collections in small numbers are excluded, the species does not exist beyond a maximum of five miles from the Lake. Further south, including extreme cases, it has never been recorded from places over twenty miles from the Bay of Bengal. It has now disappeared, either spontaneously or as a result of control measures, almost entirely from this area.

A. sundaicus has not been recorded south of the River Vamsadhara. Christophers (1933) referred to *sundaicus* (then known as *ludlowi*) as having been included in the list of Colombo mosquitoes by James in 1914 but it has not since been recorded there and is thought by Carter not to occur in Ceylon.

Thus, the *sundaicus*-infested area in the present West Bengal is only about 1,000 sq. miles, while in Orissa and North Vizagapatam areas, the extent is approximately 1,500 sq. miles.

The 200-mile length of the coast between Puri and Calcutta has not been surveyed and whether *sundaicus* is present there, either continuously or in isolated patches, is completely unknown. The only reference to malaria prevalence in the area is made by Hunter (1872) who says that "fever of low malarious type" is prevalent everywhere and specially so at the mouth of the River Damra, which is about as unhealthy as any found in Bengal and that the malaria season extends from August to October. It is also known that the port of Chandbali is highly malarious. The presence of *sundaicus* may be suspected in both the cases. It, however, appears that continuous distribution of *sundaicus* all along the coast is not very probable as suitable estuarine conditions are seldom found without considerable separatory areas. There are, at least, some known healthy spots on this coast like Contai and Digha. The infested area, if there is any, is thus unlikely

to be more than a thousand square miles in extent, making a total of 3,500 square miles.

If *sundaicus* has not extended further inland than it has already done, it is not because of natural barriers but because the mosquito is incapable of doing so owing to its evident need for an equable climate. In fact, the only trait it still exhibits in common with its original home, Indonesia, is its association with the sea shore.

For all practical purposes, therefore, *sundaicus* has a limited distribution and is subject to constitutionally imposed barriers against further expansion inland.

As the presence of *A. sundaicus* has been recorded only on the east coast of India between the Sunderbans and the mouth of the Vamsadhara River and nowhere else, either in India itself or in the countries west of India, it is definite that the area of its present prevalence is the westernmost limit of its area of distribution. *A. sundaicus* here is occupying an extremely narrow marginal zone.

The situation is, however, complicated by the presence, at least in Orissa and North Vizagapatnam areas, of two forms of *sundaicus*, one breeding in fresh waters and the other in saline waters. According to epidemiological evidence, the fresh water form appears to be a potent vector of malaria, while the other form is at best a vector of secondary importance (Senior White *et al.*, 1947).

Now, *sundaicus* has been recognized to be pre-eminently a salt water breeder everywhere. The only reference in the previous literature to fresh water *sundaicus* is made by Bonne Wepster and Swellengrebel (1953), who have stated that there was a fresh water form in Sumatra, that it was a potent vector there and that it was eliminated when its breeding places—fresh water fish ponds—were eradicated. Thus, fresh water *sundaicus* appears to have existed in Sumatra and still exists in India, which are the eastern and western margins of the area of *sundaicus* distribution.

The salt water breeding *sundaicus* has always been looked upon as a very effective carrier of malaria in the coastal regions of all countries wherever it occurs. However, Taylor (1943) observed that, owing to the lack of direct correlation between the presence of this species and malaria on all occasions in Singapore, it had been thought that *A. sundaicus* of that island might consist of more than one species or variety and that more recent work indicated that this " supposition " was correct. If, as is presumably the case, he referred to salt water breeders only, it appears that, even among them, there are forms which are vectors and others which are not. Even in the Arakan region of Burma, Lalor (1912), Feegrade (1924) and Macan and Watson (quoted by Fox, 1949) dissected altogether 908 specimens with negative results and therefore, *prima facie, sundaicus* is not a vector there too. No fresh water breeding has ever been recorded from this area.

The available information indicates that, while there may be, among salt water breeders, some non-vector forms, the fresh water forms are potent vectors wherever they exist. Subsequent work carried out by Venkat Rao and Ramakrishna (1950) in the North Vizagapatnam area has shown that the two forms can be separated morphologically owing to differences in the number and shape of the leaflets of their phallosomes, somewhat similar to those observed in the corresponding forms of Sumatra (Bonne Wepster and Swellengrebel, 1953).

It cannot perhaps be assumed that the two forms of *sundaicus* have always been present in this area; the fresh water form, which is located in recent years, should then have evolved out of the earlier salt water form. Huxley (1942) has observed that a single species may separate gradually into two or more divergent lines transcending the limits of species distinction and that the separation into two mutually infertile or otherwise distinct groups may occur suddenly though the subsequent divergence may be gradual. He goes on to state that ecological but spatially overlapping differentiation will promote divergence in general characters since more complete adaptation to two ecological niches will be advantageous to both species. Probably, when *sundaicus* is faced with the problem of heavy overpopulation, it is capable of meeting the situation by separating, more or less suddenly, into two or more groups, each occupying a different ecological zone. When they are thus separated, they may not merge again into one species through cross-mating. Though the variations in male genitalia mentioned above may not raise a mechanical barrier to copulation between the sexes of the two forms, there is an imponderable factor of a physiological nature operating against cross-mating in such cases (Fennah, 1946). However, as both forms exhibit a marked preference to the ecoclimate of the sea coast and are present together within the same areas, and as both the forms have been found to be vectors in some area or other, this question, so far as eradication is concerned, may not require further discussion.

Having shown that all the three conditions postulated for a successful eradication campaign are satisfied in a large measure in the case of *sundaicus*, the specific measures which should be undertaken for the purpose, shall now be considered. It must, however, be emphasized, as Gabaldon (1953) has pointed out, that eradication is a costly method which requires vigorous action against both larvæ and adults not always needed for successful malaria control. However, if one has to carry out indoor residual spraying continuously over a number of years, the total recurring cost might well be found as high as the cost of an eradication campaign if not higher and the problem is never solved finally. There is no reason to suppose that indoor residual spraying alone will result in ultimate eradication of *sundaicus* as it did in certain other cases. On the other hand, Crandell (1954) has reported that *sundaicus* in the Djakarta area of Indonesia has developed chemico-resistance against D.D.T., though not yet to dieldrin, after a few years' spraying indoors. Therefore, as in all other cases where eradication has been purposely aimed at, antilarval measures should constitute the primary means of attack, though indoor spraying should also be pursued simultaneously.

Fortunately, a good deal of study has already been made by various authors on the breeding habits of *sundaicus*. The vast bulk of the breeding occurs in lakes, tanks, ponds and such other still waters. The breeding in flowing waters and ricefields is minimal, being restricted to the mouths of rivers and the narrow belt of ricefields along the margins of large bodies of saline water like the Chilka Lake. The association of *sundaicus* with aquatic vegetation is very striking and the species will apparently breed in the presence of many different types of vegetation.

While dealing mainly with salt waters, Covell and Singh (1942) have pointed out that it is only when the vegetation comes above the water surface and begins to putrefy that the larvæ of this species are found. This suggests that water

polluted by rotting vegetation favours the breeding. This is in accordance with the observations of Iyengar (1931) that, besides salinity, organic pollution of the breeding place is a cardinal condition of *sundaicus* breeding. Can this be the reason for the poor vectorial status of the salt water form, at least in the Chilka area? The critical experiments of Russell and Mohan (1939;1940) have shown this to be a wrong premise but Muirhead Thomson (1951) observed that in these experiments the pollution was of animal origin and that it still remained to be seen if gross pollution of the larval environment with vegetable organic matter—a much more widespread phenomenon in nature—would have any effect on the susceptibility of infection of the resulting adults. However, Venkat Rao and Ramakrishna (1947*a*) have shown that, breeding of the fresh water form occurs in waters which are clear and good enough for the people to use for drinking purposes and that, when the vegetation is removed, the water becomes turbid and polluted and the green alga, *Microcystis auriginosa* grows in quantities and *sundaicus* disappears. It is thus seen that the salt water form breeds largely in polluted waters and the fresh water form in clean waters, which may be a significant difference in their behaviour.

Covell and Singh (1942) observed the association of *sundaicus* with thirteen types of aquatic plants and eight types of algæ; Najas, Ceratophyllum and Hydrilla among plants and Lyngbya, Anabena and Spirogyra among algæ being the most important. In fresh waters, association with the same three plants and Spirogyra was very marked. Whereas a thick cover of *Eichhornia speciosa* in the breeding place was found to inhibit *sundaicus* breeding in Bengal (Iyengar, 1946), it did not produce similar results in Orissa (Venkat Rao and Ramakrishna, 1947*b*). Breeding in waters devoid of vegetation is practically nil and deweeding of breeding places was found to be very effective in eliminating *sundaicus* from the breeding places both by Covell and Singh (1942) and Venkat Rao and Ramakrishna (1947*a*).

Control of *sundaicus* breeding may be effected by the use of paris green or naturalistic measures like deweeding. Paris green treatment of the numerous breeding places at intervals of five to six days is a very costly and difficult method. It is also likely to be inefficient during periods of heavy and continuous rainfall. Deweeding thus becomes the only reliable alternative available.

Deweeding by manual and other mechanical means was tried on a large scale in the Chilka and North Vizagapatam areas and proved very effective. The breeding is immediately checked and the breeding places remain negative for *sundaicus* larvæ as long as the weed-free condition of the water surface is maintained. However, regrowth of vegetation occurs so fast, at least during the first two or three years, that the maintenance work becomes very difficult, requiring a large labour force and proper supervision, without which the work becomes unsatisfactory.

A very much simpler and equally effective method of deweeding is offered by the recent introduction of chemical weed-killers. These are claimed to remain effective, after one application, for four to five years, in which case one application may suffice for the whole campaign.

While this method is applicable to inland waters, both fresh and saline, of limited extent, it does not apply, owing to its cost, to the large bodies of salt water like the Chilka Lake, which alone is over 500 square miles in extent. But, in this

lake, though the fauna is marine, the flora is that of fresh water (Annandale and Kemp, 1915), which can be eliminated by raising the salinity through the introduction of sea water. There may be some damage to rice cultivation in the immediate vicinity of the lake where the lake water, during periods of low salinity, is used for irrigating the ricefields but the loss is more than compensated by augmentation of fish supply, for which the lake is already noted. Some dredging is occasionally necessary to keep the mouth of the lake constantly open for the ingress of sea water, as otherwise the littoral sand drift might soon choke the mouth, resulting in the ultimate freshening of water in the lake.

Lastly, effective measures have to be undertaken to prevent re-infestation from the neighbouring countries, East Pakistan and Burma. It is now recognized that, for eradication of malaria or of vector anophelines, international cooperation is essenial. The best method would appear to be the application of eradication measures simultaneously in all the three countries.

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SOME MALARIA ERADICATION PROBLEMS AS VISUALIZED IN 1955.

BY

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(August 25, 1955.)

"The ultimate goal of a nation-wide malaria control programme is the eradication of the disease".—*W.H.O. Second Asian Malaria Conference*, 1954.

It seems strange that during the many years of malaria control by residual insecticides so little account has been taken of the destiny of the malaria parasite in man. Attention has been focused on vector control, apparently forgetting that if the vectors have no infection to transmit, their control would be superfluous. The fact that malaria infections die out spontaneously within a few years and that therefore, if no new cases occur or are imported, the population will be cleared of malaria when those few years are over, was very seldom taken into consideration when planning programmes. During the development of a programme, emphasis was put on operational techniques, on the number of houses sprayed, on variations of vector densities, less frequently on those of malariometric rates; but practically never on achieving the full interruption of transmission which might have led to the eradication of the infection in the population and to the discontinuation of insecticide spraying. This concept was presented as far back as 1948 at the Fourth International Congress of Tropical Medicine and Malaria (Pampana, 1948; 1952), but it was only after the demonstration that some anopheline species could develop resistance to chlorinated hydrocarbon insecticides that it was given serious consideration. It was then felt that resistance was such a danger that efforts should be made to shift the objective of antimalaria activities from simple malaria control, that is reduction of transmission, to malaria eradication, so that residual insecticide applications could be stopped before resistance had a chance to occur (Pampana, 1954). This principle was discussed and a new strategy eventually approved by important international conferences, both at the intergovernmental level (XIV Pan-American Sanitary Conference, Santiago, 1954) and at the technical level (Second Asian Malaria Conference, Baguio, P.I., 1954) and, finally, by the Executive Board (15th Session) of the World Health Organization

in January 1955 and the Eighth World Health Assembly (Mexico, May 1955). While readers of this Journal are probably acquainted with the Report of the Second Asian Malaria Conference, it might be useful to recollect that the Santiago Conference resolved " that it [was] of the utmost urgency that the Member Governments should convert all control programmes into eradication programmes within the shortest possible time, so as to achieve eradication before the appearance of anopheline resistance to insecticides ". Further, the Eighth World Health Assembly Resolution *inter alia* resolved to " request Governments to intensify plans of nation-wide malaria control so that malaria eradication may be achieved and the regular insecticide-spraying campaigns safely terminated before the potential danger of a development of resistance to insecticides in anopheline vector species materializes ". It may be said that there is today a large consensus of expert opinion that wherever it is planned to control malaria by residual insecticide spraying, eradication of the disease should be the goal, so that the insecticide could be discontinued when still fully effective against the vectors. Obviously eradication will require greater efforts, and larger antimalaria appropriations. Hence finance departments should be persuaded to give more funds and the two main arguments that would perhaps convince them are, (1) that although eradication would require a larger annual budget than present control programmes, this would only be required for a few years, so that in the end savings would be effected; and (2) that by continuing residual spraying from year to year resistance to the insecticides might develop and malaria control would then become a much more expensive proposition, if not practically impossible.

But even malariologists, not to mention public health administrators, may have some doubts as to the advisability, the feasibility and the urgency of malaria eradication. These doubts are quite understandable, for eradication is a new venture. We shall try in this paper to foresee some of these queries and doubts, and attempt to reply.

1. One of the reasons that prompt us to change our strategy is the development of resistance to insecticides. Is it likely that resistance, so far described only in a few species in a few countries or localities, would develop in others? Unfortunately there is perhaps no answer to this first query. But this same question came to the mind of malariologists after the report of the D.D.T.-resistance of *A. sacharovi* in Greece (Livadas, 1951; Livadas and Georgopoulos, 1953). Later also in the same country *A. maculipennis* and *A. superpictus* were reported to have developed resistance as well (Belios, 1954). A few months passed. Then in some localities of Java *A. sundaicus*, it was demonstrated (Crandell, 1954), had become D.D.T.-resistant. More months passed; and then resistance was stated to have developed in *A. stephensi* in some localities of Saudi Arabia (Daggy, 1955). If this has happened, it seems reasonable to think that in the future resistance may occur in other parts of the world and with other species. And as resistance to the chlorinated hydrocarbon insecticides might be, in our present state of knowledge, a very serious occurrence in countries which were highly malarious before the inception of control, there is ample justification for adjusting programmes in such a way as to obtain most benefit from the insecticides and to stop their application when they are still active.

2. It is stated that malaria eradication is possible because malaria infections are self-curing and do not last more than three years. But we all know of ancient and recent observations reporting that some subjects, not exposed to reinfections, have relapsed well beyond the period often believed to be the maximum duration of the respective infection. Without considering *P. malariæ* infections, which may well last a lifetime, *P. falciparum* infection, which generally does not last more than nine to twelve months, has been found to last up to 503 days—as a maximum—in experimental infections with a Panamanian strain, the average duration being however 279·5 days (±19·9) (Jeffery and Eyles, 1954). After 13 months, in Porto Rico, it had been calculated that 18·3 per cent of *falciparum* cases might still show parasites in their blood (Macdonald, 1950); and a case has been described of an American with a *falciparum* infection present four years after he had contracted it in the Congo (Logan, 1953).

As regards *vivax* infections, which generally do not last beyond two years, we shall only recollect observations on infections contracted in the South Pacific, in 4·3 per cent of which clinical attacks were still present four years after (Hill and Amatuzio, 1949).

In conclusion there certainly are infections of *P. falciparum* lasting more than one year and of *P. vivax* lasting more than three years. This is a parasitological truth; such cases, however, are rare*, and we may assume, for public health purposes, that they do not invalidate the general statement that if no new cases of malaria have occurred for three years in a given locality, its population no longer represents a source of infection for anophelines. It should not be forgotten that public health policies are based on generalizations. No vaccination gives 100 per cent protection and still vaccination has succeeded in eradicating smallpox from many a country.

If, exceptionally, a long lasting malaria infection succeeds in infecting a mosquito and in giving rise to secondary cases, the small outbreak could easily be brought under control, particularly so if residual insecticides can be effectively employed. It might be compared to an outcrop of secondary cases following the immigration from abroad of a gametocyte carrier, such as that described by Brunetti *et al.* (1954) in the U.S.A.

3. When could it be stated that malaria eradication has been achieved ? We know that among the various malariometric indices the infant parasite rate (I.P.R.) is probably the most sensitive indicator of transmission. In some malarious territories, where epidemiological and entomological conditions are particularly favourable to control by D.D.T. or other insecticides, the I.P.R. may become zero after a single spraying. If it is maintained at this level and no infective subjects come from abroad, it would be justifiable to withhold insecticide spraying in the fourth year. Case (*a*) of Figure 1 depicts this example. Of course, a zero infant parasite rate does not necessarily mean that no cases occur in the population ;

* In this connexion it may be interesting to quote certain figures relative to the *Anopheles gambiæ* eradication campaign in Brazil. Prior to the campaign, in May-November 1939 out of 11,207 subjects examined 65·7 per cent had a positive blood. In July-August 1941, *i.e.*, one year only after "practically all transmission by *gambiæ* had ceased" only 0·5 per cent were positive (84 out of 16,530 examined. Of the 84, 18 were *vivax*, 67 *falciparum*, 1 mixed) (Soper and Wilson, 1943).

therefore, we cannot say that malaria eradication has been achieved when we stop the insecticide. It will be necessary first to set up a machinery of searching for new cases, and incidentally, of applying the necessary protective measures. This machinery of epidemiological surveillance, should be set up, as recommended by the Second Asian Malaria Conference, before the discontinuation of spraying. We should assume that, as in our figure, it is working already in the year which is supposed to be the last of the spraying years. In other words, in the first example (*a*) of Figure 1, in the third year of spraying; in the second example (*b*), in the fourt year. Now let us recollect the definition of the National Malaria Society of the U.S.A.: " Malaria may be assumed to be no longer endemic in any given area when no primary indigenous case has occurred there for three years, if reporting . . . and case finding are actively promoted and adequate investigations are carried out ". A corollary of this definition (definition which had been quoted by the Fifth Report of the Expert Committee on Malaria of W.H.O.) is that *when malaria " is no longer endemic "* in any given area, it would mean that eradication had been achieved three years before. In case (*a*) of Figure 1 eradication would have been obtained in the fourth year, in case (*b*) in the sixth year. In conclusion the date of actual eradication could only be established retroactively.

4. It has often been recommended that the area of eradication should be " as large as possible " (Reports of the First and of the Second Asian Malaria Conferences). One would think it easier to eradicate malaria from a village or a group of villages rather than from a whole country. But it would be impossible to maintain those localities free of sources of infection for the vector mosquitoes, because we can neither compel the villagers to remain for at least three years in their villages, nor preclude the entrance of other people from outside. It might be possible to prevent such exchange of persons if the villages were completely isolated, as, for instance, in the case of an island. With such exceptions, the large size of an area would minimize the danger of sources of infection being imported. The larger the area is, the smaller would be the danger. Eradication on a continental scale, as it has been decided for the Americas, once achieved, will have to establish protection only against travellers coming from other continents or oceanic islands.

Still, in programmes on a continental, sub-continental or even country-wide scale, it might be expedient and even necessary to proceed by steps and to split the territory from which malaria should be eradicated into zones with different priorities. Each of these zones should, as far as possible, be large enough to reach the borders of healthy areas, or natural, or man-made barriers so that the reintroduction of sources of infection would be minimized. In some cases this might not be feasible, and one might suggest continuing the residual spraying of all localities situated at the periphery of the eradication area. But the danger of developing insecticide resistance would counterindicate this idea, which, in any case, would hardly be logical.

5. Still, if the eradication area cannot be surrounded by healthy areas or adequate barriers, some way could be devised to reduce the danger of reintroduction of sources of infection.

Let us suppose that, contiguous to eradication area '*A*' is another malarious area '*B*' and that the epidemiological conditions in '*A*' are such that it can be

FIGURE 1.

Tentative schemes of the sequence of events in malaria eradication programmes.

Years.	−1	1°	2°	3°	4°	5°	6°	7°	8°	9°	10°
Case (*a*) of conditions exceptionally favourable, in which the infant parasite rate, in infants born after the first spraying, is brought down to naught during the first year.											
	SURVEY	ATTACK	CONSOLIDATION		MAINTENANCE						
Spraying operations (on a total coverage base)		XXXXXXXXXXXXXXXXX									
Infant parasite rate ...	++	o(1)	o	o							
Epidemiological surveillance				XXXXXXXXXXXXXXXX Special surveillance carried out by malaria service.				.... General surveillance carried out by health service.			
New cases of malaria locally contracted and traced by epidemiological surveillance				++	o	o	o	↑			
								At this point malaria is no longer endemic.			
Case (*b*) of average conditions, rather favourable, in which the infant parasite rate is brought down to naught only during the second year of spraying.											
	SURVEY	ATTACK	CONSOLIDATION		MAINTENANCE						
Spraying operations (on a total coverage base)		XXXXXXXXXXXXXXXXXXXXXXXXX									
Infant parasite rate ...	++	+(?)	o	o	o						
Epidemiological surveillance					XXXXXXXXXXXXXXXXXX Special surveillance carried out by malaria service. »					.... General surveillance carried out by health service.	
New cases of malaria locally contracted and traced by epidemiological surveillance.	(?)	(?)	(?)	(?)	+	+	o	o	o	↑	
										At this point malaria is no longer endemic.	

(1) In infants born after first spraying.

foreseen that spraying can be withheld after four years of operations. It will be recollected that the Second Asian Malaria Conference agreed that " adequate surveillance of malaria incidence . . . . should be initiated well before the time of interruption of spraying ". Then in area '*A*' surveillance should be functioning not later than during the fourth year. Suppose that we provide, in the country-wide programme, for expansion of the operations to area '*B*' in this same year. Then a very large proportion of, if not all, new malaria cases in '*B*' will be prevented in the year. What will then be the reservoir of infections during the fourth year in '*B*'? Very few *falciparum* subjects, infected prior to the spraying, will still show parasites. There will, however, be a good proportion of sources of infections of *P. vivax* and of course of *P. malariæ*, contracted chiefly prior to the beginning of spraying. But it is clear that the total number of subjects infective for mosquitoes will have been greatly reduced, the more so if *falciparum* malaria had been prevalent. Therefore, among the persons from area '*B*' who in the fourth year would go into area '*A*' and would remain there after the spraying had been discontinued (*i.e.* in the fifth year) not many could be capable of infecting the anopheles of '*A*'. Moreover, as epidemiological surveillance in '*A*' is already functioning, malaria cases both in immigrants from '*B*' and in inhabitants of '*A*' (who might have become secondarily infected from the former) could be easily traced and dealt with. Finally, if spraying of '*B*' in the fourth year reduces, on the one hand, the problem which faces epidemiological surveillance of '*A*' it will also, on the other hand, protect visitors from '*A*' from getting infected in '*B*' and carry back the infection to zone '*A*'.

In conclusion, even if no adequate, natural or man-made barrier could be found, it would appear that a satisfactory protection might be secured, if, in a given area, epidemiological surveillance could start simultaneously (if not earlier) with the year for which the last spraying cycle is foreseen and if in the same year the surrounding malarious areas could be sprayed.

6. As the possibility of starting transmission again after spraying is discontinued appears as a real danger, it would be interesting to visualize how dangerous the immigration of people from malarious areas of varying endemicity into the area from which spraying has been stopped, would be. If we call '*A*' the area in which spraying has been stopped, let us call '*B*' and '*C*' the surrounding areas, which are both malarious. '*B*' is hyperendemic, or holo-endemic. Everyone of its people will probably be infected, but gametocytes will be found practically only in the blood of children. Now children,introduced in area '*A*' would represent a great danger for infecting the local mosquitoes; but children travel much less than adults, and only a few would be likely to be brought into area '*A*'; moreover, they could be easily spotted, by the surveillance teams, and efficiently treated.

Area '*C*' on the other hand, is a mild meso-endemic area; let us suppose with a spleen rate of 20 per cent. Here we would probably find gametocytes carriers among adults; but infected subjects, whatever their age, would represent only a small proportion of the total population. In the cases of both '*B*' and '*C*', therefore, the danger represented by immigration of people from malarious areas into the adjoining eradication area (if this is sufficiently large and adequately

supervised by epidemiological surveillance) is perhaps less than one would think. The danger would be greater for persons of '*A*' visiting '*C*' and particularly '*B*'; but one would think that these persons, should they develop malaria upon their return, would more easily be found by the personnel of the epidemiological surveillance and one would count on their willingness to co-operate with them.

The mode of travel across the borders of the eradication area also deserves attention. Travelling on foot, by horse, or ox-cart, by bicycle, etc., represents probably the greatest menace because these are the means employed by rural groups, such as entire families, going from village to village. This method of travel, however, does not generally cover long distances* and if the eradication area is large enough it would represent a danger only for the peripheral band, where epidemiological surveillance should, therefore, be particularly efficient. Travelling by other means, motor-bus or train, is expensive over long distances: babies and children do not travel alone, and the travel of entire families by these means would perhaps not be too frequent and might be restricted to cases of change of residence, important family events, etc. Furthermore, one would think that most of the people travelling long distances by train would come from towns and go to live in other towns; and towns often have no anophelines or else are the first localities in which malaria is controlled. This reasoning applies even more cogently to those travelling by air.

In conclusion it appears that, if the eradication programme covers an area large enough and if epidemiological surveillance is more efficient and strict in the peripheral part of it, the problem of imported gametocyte-carriers could be solved satisfactorily.

7. As shown in Figure 1, it is tentatively assumed that residual spraying may be interrupted, obviously with the necessary safeguards, after the infant parasite rate has remained at zero for three years. In order to shorten as much as possible the period of years during which insecticide spraying must be carried out, efforts should be made to bring the I.P.R. down to zero rapidly. In W.H.O.'s experience this may happen after the first spraying; but only exceptionally. More often two years of spraying, at least, will be necessary. In other cases this is not enough; or in some areas the I.P.R. becomes zero only in some sectors and not in others. Often such failures can be explained by the small size of the area, or by disregard of the need of total coverage, or by faulty techniques (including poor insecticide formulations, or rapid sorption of the insecticides by the walls). Should these three mistakes be corrected, or avoided, and transmission continue, then it may be thought that transmission is occurring, at least in part, outdoors. By repeating the spraying from year to year it is to be expected that the progressive reduction of gametocyte-carriers in the population would eventually stop transmission; but given the advisability of accelerating results with a view to discontinuing spraying before resistance may occur, it will be necessary to supplement residual spraying with other anti-malaria techniques, such as:

(*a*) Anti-imago space spraying, such as was applied before the "D.D.T.-era" in South Africa, in India, in the Netherlands.

*It will be noted that in this paper we have refrained from discussing the difficulties caused by nomadism, which is a special case and could not be sufficiently dealt with in this short article.

(*b*) Larval control, though excluding the use of the chlorinated hydrocarbon insecticides.

(*c*) Use of drugs. Very exceptionally would it be possible to submit a whole population of an area to frequently repeated administrations of drugs, for the organization of such schemes would be very difficult and costly; but ways could be found of utilizing the spraying personnel for distributing a few mass treatments to all or to selected groups of the population.

The use of drugs might also be considered in areas where the residual insecticide will succeed in fully interrupting transmission, with a view to reducing the number of years during which the insecticide spraying must be continued. It is realized, however, that with the possible exception of Pinotti's (1953) method, the systematic distribution of drugs or a systematic attempt towards radical cure of all cases of *vivax* or *malariæ* infections will be a very difficult problem.

8. In programmes aiming simply at some degree of malaria control, it is not essential, although desirable, to cover by residual spraying (or possibly to protect by other means) all houses of the area. In programmes aiming at malaria eradication total coverage is essential. Furthermore, if interruption of the spraying is to be applied as soon as possible, not only total coverage will be essential, but also a simultaneous implementation of the programme all over the area, with the same degree of efficiency everywhere (This need of coordination in space, time and efficiency was emphasized in the Report of the Second Asian Malaria Conference). These are, incidentally, the reasons why eradication programmes have a higher *per capita* cost than control programmes. If in some portions of the area people refuse to have their houses sprayed, or if in some villages where drugs should supplement the insecticide, people refuse to take them, clearly pockets of transmission will remain in the area and might make it necessary to continue the spraying operations beyond the limit that could have been foreseen had the reaction of the population been more favourable. Hence the need of providing for a suitable education of the population on the one hand, and, on the other hand, for suitable legislation.

Legislation for malaria eradication should not only provide authority to overcome the objections of the inhabitants where health education has failed; but, as for instance in countries where the operations are largely decentralized, should also make provision for allowing the central antimalaria organization to intervene if the local authorities are lagging behind the schedule of the national endeavour in which they participate.

SUMMARY.

As it appears that there is to-day a large consensus of expert opinion that the objective of malaria control by residual insecticide spraying should be that of malaria eradication, so that the spraying of insecticide could be discontinued before the local vectors develop resistance to it, some problems related to eradication have been considered. These problems are, whether D.D.T. resistance is likely to develop in future in other species; whether it is correct that from the public health standpoint eradication of the infection can be achieved after a few years,

and when. The questions of the size of the area and of the danger of reintroduction of the sources of infection in the area from which malaria has been eradicated, and the influence of the mode of travel on this danger have been considered.

Finally, techniques supplementary to insecticide residual spraying are suggested to control pockets where transmission may linger, and the need of adequate legislation in any eradication campaign is emphasized.

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OBSERVATIONS ON SOME ASPECTS OF THE NOCTURNAL BEHAVIOUR OF *ANOPHELES CULICIFACIES*.

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INTRODUCTION.

THIS paper reports observations made on some aspects of the nocturnal behaviour of *Anopheles culicifacies*, the most important vector species involved in malaria transmission in India. Such studies throw light not only on the normal pattern of behaviour of the adult mosquitoes but on possible changes which may follow the application of residual insecticides. The insect behaviour vitally affects the degree and duration of successful establishment of malaria control and, ultimately, malaria eradication. Insect behaviour not only varies with species as shown by Thomson (1950) in East Africa, Davidson (1953) in Kenya, Wharton (1951) in Malaya and Bordas and Downs (1951) in Mexico, but also with respect to the same species in different geographical areas as shown in the case of *A. fluviatilis* in India, by Nursing, Rao and Sweet (1934) in Mysore; Viswanathan, Ramachandra Rao and Rama Rao (1944) in N. Kanara District (Bombay State); Senior White, Ghosh and Venkat Rao (1945) in Orissa and Jaswant Singh and Mohan (1951) in the Nilgiris (Madras State). *A. culicifacies* is much more widely prevalent in India and transmits malaria in widely separated regions and its pattern of behaviour is, therefore, likely to be more varied. While several studies on certain aspects of the bionomics of adult *A. culicifacies*, such as those relating to resting places, feeding habits, hosts of predilection, longevity, range of flight, ovipositing behaviour, etc., have been published, there are few records on the nocturnal behaviour. Even the few that have been published are based on limited observations (Rajindar Pal, 1945; Rajindar Pal and Sharma, 1952).

METHODS.

Two experimental huts were constructed for the purpose of these studies at Vithalwadi located on the banks of the Mutha River, six miles to the west of Poona City. The river bed itself and several small streams, channels and seepages in the locality provided good breeding places for *A. culicifacies*. The huts (Figures 1 and 2) were 8 feet long and 6 feet broad and had gabled roofs, the highest point of which was $7\frac{1}{2}$ feet from the ground. The walls and roof were made of bamboo matting supported by strong bamboos at the corners. The roof was covered by thatch and the inner sides of both the roof and the walls were completely lined with mud plaster. The hut had one door 5 feet $\times$2 feet facing east and only one window 1 foot square on the north. The door could be closed fairly tightly. The window was provided on its exterior with an outlet window trap made of cloth netting. On two sides of the hut, across the middle, was a horizontal opening produced by the overlapping of the upper part of the matting over the lower part, the space between the two being about two inches. The extent of the overlap was four inches, and a mosquito in order to enter the hut through these openings had to fly four inches upwards before reaching the interior. When the door was tightly shut, the only entrance available for the mosquitoes was through these long slits. In view of the well-known tendency of mosquitoes not to fly downwards when trying to escape, these slits provided possible means of ingress when the door was kept shut but not for their egress. The dimensions of the hut were such that the entire interior could be thoroughly inspected and all resting mosquitoes easily

FIG. 1. Experimental huts built for the study of behaviour of *A. culicifacies*.

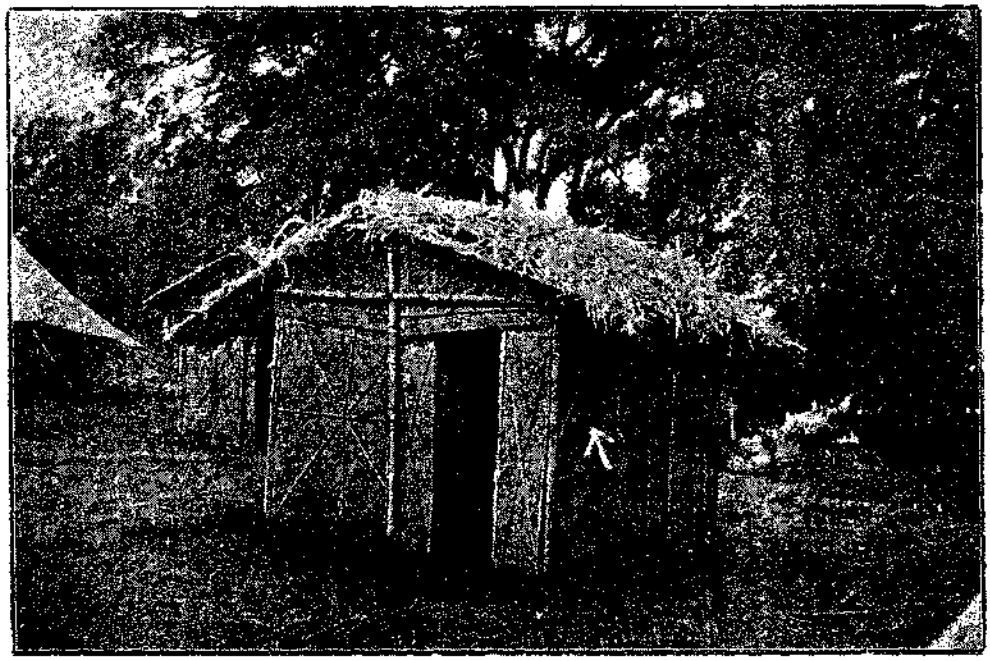

FIG. 2. Closer view of one hut showing the window trap. The arrow points to the slit entrance.

collected by hand with the suction tube. A tent was pitched 50 feet away from the huts to provide a small field laboratory for use at night. Except for a couple of rural houses about 300 yards away there were no other houses within a radius of half a mile.

A buffalo was tethered in the hut between 18.00 and 07.00 hours on every night of observation. Several preliminary attempts with human baits showed that the numbers of *A. culicifacies* attracted to man were much too small to be of any utility in these studies. This was rather a handicap because the number found dead on the floor after an application of insecticide could not be so satisfactorily determined with a buffalo inside the room as with a human bait. A clean floor is necessary to be able to spot dead insects.

OBSERVATIONS.

Observations were made in these huts on the time of entry and the time of feeding from December 1951 to May 1952, and on the effects of D.D.T. spraying in June, November and December 1952. During the monsoon months no observations were made.

MOSQUITO FAUNA.

During the course of these studies adults of the following species of mosquitoes were collected in the huts:—

Anopheles	1. *annularis*, 2. *culicifacies*, 3. *fluviatilis*, 4. *hyrcanus*, 5. *jamesi*, 6. *jeyporiensis*, 7. *stephensi*, 8. *splendidus*, 9. *subpictus*, 10. *tessellatus*, 11. *theobaldi*, 12. *turkhudi*, and 13. *vagus*.
Culex	1. *bitaeniorhynchus*, 2. *fatigans*, and 3. *vishnui*.

Further studies were, however, restricted only to *A. culicifacies*.

TIME OF ENTRY.

In order to determine the time of entry, a thorough search was first made in the huts between 17.30 and 18.00 hours and all resting mosquitoes removed by hand collection. Captures were then made in the huts every three hours by two trained Insect Collectors working simultaneously for thirty minutes, that is, between 20.30 and 21.00 hours, 23.30 and midnight, 02.30 and 03.00 hours and 05.30 and 06.00 hours. Again, a further search was invariably made between 07.00 and 07.30 hours to collect any mosquitoes which had entered the hut subsequent to the search made between 05.30 and 06.00 hours. This time schedule was observed in all seasons of the year.

In a series of 54 observations made with the door fully closed, the total number of *A. culicifacies* collected in these huts was 492 out of which 447 were females (Table I) and in another series of 27 comparative observations made with the door fully open 311 males and 1,118 females of this species, or 1,429 in all, were collected. The figures bring out the fact that nearly five times as many female *A. culicifacies* enter the huts when the door is kept open as when only the slit entrances are available.

Observations made in the three-hourly collections were compared with another series of observations in which catches were made only once between 07.00 and 07.30 hours. The data collected in 18 observations with the door closed and 17 with the door open, are given in Table II. Many of these observations were made on nights alternating with those in which three-hourly catches were made and, therefore, the data are generally comparable and the seasonal differences in the output from the breeding places do not differently affect the two types of observations. These figures reveal that when the door is kept open, the average number of *A. culicifacies* when only a single collection is made in the morning is

26·5 females and 11·6 males as compared with 41·3 and 11·6, respectively, when catches are made once in three hours and again in the early morning, indicating that most probably a certain proportion—roughly 36 per cent—of *A. culicifacies* females which enter the hut during the night and dawn leave the hut before, say, 7·30 hours. But when the door is kept closed, there is no outward movement during the night as shown by the average number collected in the single early morning catch (11·3) not being less than the total of all the quarterly collections plus the early morning catch (8·3). These figures are in accordance with the view that the slit entrances, while affording ingress to the mosquitoes, are not suitable for their egress.

TABLE I.

Number of A. culicifacies *collected in four 3-hourly collections and at 07·00 hours.*

	With door closed.	With door open.
Number of nights of observation, December 1951 to May 1952	54	27
Total number of *A. culicifacies* collected in all the four 3-hourly collections and at 07·00 hours:		
Males	45	311
Females	447	1,118
Average number collected per night:		
Males	0·8	11·6
Females	8·3	41·3

TABLE II.

Number of A. culicifacies *collected in single catches made at 07·00-07·30 hours.*

	Door closed.	Door open.
Number of observations	18	17
Total number of *A. culicifacies* collected in single catches made at 07.00-07.30 hours, December 1951 to May 1952:		
Males	13	197
Females	204	451
Average number collected per night:		
Males	0·7	11·6
Females	11·3	26·5

In Table III are given figures regarding the number and proportions of females of *A. culicifacies* entering the hut at the different quarters of the night

and the early morning before the 07.00-07.30 hours collection. In the 54 completed observations with the door shut, a total of 447 females was collected throughout the study. Of these, 74 per cent entered in the first quarter and 16 per cent in the second quarter, making a total of 90 per cent entering before midnight. Similarly, of the 1,118 females collected in 27 nights when the door was kept open, 44 per cent entered in the first quarter and 23 per cent in the second quarter, making a total of 67 per cent of entry before midnight. These figures bring out the fact that while *A. culicifacies* enter dwellings throughout the night, the bulk of entry occurs before midnight and that the first quarter is the most active period of entry. The figures further suggest that a certain proportion of those which enter houses in the earlier part of the night, leave them and re-enter later. Thus in the first quarter nearly 30 out of 74 or roughly 40 per cent leave the houses presumably in search of more congenial places of feeding or shelter. In the first and second quarters put together 23 out of 90 or roughly 25 per cent leave the houses. This shows that at least 7 out of the 30 mosquitoes which left the houses out of a batch of 74 which entered during the first quarter, re-entered during the second quarter. Only about ten per cent enter houses for the first time after midnight.

TABLE III.

Number and proportions of females of A. culicifacies *entering the hut at different quarters of the night and the early morning.*

	Door closed		Door open	
Number of nights of observations	54		27	
Females of *A. culicifacies* captured in	Number	Per cent	Number	Per cent
1st quarter	329	74	490	44
2nd quarter	71	16	254	23
3rd quarter	9	2	83	7
4th quarter	19	4	151	14
07·00-07·30 hours	19	4	140	12
Total ...	447	100	1,118	100

TIME OF FEEDING.

In Table IV are presented figures showing the proportions of female *A. culicifacies* found in different gonotrophic conditions at the time of capture. These figures are a little less than those recorded in Table III as detailed observations on gonotrophic conditions could not be made in every case. The classification of gonotrophic conditions adopted for these studies is the one used by Viswanathan and Ramachandra Rao (1944) with a further sub-classification of the classes *A* and *B*.

TABLE IV.

Proportions of A. culicifacies *females in different gonotrophic conditions.*

(A) IN COLLECTIONS MADE AT 3-HOURLY INTERVALS AND AT 07.00-07.30 HOURS

Number of nights of observations ...	Door closed.							Door open.						
	54							18*						
		Percentages							Percentages					
	Total.	*A.*	*B.*	*C.*	*D.*	*E.*	*F.*	Total.	*A.*	*B.*	*C.*	*D.*	*E.*	*F.*
1st quarter ...	349	6	78	12	2	0	2	443	14	56	29	@	0	@
2nd quarter ...	71	1	83	11	1	0	3	334	7	81	12	0	0	0
3rd quarter ...	9	0	67	33	0	0	0	30	0	93	7	0	0	0
4th quarter ...	19	0	68	5	16	0	10	66	23	68	8	1	0	0
07.00-07.30 hours ...	19	6	58	16	10	5	0	53	26	44	24	6	0	0
Total ...	447	5	78	12	3	@	2	746	14	64	22	@	0	@

@ = Less than 1 per cent.

(B) IN COLLECTION MADE ONLY AT 07·00-07·30 HOURS

Number of nights of observations ...	18							7						
	Percentages.							Percentages						
	Total.	*A.*	*B.*	*C.*	*D.*	*E.*	*F.*	Total.	*A.*	*B.*	*C.*	*D.*	*E.*	*F.*
Total ...	304	2	75	10	5	2	5	147	1	57	11	1	0	0

**Note.*—The data for nine collections made in May 1952 not included as they are incomplete in respect of gonotrophic conditions.

The classification now used is as follows:—

Class *A*. Empty abdomen; unfed:
- *A1* With no trace of old blood.
- *A2* With traces of previous bloodmeal.

Class *B*. Freshly fed:
- *B1* Fully fed, no trace of old blood, or of ovarian development.
- *B2* Fully fed, with trace of old blood but no ovarian development.
- *B3* Fully or partially fed with traces of old partially digested blood and of ovarian development.
- *B4* Partially fed without trace of old blood or of ovarian dvelopment (interrupted feeding).

Class *C.*	Partially digested blood and partial ovarian development.	
Class *D.*	Gravid, with a slight trace of old blood.	
Class *E.*	Gravid, without trace of blood.	
Class *F.*	Miscellaneous.	*F1* Unclassified. *F2* Partial digestion of blood but no ovarian development.

For purpose of Table IV, however, the sub-classifications of *A* and *B* are not used.

It will be noticed that 78 per cent of all females caught with the door closed and 64 per cent with the door open belong to the Class *B*, that is, those which had taken fresh bloodmeals. With the door closed, the proportions of specimens found with fresh blood in the four different quarters of the night also do not greatly vary, indicating that while the egress is barred *A. culicifacies* females take a bloodmeal soon after their entry. When the door is left open, there is a slightly smaller proportion of freshly fed specimens in the first and last quarters than in the second and third. This may be due to the exercise of a greater degree of choice by the mosquitoes resulting in some movement of mosquitoes ready to feed to other possible places of feeding. Altogether while feeding may take place all through the night, generally it occurs soon after entry and as the bulk of the entry takes place before midnight the bulk of feeding also occurs before midnight.

The data also show that a certain number of females in the *C* and *D* classes also enter the huts, their total proportions being as much as 15 per cent in the closed-door hut and 23 per cent in the open-door hut. When a single collection was made at 07.00-07.30 hours their proportions were 15 per cent with the door closed and 42 per cent with the door open. Such an inward movement of mosquitoes in the *C* and *D* classes occurs throughout the night including the first quarter and is not restricted to the early morning collection. Had the bulk of the *C* and *D* classes collected been largely restricted to the 07.00-07.30 hours collection, one might have explained it as due to the entry of shelter-seekers at daybreak. But the entry of the *C* and *D* classes throughout the night indicates either that there is a considerable degree of movement of mosquitoes at night involving change of shelters by mosquitoes not supposedly in a condition favourable for feeding or the number of *C* and *D* classes represent largely nulliparous females which enter houses for a second feeding before their first oviposition. In the single early morning collections, the proportion of the *C* and *D* classes together is as high as 42 per cent in the open hut while it is only 15 per cent in the closed hut. One may infer that the urge for feeding is greater and stimulates entry even with obstacles while the urge for shelter seeking is not so great as to surmount difficulties in entry.

In Table V are given the sub-classifications of 1,942 freshly fed *A. culicifacies* (Class *B*) females collected in all the captures made in these huts and in which such data were recorded, including also the 447 and 746 females which were dealt with above. Of the 1,942 females subjected to this analysis, 61 per cent were in

B1 condition, 19·6 in *B2*, 7·1 in *B3* and 12·3 in *B4*. From the details of the classification presented earlier, it will be seen that only *B1*, *B2* and *B4* are forms which actually enter the huts in *A* condition, that is, in an unfed condition with empty abdomens. *B3* are semi-gravid forms which entered in the *C* condition and took a bloodmeal before the blood of the previous meal was digested. They form roughly seven per cent of all the females with evidence of fresh bloodmeal on the night of collection. The significance of this is that roughly seven per cent of the feeding, which takes place at night, occurs among nulliparous females for the second time after their emergence or the seven per cent may be composed partly of feeding by nulliparous females and the rest represents uncommon behaviour involving double feeding among the parous females within the same gonotrophic cycle. Further those in the *B4* class, that is, those which show interrupted feeding, are roughly 12 per cent of the total and it is probable that some of them may bite again during the same night to complete their bloodmeals. No significant variations in these proportions have been noticed between the different months. The feeding by a proportion of the semi-gravid mosquitoes, within a gonotrophic cycle somewhat increases Macdonald's (1950) biting index unless in the case of parous females those that bite more than once have their gonotrophic cycle extended at least during that cycle. In the case of nulliparous females, the duration between emergence and oviposition is shown by Davidson and Draper (1953) to be at least twice as long as subsequent gonotrophic cycles. Hence the biting frequency is not altered. Multiple feeding in the same night by a mosquito on different hosts profoundly affects transmission, the definite extent of which has not yet been precisely determined.

TABLE V.

Classification of freshly fed females of A. culicifacies *in respect of all collections from January to end of May.*

Month.	B_1	B_2	B_3	B_4	Total.
January	59	18	22	11	110
February	183	13	25	52	303
March	237	94	23	40	394
April	364	110	29	78	581
May	341	146	39	58	554
Total ...	1,184	381	138	239	1,942
Per cent ...	61·0	19·6	7·1	12·3	100·0

OUTLET WINDOW TRAP.

The number of mosquitoes collected in the outlet window traps throughout the period of these studies was very small and extremely disappointing. During the entire period of study with over 120 nights of observations in unsprayed huts, only 33 adults of *A. culicifacies* (16 males and 17 females) were found. The outlet window traps were, therefore, not considered of much utility in the study of behaviour of this species.

DEGREE OF OUT-DOOR RESTING.

The observations made in the huts have also not yielded any definite information regarding the degree of outdoor resting during daytime resorted to by adults of *A. culicifacies*. While from a comparison of the number of mosquitoes collected in the three-hourly collections and the single collection made in the morning it has been inferred that roughly 36 per cent of *A. culicifacies* leave the dwellings at night, this movement may only be directed towards finding a better place of feeding or shelter. A fair number enter the dwellings in the early morning. Unless the relative numbers which actually leave the huts at night and those which enter the hut in the morning are adequately determined it would not be possible to estimate the proportion which stay away outdoors during daytime. A comparison of the relative proportions of the freshly fed and the semi-gravid mosquitoes in the early morning collections (Table IV-B Door open) shows that the number of semi-gravid females resting indoors is only slightly less than the number of freshly fed ones and the difference may be due to the natural mortality taking place. It should be remembered that all the mosquitoes reported in these studies were those which entered the hut during single nights, for the huts were thoroughly searched and all resting mosquitoes removed to depletion at 17.30 hours each evening before the commencement of the observations. What the proportion of the gonotrophic conditions would have been if the mosquitoes were left undisturbed for several days cannot be stated. From the first glance of these figures one may conclude that as the gonotrophic cycle is 48 hours practically all the females are endophilic but this will be true only if collections made in dwellings represent the total insect population. Further studies are needed in this regard.

BEHAVIOUR IN HUTS SPRAYED WITH D.D.T.

Several preliminary attempts were made to study the behaviour of mosquitoes in huts treated with D.D.T. One hut was sprayed on May 23, 1952 at a dosage of 112 mg./sq. ft. in an aromex-water emulsion and observations carried on for 11 nights thereafter till the onset of the monsoon. After the monsoon the huts were rebuilt and one of them was sprayed on November 14, 1952 at a dosage of 56 mg./sq. ft. again as an aromex-water emulsion and observations were carried on for six days thereafter. The observations in these two huts are described below:

(1) *Hut sprayed on May* 23, 1952.—No separate studies were made in this hut just prior to the spraying but the normal behaviour of the mosquitoes in unsprayed huts, as described above, held good. Collections, made on 11 consecutive nights after the day of spraying, consisted of (*i*) mosquitoes resting inside the hut, (*ii*) mosquitoes found dead on the floor and (*iii*) mosquitoes found in the outlet window trap. In all these observations the door was kept closed and only a single collection made at 07.00-07.30 hours. It was soon realized that the number of mosquitoes found dead on the floor did not represent all the dead ones because of the interference by the buffalo which was used as a bait. Therefore, the data in respect of this hut cannot be used for quantitative studies but can be employed for qualitative purposes only. In all these collections only two live *A. culicifacies*,

females, both in the unfed condition, were found resting in the hut. Perhaps they had entered the hut just prior to the collection. Eight males and 12 females of the species were found dead on the floor. Of the 12 females, 9 were unfed and 3 were freshly fed. Eleven males and eight females were found in the outlet window trap. Of the eight females, four were unfed, three freshly fed and one semi-gravid. The other species of mosquitoes collected were one female resting, three females found dead on the floor and two males and three females in the trap. Obviously *A. culicifacies* adults enter sprayed huts and some even take blood meals. The number found in the outlet window trap was slightly more than that found in the traps attached to unsprayed huts and this is suggestive of the phenomenon of excito-repellancy but all those found in the traps had died and, therefore, the number which escape death is perhaps insignificant.

(2) *Hut sprayed on November* 14, 1952.—Preliminary observations were made on four nights, before this hut was sprayed, in two of which quarterly collections were made and in the other two only a single collection in the morning was made. In all these observations the door was kept open. The results of these collections were as follows:

(*A*) *Single collection at* 07.00-07.30 *hours —Two observations.*—

	Males	Females
A. culicifacies	0	8(*B*-6; *C*-2).

(*B*) *Quarterly collections—Two observations.*—

	Males	Females
I quarter:—		
A. culcifacies	0	14(*A*-1, *B*-11, *C*-2)
II quarter:—		
A. culicifacies	0	3(*B*-3)
III quarter	Nil	Nil
IV quarter		
07.00 hour	Nil	Nil

After the hut was sprayed, collections were made subsequently for six consecutive nights. On these nights, collections were *continuously* made from 18.00 to 24.00 hours and all mosquitoes found resting on the walls or roof caught. The number of *A. culicifacies* and other mosquitoes collected were as follows:—

	Males	Females
I quarter:—		
A. culicifacies	5	68
All others	2	36
II quarter:—		
A. culicifacies	0	0
All others	0	1

The gonotrophic conditions of females of *A. culicifacies* were as follows:—

A	*B*	*C*	*D*	*E*	*F*	Total
8	58	2	0	0	0	68

These observations are admittedly limited but strongly suggest that *A. culicifacies* do enter D.D.T. sprayed dwellings and take a bloodmeal if facilities for the same are available. Most of the mosquitoes collected at night were, however, found dead before the following midday in the cloth cages to which they were transferred, indicating a high rate of mortality. In the early morning a few dead mosquitoes were also found on the floor but no numerical observations were possible. There were no observations in the outlet window because the door had been kept open.

While our huts proved very useful for the study of certain aspects of nocturnal behaviour, they were not quite satisfactory for the completion of the studies after treatment with D.D.T., mainly because man could not be used as a bait. The buffalo was satisfactory so far as studies in untreated huts were concerned but was most unsatisfactory for studies after treatment. The need now is to find a more 'cooperative' bait or to find a method of restraining the efficient bait in such a manner that it does not spoil the floor and interfere with studies after treatment.

SUMMARY AND CONCLUSIONS.

(1) Observations were carried out on the nocturnal behaviour of *A. culicifacies* in two experimental huts constructed for the purpose near Poona City from December 1951 to December 1952 with a break of four months during the monsoon. A buffalo was used as bait for these experiments.

(2) When the only available entrance into the hut was a horizontal slit and the door of the hut was closed, fewer mosquitoes entered the hut for the purpose of feeding or shelter than in a similar hut with the door kept fully open.

(3) *A. culicifacies* enter the dwellings throughout the night but the bulk of the entry takes place before mid-night. There was some evidence to show that some mosquitoes which enter in the first quarter of the night leave the dwelling and a smaller proportion amongst them re-enter subsequently. Altogether not more than ten per cent of nocturnal entry for the first time takes place after midnight.

(4) Comparing collections made throughout the night and collections made only from 07.30 to 08.00 hours, it would appear that roughly 36 per cent of the mosquitoes which enter the dwelling leave it during the course of the night. It is not, however, possible to say whether such egress is towards outdoor shelters or towards another indoor place either for shelter or for feeding. In other words, these experiments do not throw any light on the degree of endophily in respect of *A. culicifacies*.

(5) While feeding occurs all through the night and perhaps relatively early after entry, the bulk of the feeding takes place before midnight because the bulk of the entry also takes place before that time.

(6) When the door is closed and horizontal slits are provided for entry, there is a greater degree of bar to entry of mosquitoes which do not have an urge for feeding. However, even with such obstacles mosquitoes not supposedly in a condition ready for feeding do enter houses during the night. On an average about 23 per cent of mosquitoes which enter at night represent those that are not in a condition ready for feeding.

(7) About seven per cent of the mosquitoes with fresh blood were found with their ovaries half developed and the previous bloodmeal partially digested. As no dissections were made on these mosquitoes it cannot be stated what proportion amongst them represent nulliparous females.

(8) About 12 per cent of the mosquitoes which were ready for feeding and which had started feeding showed evidences of interruption. This has a great bearing on the extent of malaria transmission as multiple feeding increases the chances of mosquitoes getting infected on the one hand and transmitting infection on the other.

(9) Outlet window traps did not prove successful for the study of the behaviour of *A. culicifacies.*

(10) Preliminary observations made on behaviour of mosquitoes in huts treated with D.D.T. showed that it offered no bar to their entry into treated huts and their taking a bloodmeal. There is a very slight evidence of an excito-repellant mechanism after contact with treated surfaces but on the whole even those which were excited and repelled have apparently picked up sufficient dose of insecticide to be lethal.

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MALARIA CONTROL BY THE USE OF INSECTICIDES.

A Global Review.

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INTRODUCTION.

THE advent of insecticides has marked a new stage in the long history of malaria as their application has demonstrated unprecedented success in the control of this disease and the economic feasibility of nation-wide control programmes throughout the world. Pyrethrum sprays were introduced on a large scale in 1937, followed later in 1945 by the synthetic insecticides. The object of this paper is chiefly to review up to date observations made on the use of insecticides in malaria control and particularly their effect on the vector anopheline mosquitoes in different parts of the world.* This has helped to point out certain lacunæ in our knowledge in respect of some of the species. It is hoped that the review will be of some use as a ready reference to the workers in the field.

RATIONALE OF THE METHOD—THEORETICAL CONSIDERATIONS.

Ronald Ross in 1899 put forward the concept of malaria control by mosquito reduction. Subsequently, various workers found that total eradication of a vector species was not essential to eliminate malaria transmission in an area, but could be achieved by reducing the number of vector anopheline below a certain figure

*The results in term of malaria control have already been admirably summarized by Simmons and Upholt (1951), Pampana (1951) and Russell (1952*a*).

which was referred to as the critical density of the vector species. With the introduction of insecticides, however, a new concept has been put forward that even in the absence of mosquito reduction, malaria reduction can be achieved through interception of the vector. A mosquito is able to transmit malaria only ten to twelve days after taking an infected blood feed. If this mosquito could be destroyed before the parasite had time to develop to the infective stage, transmission of malaria would not be possible. The reduction in the longevity of mosquitoes is achieved through any of the adulticidal methods whether by space or residual spraying.

Precisely, what effect the application of insecticides has on the mosquito would depend upon the degree of contact between the mosquitoes and the treated surfaces. The mosquitoes according to their resting and feeding habits have been grouped as follows (Gabaldon, 1953*a*):—

(*a*) *Indoor biters and indoor resters* which are most associated with man and are consequently the best malaria vectors.

(*b*) *Indoor biters and outdoor resters.*

(*c*) *Outdoor biters and indoor resters.*—Both (*b*) and (*c*) have varying degree of association with man and are consequently malaria vectors of secondary importance.

(*d*) *Outdoor biters and outdoor resters* which are the least associated with man and are consequently less important in malaria transmission.

It would be obvious that mosquitoes in groups (*a*) and (*b*) have the best chances of contact with the treated surfaces as they rest or feed indoors, thereby coming into contact with the treated surfaces. In the third group, the mosquitoes may come into contact with the treated surface if they rest in houses after the feed, thus giving a varying degree of malaria control, whereas in the fourth group control by the application of insecticides may not at all be possible.

In the first group of mosquitoes reduction or near eradication of the vector may result whereas in the other groups, there may or may not be appreciable reduction in mosquito density although malaria control may be achieved through interception of the vector anopheline.

These statements are, however, oversimplified because whether the species is a zoophilic or anthropophilic and what percentage of the mosquitoes rest indoors or outdoors would affect their control by the application of residual insecticides inside the houses.

PRACTICAL RESULTS ACHIEVED.

One thing is clear that the application of insecticides has a profound effect on the control of malaria under widely different conditions. Malaria reduction to a greater or lesser degree has followed premises spraying with residual insecticides wherever it has been efficiently carried out in spite of the diversified habits of the vector species. A good example may be cited of the two most notorious vectors of malaria in India; *A. culicifacies* is a domestic species with large numerical prevalence, predominantly zoophilic with poor infectivity rate and on the contrary *A. fluviatilis* is a sparse species resting mostly outdoors,predominantly anthropophilic

with high sporozoite rate, yet malaria transmitted by both has been equally well controlled. There are, however, a few exceptions where malaria control may be doubtful due to one or the other of the following factors:—

(*i*) *Due to the peculiar habits of the vector species.*—The mosquitoes belonging to group (*d*) mentioned above may not be vulnerable to this method of attack. Gabaldon (1953*a*) has made reference to failure of malaria control with residual insecticides in case of *A. bellator* in Trinidad, and also *A. minimus flavirostris* in Philippines. The malaria transmitted by the former species in Brazil and the latter species in parts of Philippines has, however, been controlled to a certain degree.

(*ii*) *Due to the repellent effect of residual insecticides.*—For example, *A. gambiæ*, and *A. melas* in Africa are repelled by D.D.T., but are successfully controlled by B.H.C. In the oriental region, Bertram (1951) reported unsuccessful results with D.D.T. on *A. minimus* in Assam and Reid (1951) on *A. letifer*. These findings have not been supported by other workers—Dowling (1951); Hamon and Dufour (1952) as quoted by Gabaldon (1953*a*) in the case of *A. gambiæ*; Gilroy (1951) in the case of *A. minimus* and Nair (1951) in the case of *A. letifer*.

(*iii*) *Due to the development of resistance to residual insecticides.*—The development of resistance in vector species may jeopardize control of malaria. So far only a few authentic cases of resistance have been reported, namely, *A. sacharovi* in Greece, *A. albimanus* in Panama and *A. sundaicus* in Indonesia, but not to an extent sufficient to interfere very seriously with control programmes.

Near eradication of malaria has been reported in the case of *A. quadrimaculatus** in U.S.A., *A. darlingi* and *A. albimanus* in Venezuela; *A. fluviatilis* in Bombay State, India. On the contrary only slight reduction in malaria has been achieved in the case of following species: *A. aquasalis* in South America, *A. vestitipennis* in Mexico; *A. sacharovi*, *A. sergenti* and *A. superpictus* in Jordan, *A. moucheti moucheti* in Belgian Congo; *A. albimanus* in Mexico.

It would also be obvious from the following pages that uniform results on the same vector species in different areas have not been obtained, perhaps due to the varying degree of efficiency with which control programme has been carried out, the differences in the habits of the same species in different areas, and other local factors and circumstances.

The disparity in the results obtained with different vector species with similar resting and feeding habits is, however, not clear, unless it could be shown that in details the habits were variable. Presumably the disparity may be due to the varying proportion of indoor and outdoor resters and biters, and whether zoophilic or anthropophilic. It would be evident that, in case of all the seven species referred to above where only slight reduction of malaria has been reported there are local variations in their resting and feeding habits and food preferences.

It may, however, be pointed out that in spite of the fact that insecticides have been used for the control of malaria for about ten years, there are a number of species about which no data is available (Table I). It would seem desirable that investigations on these species should be given priority.

*Very marked acquired resistance has been reported in the case of *A. quadrimaculatus* in certain parts of U. S. A. (Personal communication—K. D. Quarterman, 1955).

TABLE I.

LIST OF SPECIES ON WHICH DATA IS NOT AVAILABLE.

A. pulcherrimus, A. gambiæ melas, A. hancocki, A. hargreavesi, A. moucheti nigeriensis, A. pharoensis, A. rufipes, A. barbirostris, A. hyrcanus nigerrimus, A. jeyporiensis candidiensis, A. leucosphyrus leucosphyrus, A. novumbrosus, A. stephensi mysorensis, A. subpictus subpictus, A. umbrosus, A. bancrofti bancrofti, A. punctulatus, A. kochi, A. punctimaculatus, A. brunnipes, A. funestus imeriensis, A. nili, A. hyrcanus williamsoni, A. ludlowi torakala, and *A. tessellatus.*

The actual results achieved in the field on the effect of insecticides on vector anopheline mosquitoes are briefly summarized in the following pages. The data on the resting and feeding habits of mosquitoes and their sphere of influence has also been included.*

GROUP (*A*).

INDOOR BITERS AND INDOOR RESTERS.

1. *A.* (*M.*) *aconitus* Donitz, 1902.—Vector in Indonesia and Indo-China.

Adult females, as a rule, feed and rest indoors. They readily feed on man. Anthropophilic indices of 11·2 (caught from houses) and 0·5 (caught from stables) per cent have been recorded from Indo-China and 12·0 (cattle present) and 61·0 (cattle scarce) per cent from Indonesia.

In Indonesia, Van Goor and Lodens (1952) reported reduction in the vector population and malaria morbidity with D.D.T. residual spray applied at the rate of 200 mg./sq. ft.

2. *A.* (*N.*) *albitarsis* Lynch Arribalzaga, 1878.—Vector in Brazil, Columbia and Venezuela.

Adults in certain areas may occur in houses and show marked preference for human blood; in other areas they do not appear to occur in houses, and they may have preference for animal blood.

In Venezuela, Gabaldon and Berti (1954) have reported malaria eradication. Pinotti (1951) in Brazil, reported reduction in malaria morbidity with D.D.T. residual spray applied at the rate of 200 mg./sq. ft. though there was no effect on the vector density.

3. *A.* (*M.*) *annularis* Van der Wulp, 1884.—Vector in Eastern India, Pakistan and Burma.

Adults are found in large numbers in cattlesheds, and sometimes in houses also. Usually they feed and rest indoors. They are markedly zoophilic but feed on man as well. Anthropophilic indices of 10·0 (cattle present) and 50·0

*Publications by Covell (1927 ; 1931); Russell *et. al.* (1943 ; 1946); Boyd (1949); Russell (1952*b*) and Gabaldon (1953*b*) have been freely consulted.

(cattle scarce) per cent have been recorded from Indonesia and 1·6 per cent from Assam, India.

In India, Hajra (1948) and Adhikari and Ganguli (1949) recorded reduction in the vector density and malaria morbidity with D.D.T. residual spray applied at the rate of 38-50 mg./sq. ft.

4. *A.* (*N.*) *aquasalis* Curry, 1932.—Vector in Venezuela, Brazil, Trinidad, Grenada and Santa Lucia.

Adults in some localities are anthropophilic and house frequenting and in other zoophilic and sylvan. Zoophilic species seldom found in houses when domestic animals are abundant. Its vectorial activities may be limited because of this fact.

In Brazil, Trinidad, Tobago and Venezuela, Pinnotti (1951) and Gillette (1953) recorded reduction in malaria morbidity with D.D.T. residual spray applied at the rate of 200 mg./sq. ft., though there was no effect on the vector density. Senior White (1951) has observed in Trinidad that *A. aquasalis*, an indoor rester, has been found resting outdoors.

5. *A.* (*A.*) *atroparvus* Van Thiel, 1927.—Vector in Baltic, Netherlands, Germany, Portugal, Spain and Rumania.

Domestic mosquito; spends most of its life indoors; is powerfully attracted to stabled animals almost to the exclusion of man, but may overflow into human habitations in search of food under various circumstances, among which are disproportionate density of anophelines or scarcity of animals or for other reasons connected possibly with temperature, humidity or odour. Although bites man less readily, yet anthropophilic indices 6·09 and 40·0 per cent in Spain and 84 per cent in Holland have been recorded.

In Netherlands, Van Thiel (1953) as quoted by Gabaldon (1953*a* : 1953*b*), recorded reduction in vector density and malaria morbidity with D.D.T. residual spray applied at the rate of 100-200 mg./sq. ft.

6. *A.* (*A.*) *aztecus* Hoffman, 1935.—Vector in Mexico.

Adults feed indoors as a rule. Feed on man or animal without much preference. Rest indoors as well as outdoors.

In Mexico, Downs and Bordas (1951) reported reduction in malaria morbidity with D.D.T. residual spray applied at the rate of 200 mg./sq. ft.

7. *A.* (*A.*) *claviger*.—Vector in the Near East, Baku Region, Cyprus and Palestine.

A domestic species in Palestine, where it commonly enters houses and freely bites human beings and rests in indoor shelters. In some countries it is considered to be wild species and it rarely enters houses.

In Greece and Italy, Livadas (1950) and Missiroli *et al.* (1948) recorded reduction in malaria morbidity with D.D.T. residual spray applied at the rate of 180-200 mg./sq. ft. They did not observe any effect on the vector density.

8. *A.* (*K.*) *cruzi* Dyar and Knab, 1908.—Vector in South East Brazil.

Adults feed and rest indoors and outdoors and prefer animal blood.

In Brazil, Pinnotti (1951) observed no effect on vector density but there was reduction in malaria morbidity with D.D.T. residual spray applied at the rate of 200 mg./sq. ft.

9. *A.* (*M.*) *culicifacies* Giles, 1901.—It is an important vector in India, West Pakistan, Afghanistan, Ceylon and Burma.

Adult females feed and rest indoors and they bite man freely but the anthropophilic index is usually low, except during an epidemic. Anthropophilic indices of 1·6 and 2·5 per cent have been recorded from India.

In India, reduction in vector density and malaria morbidity was recorded by the application of D.D.T. at 50-60 mg./sq. ft. and of B.H.C. at 10 mg. gamma isomer/sq. ft. (Jaswant Singh and Dalip Singh, 1949; Viswanathan, 1950; Jaswant Singh *et al.*, 1951). Similar results were obtained in Pakistan and Ceylon with D.D.T. applications at 25-38 mg./sq. ft. (Afridi and Bhatia, 1947; Puri and Bhatia, 1947) and 50-200 mg./sq. ft. (Rajendram and Jayewickreme, 1947), respectively.

10. *A.* (*N.*) *darlingi* Root, 1926.—Vector in British Honduras, Columbia, Venezuela, Guianas, Brazil, North West Argentine, Bolivian foot hills, Equador and Peru.

This species is markedly anthropophilic, and readily enters houses from dusk to any time at night. As a rule, rests in indoor shelters. In Venezuela, out of 100 mosquitoes of this species caught 90 to 98 were from human dwellings. Anthropophilic index of 63 per cent has been recorded from Brazil.

In Brazil, with D.D.T. (200 mg./sq. ft.) application though there was little effect on vector density, there was reduction in malaria morbidity (Pinnotti, 1951). Similar applications of D.D.T. in Argentina and French Guiana also resulted in reduction in malaria morbidity (Alvarado, 1953, as quoted by Gabaldon, 1953*a*: 1953*b*; and Floch, 1952). There was reduction or eradication of the vector as well as malaria in Bolivia (Moscose-Carrasco, 1953, as quoted by Gabaldon, 1953*a*: 1953*b*) and Columbia (Ranjifo and de Zulueta, 1952) and in Venezuela with D.D.T. applications at 200 mg./sq. ft.

11. *A.* (*M.*) *farauti* Lavern, 1902.—Vector in Australia, New Guinea, Solomons, Hebrides, New Britain to Eastern Celebes.

Adult females may feed and rest indoors or outdoors and take human or cattle blood without much preference.

In New Hebrides islands and New Guinea, reduction in vector density and malaria morbidity was recorded with D.D.T. application at the rate of 100 mg./sq. ft. (Yust, 1947; and Bang *et al.*, 1947).

12. *A.* (*A.*) *freeborni* Aitken, 1939.—Vector in West U.S.A., and North West Mexico.

Adults feed and rest indoors. They readily feed on man, show progressively higher prevalence of human, pig, horse and cattle blood in their precipitin tested

stomach contents. Malaria has practically disappeared in territories occupied by this vector (Hackett, 1952).

13. *A.* (*M.*) *funestus funestus* Giles, 1900.—Vector in East, West, Central and South Africa, Mauritius and Madagascar.

Adult females feed and rest indoors and are anthropophilic. It has been shown that the species prefers to bite indoors and that an increase in number of occupants in a hut causes an increase in the number of adult mosquitoes which enter it. Anthropophilic index of 61·3 per cent has been recorded in Kenya.

Reduction in malaria was observed in Southern Rhodesia (Alves, 1951) with B.H.C. (46 mg. gamma isomer/sq. ft.) and in Ruanda Urandi (Jadin, 1951) with D.D.T. (200 mg./sq. ft.) application. Eradication and reduction of the vector and reduction in malaria morbidity has been recorded in Mauritius (D.D.T. 170-260 mg./sq. ft.), Reunion and Belgian Congo (D.D.T. 200 mg./sq. ft.), with D.D.T. applications (Dowling, 1951, Hamon and Dufour, 1952 as quoted by Gabaldon, 1953*a*:1953*b* and Vincke, 1948). There is a very remarkable control scheme in Madagascar; larger than any of the others in which local eradication of this species has been obtained together with *A. gambiæ* (Personal communication from Prof. G. Macdonald, 1954).

14. *A.* (*M.*) *gambiæ gambiæ* Giles, 1900.—Vector in East, West, Central and South Africa, Mauritius, Madagascar and Reunion.

Adult females feed and rest indoors and definitely prefer human blood. High anthropophilic indices of about 82 per cent have been recorded from West Africa and Kenya.

Jadin (1951) in Ruanda Urundi observed reduction in malaria morbidity with D.D.T. (200 mg./ sq. ft.) application. Dowling (1951) in Mauritius (D.D.T. 170-260 mg./sq. ft.), Hamon and Dufour (1952) as quoted by Gabaldon (1953*a*: 1953*b*) in Reunion, and Vincke (1948) in Belgian Congo (D.D.T. 200 mg./sq. ft.) observed that there was no effect on vector density though there was reduction in malaria morbidity with insecticidal applications.

15. *A.* (*M.*) *gambiæ melas* Theobald, 1903.—Vector in West Africa (coastal region).

It feeds and rests indoors and prefers human blood.

Data on its control by the use of insecticides not available.

16. *A.* (*M.*) *hancocki* Edwards, 1929.—Vector in East, West and Central Africa.

It feeds and rests indoors and takes human or animal blood without much preference.

Data on its control by the use of insecticides not available.

17. *A.* (*A.*) *hyrcanus sinensis* Wiedemann, 1928.—Vector in the plains of China and Formosa.

It feeds and rests indoors and takes human or cattle blood without much preference. In parts of China, it is a voracious human feeder. Anthropophilic indices as high as 95 per cent and as low as 0·9 per cent have been recorded from Shanghai and Indo-China, respectively.

Reduction in vector density was recorded in Nanking Region, China, with D.D.T. applied at the rate of 150 mg./sq. ft. (*Agr. Res. Council Abstracts*, 1950, as quoted by Jaswant Singh *et al.*, 1954).

18. *A.* (*M.*) *Jeyporiensis candidiensis* Koidzumi, 1924.—Vector in Travancore (India), Burma, Indo-China and South China.

Adult females feed indoors but may rest indoors or outdoors and are strongly anthropophilic. Anthropophilic indices of 59·0 and 70·0 per cent have been recorded from Indo-China.

Data on its control by the use of insecticides not available.

19. *A.* (*M.*) *kochi* Donitz, 1901.—Vector in Indonesia. Adults are moderately domestic, frequenting houses and stables and feeding on man and animals.

Data on its control by the use of insecticides not available.

20. *A.* (*A.*) *labranchiæ* Falleroni, 1926.—Vector in Spain, Morocco, Algeria, Tunisia, Italy, Corsica, Sardinia and Sicily.

Adult females enter houses in large numbers. They are not known anywhere to be effectively deviated from man by domestic animals. They prefer to feed, rest and hibernate in buildings. This makes house-spraying with insecticides specially effective.

In Italy Missiroli *et al.* (1948) reported reduction in vector density and malaria morbidity with D.D.T. (200 mg./sq. ft.) residual spray.

21. *A.* (*A.*) *maculipennis maculipennis* Meigen, 1818.—Vector in Turkey and Bulgaria.

Adult females feed and rest indoors and outdoors and prefer animal blood but at times may feed on man.

Livadas (1950) recorded reduction and eradication of vector and reduction in malaria morbidity in Greece with D.D.T. (180 mg./sq. ft.) residual spray. Missiroli *et al.* (1948) in Italy observed reduction in malaria morbidity but little effect on vector density with D.D.T. applied at the rate of 200 mg./sq. ft.

22. *A.* (*M.*) *mangynus* Banks, 1906.—Vector in Philippines.

It bites indoors but may rest indoors or outdoors.

In Philippines, Bhatia (1953) as quoted by Jaswant Singh *et al.* (1954) recorded reduction in its density with D.D.T. applied at the rate of 200 mg./sq. ft.

23. *A.* (*A.*) *messae* Falleroni, 1926.—Vector in Hungary, Germany, Poland, U.S.S.R., Balkans and Manchuria.

It is powerfully attracted to stabled animals almost to the exclusion of man, but may overflow into human habitation in search of food under various circumstances among which are a disproportionate density of anophelines, or scarcity of animals, or for other reasons connected possibly with temperature, humidity or odour. Anthropophilic index of 63·0 per cent has been recorded in Holland.

In Netherlands, Cseh Firtos (1952) recorded reduction in vector density and malaria morbidity with D.D.T. residual spray applied at the rate of 200-300 mg./sq. ft.

24. *A.* (*M.*) *minimus minimus.*—Vector in North-east India, Burma, Indo-China, South China, Formosa, Indonesia, Hong Kong, Thailand, Amoy Island, Sumatra, Celebes and Moluccas.

Mainly a domestic species with a high preference for human blood. It feeds indoors but may rest indoors or outdoors. Anthropophilic indices as high as 85·7 and 92·4 per cent have been recorded from India.

In India, D.D.T. applied at the rate of 50-120 mg./sq. ft. (Puri and Krishnaswami, 1947; Kar, 1950; and Krishnaswami, 1952) and in Thailand at 200 mg./sq. ft. (Bhatia, 1953, as quoted by Jaswant Singh *et al.*, 1954) resulted in reduction of vector density and malaria morbidity.

25. *A.* (*M.*) *moucheti moucheti* Evans, 1925.—Vector in Belgian Congo and Uganda.

It feeds and rests indoors and takes human or animal blood without much preference.

Davidson (1950) as quoted by Gabaldon (1953*a*:1953*b*) recorded slight reduction in malaria morbidity with B.H.C. applied at the rate of 10 mg. gamma isomer/sq. ft.

26. *A.* (*M.*) *moucheti nigeriensis* Evans, 1931.—Vector in Nigeria.

It feeds and rests indoors and takes human or animal blood without much preference. Data on its control by the use of insecticides not available.

27. *A.* (*M.*) *nili* Theobald, 1901.—Vector in Belgian Congo, Liberia, Sierra Leone.

In some parts, this species enters houses, bites man and may be an important vector of malaria; whereas in others the insect is rare and is of no importance.

Data on its control by the use of insecticides not available.

28. *A.* (*M.*) *philippinensis* Ludlow, 1902.—Vector in Bengal, India and East Pakistan.

Adults chiefly rest in houses usually 1½ ft. off the floor, biting at night between 8 p.m. and 4 a.m. In Assam this species appears to be zoophilic. An anthropophilic index of 6·4 per cent has been recorded from Assam, India.

In East Pakistan, Nasir-ud-din (1952) recorded reduction in malaria morbidity with D.D.T. applied at the rate of 106 mg./sq. ft.

29. *A.* (*A.*) *pseudopunctipennis* Theobald, 1901.—Vector in Mexico, Columbia, Bolivia, Peru, Chile, Argentina, probably Gautemala, Venezuela and Equador.

As a rule, adult females feed and rest indoors and take human or animal blood without much preference. Anthropophilic indices of 50 to 67·6 per cent in Argentina and 2·5 per cent in Venezuela have been recorded.

In Mexico, Downs *et al.* (1950) observed reduction in vector density and malaria morbidity with D.D.T. application (200 mg./sq. ft.). Similarly D.D.T. (200 mg./sq. ft.) residual spray in Columbia, Ecuador, Peru (Montalvan, 1953) and Argentina (Alvarado, 1953 as quoted by Gabaldon, 1953*a*:1953*b*) resulted

in the reduction of malaria morbidity. In Bolivia and Venezuela, D.D.T. applied at the rate of 200 mg./sq. ft. reduced malaria morbidity but had little effect on vector density (Downs *et al.*, 1950 and Moscoso-Carrasco, 1953, as quoted by Gabaldon, 1953*a*:1953*b*).

30. *A.* (*M.*) *pulcherrimus* Theobald, 1902.—Vector in Caucasus and Iraq.

The females of this species like to concentrate around settlements, but they occur also far from man and his herds. As resting places, they choose buildings as well as outdoor places, burrows, vegetation and the like. They viciously bite man and animals. It is a bold feeder attacking animals and man in the open by day or by night.

Data on its control by the use of insecticides not available.

31. *A.* (*M.*) *punctulatus* Donitz, 1901.—Vector in New Guinea, Solomons to Halmahera.

Adult females feed and rest indoors or outdoors and take human or cattle blood without much preference.

Data on its control by the use of insecticides not available.

32. *A.* (*A.*) *quadrimaculatus* Say, 1924.—Vector in Central, South and East U.S.A.

Adults are most active at night, females fly as far as one mile to obtain animal or human blood meal, and readily enter houses where they often spend daylight hours in dark corners. It is the most abundant anopheline found in houses and other man-made shelters; prefers bovine hosts; but at times or in places feeds on man.

Andrews (1951) recorded reduction in vector density and malaria morbidity in U.S.A., with D.D.T. residual sprays (200 mg./sq. ft.).

33. *A.* (*M.*) *rufipes* Gough, 1910.—Vector in West Africa, coastal region.

Adults have been found in human dwellings, cowsheds, and outdoor haunts including rock clefts and cavities in banks along streams.

Data on its control by the use of insecticides not available.

34. *A.* (*A.*) *sacharovi* Favre, 1903.—It is an important vector in Balkans, Near East, Central Russia and West China.

Adults enter human habitations where they persistently bite man. They sometimes feed indiscriminately on man and domestic animals and rest indoors or outdoors.

Reduction or eradication of the vector species and reduction in malaria morbidity has been recorded from Greece (D.D.T. 100 mg./sq. ft.) (Livadas, 1950); Italy (D.D.T. 200 mg./sq. ft.) (Missiroli *et al.*, 1948) and Jordan (D.D.T. 200 mg./sq. ft.) (Farid, 1953) with D.D.T. residual sprays.

35. *A.* (*M.*) *sergenti* Theobald, 1907.—Vector in Canary Islands, Egyptian oases, Transjordan and Israel.

Adults readily enter houses, and bite most frequently after dark, prefer animal blood but at times bite man. They are found resting in caves, karezes, also in houses and tents.

In Jordan, Farid (1953) observed slight reduction in malaria morbidity but little effect on vector density with D.D.T. applied at the rate of 200 mg./sq. ft.

36. *A. (M.) stephensi mysorensis* Sweet and Rao, 1937.—Vector in South India. Considered less important as vector than the type form, but Senior White as quoted by Boyd (1949) considered it to be chief rural carrier in Vizagapatam (India).

This species is less hardy than the type form and has more zoophilic tendencies.

Data on its control by the use of insecticides not available.

37. *A. (M.) stephensi stephensi* Lisbon, 1901.—Vector in Persian Gulf area and India.

It feeds and rests indoors. Adults are commonly found in houses, barracks and cowsheds. Feeds avidly on man.

In West Pakistan, Afridi and Bhatia (1947) recorded reduction in vector density and malaria morbidity with D.D.T. sprayed at the rate of 25-38 mg./sq. ft. In India similar results were achieved by Adhikari and Ganguli (1949) with D.D.T. applications (100 mg./sq. ft.).

38. *A. (M.) subpictus subpictus* Grassi, 1899.—Vector in Celebes.

It feeds indoor but may rest indoors or outdoors. Adults are found in large numbers in houses and in cattlesheds. They may feed on man, but apparently prefer cattle. Anthropophilic indices as low as zero and as high as 25·0 per cent have been recorded from India.

Data on its control by the use of insecticides not available.

39. *A. (M.) sundaicus* Rodenwaldt, 1926.—Vector in North-east India, East Pakistan, Indonesia, Andaman, Malaya, Sarawak and Borneo.

They rest in houses and cowsheds and are voracious feeders, biting by day as well as by night. They may bite indoors or outdoors. In Indonesia it shows an overwhelming preference for human blood even in the presence of cattle. Anthropophilic indices of 94·0 and 86·0 per cent have been recorded from Indonesia.

In India (D.D.T. 100 mg./sq. ft.) and Indonesia (D.D.T. 200 mg./sq. ft.) reduction in vector density and malaria morbidity was recorded with D.D.T. residual sprays (Adhikari and Ganguli, 1949 and Van Thiel and Winoto, 1951).

40. *A. (M.) superpictus* Grassi, 1899.—Vector in Eastern Mediterranean countries including southern Italy, Near East, U.S.S.R., and Baluchistan (Pakistan).

Adults readily enter houses, tents and barracks, and females prefer human blood. They are found in large numbers in outside resting places also (caves and karezes). Anthropophilic indices of 48·1, 29·7, and 23·3 per cent of those captured in stables were recorded from Yugoslavia, Greece and Cyprus, respectively.

In Greece, (D.D.T. 180 mg./sq. ft.), Pakistan (D.D.T. 25-50 mg./sq. ft.) and Afghanistan with D.D.T. residual sprays (D.D.T. 112-200 mg. per sq. ft.) reduction in vector density and malaria morbidity was recorded (Livadas, 1950; Afridi and Bhatia, 1947; Puri and Bhatia, 1947; and Rao, 1951).

41. *A.* (*M.*) *varuna* Iyengar, 1924.—Vector of local importance in some hilly and foot-hill areas of East Central India.

The adults are found both in cowsheds and human habitations, and feed readily on man.

Senior White (1945) obtained effective control of this species for eight weeks in Jeypore hill tracts, India, with D.D.T. applied at the rate of 50 mg./sq. ft.

42. *A.* (*A.*) *vestitipennis* Dyar and Knab, 1906.—Vector in Mexico and British Honduras.

Adult females readily enter houses and feed indoors but may rest in indoor or outdoor shelters. They take human or animal blood without much preference.

In Mexico with D.D.T. applied at the rate of 200-250 mg. per sq. ft., Salinas Dopez and Roquet Perez (1950) obtained slight reduction in malaria morbidity but no effect on vector density.

GROUP (*B*).

Indoor biters and outdoor resters.

1. *A.* (*N.*) *albimanus* Wiedemann, 1921.—Vector in Central America, West Indies, Caribbean Coast, Venezuela, Columbia, Pacific Coast and Ecuador.

It feeds indoors but rests in outdoor shelters. Adults are nocturnal in habits and avid feeders on man who may be the preferred host, or on horses, cows, goats or pigs. They invade houses in large numbers but do not, as a rule, remain in houses after sunrise. Its domesticity is low in Venezuela. Out of 100 mosquitoes of this species caught, two to five come from houses. Anthropophilic index of 34 per cent has been recorded in Venezuela.

With D.D.T. residual sprays, reduction in vector density and malaria morbidity has been recorded in Puerto Rico (D.D.T. 150-200 mg./sq. ft.) (Gahan and Lindquist, 1945); Ecuador (D.D.T. 150-200 mg./sq. ft.) (Montalvan, 1953); Venezuela (D.D.T. 200 mg./sq. ft.) (Gabaldon, 1953*a*:1953*b*) and Panama (D.D.T. 200 mg./sq. ft.) (Galindo and Gallardo, 1947 as quoted by Gabaldon, 1953*a*:1953*b*). In Mexico, there was slight reduction in malaria morbidity but little effect on vector density with D.D.T. applied at the rate of 200-250 mg./sq. ft.

2. *A.* (*M.*) *fluviatilis* James, 1902.—Vector in hill and foot-hill regions of India.

They feed indoors and a significant fraction of population is in outdoor resting places during the day. The species seems to be composed of two biological races; one is strongly anthropophilic, while the other feeds almost exclusively on cattle. Anthropophilic indices as high as 87·0 per cent and as low as 1·0 per cent have been recorded in India.

With D.D.T. residual sprays (50-60 mg./sq. ft.) reduction in vector density and malaria morbidity has been recorded in India (Ramakrishnan *et al.*, 1948; Vedamanikkam, 1949; Jaswant Singh and Kariappa, 1949; Viswanathan, 1950; and Srivastava and Chakrabarti, 1952).

3. *A.* (*M.*) *lelifer* Gater, 1941; Sandosham, 1945.—Vector in Malaya.

Adults are found in deeply shaded places in jungle and also in houses. It feeds indoors and outdoors but rests in outdoor shelters. It has marked preference for human blood and seldom attacks cattle (Nair, 1947*a*).

With D.D.T. application at the rate of 100 mg./sq. ft., Nair (1947*b*:1951) recorded reduction in vector density and malaria morbidity.

4. *A.* (*M.*) *leucosphyrus leucosphyrus* Donitz, 1901.—Vector in North-east Assam (India), Borneo, Indonesia and Burma.

Adults are wild and naturally occur in dense jungle, but may be found in houses also. It feeds indoors but rests outdoors. Adults appear to take human or animal blood without much preference. Anthropophilic index of 75·5 per cent has been recorded in India.

Data on its control by the use of insecticides not available.

5. *A.* (*M.*) *maculatus maculatus* Theobald, 1901.—Vectors in Malaya, Indonesia, Yunnan and Hong Kong.

Adults enter houses readily, bite man between 9 p.m. and 2 a.m. and during the day are found in houses, cattlesheds and outdoor resting places. Apparently the feeding habits of this species differ in different parts. In Assam, Indo-China, South China and Philippines it appears to be zoophilic while in Malaya and Indonesia it is anthropophilic. Anthropophilic indices as high as 97·0 and as low as 2·8 per cent have been recorded in Indonesia and Philippines, respectively.

Schiphorst (1952) (as quoted by Gabaldon, 1953*a*:1953*b*), obtained reduction in malaria morbidity with D.D.T. residual application (200 mg./sq. ft.) whereas Wallace (1948) recorded reduction in vector density with little effect on malaria morbidity with D.D.T. applied at the rate of 100 mg./sq. ft.

6. *A.* (*M.*) *minimus flavirostris* Ludlow, 1914.—Vector in Philippines, Borneo, Java and Celebes.

It bites indoors but rests in outdoor shelters. Adults bite man and cattle without much discrimination. They enter houses at night to feed but do not rest inside. During the day, they frequently rest under overhanging stream banks.

In Philippines, Smith and Dy (1949) obtained little effect on vector density with D.D.T. applied at the rate of 200 mg. per sq. ft. Bhatia (1953) (as quoted by Jaswant Singh *et al.*, 1954) recorded reduction in vector density and malaria morbidity with D.D.T. residual spray applied at the rate of 200 mg. per sq. ft.

7. *A.* (*A.*) *novumbrosus* Strickland, 1916.—Vector in Malaya.

It may feed indoors or outdoors but it rests in outdoor shelters. There is insufficient evidence regarding its preference for human or cattle blood.

Data on its control by the use of insecticides not available.

8. *A.* (*M.*) *pharoensis* Theobald, 1901.—Vector in Egyptian delta and oases and Uganda Congo.

It feeds indoors but rests in outdoor shelters. It may be strongly (South Nigeria) or weakly (Kenya) anthropophilic. In Egypt, anthropophilic index of

those caught from houses and tents was 97·5 per cent and those from stables 10·8 per cent. Apparently spends the day in ricefields as none are found in stables, houses or similar shelters (Nile Valley). Prof. G. Macdonald's experience is very much against this observation (personal communication, 1954).

Data on its control by the use of insecticides not available.

9. *A.* (*A*). *punctimaculatus* Dyar and Knab, 1906.—Vector in Columbia, Peru and Panama.

It feeds and rests indoors and outdoors and prefers cattle blood. Adults are abundant in undrained jungle areas and the females engage in flights, invade dwellings and feed on human blood.

Data on its control by the use of insecticides not available.

10. *A.* (*A.*) *umbrosus* Theobald, 1903.—Vector in Malaya and Indonesia.

It feeds indoors or outdoors but rests outdoors. Adults are fierce biters and are found in deeply shaded places in dense forests and also in houses. They have marked preference for human blood. Anthropophilic index of 95·0 per cent has been recorded from Malaya.

Data on its control by the use of insecticides not available.

GROUPS (*C*) AND (*D*).

Outdoor biters and indoor resters; outdoor biters and outdoor resters.

1. *A.* (*A.*) *bancrofti bancrofti* Giles, 1902.—Vector in Australia and Dutch New Guinea.

Adults rarely frequent houses. It is said to vary a great deal in its feeding habits in different areas. In South Queensland, it attacks human beings in the bush in the daytime while at Cairns it is said to show some definite discrimination against man.

Data on its control by the use of insecticides not available.

2. *A.* (*A.*) *barbirostris* Vander Wulp, 1884.—Vector in Malaya and Indonesia.

To a large extent this is a wild species. It is more frequently found in houses in the Indian areas than further east. It is the common species in houses in Celebes. The form occurring in India seems to prefer blood of domestic animals, but in Celebes this species is apparently anthropophilic. Anthropophilic index of 9·0 (cattle present) and 31·0 (cattle scarce) per cent have been recorded from Indonesia and 95 per cent from Malaya.

Data on its control by the use of insecticides not available.

3. *A.* (*K.*) *bellator* Dyar and Knab, 1906.—Vector in Brazil and Trinidad.

It may feed indoors or outdoors but rests in outdoor shelters. In Trinidad, it does not enter houses in numbers and transmission of malaria is undoubtedly almost entirely out of doors. It prefers animal blood but may feed on man.

In Brazil, Pinotti (1951) recorded reduction in malaria morbidity but little effect on vector density with D.D.T. applied at the rate of 200 mg./sq. ft.

4. *A.* (*M.*) *hargreavesi* Evans, 1927.—Vector in West Africa inland.

It feeds outdoors but may rest in indoor or outdoor shelters. Takes human or animal blood without much preference.

Data on its control by the use of insecticides not available.

5. *A.* (*A.*) *hyrcanus nigerrimus* Giles, 1900.—Vector in Indo-China, Malaya and Indonesia.

Adults are rarely found in houses but are found more often in cattlesheds. They feed and rest outdoors. They bite man outside in the evening and even in the shade during the day. This is considered to be a zoophilic species. Anthropophilic indices of 83·0, 30·2, and 3·8 per cent have been recorded from Indonesia, Malaya and India, respectively.

Data on its control by the use of insecticides not available.

6. *A.* (*A.*) *hyrcanus williamsoni* Baisas and Hu, 1936.—Vector in Java and Celebes.

It feeds and rests indoors or outdoors. There is insufficient evidence regarding its preference for human or cattle blood.

Data on its control by the use of insecticides not available.

7. *A.* (*N.*) *nuneztovari* Gabaldon, 1940.—Vector in Venezuela.

Authentic data on its habits not available.

Gabaldon (1953*a*:1953*b*) with D.D.T. application (200 mg./sq. ft.) in Venezuela, obtained slight reduction in malaria morbidity but little effect on vector density.

8. *A.* (*M.*) *tessellatus* Theobald, 1901.—Vector in Maldiv islands.

Adults are found resting among trees in the jungle, along banks of streams, but have been taken also in houses and cowsheds.

Data on its control by the use of insecticides not available.

SUMMARY.

The observations on malaria control *vis-a-vis* the application of insecticides has been reviewed from the global standpoint.

(*a*) Use of residual insecticides has resulted in effective control of malaria transmitted by the following vector species:

A. aconitus; A. albitarsis; A. annularis; A. aquasalis; A. atroparvus; A. aztecus; A. claviger: A. cruzi; A. culicifacies; A. darlingi; A. farauti; A. freeborni; A. funestus funestus; A. gambiæ gambiæ; A. hyrcanus sinensis; A. labranchiæ; A. maculipennis maculipennis; A. mangynus; A. messæ; A. philippinensis; A. pseudopunctipennis; A. quadrimaculatus; A. sacharovi; A. stephensi stephensi; A. sundaicus; A. albimanus; A. fluviatilis; A. letifer; A. maculatus maculatus and *A. minimus minimus.*

(*b*) In some countries, use of residual insecticides has not resulted in effective control of malaria in respect of the following vector species due to the various factors outlined.

A. bellator; A. nuneztovari; A. minimus flavirostris; A. moucheti moucheti; A. sergenti; A. vestitipennis and *A. maculatus maculatus.*

(*c*) It may, however, be pointed out that in spite of the fact that insecticides have been used for the control of malaria for about ten years, there are a number of species listed below about which no data is available.

A. pulcherrimus; A. gambiæ melas; A. hancocki, A. hargreavesi; A. moucheti nigeriensis; A. pharoensis; A. rufipes; A. barbirostris; A. hyrcanus nigerrimus; A. jeyporiensis candidiensis; A. leucosphyrus leucosphyrus; A. novumbrosus; A. stephensi mysorensis; A. subpictus subpictus; A. umbrosus; A. bancrofti bancrofti; A. punctulatus; A. kochi; A. punctimaculatus; A. brunnipes; A. funestus imeriensis; A.nili; A. hyrcanus williamsoni; A. ludlowi torakala and *A. tessellatus.*

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