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Director, Malaria Institute of India.

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MALARIA CONTROL ON BENGAL-NAGPUR RAILWAY FROM 1925 TO 1948.*

BY

A. K. ADHIKARI,

AND

N. GANGULI.

(*Malaria Section, Medical Department, B.-N. Railway.*)

[March 5, 1949.]

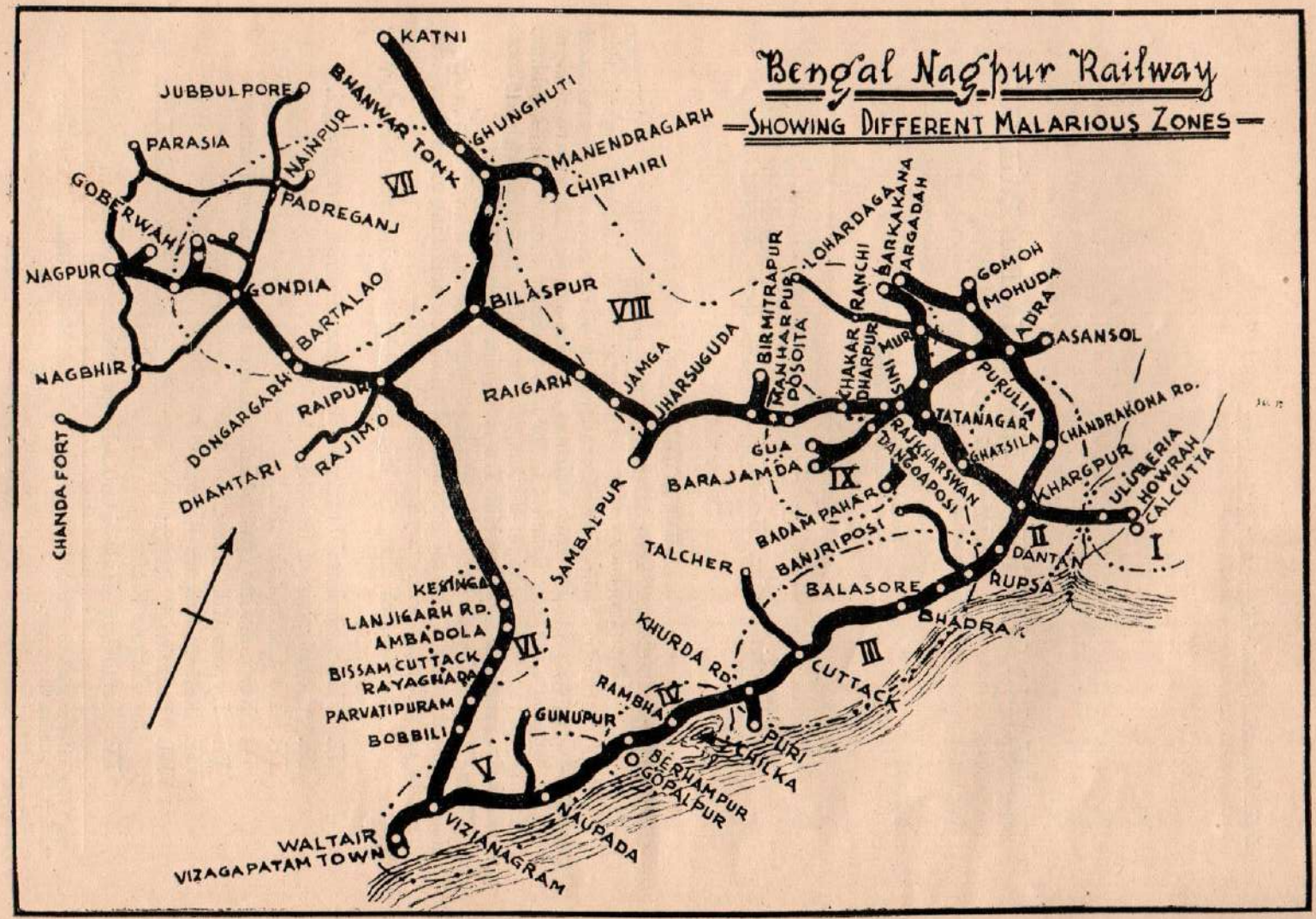
INTRODUCTION.

THE Malaria Section of the Medical Department of the Bengal-Nagpur Railway was created in the year 1925. The necessity of a malaria organization was felt most acutely at the time of construction of the Sarandah Tunnel, west of Chakardharpur, 195 miles from Calcutta ; working of the locomotive shed at Dangoaposi, 182 miles from Calcutta in the hyperendemic areas of the Singhbhum Hills, and survey of the Raipur-Vizianagram line which passes through the most notoriously malarious Jeypore Hill Tracts (Senior White, 1928).

The Bengal-Nagpur Railway traverses portions of West Bengal, Orissa, Madras, Bihar and the Central Provinces of which the following areas have been found to be malarious with varying intensity (Map)—

I. Deltaic area along the River Hooghly in the Howrah District, West Bengal.
II. Flat agricultural country of Midnapore District, West Bengal.
III. Coastal plain of Orissa.
IV. The Chilka Lake area.
V. North Madras coast and coastal area around Vizagapatam.
VI. Jeypore Hill Tracts.
VII. Eastern Satpura Ranges.
VIII. Hazaribagh Ranges and Ranchi Plateau which touch Korea and Sarguja States.
IX. Singhbhum Hills of Chota Nagpur Division, Bihar.

* The authors wish to record their thanks to the Chief Medical Officer of this railway for permission to publish this paper.


Bengal Nagpur Railway
SHOWING DIFFERENT MALARIOUS ZONES
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JUBBULPORE
PARASIA
NAINPUR
BHANWAR TONK
GHUNGHUTI
MANENDRAGARH
CHIRIMIRI
GOBERWAH
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RAMBHA
GUNUPUR
PURI
KHALIKA
BERHAMPUR
GOPALPUR
NAUPADA
WALTAIR
VIZAGAPATAM TOWN
VIZIANAGRAM
I
II
III
IV
V
VI
VII
VIII
IX

I. Deltaic area along the River Hooghly in the Howrah District, West Bengal.—This area had all along been healthy till 1930 when an epidemic of malaria of a severe type broke out at Chengail and Bauria. Iyengar (1931) incriminated *A. sundaicus* to be the important vector here owing to the close correlation between the incidence of malaria and proximity of the breeding places of *A. sundaicus* coupled with an infectivity rate of 4·2 per cent in the species caught in nature. By 1931, the species had crept up to Uluberia and retained its activity in the four stations between Uluberia and Bauria, till 1944. In 1945, it extended its range both eastwards up to Andul and westwards up to Deulti from the endemic Uluberia-Bauria focus. It was only in the last year that it was first found to have crossed the River Rupnarain.

Dissections of *A. sundaicus* caught from eleven localities in this area were carried out during the years 1945, 1946 and 1947 and the results have been tabulated in Table I. All the infected specimens were collected from four places, namely Bauria, Fuleshwar, Uluberia and Deulti. Infection was found in both the house and cattleshed catches.

Table II shows the combined results of dissection of the area. Natural infection was found only in the months of October and November and that too in the first two years only.

II. Flat agricultural country of Midnapore District, West Bengal.—*A. philippinensis* is found to be responsible for malaria both to the north-east and south of Khargpur (Sur, 1929 ; Ganguli, 1947).

III. Coastal plain of Orissa.—The principal vector of the plains appears mainly to be *A. annularis* though *A. aconitus* plays a very minor rôle in certain areas (Senior White *et al.*, 1943 ; Senior White, 1946).

IV. Chilka Lake area of Orissa.—Investigations regarding malaria conditions in the coastal belt of Orissa reveal that *A. sundaicus* is the only malaria vector throughout the area (Senior White and Adhikari, 1939 ; Covell and Singh, 1942).

V. North Madras coast and coastal area around Vizagapatam.—*A. sundaicus* appears to have invaded the North Madras coastal areas between 1938 and 1945 from an isolated coastal focus and in certain parts of these areas *A. stephensi* is known to be the vector (Senior White *et al.*, 1947). Around Vizagapatam, endemicity is maintained mainly by *A. stephensi* (both races) (Senior White and Rao, 1943 ; Senior White, 1946).

VI. Jeypore Hill Tracts.—Investigations in the Jeypore Hills fully confirm that *A. fluviatilis*, *A. varuna* and *A. minimus* play a most important part in the malaria transmission in that locality (Senior White, 1937, 1938, 1946).

VII. Eastern Satpura Ranges.—The principal vectors of the hyperendemic tracts of this area are *A. fluviatilis* and *A. varuna*. *A. culicifacies* plays a very minor rôle in malaria transmission in these hills but is of importance in the foothill areas of this zone (Senior White and Adhikari, 1940 ; Senior White, 1946).

VIII. Hazaribagh Ranges and Ranchi Plateau which touch Korea and Sarguja States.—*A. fluviatilis* is the principal malaria carrier in this area with *A. culicifacies* transmitting in the earlier part of the season only (Senior White, 1943 ; 1946).

IX. Singhbhum Hills of Chota Nagpur Division, Bihar.—The three species—*A. fluviatilis*, *A. varuna* and *A. minimus*—are the malaria vectors in the Singhbhum Hills and there is a complete lack of malaria significance in *A. culicifacies* (Senior White and Das, 1938 ; Senior White and Narayana, 1940).

ORGANIZATION.

At the beginning of 1925, malaria investigation and control were carried out by an Assistant Surgeon assisted by Sanitary Inspectors. After some months in the same year, a Senior Malaria Officer* was appointed to this section. During the succeeding 24 years, the organization has become more and more elaborate every year and has at present a sanctioned staff of different categories as below :—

Technical.		*Ministerial.*	
Malariologist	1	Head Clerk	1
Asstt. Medical Officer (Malaria) ...	1	Stenographer	1
Asstt. Malariologist	1	Typist	1
Malaria Inspectors (Grade I) ...	4	Clerks	10
Malaria Inspectors (Grade II) ...	3	Record Searcher	1
Laboratory Assistants ...	3	Peons	12
Asstt. Malaria Inspectors ...	14		
Sub-Asstt. Malaria Inspectors ...	16		
Malaria Educated Mates ...	23		
Malaria Jemadars	17		
Mosquito-men	262		
Trolley-men	23		

In 1925, malaria control was started in one open line station at Dangoaposi, and in 1949, 105 stations have been under malaria control.

METHOD OF CONTROL.

It has consisted mainly of (*a*) antilarval measures, (*b*) antiadult measures, (*c*) personal protection, and (*d*) drug prophylaxis. Comparatively large colonies in endemic areas have been generally protected by antilarval measures only while smaller railway colonies in endemic areas situated in the belts of periodical epidemics and in hyperendemic areas have been controlled by both antilarval and antiadult measures. Very small wayside malarious stations were controlled by antiadult measures supplemented by drug prophylaxis. The running staff, such as drivers, guards, khalashies, etc., whose duties keep them away from the protected areas, are supplied with repellents, such as dimethyl phthalate (D.M.P.) ; and paludrine as prophylaxis once or twice weekly according to the degree of malariousness of the country over which they have to travel.

* This Senior Malaria Officer was Mr. R. Senior White, F.R.S.E., F.R.E.S., F.R.S.T.M. & H., M.R.san.I., who rendered remarkably good and splendid service as Malariologist to the B.-N. Railway for long twenty-one years since joining it in September 1925. His reputation for meritorious work was not confined to this railway alone ; it extended over the whole of India and even outside. The writers think themselves to be fortunate that they had the privilege to work with him during his stay in this railway.

In large colonies like the big stations of Khargpur, Tatanagar, etc., where the population is 10,000 or more, antilarval measures were in vogue though selective spraying of D.D.T. and pyrethrum was resorted to, particularly in the fringe areas when such is indicated. Even in the smaller colonies, antiadult measures have not been applied very thoroughly, as some quarters have often to be left over owing to houses being locked when the staff were on duty or on leave.

(*a*) *Antilarval measures.*—In 1925, diesel oil with 2·5 per cent cresol was being applied as the only larvicide. By the end of 1925, paris green was used for the first time on this railway. All breeding places were treated with diesel oil or paris green and this continued up to 1936 when control by means of 'species sanitation' was introduced by 'herbage packing' and by other methods (Senior White, 1936); this, although eliminated the vector species successfully, led to a great increase in the number of nuisance mosquitoes and left a large proportion of mosquito fauna untouched. All breeding places were therefore put under weekly treatment with malariol or 2 per cent paris green in soapstone powder.

Experiments with malariol mixed with D.D. T.and gammexane in soapstone powder as larvicides are now being tried out.

(*b*) *Antiadult measures.*—The daily use of pyrethrum spraying was started at one station Nagarwara in the Eastern Satpuras in June 1937. The village huts in the vicinity were also included. The results were very satisfactory and more and more stations were gradually put under antiadult control. Later, in some of the bigger stations, antilarval measures were supplemented by spraying of pyrethrum on alternate days.

D.D.T. was first applied on this railway in 1944 (Senior White, 1945). It was found cheaper and more effective than pyrethrum. In 1946, only some stations had D.D.T. applications and it was only from the malaria season of 1947 that D.D.T. was regularly used for its residual effect as an antiadult measure.

It was found that the residual effect of 5 per cent D.D.T. solution in red kerosene oil persisted for 6 to 8 weeks when applied by stirrup pump with a nozzle bore of 1/64 inch or a Ross pattern knapsack or Hydra sprayer with fine bore at the rate of 1 quart per 600 sq. ft. The applications were repeated every 6 to 8 weeks during each malaria season.

Railway public opinion about this newly introduced D.D.T.-M.K.E. emulsion was not very encouraging at its initial stage. None the less, from a short experience it was found that there was very little difference between the two in their killing effects on insects and the opinion is changing for the better.

As mentioned elsewhere, no single method of antimalaria measure was found to be perfect, and as such, one had often to be supplemented by another. The squatters' huts, growing bustees and the villages are sometimes so intimately mixed up with the railway colonies, and the possibilities of infiltration of infected anophelines are so apparent that no one orthodox and water-tight method of adult control can be considered safe. Then again at the time of application of D.D.T., much of the household articles are removed and subsequently brought in facilitating mosquitoes to sit on unsprayed surface. Because of such difficulties as mentioned above, spraying of pyrethrum could not be altogether omitted from

the antiadult measures. Pyrethrum insecticide is, therefore, sprayed twice a week in all railway quarters where application of D.D.T. can neither be made very thorough nor the possibilities of infiltration of infected mosquitoes eliminated.

(*c*) *Repellents.*—Repellents of different formulæ with citronella as the main ingredient were distributed to the running staff, such as drivers, guards, coal-men, khalashies, etc., in the old days. During the war, the repellent with pyrethrum and citronella in white vaseline base was distributed. Now D.M.P. is used as it is available in bulk from the disposals. It appears to be better than any other substance used previously. Attention of the staff was necessarily drawn to the fact that its effect does not last more than 3 to 4 hours. Shortcomings of its use were pointed out by explaining to the individuals that it is not a substitute for mosquito net and that they are liable to be bitten while asleep after its application.

(*d*) *Drug prophylaxis.*—Quinine had not been extensively used as a suppressive in this railway before the synthetic antimalarials came into use. An experiment was carried out to determine whether the incidence of malaria at Dangoaposi near Chaibasa could further be reduced by a single blanket treatment with plasmochin to all children immediately prior to the malaria season of 1933. It was found that no permanent improvement could be achieved by a single blanket treatment (Senior White and Adhikari, 1934). In the year 1935, this antigametocyte treatment was, however, repeated at Sarandah near Chakardharpur in a different way giving two tablets (0·01 gm.) weekly for 6 months to all the adults of the labour camp. There too, however, no additional benefit over the antilarval measures was derived (Senior White and Adhikari, 1937).

In the malaria season of 1946, paludrine prophylaxis had been carried out at the rate of 1 tablet (100 mg.) per week per person at five hyperendemic stations with encouraging results. In September 1947, larger number of stations were brought under this regime with equally satisfactory results. From the very beginning of the malaria season of 1948, all wayside stations in the hyperendemic areas and the running staff, who are likely to travel on duty to hyperendemic areas, have been brought under paludrine prophylaxis. The results so far appear to be very satisfactory.

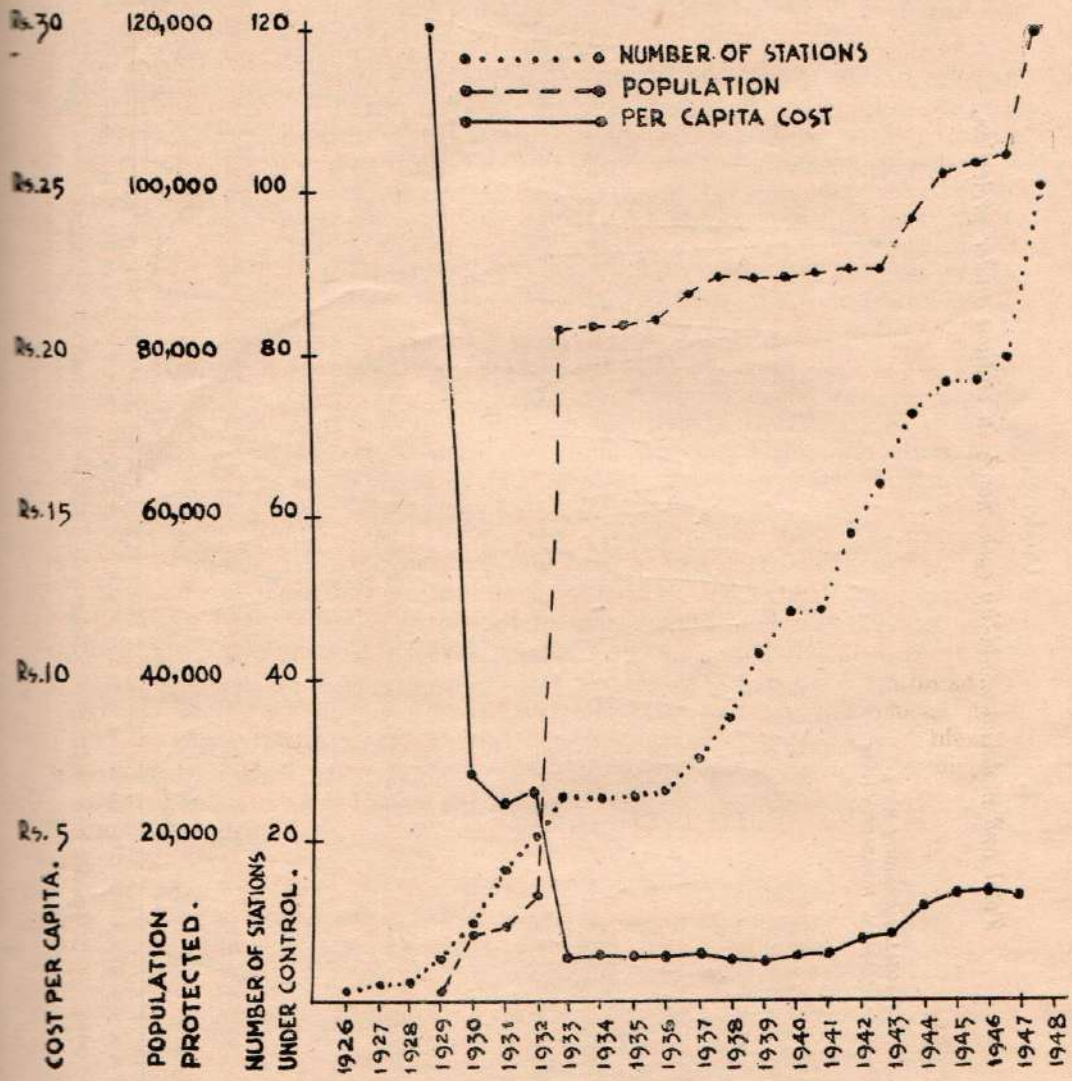
Chemoprophylaxis of malaria for staff posted at malarious stations who have not been provided with quarters and therefore have to live in villages, and running staff who have to travel and wait in unprotected areas, will be necessary for some years to come.

COSTS.

The annual expenditure and the number of stations and population protected each year are given in Table III and graphically represented in Chart 1. Expenditure figures prior to 1929 are not shown as they are not separable from general medical expenses. The *per capita* cost which was little above Rs. 30 in 1929 came down to Re. 1-3-0 just before the war-time rise in prices. With the present all-round rise in prices, enhanced rates of post-war scales of pay and simultaneous launching of different antimosquito measures throughout the year, the *per capita* cost is now a little over Rs. 3.

CHART 1.

Number of stations and population under protection and per capita *cost.*

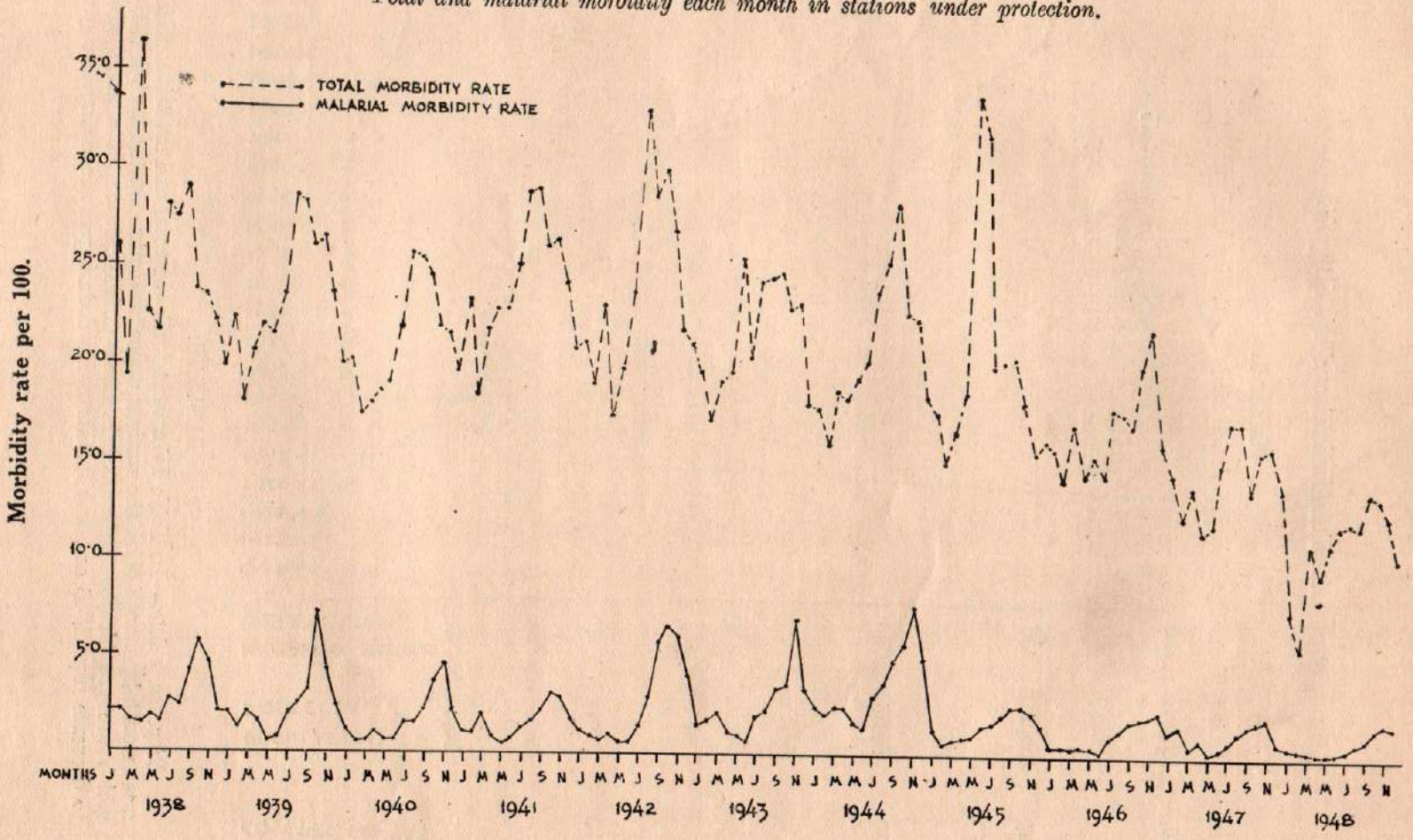


RESULTS.

Dispensary data.—In Table IV are set forth the total and malaria morbidity rates month by month of all the stations under protection for the period 1938–48 which are graphically represented in Chart 2. Incidence of malaria started to decrease from 1945 and was further reduced during the following two years.

CHART 2.

Total and malarial morbidity each month in stations under protection.



On an average more than 5 per cent of the population used to suffer from malaria in the peak months previously but now it has come down to less than 2 per cent.

Reduction in malaria which is a bulk disease has resulted in a corresponding decrease in the general sickness rate as well.

Density of Anopheles mosquitoes.—The results of observations for the period 1944–48 on the prevalence of mosquitoes in all the malarious zones excepting the area in the Midnapore District of West Bengal have been shown in eight graphs (Charts 3 to 10), and the collections of mosquito vector or vectors and all anophelines are given in Tables V to XII. These are based on the adult collections obtained in the course of weekly routine check catches from the staff quarters which formed regular catching stations. It will be observed that the total time spent in collection is not uniform but varies in different localities and also in different years for the same locality; but the time factor has been reduced to 5 man-hours' catch.

The number of mosquitoes in 1947 and 1948 remained very low practically throughout the period. Of the vectors, *A. fluviatilis* group and *A. sundaicus* have been found to be very sensitive to the D.D.T. residue. There was also a distinct reduction in numbers of *A. culicifacies* during the same period. Susceptibility of *A. stephensi* to D.D.T. could not be assessed as the number of this species caught in nature had always been very small.

SUMMARY.

1. A brief account of the development and activities of the malaria organization of the B.-N. Railway for the last 24 years is given.

2. The malarial morbidity rate and density of Anopheles adults have been brought down to a significantly low level, specially during the last two years.

3. Initial *per capita* cost of Rs. 30·16 was reduced to Rs. 1·29 in four years which remained fairly steady up to the outbreak of World War II. Due to increase in cost of material and labour, the *per capita* cost has since risen to Rs. 3·1 in 1948.

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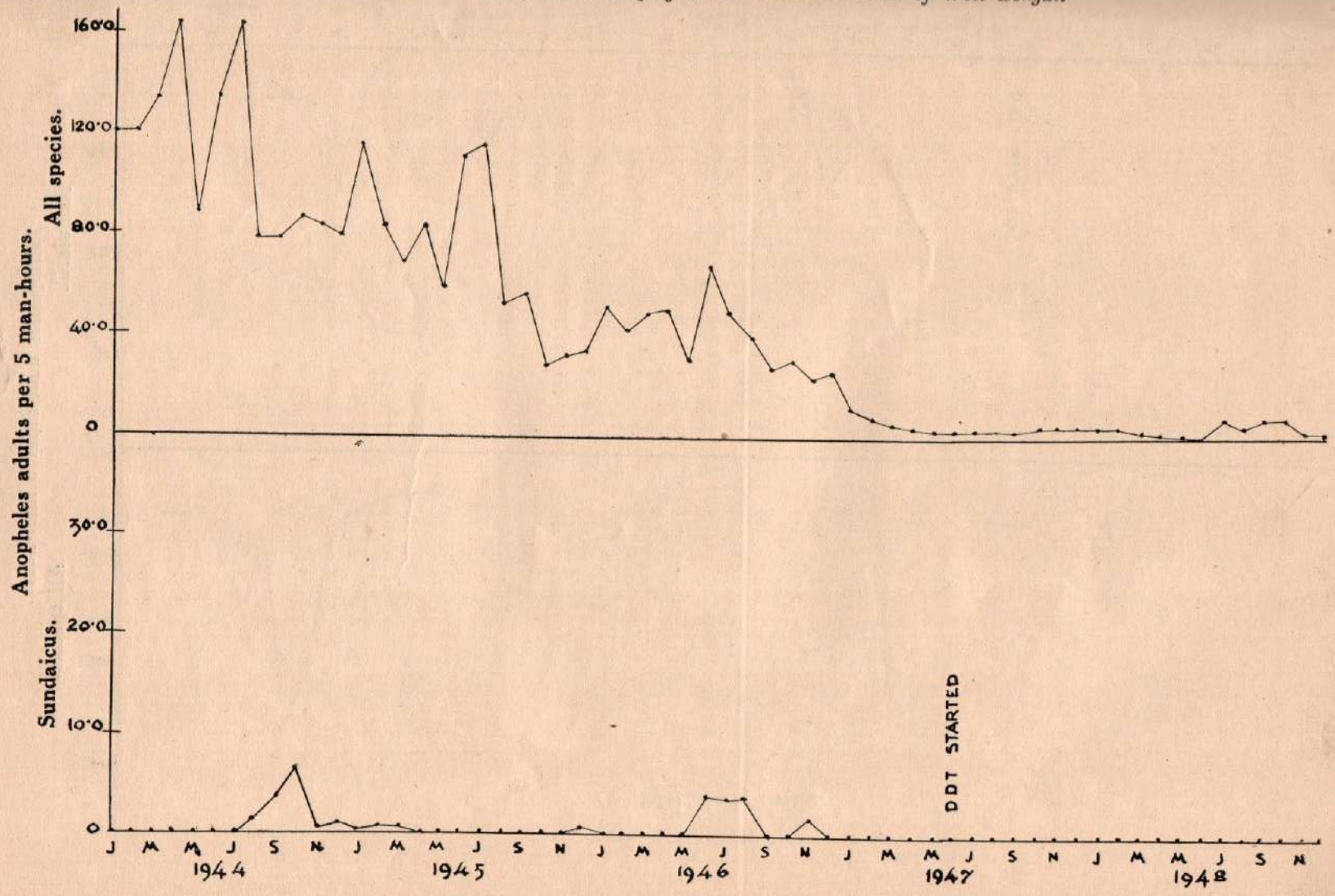
CHART 5.

Deltaic area along River Hooghly in the Howrah District of West Bengal.



HART 4.

Coastal plain of Orissa.

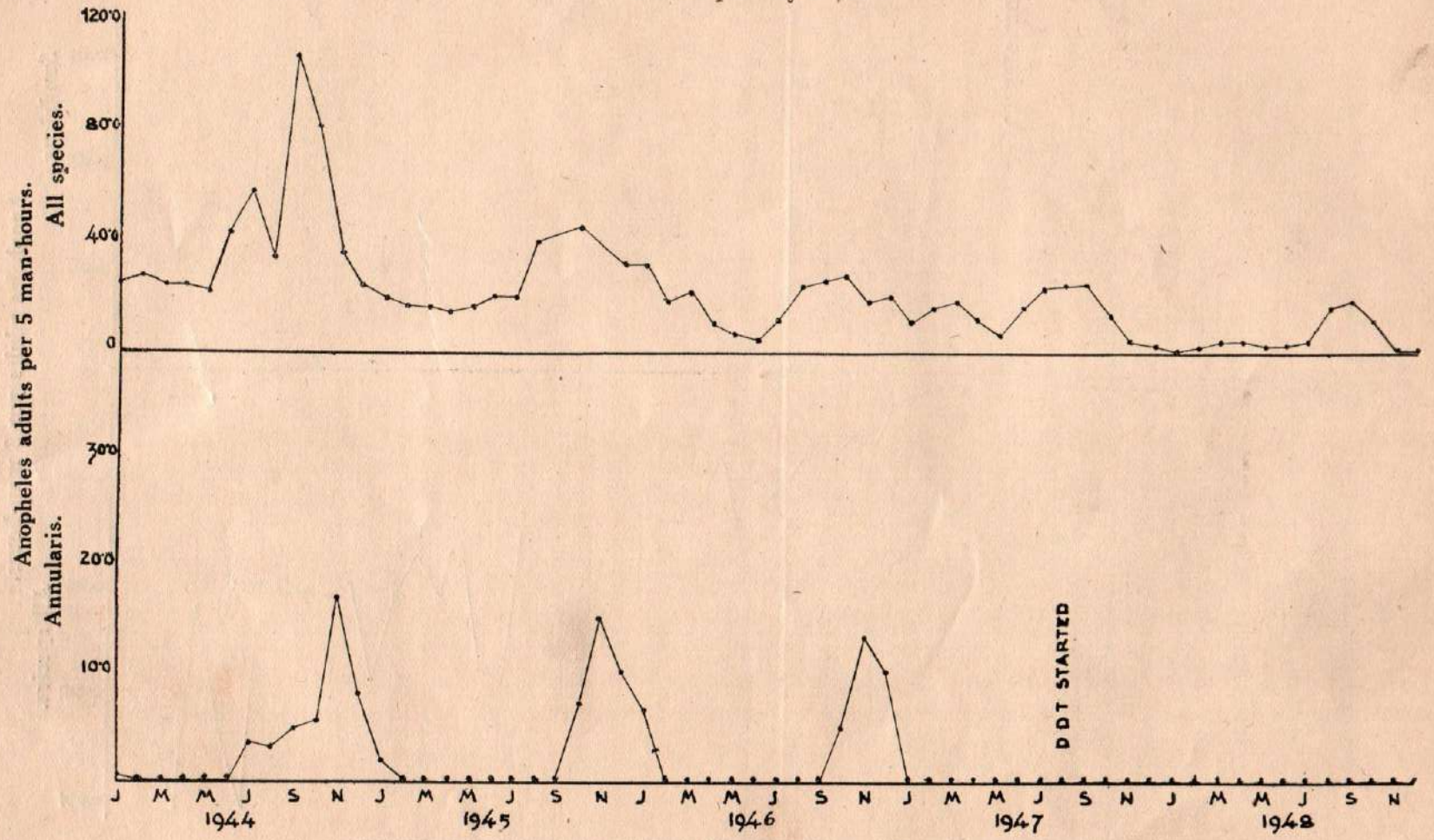


CHART 5.

Chilka Lake area.

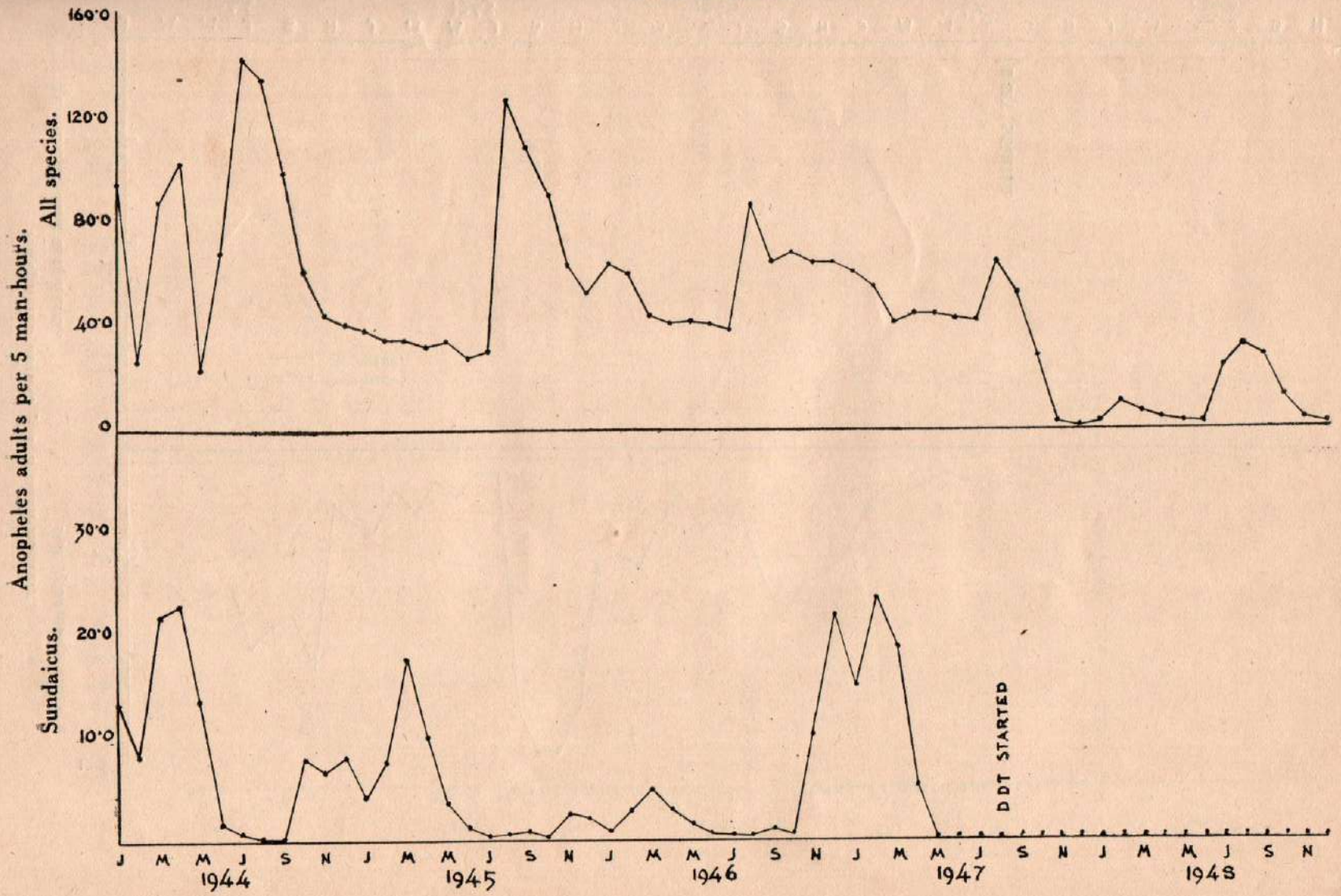


CHART 6.

North Madras Coast and coastal area around Vizagapatam.

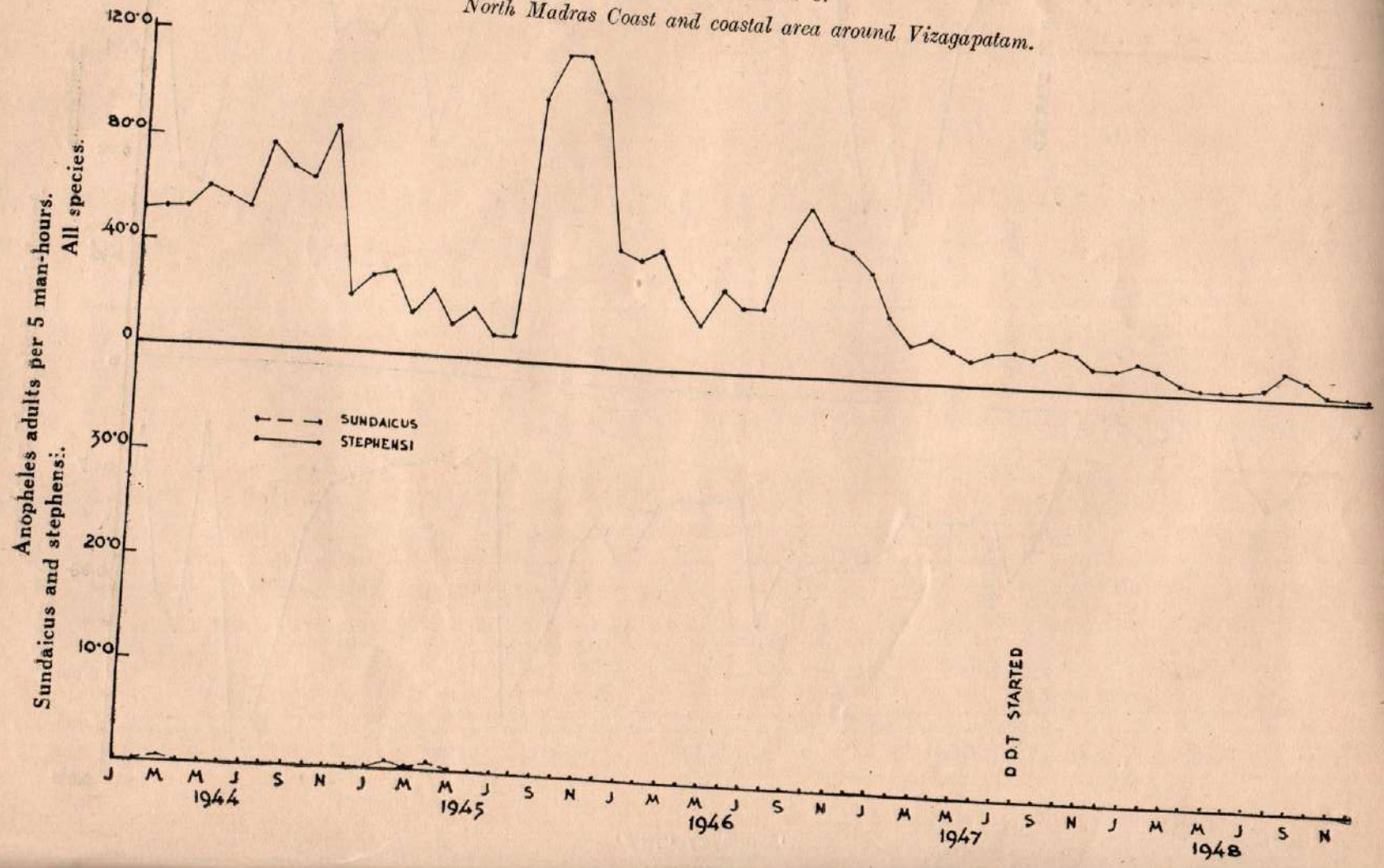


CHART 7.

Jeypore Hill Tracts.

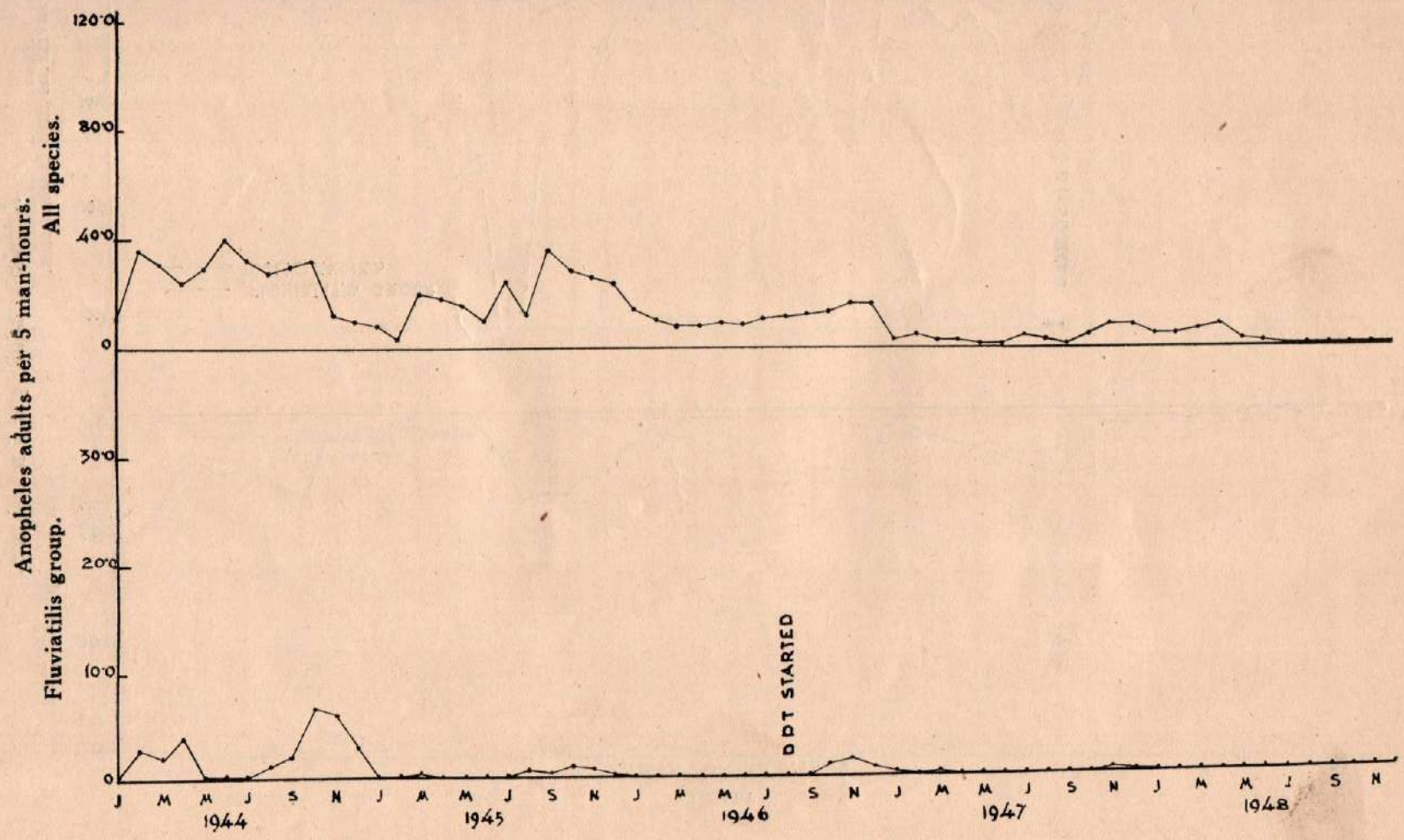


CHART 8.

Eastern Satpura Ranges.

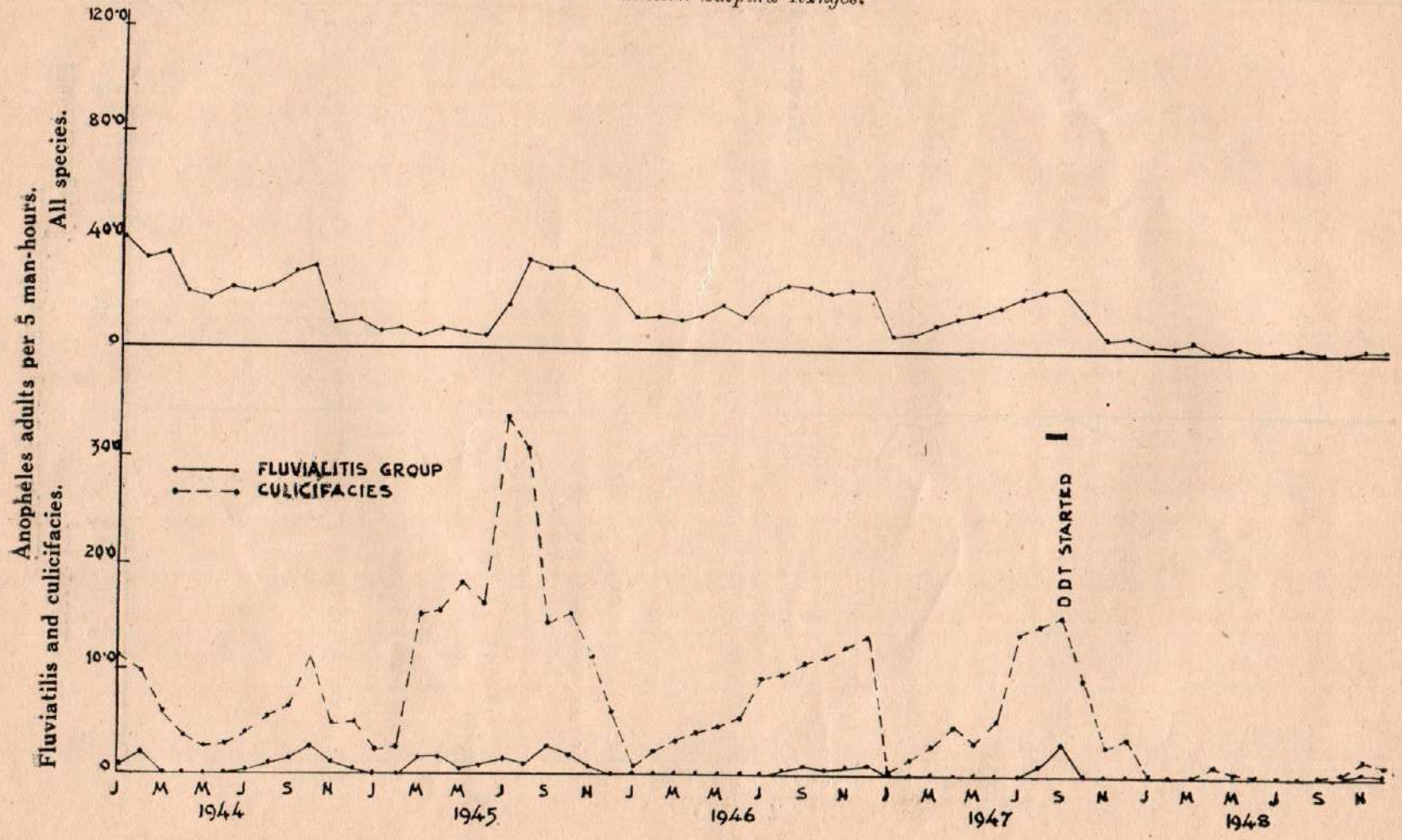


CHART 9.

Hazaribagh Ranges and Ranchi Plateau.

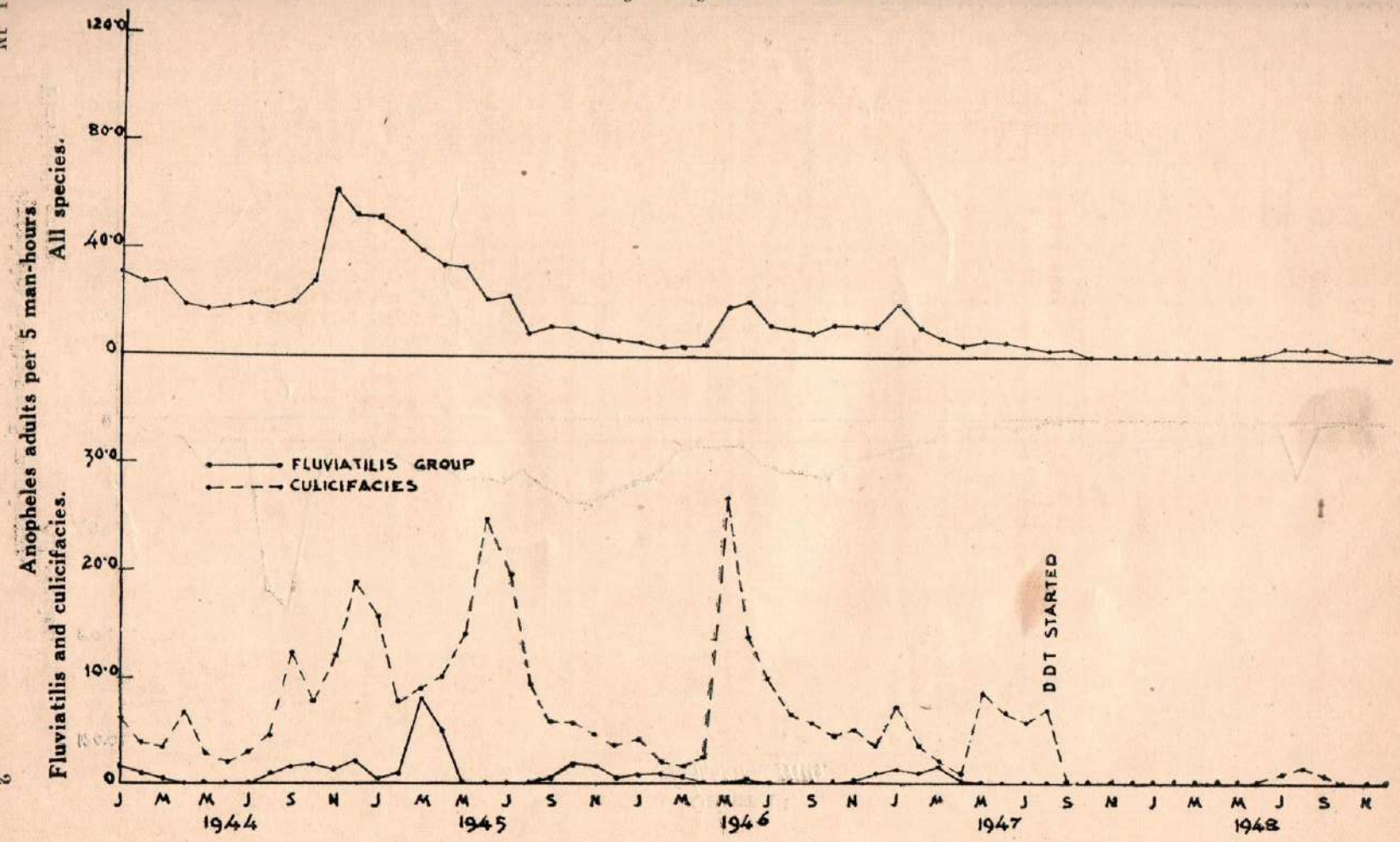


CHART 10.

Singhbhum Hills.

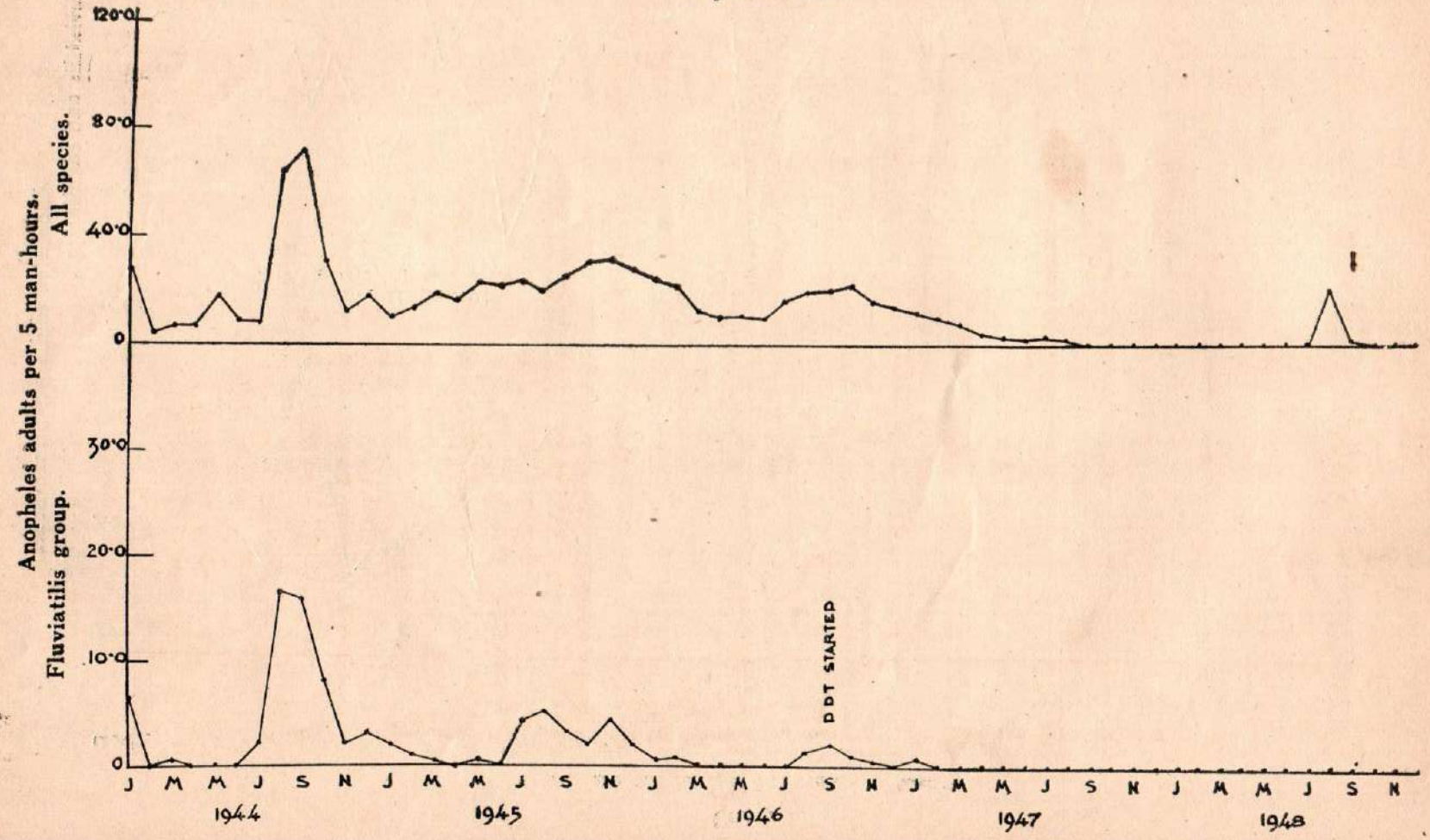


TABLE I.

Results of dissections of A. sundaicus *in deltaic area along the River Hooghly in Howrah District, West Bengal, 1945–47.*

1945

Month.	ANDUL HOUSES. (1)			SANKRAIL HOUSES. (2)			NALPUR HOUSES. (3)			BAURIA HOUSES. (4)			BAURIA CATTLESHED. (5)			CHENGAIL HOUSES. (6)			FULESHWAR HOUSES. (7)		
	Number dissected.	G+	Gl+	Number dissected.	G+	Gl+	Number dissected.	G+	Gl+	Number dissected.	G+	Gl+	Number dissected.	G+	Gl+	Number dissected.	G+	Gl+	Number dissected.	G+	Gl+
Sep. ...	3	0	0	0	0	0	1	0	0	5	0	0	0	0	0	0	0	0	3	0	0
Oct. ...	1	0	0	0	0	0	0	0	0	7	0	0	32	1	0	1	0	0	4	0	0
Nov. ...	0	0	0	2	0	0	0	0	0	2	0	0	4	0	0	0	0	0	0	0	0
TOTAL ...	4	0	0	2	0	0	1	0	0	14	0	0	36	1	0	1	0	0	7	0	0
INFECTION RATE, PER CENT.	...	0	0	...	0	0	...	0	0	...	0	0	...	2·8	0	...	0	0	...	0	0

G+ = gut infected. Gl+ = salivary glands infected.
0 = a search was made, but no specimens found. ... = no collection.

TABLE I—*contd.*

1945

Month.	FULESHWAR CATTLESHEDS. (8)			ULUBARIA HOUSES. (9)			ULUBARIA CATTLESHEDS. (10)			BIRSHIBPUR HOUSES. (11)			KULGACHIA HOUSES. (12)			BAGNAN HOUSES. (13)			DEULTI HOUSES. (14)		
	Number dissected.	G+	Gl+	Number dissected.	G+	Gl+	Number dissected.	G+	Gl+	Number dissected.	G+	Gl+	Number dissected.	G+	Gl+	Number dissected.	G+	Gl+	Number dissected.	G+	Gl+
Sep. ...	0	0	0	0	0	0	0	0	0	...	...	...	...	...	...	...	...	...	...	...	...
Oct. ...	25	0	2	0	0	0	5	0	0	...	...	...	...	...	...	...	...	...	...	...	...
Nov. ...	13	0	0	1	0	0	18	1	0	0	0	0	0	0	0	7	0	0	2	0	0
TOTAL ...	38	0	2	1	0	0	23	1	0	0	0	0	0	0	0	7	0	0	2	0	0
INFECTION RATE, PER CENT.	...	0	5·2	...	0	0	...	4·3	0	...	0	0	...	0	0	...	0	0	...	0	0

G+ = gut infected.
0 = a search was made, but no specimens found.
Gl+ = salivary glands infected.
... = no collection.

TABLE I—*contd.*

1946

Month.	ANDUL HOUSES. (1)			SANKRAIL HOUSES. (2)			NALPUR HOUSES. (3)			BAURIA HOUSES. (4)			CHENGAIL HOUSES. (5)			FULESHWAR HOUSES. (6)		
	Number dissected.	G+	Gl+	Number dissected.	G+	1+	Number dissected.	G+	Gl+	Number dissected.	G+	Gl+	Number dissected.	G+	Gl+	Number dissected.	G+	Gl+
Jul. ...	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0
Aug. ...	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Sep. ...	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oct. ...	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
Nov. ...	0	0	0	0	0	0	0	0	0	7	0	0	1	0	0	2	0	0
TOTAL ...	3	0	0	0	0	0	1	0	0	8	0	0	2	0	0	4	0	0
INFECTION RATE, PER CENT.	...	0	0	...	0	0	...	0	0	...	0	0	...	0	0	...	0	0

G+ = gut infected.
0 = a search was made, but no specimens found.
Gl+ = salivary glands infected.
... = no collection.

TABLE I—*contd.*

1946

Month.	ULUBARIA HOUSES. (7)			BIRSHIBPUR HOUSES. (8)			KULGACHIA HOUSES. (9)			BAGNAN HOUSES. (10)			DEULTI HOUSES. (11)		
	Number dissected.	G+	Gl+	Number dissected.	G+	Gl+	Number dissected.	G+	Gl+	Number dissected.	G+	Gl+	Number dissected.	G+	Gl+
Jul. ...	0	0	0	1	0	0	3	0	0	5	0	0	3	0	0
Aug. ...	2	0	0	0	0	0	0	0	0	3	0	0	2	0	0
Sep. ...	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oct. ...	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nov. ...	2	0	0	0	0	0	0	0	0	1	0	0	6	0	1
TOTAL ...	4	0	0	1	0	0	3	0	0	9	0	0	11	0	1
INFECTION RATE, PER CENT.	...	0	0	...	0	0	...	0	0	...	0	0	...	0	9·1

G+ = gut infected.
0 = a search was made, but no specimens found.
Gl+ = salivary glands infected.
... = no collection.

TABLE I—*contd.*

1947

Month.	ANDUL HOUSES. (1)			SANKRAIL HOUSES. (2)			NALPUR HOUSES. (3)			BAURIA HOUSES. (4)			CHENGAIL HOUSES. (5)		
	Number dissected.	G+	Gl+	Number dissected.	G+	Gl+	Number dissected.	G+	Gl+	Number dissected.	G+	Gl+	Number dissected.	G+	Gl+
Jun. ...	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0
Jul. ...	0	0	0	0	0	0	0	0	0	9	0	0	4	0	0
Aug. ...	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sep. ...	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oct. ...	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nov. ...	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL ...	3	0	0	0	0	0	0	0	0	14	0	0	4	0	0
INFECTION RATE, PER CENT.	...	0	0	...	0	0	...	0	0	...	0	0	...	0	0

G+ = gut infected.
0 = a search was made, but no specimens found.
Gl+ = salivary glands infected.
... = no collection.

TABLE I—*concld.*

1947

Month.	FULESHWAR HOUSES. (6)			BIRSHIBPUR HOUSES. (7)			KULGACHIA HOUSES. (8)			BAGNAN HOUSES. (9)			DEULTI HOUSES. (10)		
	Number dissected.	G+	Gl+	Number dissected.	G+	Gl+	Number dissected.	G+	Gl+	Number dissected.	G+	Gl+	Number dissected.	G+	Gl+
Jun. ...	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jul. ...	2	0	0	0	0	0	0	0	0	2	0	0	0	0	0
Aug. ...	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sep. ...	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oct. ...	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nov. ...	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL ...	6	0	0	0	0	0	0	0	0	2	0	0	0	0	0
INFECTION RATE, PER CENT.	...	0	0	...	0	0	...	0	0	...	0	0	0	0	0

G+ = gut infected.
0 = a search was made, but no specimens found.
Gl+ = salivary glands infected.
... = no collection.

TABLE II.

Combined data of seasonal incidence of natural infection in A. sundaicus *in the deltaic area along the River Hooghly in the Howrah District of West Bengal, 1945–47.*

Month.	1945					1946					1947				
	Number dissected.	G+	Gl+	Oöcyst rate, per cent.	Sporozoite rate, per cent.	Number dissected.	G+	Gl+	Oöcyst rate, per cent.	Sporozoite rate, per cent.	Number dissected.	G+	Gl+	Oöcyst rate, per cent.	Sporozoite rate, per cent.
Jun. ...	...	...	...	...	...	...	...	...	...	...	5	0	0	0	0
Jul. ...	...	...	...	...	...	16	0	0	0	0	17	0	0	0	0
Aug. ...	...	...	...	...	...	9	0	0	0	0	0	0	0	0	0
Sep. ...	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oct. ...	75	1	2	1·3	2·6	2	0	0	0	0	4	0	0	0	0
Nov. ...	49	1	0	2·0	0	19	0	1	0	5·3	3	0	0	0	0
TOTAL ...	136	2	2	1·5	1·5	46	0	1	0	2·2	29	0	0	0	0

G+ = gut infected.
0 = a search was made, but no specimens found.
Gl+ = salivary glands infected.
... = no collection.

TABLE III.

Number of stations and population thereof under protection with annual expenditure during the period, 1926–48.

Year.	Number of stations under control.	Population protected.	Cost of protection (Actuals). Rs.	Cost per head protected. Rs.
1926	1	207	Figures for these years are not separable from general medical expenses.	
1927	2	905		
1928	2	1,109		
1929	5	1,416	42,703	30·16
1930	9	8,467	60,193	7·1
1931	16	9,568	57,741	6·03
1932	20	13,678	89,895	6·57
1933	25	83,437	1,07,596	1·29
1934	25	83,879	1,18,589	1·41
1935	25	83,656	1,16,054	1·39
1936	26	83,836	1,22,064	1·45
1937	30	86,596	1,29,568	1·5
1938	35	88,797	1,16,465	1·31
1939	43	89,995	1,08,489	1·2
1940	48	89,897	1,27,489	1·41
1941	48	89,880	1,33,593	1·48
1942	57	89,978	1,70,109	1·89
1943	63	90,469	1,91,832	2·12
1944	72	96,352	2,87,784	2·99
1945	76	102,090	3,24,484	3·18
1946	76	103,412	3,30,160	3·2
1947	79	104,382	3 21,715	3·1
1948	100	119,747	Not available yet.	

TABLE IV.

Statement of total and malarial morbidity each month in stations under protection.

Month.	1938		1939		1940		1941	
	Incidence rate of all diseases, per cent.	Incidence rate of malaria, per cent.	Incidence rate of all diseases, per cent.	Incidence rate of malaria, per cent.	Incidence rate of all diseases, per cent.	Incidence rate of malaria, per cent.	Incidence rate of all diseases, per cent.	Incidence rate of malaria, per cent.
Jan. ...	25·8	1·9	22·1	1·7	20·0	1·1	23·3	1·0
Feb. ...	19·2	1·9	17·9	1·1	17·3	0·4	18·4	0·9
Mar. ...	36·2	1·4	20·4	1·7	17·8	0·5	21·7	1·8
Apr. ...	22·4	1·2	21·7	1·4	18·5	0·8	22·8	0·7
May ...	21·5	1·6	21·4	0·4	18·9	0·5	22·8	0·3
Jun. ...	27·9	1·3	23·3	0·6	21·7	0·5	25·1	0·6
Jul. ...	27·2	2·4	28·4	1·7	25·5	1·4	28·7	1·1
Aug. ...	28·7	2·2	28·1	2·2	25·4	1·4	29·1	1·5
Sep. ...	23·5	3·8	25·8	3·1	24·5	2·0	26·1	2·1
Oct. ...	23·3	5·4	26·3	7·1	21·9	3·5	26·4	3·0
Nov. ...	21·9	4·3	23·5	4·0	21·5	4·4	24·2	2·7
Dec. ...	19·7	1·7	19·9	2·2	19·6	2·0	20·8	1·6

TABLE IV—*contd.*

Month.	1942		1943		1944		1945	
	Incidence rate of all diseases, per cent.	Incidence rate of malaria, per cent.	Incidence rate of all diseases, per cent.	Incidence rate of malaria, per cent.	Incidence rate of all diseases, per cent.	Incidence rate of malaria, per cent.	Incidence rate of all diseases, per cent.	Incidence rate of malaria, per cent.
Jan. ...	21·2	1·1	19·7	1·4	18·1	2·3	17·8	1·2
Feb. ...	19·1	0·7	17·2	1·6	16·2	1·8	15·3	0·6
Mar. ...	23·1	0·6	19·3	2·0	18·9	2·3	16·9	0·7
Apr. ...	17·5	0·8	19·7	1·1	18·6	2·2	18·8	0·8
May ...	19·9	0·5	25·5	0·8	19·6	1·6	33·9	0·9
Jun. ...	23·7	0·5	20·6	0·6	20·3	1·3	31·8	1·4
Jul. ...	33·1	1·3	24·4	1·7	23·9	2·8	20·1	1·6
Aug. ...	28·7	2·7	24·6	2·1	25·3	3·6	20·4	1·8
Sep. ...	30·2	5·6	24·8	3·2	28·3	4·6	20·5	2·4
Oct. ..	26·8	6·4	23·1	3·4	22·8	5·5	18·3	2·4
Nov. ...	21·9	5·8	23·3	6·7	22·5	7·5	15·7	2·1
Dec. ...	21·2	3·8	18·2	3·2	17·5	4·8	16·4	1·5

TABLE IV—*concld.*

Month.	1946		1947		1948	
	Incidence rate of all diseases, per cent.	Incidence rate of malaria, per cent.	Incidence rate of all diseases, per cent.	Incidence rate of malaria, per cent.	Incidence rate of all diseases, per cent.	Incidence rate of malaria, per cent.
Jan. ...	15·8	0·5	14·7	1·2	7·4	0·6
Feb. ...	14·4	0·5	12·3	1·6	5·6	0·5
Mar. ...	17·1	0·4	13·8	0·6	10·7	0·4
Apr. ...	14·6	0·5	11·5	0·8	9·3	0·3
May ...	15·5	0·4	11·9	0·3	10·8	0·3
Jun. ...	14·5	0·2	15·0	0·4	11·9	0·3
Jul. ...	17·8	1·0	17·1	0·7	12·1	0·5
Aug. ...	16·7	1·3	17·1	1·3	11·8	0·7
Sep. ...	17·0	1·7	13·6	1·6	13·5	1·2
Oct. ...	20·0	1·9	15·6	1·7	13·2	1·5
Nov. ...	21·8	2·0	15·8	2·0	12·3	1·7
Dec. ...	16·1	2·2	13·7	0·7	10·2	1·6

TABLE V.

Density of Anopheles adults in human dwellings of deltaic area along River Hooghly in the Howrah District of West Bengal.

Month.	Time spent in hours.	Per 5 man-hours.		Time spent in hours.	Per 5 man-hours.	
		A. sundaicus.	All anophelines.		*A. sundaicus.*	All anophelines.
	1944			**1945**		
Jan. ...	6⅓	0·0	120·7	18⅔	0·0	116·8
Feb. ...	6⅓	0·0	121·0	18⅔	0·5	83·5
Mar. ...	6⅓	0·0	134·4	18⅔	0·3	68·5
Apr. ...	6⅓	0·0	165·8	18⅔	0·0	83·0
May ...	6⅓	0·0	89·2	18⅔	0·0	58·1
Jun. ...	6⅓	0·0	134·2	18⅔	0·0	111·1
Jul. ...	6⅓	0·0	163·4	18⅔	0·0	115·5
Aug. ...	9⅓	1·5	78·4	37	0·0	53·9
Sep. ...	9⅓	3·6	79·6	37	0·0	56·7
Oct. ...	9⅓	6·2	87·9	37	0·0	29·1
Nov. ...	18⅔	0·0	84·6	37	0·0	33·4
Dec. ...	18⅔	1·07	79·8	37	0·1	34·2
	1946			**1947**		
Jan. ...	37	0·0	53·1	37	0·0	10·0
Feb. ...	37	0·0	42·8	37	0·0	7·5
Mar. ...	37	0·0	50·3	37	0·0	4·5
Apr. ...	37	0·0	51·8	37	0·0	3·0
May ...	37	0·0	29·4	37	0·0	2·0
Jun. ...	37	3·8	68·4	37	0·0	2·0
Jul. ...	37	3·4	48·4	37	0·0	3·0
Aug. ...	37	3·5	40·4	37	0·0	2·0
Sep. ...	37	0·3	26·8	37	0·0	2·5
Oct. ...	37	0·1	30·7	37	0·0	4·0
Nov. ...	37	1·5	23·2	37	0·0	3·8
Dec. ...	37	0·0	25·5	37	0·0	4·2

Month.	Time spent in hours.	Per 5 man-hours.	
		A. sundaicus.	All anophelines.
	1948		
Jan. ...	37	0·0	4·0
Feb. ...	37	0·0	4·0
Mar. ...	37	0·0	1·6
Apr. ...	37	0·0	0·2
May ...	37	0·0	0·0
Jun. ...	37	0·0	0·1
Jul. ...	37	0·0	7·2
Aug. ...	37	0·0	5·6
Sep. ...	37	0·0	6·6
Oct. ...	37	0·0	6·6
Nov. ...	37	0·0	2·2
Dec. ...	37	0·0	2·6

TABLE VI.

Density of Anopheles adults in human dwellings in the coastal plain of Orissa.

Month.	Time spent in hours.	Per 5 man-hours.		Time spent in hours.	Per 5 man-hours.	
		A. annularis.	All anophelines.		*A. annularis.*	All anophelines.
	1944			**1945**		
Jan. ...	12	0·8	25·6	12	2·0	19·0
Feb. ...	12	0·0	28·8	12	0·0	17·0
Mar. ...	12	0·0	25·6	12	0·0	15·0
Apr. ...	12	0·0	24·4	12	0·0	14·0
May ...	12	0·0	22·4	12	0·0	16·0
Jun. ...	12	0·0	45·6	12	0·0	21·0
Jul. ...	12	3·6	58·0	12	0·0	19·0
Aug. ...	12	3·2	34·0	12	0·0	40·0
Sep. ...	12	4·8	108·0	12	0·0	43·0
Oct. ...	12	5·6	82·0	12	6·5	36·5
Nov. ...	12	17·0	36·0	12	15·0	38·0
Dec. ...	12	8·0	24·0	12	10·0	31·5
	1946			**1947**		
Jan. ...	12	6·5	33·0	12	0·0	10·0
Feb. ...	12	0·0	18·0	12	0·0	15·0
Mar. ...	12	0·0	23·0	12	0·0	18·0
Apr. ...	12	0·0	10·0	12	0·0	11·5
May ...	12	0·0	6·5	12	0·0	6·5
Jun. ...	12	0·0	5·0	12	0·0	15·0
Jul. ...	12	0·0	11·5	12	0·0	23·0
Aug. ...	12	0·0	24·0	12	0·0	25·0
Sep. ...	12	0·0	26·5	12	0·0	21·5
Oct. ...	12	5·0	28·0	12	0·0	13·7
Nov. ...	12	13·0	18·0	12	0·0	3·9
Dec. ...	12	10·0	25·0	12	0·0	2·3

Month.	Time spent in hours.	Per 5 man-hours.	
		A. annularis.	All anophelines.
	1948		
Jan. ...	12	0·0	0·0
Feb. ...	12	0·0	1·5
Mar. ...	12	0·0	3·1
Apr. ...	12	0·0	3·1
May ...	12	0·0	1·5
Jun. ...	12	0·0	1·5
Jul. ...	12	0·0	4·7
Aug. ...	12	0·0	17·3
Sep. ...	12	0·0	19·2
Oct. ...	12	0·0	11·7
Nov. ...	12	0·0	0·0
Dec. ...	12	0·0	0·0

TABLE VII.

Density of Anopheles adults in human dwellings in Chilka Lake area.

Month.	Time spent in hours.	Per 5 man-hours.		Time spent in hours.	Per 5 man-hours.	
		A. sundaicus.	All anophelines.		*A. sundaicus.*	All anophelines.
	1944				**1945**	
Jan. ...	12	13·7	96·4	24	4·0	38·7
Feb. ...	12	8·0	26·7	24	7·5	34·3
Mar. ...	12	22·1	89·6	24	17·5	35·2
Apr. ...	12	22·9	105·4	24	10·0	32·5
May ...	12	13·7	22·0	24	3·5	34·8
Jun. ...	12	1·7	68·7	24	1·0	28·3
Jul. ...	12	0·8	145·0	24	0·0	30·6
Aug. ...	12	0·0	137·1	24	0·2	128·1
Sep. ...	12	0·0	100·0	24	0·8	109·5
Oct. ...	12	7·9	60·8	24	0·0	91·0
Nov. ...	24	6·5	45·0	24	2·5	65·8
Dec. ...	24	8·0	41·0	24	2·0	53·3
	1946				**1947**	
Jan. ...	24	0·8	65·8	24	15·0	61·8
Feb. ...	24	2·7	60·2	24	23·5	55·2
Mar. ...	24	5·0	45·0	24	18·7	40·8
Apr. ...	24	2·8	41·0	24	5·6	44·8
May ...	24	1·4	42·9	24	0·0	44·7
Jun. ...	24	0·6	41·7	24	0·0	42·9
Jul. ...	24	0·4	38·6	24	0·0	41·2
Aug. ...	24	0·2	86·6	24	0·0	65·8
Sep. ...	24	1·0	64·3	24	0·0	53·3
Oct. ...	24	0·2	67·7	24	0·0	28·0
Nov. ...	24	11·7	65·8	24	0·0	1·7
Dec. ...	24	22·1	64·3	24	0·0	0·0

Month.	Time spent in hours.	Per 5 man-hours.	
		A. sundaicus.	All anophelines.
	1948		
Jan. ...	24	0·0	2·1
Feb. ...	24	0·0	10·0
Mar. ...	24	0·0	5·8
Apr. ...	24	0·0	3·3
May ...	24	0·0	1·7
Jun. ...	24	0·0	1·2
Jul. ...	24	0·0	23·3
Aug. ...	24	0·0	31·2
Sep. ...	24	0·0	28·8
Oct. ...	24	0·0	12·5
Nov. ...	24	0·0	3·3
Dec. ...	24	0·0	0·2

TABLE VIII.

Density of Anopheles adults in human dwellings in North Madras Coast and coastal area around Vizagapatam.

Month.	Time spent in hours.	Per 5 man-hours.			Time spent in hours.	Per 5 man-hours.		
		A. sundaicus.	*A. stephensi.*	All anophelines.		*A. sundaicus.*	*A. stephensi.*	All anophelines.
		1944				**1945**		
Jan. ...	12	0·0	0·0	50·0	26⅔	0·0	0·0	30·7
Feb. ...	12	0·0	0·0	51·3	26⅔	0·0	0·4	14·6
Mar. ...	12	0·3	0·0	52·7	26⅔	0·0	0·0	24·0
Apr. ...	12	0·0	0·0	59·7	26⅔	0·2	0·0	11·1
May ...	12	0·0	0·0	56·3	26⅔	0·0	0·0	18·0
Jun. ...	12	0·0	0·0	53·3	26⅔	0·0	0·0	8·1
Jul. ...	12	0·0	0·0	77·7	26⅔	0·0	0·0	13·1
Aug. ...	12	0·0	0·0	68·7	26⅔	0·0	0·0	98·2
Sep. ...	12	0·0	0·0	65·7	26⅔	0·0	0·0	116·2
Oct. ...	12	0·0	0·0	83·3	26⅔	0·0	0·0	116·6
Nov. ...	26⅔	0·0	0·0	21·4	26⅔	0·0	0·0	101·4
Dec. ...	26⅔	0·0	0·0	28·1	26⅔	0·0	0·0	45·5
		1946				**1947**		
Jan. ...	26⅔	0·0	0·0	39·0	26⅔	0·0	0·0	23·0
Feb. ...	26⅔	0·0	0·0	45·7	26⅔	0·0	0·0	13·5
Mar. ...	26⅔	0·0	0·0	29·1	26⅔	0·0	0·0	16·7
Apr. ...	26⅔	0·0	0·0	17·3	26⅔	0·0	0·0	12·5
May ...	26⅔	0·0	0·0	30·2	26⅔	0·0	0·0	8·6
Jun. ...	26⅔	0·0	0·0	24·7	26⅔	0·0	0·0	11·1
Jul. ...	26⅔	0·0	0·0	24·2	26⅔	0·0	0·0	12·0
Aug. ...	26⅔	0·0	0·0	50·8	26⅔	0·0	0·0	9·9
Sep. ...	26⅔	0·0	0·0	63·0	26⅔	0·0	0·0	14·2
Oct. ...	26⅔	0·0	0·0	50·0	26⅔	0·0	0·0	12·0
Nov. ...	26⅔	0·0	0·0	48·0	26⅔	0·0	0·0	9·2
Dec. ...	26⅔	0·0	0·0	39·7	26⅔	0·0	0·0	8·6

Month.	Time spent in hours.	Per 5 man-hours.		
		A. sundaicus.	*A. stephensi.*	All anophelines.
		1948		
Jan. ...	26⅔	0·0	0·0	10·1
Feb. ...	26⅔	0·0	0·0	7·5
Mar. ...	26⅔	0·0	0·0	3·7
Apr. ...	26⅔	0·0	0·0	1·7
May ...	26⅔	0·0	0·0	1·7
Jun. ...	26⅔	0·0	0·0	1·7
Jul. ...	26⅔	0·0	0·0	2·8
Aug. ...	26⅔	0·0	0·0	9·0
Sep. ...	26⅔	0·0	0·0	6·9
Oct. ...	26⅔	0·0	0·0	1·3
Nov. ...	26⅔	0·0	0·0	0·4
Dec. ...	26⅔	0·0	0·0	0·2

Table IX.

Density of Anopheles adults in human dwellings in Jeypore Hill Tracts.

Month.	Time spent in hours.	Per 5 man-hours.		Time spent in hours.	Per 5 man-hours.	
		A. fluviatilis.	All anophelines.		*A. fluviatilis.*	All anophelines.
	1944			**1945**		
Jan. ...	35	0·1	11·1	35	0·0	8·6
Feb. ...	35	3·8	36·8	35	0·0	3·1
Mar. ...	35	2·0	30·2	35	0·1	20·1
Apr. ...	35	4·3	24·7	35	0·0	18·6
May ...	35	0·1	29·7	35	0·0	16·0
Jun. ...	35	0·2	40·0	35	0·0	10·1
Jul. ...	35	0·2	33·1	35	0·0	25·6
Aug. ...	35	1·4	27·7	35	0·9	12·0
Sep. ...	35	2·7	30·4	35	0·3	36·6
Oct. ...	35	6·4	33·4	35	1·1	28·0
Nov. ...	35	6·1	13·3	35	0·9	26·2
Dec. ...	35	3·0	10·7	35	0·3	23·0
	1946			**1947**		
Jan. ...	35	0·0	14·1	35	0·3	3·6
Feb. ...	35	0·0	10·7	35	0·0	4·8
Mar. ...	35	0·0	8·7	35	0·3	2·1
Apr. ...	35	0·0	8·0	35	0·0	2·2
May ...	35	0·0	9·0	35	0·0	1·4
Jun. ...	35	0·0	8·8	35	0·0	1·2
Jul. ...	35	0·0	10·8	35	0·0	4·8
Aug. ...	35	0·0	11·1	35	0·0	1·9
Sep. ...	35	0·0	12·0	35	0·0	0·6
Oct. ...	35	1·1	13·0	35	0·0	4·2
Nov. ...	35	1·7	16·5	35	0·5	8·4
Dec. ...	35	0·9	16·0	35	0·1	8·2

Month.	Time spent in hours.	Per 5 man-hours.	
		A. fluviatilis.	All anophelines.
	1948		
Jan. ...	35	0·0	4·6
Feb. ...	35	0·0	4·6
Mar. ...	35	0·0	6·2
Apr. ...	35	0·0	8·2
May ...	35	0·0	3·6
Jun. ...	35	0·0	1·2
Jul. ...	35	0·0	0·0
Aug. ...	35	0·0	0·0
Sep. ...	35	0·0	0·0
Oct. ...	35	0·0	0·0
Nov. ...	35	0·0	0·0
Dec. ...	35	0·0	0·0

TABLE X.

Density of Anopheles adults in human dwellings in Eastern Satpura Ranges.

Month.	Time spent in hours.	Per 5 man-hours.			Time spent in hours.	Per 5 man-hours.		
		A. fluviatilis.	*A. culicifacies.*	All anophelines.		*A. fluviatilis.*	*A. culicifacies.*	All anophelines.
		1944				**1945**		
Jan. ...	32	1·0	11·1	41·5	32	0·0	2·4	6·5
Feb. ...	32	2·1	9·7	33·3	32	0·0	2·6	7·3
Mar. ...	32	0·0	5·8	35·5	32	1·7	15·0	5·1
Apr. ...	32	0·0	3·5	20·3	32	1·8	15·4	7·1
May ...	32	0·0	2·5	18·8	32	0·9	18·1	6·6
Jun. ...	32	0·0	2·8	22·7	32	1·2	16·2	5·6
Jul. ...	32	0·6	4·0	20·8	32	1·7	33·7	17·8
Aug. ...	32	1·1	5·5	21·8	32	1·2	30·9	33·1
Sep. ...	32	1·5	6·6	27·1	32	2·8	14·3	30·9
Oct. ...	32	1·8	11·0	30·8	32	2·1	15·3	29·3
Nov. ...	32	1·2	4·8	9·2	32	0·9	11·2	23·4
Dec. ...	32	0·6	5·4	10·0	32	0·0	6·2	22·9
		1946				**1947**		
Jan. ...	32	0·0	1·2	11·2	32	0·0	0·6	5·9
Feb. ...	32	0·0	2·4	13·7	32	0·0	1·8	7·8
Mar. ...	32	0·0	3·4	11·5	32	0·0	2·8	10·6
Apr. ...	32	0·0	4·2	13·7	32	0·0	5·0	13·7
May ...	32	0·0	4·8	17·5	32	0·0	3·4	14·3
Jun. ...	32	0·0	5·3	13·7	32	0·0	5·3	17·5
Jul. ...	32	0·0	9·1	21·5	32	0·0	13·4	21·5
Aug. ...	32	0·6	9·6	24·6	32	1·2	14·1	22·8
Sep. ...	32	2·1	10·6	23·7	32	3·1	15·9	23·1
Oct. ...	32	0·6	11·2	21·5	32	0·0	9·0	14·0
Nov. ...	32	0·9	12·1	22·9	32	0·0	2·7	5·4
Dec. ...	32	1·1	13·1	22·8	32	0·0	3·9	6·8

Month.	Time spent in hours.	Per 5 man-hours.		
		A. fluviatilis.	*A. culicifacies.*	All anophelines.
		1948		
Jan. ...	32	0·0	0·0	3·4
Feb. ...	32	0·0	0·0	1·9
Mar. ...	32	0·0	0·0	4·6
Apr. ...	32	0·0	1·0	0·0
May ...	32	0·0	0·3	2·6
Jun. ...	32	0·0	0·0	0·0
Jul. ...	32	0·0	0·0	0·0
Aug. ...	32	0·0	0·0	1·4
Sep. ...	32	0·0	0·0	0·0
Oct. ...	32	0·0	0·0	0·0
Nov. ...	32	0·6	1·6	1·1
Dec. ...	32	0·2	1·1	0·5

TABLE XI.

Density of Anopheles adults in human dwellings in Singhbhum Hills of Chota Nagpur Division of Bihar.

Month.	Time spent in hours.	Per 5 man-hours. *A. fluviatilis.*	*A. culicifacies.*	All anophelines.	Time spent in hours.	Per 5 man-hours. *A. fluviatilis.*	*A. culicifacies.*	All anophelines.
		1944				**1945**		
Jan. ...	29½	1·5	6·3	30·8	29⅓	0·5	15·5	51·4
Feb. ...	29⅓	1·0	4·0	27·5	29⅓	1·1	7·5	46·1
Mar. ...	29⅓	0·3	3·5	28·0	29½	8·0	9·1	39·1
Apr. ...	29⅓	0·0	6·6	18·5	29⅓	4·8	10·1	34·2
May ...	29⅓	0·0	2·7	17·5	29⅓	0·0	13·6	33·1
Jun. ...	29⅓	0·0	2·1	17·8	29⅓	0·0	24·6	21·9
Jul. ...	29⅓	0·0	3·1	19·0	29⅓	0·0	19·8	23·5
Aug. ...	29⅓	0·8	4·7	18·3	29⅓	0·0	9·6	9·4
Sep. ...	29⅓	1·5	12·3	21·0	29⅓	0·5	5·9	11·2
Oct. ...	29⅓	1·8	7·8	28·0	29⅓	1·8	5·6	10·4
Nov. ...	29⅓	1·07	11·7	62·1	29⅓	1·6	4·6	7·7
Dec. ...	29⅓	2·1	18·7	52·5	29⅓	0·5	3·7	6·9
		1946				**1947**		
Jan. ...	29⅓	0·8	4·2	5·1	29⅓	1·3	7·2	21·1
Feb. ...	29⅓	0·8	2·1	3·7	29⅓	1·0	3·7	11·2
Mar. ...	29⅓	0·5	1·6	4·8	29⅓	1·6	2·1	6·9
Apr. ...	29⅓	0·0	2·4	5·6	29⅓	0·2	1·1	4·2
May ...	29⅓	0·0	26·5	18·4	29⅓	0·0	8·5	6·1
Jun. ...	29⅓	0·2	13·9	21·1	29⅓	0·0	6·4	5·6
Jul. ...	29⅓	0·0	9·6	11·5	29⅓	0·0	5·6	4·0
Aug. ...	29⅓	0·0	6·4	10·4	29⅓	0·0	6·9	2·4
Sep. ...	29⅓	0·0	5·6	9·6	29⅓	0·0	0·0	2·6
Oct. ...	29⅓	0·0	4·5	12·3	29⅓	0·0	0·0	0·0
Nov. ...	29⅓	0·2	5·1	11·2	29⅓	0·0	0·0	0·0
Dec. ...	29⅓	0·8	3·2	11·7	29⅓	0·0	0·0	0·0

Month.	Time spent in hours.	Per 5 man-hours. *A. fluviatilis.*	*A. culicifacies.*	All anophelines.
		1948		
Jan. ...	29⅓	0·0	0·0	0·0
Feb. ...	29⅓	0·0	0·0	0·0
Mar. ...	29⅓	0·0	0·0	0·0
Apr. ...	29⅓	0·0	0·0	0·0
May ...	29⅓	0·0	0·0	0·0
Jun. ...	29½	0·0	0·0	0·6
Jul. ...	29⅓	0·0	1·2	3·6
Aug. ...	29⅓	0·0	1·4	4·0
Sep. ...	29⅓	0·0	0·8	4·0
Oct. ...	29⅓	0·0	0·0	0·6
Nov. ...	29⅓	0·0	0·0	1·4
Dec. ...	29⅓	0·0	0·0	0·0

TABLE XII.

Density of Anopheles adults in human dwellings in Hazaribagh Ranges and Ranchi Plateau.

Month.	Time spent in hours.	Per 5 man-hours. *A. fluviatilis.*	Per 5 man-hours. All anophelines.	Time spent in hours.	Per 5 man-hours. *A. fluviatilis.*	Per 5 man-hours. All anophelines.
	1944				**1945**	
Jan. ...	6⅔	6·7	27·7	13⅓	2·2	10·1
Feb. ...	6⅔	0·0	3·7	13⅓	1·1	14·6
Mar. ...	6⅔	0·7	6·7	13⅓	0·4	19·1
Apr. ...	6⅔	0·0	6·7	13⅓	0·0	17·6
May ...	6⅔	0·0	18·0	13⅓	0·4	23·6
Jun. ...	6⅔	0·0	9·0	13⅓	0·0	22·1
Jul. ...	6⅔	2·2	8·2	13⅓	4·4	24·7
Aug. ...	6⅔	16·5	63·7	13⅓	6·2	20·2
Sep. ...	6⅔	15·7	72·0	13⅓	3·3	26·6
Oct. ...	6⅔	8·2	31·0	13⅓	2·2	31·1
Nov. ...	13⅓	2·2	13·5	13⅓	4·4	31·5
Dec. ...	13⅓	3·0	18·0	13⅓	2·2	29·6
	1946				**1947**	
Jan. ...	13⅓	0·4	24·3	13⅓	0·8	12·7
Feb. ...	13⅓	0·8	22·8	13⅓	0·0	9·7
Mar. ...	13⅓	0·0	13·5	13⅓	0·0	8·2
Apr. ...	13⅓	0·0	10·8	13⅓	0·0	4·1
May ...	13⅓	0·0	11·6	13⅓	0·0	3·3
Jun. ...	13⅓	0·0	10·1	13⅓	0·0	2·2
Jul. ...	13⅓	1·5	17·2	13⅓	0·0	3·0
Aug. ...	13⅓	2·2	19·9	13⅓	0·0	2·1
Sep. ...	13⅓	1·1	21·0	13⅓	0·0	0·0
Oct. ...	13⅓	0·4	22·8	13⅓	0·0	0·0
Nov. ...	13⅓	0·0	16·1	15	0·0	0·0
Dec. ...	13⅓		14·6	15	0·0	0·0

Month.	Time spent in hours.	Per 5 man-hours. *A. fluviatilis.*	Per 5 man-hours. All anophelines.
	1948		
Jan. ...	15	0·0	0·0
Feb. ...	15	0·0	0·0
Mar. ...	15	0·0	0·0
Apr. ...	15	0·0	0·0
May ...	15	0·0	0·0
Jun. ...	15	0·0	0·0
Jul. ...	15	0·0	5·3
Aug. ...	15	0·0	0·4
Sep. ...	15	0·0	0·0
Oct. ...	15	0·0	0·0
Nov. ...	15	0·0	0·0
Dec. ...			

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SOME OBSERVATIONS ON THE PREVALENCE OF MALARIA AND FILARIASIS IN SRI HARIKOTTA ISLAND, NELLORE, MADRAS PRESIDENCY.

BY

N. G. S. RAGHAVAN,

AND

K. S. KRISHNAN.

(*Malaria Institute of India, Delhi.*)

[April 2, 1949.]

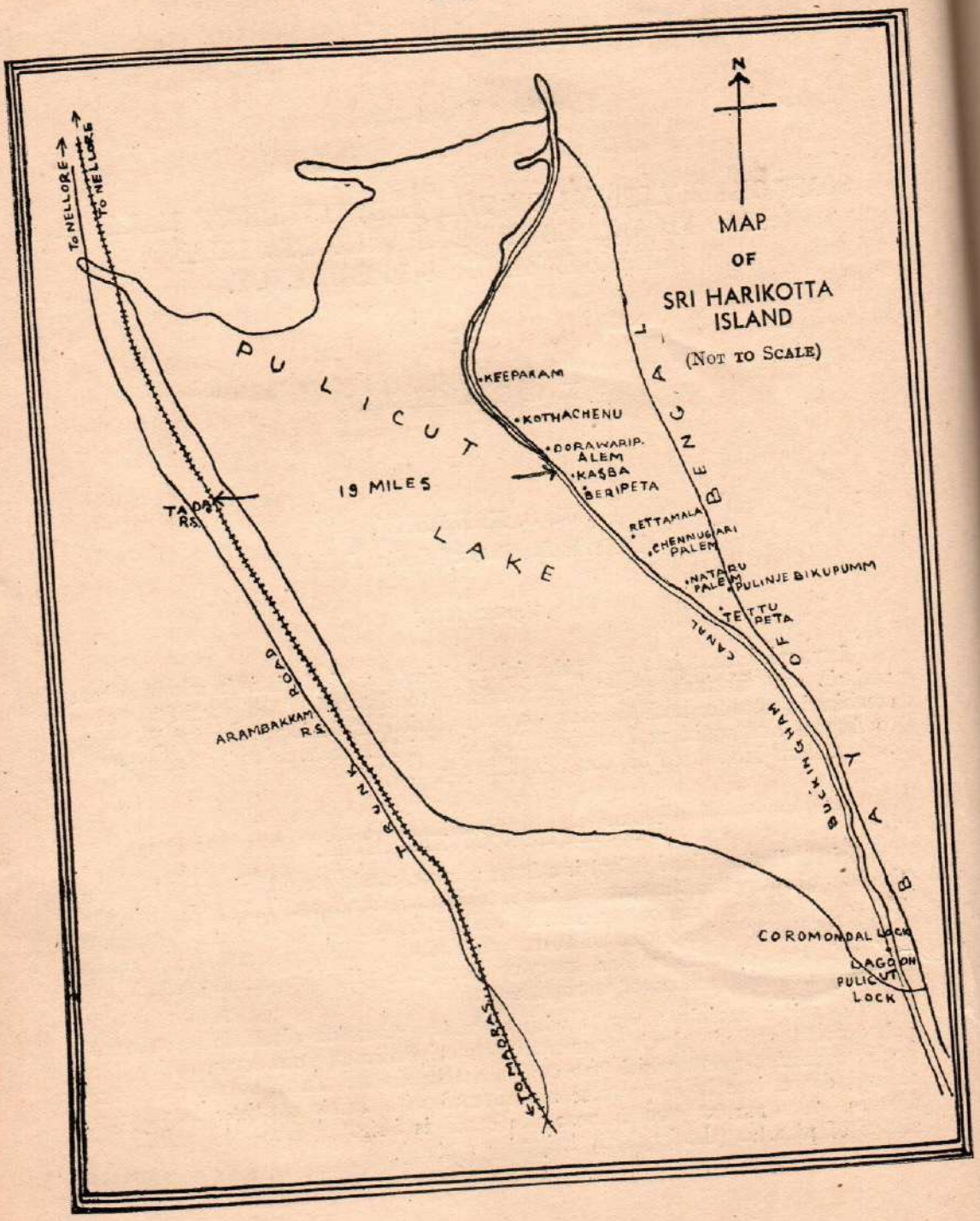
INVESTIGATIONS into the epidemiological features of filariasis in an area where malaria and filariasis co-exist, particularly to determine the possibility of a combined vector for both the diseases, were carried out during the period February to August and again in December 1948 in Sri Harikotta Island. Some of the observations have been set forth in this paper.

DESCRIPTION OF THE AREA.

Sri Harikotta Island (Map) is a long, narrow, sandy stretch, situated between the Bay of Bengal in the east and the Buckingham Canal and Pulicut Lake in the west. It forms a part of the Ennore-Nellore coastal area, of about 50 square miles and lies 50 miles to the north-east of Madras. In the absence of any roads and railways, communications are very poor and primitive country crafts are the only available means of transport.

The soil is mainly unfertile with extensive casuarina plantations, coco-nut palm groves, and pitted with numerous pits originally dug out for watering the young casuarina plants which are at present in disuse. These as well as other natural depressions holding water are covered with *Pistia stratiotes* in varying degrees. The narrow stretch near the canal is irrigated from the ponds by means of picottas (the local lift irrigation system).

MAP.


N
MAP
OF
SRI HARIKOTTA
ISLAND
(NOT TO SCALE)
TO NELLORE
TO NELLORE
PULICUT
LAKE
19 MILES
KEEPARAM
KOTHACHENU
DORAWARIP-
ALEM
KASBA
BERIPETA
RETTAMALA
CHENNUGARI
PALEM
NATARU
PALEM
PULINJE BIKUPUMM
TETTU
PETA
CANAL
BUCKINGHAM
CANAL
TADA
RS.
ARAMBAKKAM
RS.
ROAD
TRUNK
TO MADRAS
COROMONDAL LOCK
LAGOON
PULICUT
LOCK

POPULATION AND ECONOMIC IMPORTANCE OF THE ISLAND.

According to the 1941 census, the population was only 5,492. Due to the ravages of malaria and filaria, together with malnutrition, high death and low birth rates, it has now dwindled down to about 3,900 and can be divided as follows :—

(*a*) indigenous labour class, called yenadis ;

(*b*) fishermen ;

(*c*) Government servants and businessmen.

The former two classes, either engaged as labourers in jungle clearing, casuarina cutting, etc., or in fishing, constitute the major proportion of the population which is more or less static.

The majority of the indigenous population lives in groups in thatched mud houses chiefly on the banks of the Buckingham Canal and only a few groups are found in the interior. Many of the hamlets have been deserted.

There is no protected water supply and the only sources are a few wells and the water collections referred to above. In the absence of cattlesheds, the cattle move about freely.

The economic importance of the island depends on fishing, jungle produce, coco-nut plantations and supplying enormous quantities of casuarina equestifolia used as firewood for the markets in Madras.

HISTORY OF MALARIA AND FILARIA IN THE ISLAND.

Various workers have reported on the prevalence of malaria since 1913 but only a casual mention has been made of the high incidence of elephantiasis (Rao, 1913, 1915 ; Russell and Jacob, 1939). *A. culicifacies* breeding in the dug-outs originally meant for watering the young casuarina but now in disuse has been incriminated as a vector of malaria. Russell and Jacob (*loc. cit.*) reported two specimens of *A. culicifacies* infected with both malaria and filaria at Ennore. Ennore being non-filarial, they attributed the source of filarial infections to the wood-cutters from Sri Harikotta Island, then working at Ennore. They also reported two specimens of *A. subpictus* with oöcysts out of 4,013 specimens dissected from the Ennore-Nellore coastal area and another with filarial infection (thorax) at Tammennapatnam (north of the area now surveyed) where it is not uncommon to see elephantiasis of the extremities.

The island seems to have been regarded as healthy before 1913 and since then epidemic form of malaria has been reported to be a regular feature and the last epidemic occurred in 1946. According to the information available from the local sources, malaria is of a much more recent origin than filariasis, due presumably to the fact that casuarina plantations are of recent origin. This has now been confirmed.

The casuarina plantations round Kothachenu were first started in 1911 and abandoned in 1913, only to be revived in 1929 (Rangaswami Aiyangar, 1949).

The island used to be a sub-magistrate's jurisdiction and a post office, dispensary and a school were located at Kasba. The various buildings are now dilapidated and deserted.

CLIMATE.

Table I sets out the meteorological conditions. The maximum temperature seldom exceeds 105°F. and minimum temperature seldom reaches 70°F. Relative humidity is usually above 60 per cent. The average annual rainfall is about 50 inches and is mainly dependent on the north-east monsoon (September–December). The 1947 monsoon was deficient, being only 29 inches and droughty conditions existed at the time of the survey when majority of the breeding places were dry or fast drying up.

TABLE I.

Meteorological conditions—monthly means for 1948.

Month.	Temperature, °F.		Relative humidity, per cent.	Rainfall, inches.
	Maximum.	Minimum.		
Jan. ...	83·5	69·7	89	0·5
Feb. ...	88·1	71·3	86	0·4
Mar. ...	91·3	74·2	79	...
Apr. ...	95·0	78·3	76	...
May ...	102·0	83·7	62	...
Jun. ...	102·1	83·4	58	...
Jul. ...	96·4	79·8	68	1·2
Aug. ...	94·5	78·7	71	1·2
Sep. ...	94·1	77·6	75	0·6
Oct. ...	89·7	75·8	85	5·0
Nov. ...	86·4	74·8	88	14·5
Dec. ...	84·1	68·9	87	0·4

MORBIDITY.

Medical aid is completely lacking as even the single dispensary that used to function previously has now been closed altogether. Table II shows the hospital figures from 1935 onwards. The infant mortality is said to be very high and the birth rates low. Reliable figures are however not available.

TABLE II.

Cases treated at Sri Harikotta Dispensary.

Year.	Total admission.	Filariasis.	Malaria.
1935 ...	2,267	46	524
1936 ...	3,868	24	1,441
1938 ...	8,070	191	2,865
1939 ...	6,338	77	2,178
1940 ...	8,536	70	3,373
1942 ...	10,548	14	3,848
1943 ...	11,559	17	5,660
1945 ...	...	50	865
1946* ...	...	...	...
1947* ...	...	12	460
1948 (3 months)	...	...	230

* In 1946 and 1947 the dispensary functioned for only a few months each year.

PRESENT SURVEY.

As it was not possible to examine all individuals in the island, men, women and children in selected areas were examined without missing any one during the hours 8-30 p.m. to 11-30 p.m.

Of the total population (about 3,900), 927 were examined for any evidence of disease and other manifestations but only 709 were put through an exhaustive examination.

Each person was interrogated in detail and conditions of lymphangitis, lymphadenitis, swelling of extremities and other parts were all recorded. In the case of women this was more difficult, as a thorough examination was not possible in all cases.

Approximately 20 c.mm. of peripheral blood was drawn from each person for making uniform thick smears and a thin film was also made in each case.

Staining methods adopted.—The smears were stained the following day by an aqueous solution of methylene blue (15 mg. to the litre), allowing the strain to stand for 2 hours and then examining the slides wet as suggested by Iyengar (1938). But the disadvantages were, (*a*) likely plasmodia co-existing with the microfilariæ were not stained and (*b*) the slides did not keep well. This was overcome by adopting J. S. B. technique (Jaswant Singh and Bhattacharjee, 1944). The plasmodiæ and microfilariæ were well stained and the slides kept better even after several months of storage under field conditions.

The results of filaria survey by age groups are shown in Table III and the distribution of filarial disease manifestations in Table IV.

TABLE III.

Details of filaria survey by age groups.

Age groups, years.	Number examined.	Number with micro-filaria.	Filarial infection rate, per cent.	Number with disease mani-festation.	Filarial disease rate, per cent.	Number with filarial disease or infection or both.	Filarial endemicity rate, per cent.
Less than 1	3	...	...	...	...	...	...
1– 5	42	12	28·6	...	...	12	28·6
6–10	104	13	12·5	...	...	13	12·5
11–15	36	9	25·0	12	33·3	21	58·33
16–20	87	18	20·7	12	13·8	30	34·5
21–25	60	12	20·0	3	5·0	15	25·0
26–30	126	42	33·3	15	11·9	57	47·6
31–35	87	21	24·1	21	24·1	42	48·3
36–40	45	10	22·2	12	26·66	22	48·9
41–45	15	3	20·0	5	33·33	8	53·3
46–50	75	12	16·0	15	20·0	27	56·6
51–55	18	4	22·2	4	22·2	8	44·4
56–60	7	1	14·14	2	28·6	3	42·8
Above 60	4	...	...	1	25·0	1	25·0
TOTAL ...	709	175	22·14	102	14·38	259	36·5

The filarial indices for the community examined were as follows :—

Filarial infection rate ... ... 22·14 per cent.
Filarial disease rate ... ... 14·38 per cent.
Average infestation—24 microfilariæ per 20 c.mm.
Number of persons with disease manifestations 102.

TABLE IV.

Distribution of filarial disease manifestations.

Part or parts affected.	Numbers.	REMARKS.
Either lower extremities ...	56	
Both lower extremities ...	21	
Either upper extremities ...	11	
Both upper extremities ...	7	
Upper and lower extremities ...	5	
Hydrocoele	1	Resident for a long period outside island in a *W. bancrofti* area and arrived in the island recently.
Chyluria	1	
TOTAL ...	102	

Clinical findings.—Genital lesions, chyluria or lymph varix were conspicuously absent amongst the permanent residents there, but in only 2 persons who had recently come from a *W. bancrofti* area, hydrocoele and chyluria were noted.

A number of people without filarial manifestations complained of peculiar burning sensation in the epigastric and hypogastric regions (heart-burn).

Parasitological findings.—Out of 102 persons with established disease, not a single case showed microfilariæ in the peripheral circulation.

No mixed filarial infections were noted in any of the slides examined.

In a hamlet round about Peddaratamala (near the southern extremity of the island), in 61 persons examined in detail, of a population of about 160, not a single person had disease manifestations but 21 showed microfilariæ in their blood.

Results of examination of blood smears taken at night are shown below.

TABLE V.

Analysis of results of blood smears taken at night.

Number of smears examined.	Number of cases with filarial disease manifestation.	Number of persons showing microfilariæ.	Number of cases with plasmodia and details of plasmodia.	Number of persons showing microfilariæ and plasmodiæ.	REMARKS.
102	102	...	4 (1 P.F.; 3 P.V.)	...	Total = 29 (11 P.F.; 14 P.V.; 4 P.M.)
183	...	183	7 (2 P.F.; 5 P.V.)	7	
424	...	...	18 (8 P.F.; 6 P.V.; 4 P.M.)	...	
TOTAL 709	102	183	29	7	

P.F. = *P. falciparum*. P.V. = *P. vivax*. P.M. = *P. malariæ*.

Table V shows that malaria parasites were detected in individuals with microfilaria, without microfilaria but with established filariasis, and also in persons from the same community but with no evidence of filarial infections.

Blood smears of domestic animals and birds were also examined but with negative results for either microfilariæ or plasmodial infection.

Other findings.—1. The type of filarial infection:—The microfilariæ found in the peripheral circulation was morphologically similar to *W. malayi* described by Brug (1927).

2. *M. annulifera*, breeding among the *Pistia stratiotes* in ponds contaminated with organic matter, was found to be the vector in nature (Table VI) and also experimentally (Appendix I).

3. Amongst the permanent residents of the island, genital lesions, chyluria and lymph varix were conspicuous by their absence.

4. Experimental infections in *C. fatigans* (Appendix I) proved characteristically refractory, thus confirming refractoriness quoted by Brug (1927) from Lichtenstein (1927) and Iyengar (1938).

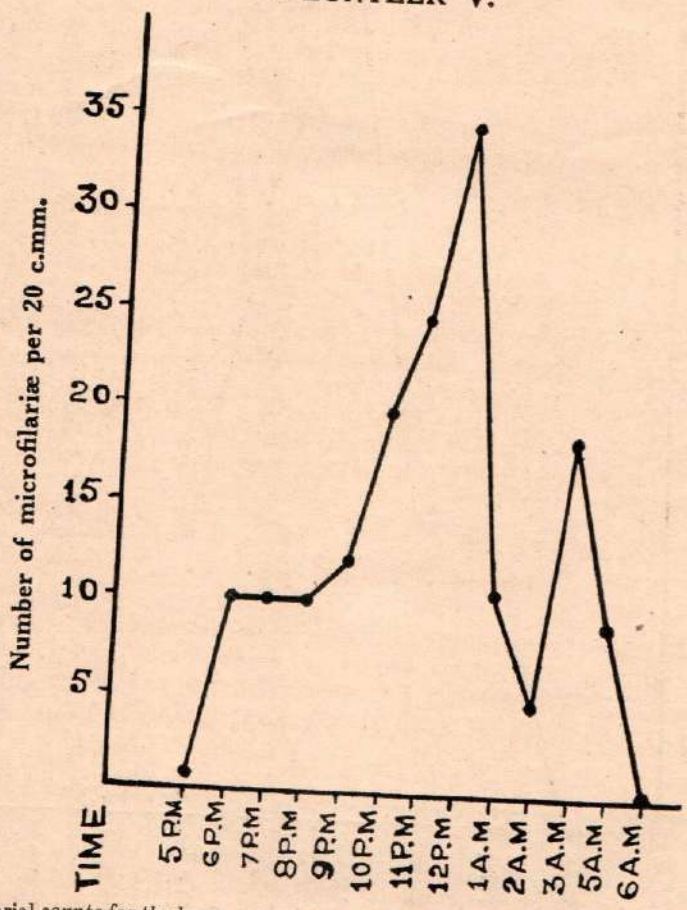
5. Results of spleen and parasite surveys are set out in Appendix II and III respectively.

6. Periodicity of microfilaria was worked out in 3 cases and is represented in Charts 1, 2 and 3.

CHART 1.

Periodicity of Mf. malayi.

VOLUNTEER V.



Microfilarial counts for the hours not shown in the 24 hours denote that there were none.

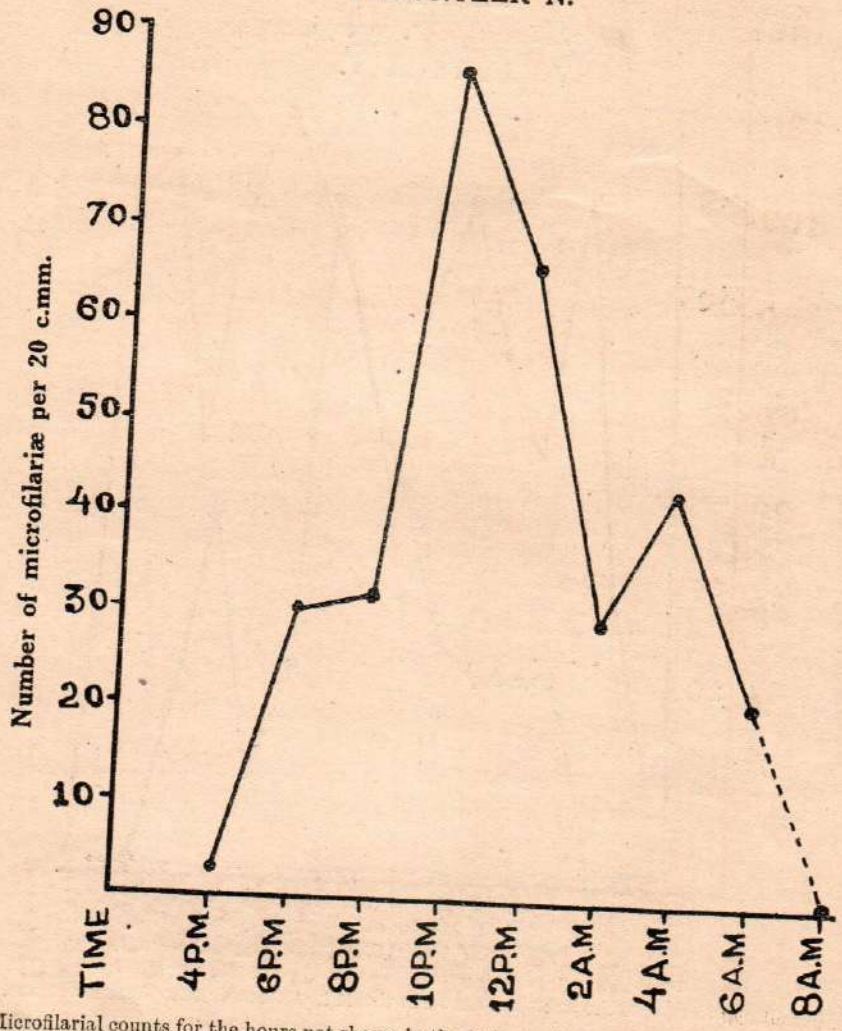
An index $\frac{\text{Midday counts}}{\text{Midnight counts}}$ (suggested by Brug, 1927) was worked out in 3 cases and it was 0/80, 0/63 and 0/35. The two peaks supposed to be occurring characteristically at 8 p.m. and 4 a.m. according to Brug (*loc. cit.*) were

not observed which in these cases occurred at 2 a.m. and 5 a.m., 10 p.m. and 4 a.m., midnight and 3 a.m., respectively. This is in conformity with Iyengar (1938), who suggested an index $\frac{\text{Average of counts between 9 a.m. and 4 p.m.}}{\text{Average of counts between 9 p.m. and 4 a.m.}} \times 100$.

CHART 2.

Periodicity of Mf. malayi.

VOLUNTEER N.



Microfilarial counts for the hours not shown in the 24 hours denote that there were none.

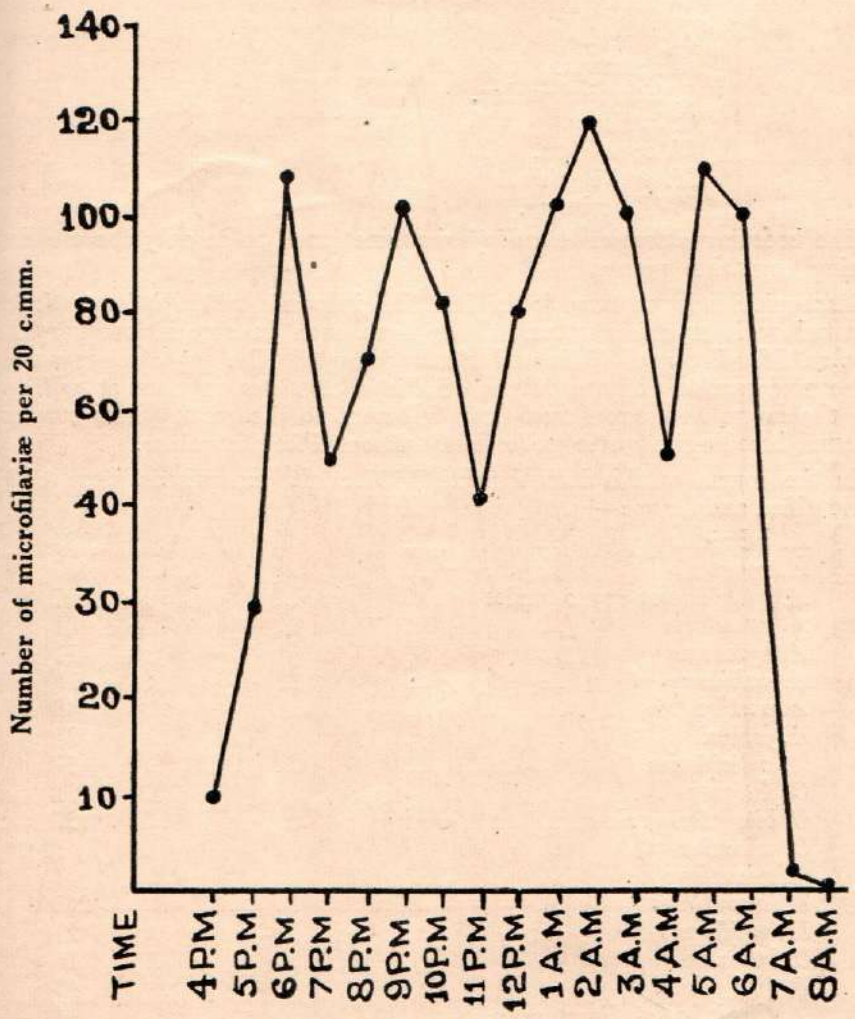
which worked out to be 0·100, 0·100 and 0·100. The above indices bring out the true nocturnal periodicity of the local filaria.

Early occurrence of filarial infections and disease.—While the earliest age at which microfilariæ were observed in the peripheral circulation was in the case of

CHART 3.

Periodicity of Mf. malayi.

VOLUNTEER K.



Microfilarial counts for the hours not shown in the 24 hours denote that there were none.

a boy of 23 months old, disease manifestations were encountered in an individual (boy) aged 7½ years and established disease was noted in a boy of 10¼ years old.

Entomological data.—Larvæ were collected from the dug-outs originally meant for watering the young casuarina, irrigation ponds and wells. On account of the droughty conditions, many of the natural depressions were dry at the time of the survey and as such the collections were restricted to only the former group of breeding places.

Larvæ of the following species were collected :—

A. culicifacies.
A. subpictus.
A. annularis.
A. jamesi.
A. hyrcanus.
C. bitaeniorhynchus.
C. lutzia sp.
M. annulifera.
M. uniformis.
F. (F.) minima.

The breeding places of *A. culicifacies* were found to be only the pits dug for watering casuarina plants.

Mansonioides larvæ were found breeding in ponds with *Pistia stratiotes* contaminated with organic matter. Other flora seen in association were *Lemna polyrrhza*, *Marsilea quadrifolia* and *Azolla pinnata*. The egg clusters were seen on the undersurface of the leaves of *Pistia stratiotes* in contact with water while the larvæ and pupæ were seen adherent to the roots. This species was not found breeding in water collections without *Pistia stratiotes*.

In the course of this investigation 2,667 adult mosquitoes were collected and identified as follows :—

A. subpictus	1,284
A. vagus	447
A. subpictus or *vagus* (males)	397
A. annularis	31
A. hyrcanus	11
A. pallidus	10
A. barbirostris	6
A. jamesi	25
A. culicifacies	57
C. fatigans	21
C. bitaeniorhynchus	27
C. mimulus	5
C. mimeticus	4
M. annulifera	261
M. uniformis	21
C. lutzia sp.	11
F. (F.) minima	43
A. (banksinella) lineatopennis	4
Ædes ægypti	2

Mansonioides were caught mainly from the thatch and on a few occasions from the hanging clothes. Many of the adults were captured from houses close

to the breeding places between 6 and 10 p.m. and 5 and 6-30 a.m. This mosquito feeds readily to repletion in a very short time.

Dissections.—Mosquitoes collected from dwellings were dissected for malarial and filarial infections. The results of dissections are given in Table VI.

TABLE VI.

Results of dissections.

Species of mosquito.	Number dissected.	Positive for malaria.		Positive for filaria.			Remarks.
		Gut.	Gland.	Abdomen.	Thorax.	Head/proboscis infected.	
A. subpictus ...	1,234	...	...	...	...	...	
A. vagus	407	...	...	...	...	...	
A. hyrcanus ...	7	...	...	...	...	...	
A. barbirostris ...	3	...	...	...	...	...	
A. annularis ...	29	...	...	...	...	...	
A. pallidus	8	...	...	...	...	...	
A. culicifacies ...	54	1	...	...	...	...	1/54 oöcysts.
A. jamesi	18	...	...	...	...	...	
A. (banksinella) lineato-pennis.	2	...	...	...	...	...	
Ædes ægypti ...	2	...	...	...	...	...	
C. bitaeniorhynchus ...	21	...	...	...	...	...	
C. lutzia sp. ...	8	...	...	...	...	...	
C. fatigans	19	...	...	...	...	...	
C. mimeticus ...	2	...	...	...	...	...	
C. mimulus	3	...	...	...	...	...	
F. (F.) minima ...	26	...	...	...	...	...	
M. annulifera ...	169	...	...	7	14	21	24·4 per cent.
M. uniformis ...	18	...	...	...	2	1	16·16 per cent.
TOTAL ...	2,030	1	...	7	16	22	

It will be seen that *M. annulifera* and *M. uniformis* are the vectors for *W. malayi* infections and the one positive gut infection in *A. culicifacies* is suggestive of its being the vector for malaria. Filarial infections were not noted in any of the other mosquitoes. No combined infection was noted. It will be worth recalling that Russell and Jacob (*loc. cit.*) reported a specimen of *A. subpictus* with oöcysts out of 4,083 dissections. In the present series, 1,284 *A. subpictus* and 447 *A. vagus* yielded negative results.

Evidence of passive transportation by boat plying on the Buckingham Canal.— There are numerous country boats plying between various places all along the Buckingham Canal to and from the areas known for their endemicity for malaria and filaria. They have to stop at various places, waiting for a favourable breeze or for loading or unloading. Under these conditions passive transmission of disease vectors forms a potential danger on account of the proximity of the breeding places, habitations and the halting places of boats. Many of these boats are covered, affording suitable shelter for the mosquitoes.

A sample catch of mosquitoes for 30 minutes in 2 such covered boats which had to stay at a particular place known for its high endemicity of both the diseases was obtained and Table VI shows the mosquitoes collected and the results of dissections.

TABLE VII.

Collection of mosquitoes for 30 minutes in 2 covered boats and the result of their dissection.

Species of mosquito.	Number collected.	Number dissected.	Positive for malaria.		Positive for filaria.		
			Gut infected.	Gland infected.	Abdomen infected.	Thorax infected.	Head/ proboscis infected.
A. culicifacies ...	2	2	1	...	...	...	...
A. subpictus	27	27	...	...	...	...	...
A. vagus	3	3	...	...	...	...	...
A. vagus or *subpictus* ♂ ...	3	...	...	...	...	...	...
Ædes ægypti	1	1	...	...	...	...	...
M. annulifera ...	11	11	...	...	...	1	...

The effect of removal of *Pistia stratiotes* in ponds at Kothachenu, the head-quarters of the forest officials, is apparent from the reduction of *M. annulifera* from 15·4 to 4·16 per cent. At Beripeta where no such attempt was made no reduction was evident (Table VIII).

TABLE VIII.

Effect of Pistia stratiotes *removal.*

Name of place.	Month of survey.	Pistia removed or not.	Collection.		
			All mosquitoes per man-hour.	Mansonioides per man-hour.	Proportion of mansonioides to all mosquitoes per man-hour, per cent.
	1948.				
Kothachenu ...	May	No	1·3	0·2	15·4
	December	Yes (mostly).	6·0	0·25	4·16
Beripeta ...	May	No	2·25	1·5	66·6
	December	No	11·75	8·25	70·2

Co-existence of malaria and filaria in the island.—Table IX shows the results of 1948 survey.

TABLE IX.

Co-existence of malaria and filariasis.

Name of place.	Spleen rate, per cent.	Parasite rate, per cent.	Filarial infection rate, per cent.	Filarial disease rate, per cent.	Filarial endemicity rate, per cent.
Kothachenu ...	50·0	14·3	28·16	7·14	35·7
Kilivedu ...	63·6	27·2	54·5	18·8	73·3
Dorawaripalem ...	58·0	10·5	26·7	13·3	40·0
Kasba Kuppam ...	74·3	14·3	21·9	9·38	31·25
Beripeta ...	36·3	9·1	30·4	26·1	56·5
Chennugaripalem ...	48·4	6·4	15·1	21·1	36·2
Joningapalem ...	81·0	14·3	31·25	20·1	51·25
Palliveedhi ...	63·1	15·1	16·7	12·92	29·6

SUMMARY.

1. In Sri Harikotta Island, Nellore District, Madras Presidency, malaria and filaria are co-existent; the former in a hyperendemic and the latter in an endemic form.

2. Plasmodia and microfilariæ were found in the same person's blood in 7 cases and plasmodia were found in 4 persons with elephantoid conditions.

3. The filarial infection in the island is due to *W. malayi* which exhibits strict nocturnal periodicity.

4. *M. annulifera* and *M. uniformis* breeding amongst *Pistia stratiotes* in ponds contaminated with organic matter are the vectors for filaria.

5. In cases with established disease, microfilariæ were not found in the peripheral circulation.

6. Experimental infections of *Mf. malayi* are refractory in *C. fatigans.*

7. Clean-weeding of *Pistia stratiotes* resulted in definite reduction in the prevalence of mansonioides.

8. The danger of passive transportation by boats has been apprehended.

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APPENDIX I.

Results of experimental infections of mosquitoes with W. malayi.

Species of mosquito.	Number dissected.	Number positive.	Remarks.
M. annulifera ...	65	40	
A. stephensi (type) ...	168	...	Degenerative thoracic forms seen in 3 specimens. Unlikely to develop further.
C. fatigans	229	...	
Aëdes aegypti ...	68	...	
A. vagus	32	...	
A. subpictus ...	45	...	
A. annularis ...	11	...	
A. vittifacies ...	10	...	
A. furcatilis ...	49	...	

Note.—The volunteers had microfilarial counts varying from 25 to 140 per 20 c.mm. of peripheral blood. Average of 5 serial slides of 20 c.mm. taken as the count.

APPENDIX II.

Results of spleen examinations.

Month 1948.	Name of village.	Number of children examined.	Number with enlarged spleen.	Spleen rate, per cent.	Average enlarged spleen.
Feb. ...	Kilivedu	11	7	63·6	8·3
Dec. ...	,,	6	4	66·66	8·1
Feb. ...	Dorawaripalem	38	22	58·0	7·5
Feb. ...	Kothachenu	15	8	53·3	9·2
Dec. ...	,,	12	7	58·33	9·0
Mar. ...	Kasba Kuppam	35	26	74·3	9·3
Dec. ...	,,	22	17	77·27	9·1
Mar. ...	Palliveedhi	19	12	63·1	9·0
May ...	Joningapalem	21	17	81·0	8·0
Dec. ...	,,	24	20	83·33	7·8
May ...	Rettamala (Pedda)	18	15	83·3	9·2
Dec. ...	,	12	10	83·3	9·1
May ...	Chennugaripalem	31	15	48·4	9·3

APPENDIX III.

Results of blood smear examination.

Month 1948.	Name of village.	Number examined.	Number with malaria parasites.	Parasite rate, per cent.	Species of plasmodia.			
					falciparum.	*vivax.*	*malariæ.*	Mixed *falciparum* and *vivax.*
Feb. ...	Kilivedu	11	3	27·2	...	...	2	1
Dec. ...	"	6	2	33·3	...	1	1	...
Feb. ...	Dorawaripalem	38	4	10·5	...	2	2	...
Mar. ...	Kothachenu	15	2	13·3	1	1	...	...
Dec. ...	"	12	2	16·6	1	1	...	...
Mar. ...	Kasba Kuppam	35	5	14·3	1	2	1	1
Dec. ...	"	22	3	13·6	...	...	...	...
May ...	Joningapalem	21	3	14·3	1	2	...	...
Dec. ...	"	24	4	16·16	1	1	...	2
May ...	Rettamala (Pedda)	18	3	16·7	...	3	....	...
Dec. ...	"	12	3	25·0	1	2	...	...
May ...	Chennugaripalem	31	2	6·4	...	2	...	...

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A PRELIMINARY NOTE ON THE USE OF BENZENE HEXACHLORIDE AS A RESIDUAL INSECTICIDE COMPARED WITH DICHLORO-DIPHENYL-TRICHLORETHANE.

BY

D. K. VISWANATHAN,

T. RAMACHANDRA RAO,

AND

M. R. JUNEJA.

(*Malaria Organization, Bombay Province.*)

[May 5, 1949.]

INTRODUCTION.

THIS paper records the results of comparative field trials with benzene hexachloride (B.H.C.) and dichloro-diphenyl-trichlorethane (D.D.T.) in their residual insecticidal action against anopheline mosquitoes and effects on malaria transmission. This account is only of the nature of a preliminary note, as the experiments were subject to not a few limitations, principal among which are the lack of adequate data regarding the vector species, their densities and their vectorial capacity in the past and the commencement of the first round of spraying several weeks after transmission of malaria had commenced.

AREA SELECTED FOR THE EXPERIMENT.

The area selected lies in Mokhada Taluk, Thana District, Bombay Province, about 80 miles north of Bombay City, with a total population of 35,560 living in 69 villages. It was divided into three approximately equal portions, one of which was kept for comparison, another treated with benzene hexachloride and

the third with D.D.T. The villages selected in each area for detailed study and their population in 1941 are given below :—

Comparison villages.		Benzene hexachloride villages.		D.D.T. villages.	
Village.	Population.	Village.	Population.	Village.	Population.
1. Chas	692	1. Vashala	1,018	1. Karegaon	715
2. Gonda B	679	2. Saturli	687	2. Gomghar	759
3. Morhande	924	3. Mokhada	3,157	3. Suryamal	579
		4. Poshere	1,283		
		5. Khodala	962		

ANOPHELINE FAUNA.

Altogether 16 different species of Anopheles have been met with. They are *annularis*, *barbirostris*, *culicifacies*, *fluviatilis*, *jamesii*, *jeyporiensis*, *maculatus*, *moghulensis*, *pallidus*, *philippinensis*, *splendidus*, *subpictus*, *tessellatus*, *theobaldi*, *vagus* and *varuna*. Among these, in previous surveys in other parts of the district, *fluviatilis* and *culicifacies* have been incriminated as vectors. No mosquito infection was met with during the period of experiment even in the comparison area ; hence the above two species have to be assumed as only possible vectors on the analogy of their behaviour in the rest of the district and in the adjoining district of Nasik.

Altogether 150 *fluviatilis* and 226 *culicifacies* were dissected during the period of experiment with negative findings even in the unsprayed villages. Hence it would appear that the vectorial capacity of *fluviatilis* in this area cannot be of the same high order as in Kanara District or even in the foothill regions in the rest of the district where, in a survey in 1943, 23 out of 574 *fluviatilis* dissected showed infections (18 guts and 5 glands). In that survey only one *culicifacies* out of 174 dissected showed gut infection. The densities of *fluviatilis* in the present experimental area are very high but as observed in the Deccan Plateau, the vectorial capacity of this species seemed to be inversely proportional to its densities in daytime resting places. In any case, in the absence of a definite determination of the local vector species, it would be safe to reckon both the species as suspect.

SPLEEN AND PARASITE RATES.

In the first group (comparison villages), 94 out of 125 children between 2 and 10 years age had enlarged spleen with a spleen rate of 75·2 per cent early in September 1948. During the same period the spleen rates in the second (B.H.C.)

and third (D.D.T.) groups were 50·3 per cent (97 out of 193) and 42·1 per cent (59 out of 140) respectively. While in the comparison group the spleen rate is significantly higher, there is no significant difference between the spleen rates of the other two groups. The parasite rates in the three groups in September 1948 were 15·0 per cent (18 out of 120), 5·7 per cent (11 out of 192) and 6·7 per cent (9 out of 134) respectively. From a knowledge of the epidemiological conditions of the district, it may be stated that transmission of malaria would have just commenced early in September when the above spleen and blood surveys were carried out. The infant parasite rates in the three groups at this period were 7·4 per cent (2 out of 27), 2·4 per cent (2 out of 84) and nil (out of 33) respectively.

DISPENSARY FIGURES.

In the only Government dispensary in the area situated in Mokhada, 511 cases of malaria were treated in 1946 and 1,394 in 1947. In 1948, the number thus treated was 1,155. Table I below shows the details for each month.

TABLE I.

Number of malaria cases treated at the out-patient department of Mokhada Dispensary, 1946–48.

Month.	1946.	1947.	1948.
Jan.	30	144	197
Feb.	27	90	146
Mar.	22	88	141
Apr.	19	48	75
May	14	56	54
Jun.	11	36	38
Jul.	25	48	49
Aug.	33	32	71
Sep.	48	103	118
Oct.	63	112	146
Nov.	81	250	90
Dec.	138	287	30
TOTAL FOR THE YEAR ...	511	1,294	1,155

Dates of spraying—1st * Oct. 7 to Oct. 16, 1948.
2nd Nov. 17 to Nov. 26, 1948.

* Dates of spraying refer to the entire area served by Mokhada Dispensary.

SPRAYING DETAILS.

Benzene hexachloride was supplied for the purpose of the experiment by the Imperial Chemical Industries (India) Ltd., in the form of a wettable powder labelled by them as ' Gammexane P 520 ' and said to contain 50 per cent benzene hexachloride or about 6·5 per cent gamma isomer. In one village Poshere, 4 oz. of the powder mixed with a gallon of water was applied at the rate of one gallon per 2,000 sq. feet, which gives a dosage of 3·6 mg. of gamma isomer per sq. foot. In another village Khodala, 8 oz. per gallon was used (7·3 mg. per sq. foot) and in the others 12 oz. per gallon (11 mg. per sq. foot). D.D.T. was applied in accordance with our usual technique of one gallon of a 5 per cent soap and water emulsion made out of its preliminary solution in medium kerosene extract (or aromex as it is now termed by the suppliers, the Assam Oil Company Ltd.) per 4,000 sq. feet which gives a dosage of 56 mg. per sq. foot. Two rounds were sprayed, the first between October 10 and 12 and the second between November 17 and 20. Stirrup pumps with oil-resisting hose, brass lance and a nozzle 3/64 inch in diameter, delivering 600 c.c. per minute in a cone-shaped spray were used. In Mokhada and Karegaon villages, the spraying was fully supervised by one or other of the authors and in other villages by other members of the organization. All structures including human dwellings, mixed dwellings and cattlesheds were sprayed.

RESULTS.

(*a*) *Anopheles densities.*—Although collections were made from both cattlesheds and mixed dwellings (in which both men and animals are housed), cattleshed collections were made by new recruits and they were found to be erratic. In some cases collections made by trained men revealed much higher densities after scanty densities had been recorded by new recruits. Hence the few collections made in cattlesheds have been ignored. Collections by trained men were made from October 21 to 29 (11 to 17 days after first spraying or after an average interval of a fortnight after first spraying), from November 10 to 19 (4 to 5 weeks after spraying), December 13 to 18 (4 weeks after second spraying) and again from January 12 to 15 (8 weeks after second spraying).

Tables II and III give the numbers of anophelines collected as well as of *culicifacies* and *fluviatilis* on each of these collections in all villages and in Chas (representing unsprayed villages), Mokhada (B.H.C.-gamma isomer 11 mg. per sq. foot) and Karegaon (D.D.T. village 56 mg. per sq. foot), where both the spraying and the entomological collections were supervised by one or other of the authors.

It would appear from the above tables and particularly from Table III which gives more reliable data that in the case of all anophelines, their re-appearance at the end of the second week after the first round of spraying has been considerable, almost half the density level of unsprayed villages being reached after that interval. There has been a further fall in density at the end of 4 weeks but there has been a similar fall in the unsprayed village as well, indicating a seasonal change. There is no appreciable difference between the D.D.T. and B.H.C. villages in total Anopheles densities. Four weeks after the second round of spray, however, the density of total anophelines has kept very low in the

TABLE II.

Densities of anophelines in mixed dwellings (collections made from 8 a.m. to 12 noon).

Period of collection.	UNSPRAYED VILLAGES.				B.H.C.-SPRAYED VILLAGES.				D.D.T.-SPRAYED VILLAGES.			
	1948.			1949.	1948.			1949.	1948.			1949.
	Oct. 21–29.	Nov. 10–19.	Dec. 13–18.	Jan. 12–15.	Oct. 21–29.	Nov. 10–19.	Dec. 13–18.	Jan. 12–15.	Oct. 21–29.	Nov. 10–19.	Dec. 13–18.	Jan. 12–15.
Time spent hrs. mts.	37–25	23–30	19–30	7–00	24–40	10–00	25–00	11–00	23–00	16–00	22–00	12–00
Actual numbers.												
Culicifacies	609	761	581	925	31	28	306	600	54	125	199	233
Fluviatilis	177	306	59	18	44	67	51	29	14	9	7	8
Others*	1,866	511	782	102	1,075	141	664	243	280	98	183	38
All anophelines ...	2,652	1,578	1,422	1,045	1,150	236	1,021	872	348	232	389	279
Per 10 man-hours.												
Culicifacies	162·8	223·8	297·9	1,321·4	12·6	28·0	122·4	545·5	25·2	78·1	90·5	210·8
Fluviatilis	47·3	130·2	30·3	25·7	17·8	67·0	20·4	26·4	6·0	5·6	3·1	6·7
All anophelines ...	708·7	671·5	729·2	1,492·8	466·2	236·0	408·4	792·7	151·3	145·0	176·8	232·5

* The other species are roughly in the order of abundance :—
jeyporiensis, pallidus, jamesii, moghulensis, subpictus, theobaldi, annularis, vagus, varuna, maculatus, philippinensis, splendidus, tessellatus and *barbirostris.*

1st round of spray—October 10–12, 1948; 2nd round of spray—November 17–20, 1948.

TABLE III.

Densities of Anopheles adults only in mixed dwellings in Chas, Mokhada and Karegaon villages only, which were personally supervised by one of the authors during the second round.

	Chas (unsprayed).				Mokhada (B.H.C. sprayed).				Karegaon (D.D.T. sprayed).			
	1948.		1949.		1948.		1949.		1948.		1949.	
	Oct. 22–28.	Nov. 10–17.	Dec. 13.	Jan. 15.	Oct. 24–27.	Nov. 10–13.	Dec. 13–15.*	Jan. 15.	Oct. 26.	Nov. 15.	Dec. 17.*	Jan. 13.
Actual numbers.												
Culicifacies	443	479	80	925	15	12	105	241	45	81	1	29
Fluviatilis	75	167	23	18	21	30	37	8	8	3	0	0
All species	1,774	936	459	1,045	751	153	593	355	156	91	8	30
Per 10 man-hours.												
Time spent hrs. mts. ...	13–45	9–30	4–00	7–00	11–10	5–00	10–00	4–00	2–25	3–30	4–00	3–00
Culicifacies	322	504	200	1,321	13	24	105	502	180	231	3	97
Fluviatilis	55	176	58	26	19	60	37	20	32	1	0	0
All species	1,290	985	1,148	1,493	673	306	593	888	624	260	20	100

* Both in Mokhada and Karegaon, no anophelines were found in a search of 4 man-hours in mixed dwellings, on the day subsequent to the date of actual spraying of the second round.

1st round of spray—October 10–12, 1948; 2nd round of spray—November 17–20, 1948.

D.D.T. village (only 20 per 10 man-hours) but it has gone up to 593 per 10 man-hours in B.H.C. village and 1,148 per 10 man-hours in the unsprayed village. Eight weeks after the second round, total Anopheles density is still so low as 100 per 10 man-hours in D.D.T. village, while it has gone up to 888 in B.H.C. village and 1,493 in the unsprayed village. Taking all anophelines, D.D.T. definitely appears to have a much more marked residual action than B.H.C. The same differences are even more marked in the case of *fluviatilis* but as regards *culicifacies* while a similar difference in favour of D.D.T. is marked after the second round of spray, B.H.C. appears to have had a much better effect on this species after the first round. But in the absence of definite information about relative densities of different species in the different areas in previous years, it will be fallacious to draw precise conclusions and total Anopheles densities should provide a more conclusive index of relative periods of residual action.

(*b*) *Spleen and parasite rates.*—Table IV gives the above rates in all villages before and after spraying, that is early in September 1948 when transmission had just commenced, and in January 1949 at the end of the transmission season.

The spleen and parasite rates do not show any striking differences between D.D.T. and B.H.C. villages. In the unsprayed villages, there is a slight but insignificant rise in spleen rates at the end of the season. The parasite rate has gone up from 15 to 29·9 per cent. In the B.H.C. group, there is a slight but insignificant fall in the spleen rate but the parasite rate has risen from 5·7 to 17·3 per cent. In the D.D.T. group also, there is a slight but insignificant fall in spleen rates and the parasite rate has risen from 6·7 to 12·9 per cent. The rise has been least in D.D.T. group which is consistent with the mosquito densities.

(*c*) *Infant parasite rates.*—In the unsprayed villages, this rate had risen from 7·4 to 50 per cent (17 out of 34). In the B.H.C. group, it had risen from 2·4 to 9·5 per cent (8 out of 84) and in the D.D.T. group, it had risen to 4 per cent (1 out of 25) from nil. Neither the September nor the January figures for the last two groups are significantly different from each other but in January the infant parasite rate in both groups is significantly lower than in the comparison group. Only four infants showed plasmodia at the beginning of the season, 2 *vivax* and 2 *falciparum*. At the end of the season, 14 were *vivax* and 12 *falciparum*.

(*d*) *Dispensary figures.*—It will be seen from Table I that in Mokhada Dispensary there has been an abrupt drop in the number of malaria cases treated in November and December 1948 from 146 in October to 90 and 30 in November and December respectively, whereas in the previous two years it has gone on rising in the latter two months. There has thus been a very definite reduction in transmission in this village treated with B.H.C. There are no comparative figures available for the D.D.T. group or the comparison group of villages.

DISCUSSION.

The data presented in this paper do not warrant the drawing of any but tentative conclusions of the relative merits of B.H.C. and D.D.T. as residual

TABLE IV.

Spleen and parasite rates in children between 2 and 10 years of age.

Village.	SEPTEMBER 1948.				JANUARY 1949.			
	Number examined.	Spleen rate, per cent.	Number examined.	Parasite rate, per cent.	Number examined.	Spleen rate, per cent.	Number examined.	Parasite rate, per cent.
Unsprayed villages.								
Chas ...	30	70·0	30	13·3	33	93·9	24	12·5
Gonda B ...	50	70·0	45	8·9	56	75·0	19	31·6
Morhande ...	45	84·4	45	22·2	63	82·6	44	38·6
	125	75·2	120	15·0	152	82·8	87	29·9
	vivax—4, *falciparum*—14.				*vivax*—7, *falciparum*—19.			
B.H.C. villages.								
Mokhada ...	50	54·0	50	8·0	41	51·2	37	16·2
Vashala and Saturli ...	28	42·8	28	7·1	66	33·3	56	17·9
Poshere ...	65	60·0	64	4·7	53	54·7	40	27·5
Khodala ...	50	38·0	50	4·0	47	40·6	40	7·5
	193	50·3	192	5·7	207	44·4	173	17·3
	vivax—2, *falciparum*—9.				*vivax*—15, *falciparum*—15.			
D.D.T. villages.								
Karegaon ...	60	50·0	60	8·3	49	36·7	40	10·0
Gomghar ...	55	34·5	50	6·0	39	35·9	31	25·8
Suryamal ...	25	40·0	24	4·3	25	32·0	22	0·0
	140	42·1	134	6·7	113	35·4	93	12·9
	vivax—1, *falciparum*—8.				*vivax*—5, *falciparum*—7.			

insecticides inasmuch as no precise information is available of mosquito densities in the past, and also because by the time the first round of spraying was commenced, transmission of malaria had already started and subsequent parasite rates may, in part at least, be due to the effect of the earlier transmission. However, the experiment gives us some idea of the relative merits of the two insecticides and a pointer to the manner in which further experiments should be carried out.

The dispensary figures in Mokhada very definitely point to a considerable reduction in transmission of malaria in November and December. The diagnosis is no doubt made only on clinical grounds but so it was in the past. Even if some bias in favour of a lower record is conceded on account of the medical officer being fully aware of the experiments in progress, the reduction is too striking to be due to any cause but a factual reduction in transmission. The dispensary caters principally to the residents of Mokhada, the taluk headquarters. Among the total patients, less than 5 per cent are annually treated from among residents other than of Mokhada. Hence the dispensary figures show that B.H.C. applied in a dosage of 11 mg. of gamma isomer per sq. foot definitely reduces malaria transmission. There are no similar data for D.D.T. and comparison groups of villages.

The infant parasite rates are very revealing. In the unsprayed villages, the rate is 50 per cent in January at the end of the transmission. Such a high rate compared with a smaller rate of 29·9 per cent among children between 2 and 10 years is a little intriguing. While the latter rate has increased from 15·0 per cent at the beginning of transmission to 29·9 per cent at the end, the former has increased from 7·4 to 50·0 per cent. From a single year's experience with small numbers of infants examined, it will not be safe to draw any valid conclusion regarding the relative immunity status of the childhood and infant populations. The infant rates, however, show that during the experimental period malaria transmission had been very active. In the B.H.C. group, the infant parasite rate had risen from 2·4 per cent to 9·5 per cent and in the D.D.T. group, from nil to 4 per cent. These rates are not significantly different from each other either at the beginning or the end of the transmission season. In the absence of detailed history of each infant, it cannot be stated when the infants showing parasites at the end of the transmission season actually acquired the infection, whether before or after the insecticides were sprayed. On the whole, in both groups of villages there has been a far smaller rise in infant parasite rate at the end of the transmission season when compared with the unsprayed villages. The B.H.C. group show a slightly higher infection rate among the new-born infants than the D.D.T. group, but the difference is not statistically significant.

The recorded mosquito densities are best elucidated with respect to the data provided in Table III as they were checked by one or other of the authors. There is nothing to assume that among the local anophelines there is any considerable difference in species vulnerability to insecticidal action. It is more plausible to assume that differences in species densities are more due to differences in their relative local numerical prevalence. Taking all anophelines, D.D.T. has proved to have a much longer residual insecticidal action than B.H.C. *Fluviatilis* densities are even more markedly in conformity with the above

conclusion. As regards *culicifacies*, after the first round B.H.C. appears to have had a better effect but after the second round the reverse is the case. This is probably due to the seasonal changes in mosquito densities and not due to any reduced insecticidal action of B.H.C.

The determination of the interval of spraying has been made in this country on the basis of re-appearance of vector species densities above assumed critical thresholds for communal transmission. On that basis the experience of the second round of spraying (Table III) would indicate that B.H.C. in a dosage of 11 mg. of gamma isomer per sq. foot would need to be repeated at intervals not longer than 4 weeks inasmuch as the density of *culicifacies* has gone up to 105 per 10 man-hours (critical density of *culicifacies* is assumed to be 50 per 10 man-hours) in the middle of December, the last spraying having been carried out in November 17 to 20. After D.D.T. spray, it is only at the 8th week that the density has gone up to 97 per 10 man-hours. This is in conformity with our experience elsewhere that 6 weeks after D.D.T. spray (60 mg. per sq. foot) density of this species exceeds 50 per 10 man-hours. Two very relevant points arise for consideration, viz. whether the densities of mosquitoes caught during the mornings in sprayed daytime resting places are of any significance as regards their vectorial capacity and whether after insecticidal spray and effective reduction of transmission for some period a much larger critical density of the vector species is not necessary for effective communal transmission of the disease due to the diminution of the reservoirs of infection. As regards the first it is argued that mosquitoes caught in the morning in resting places which have been sprayed are inevitably destined to die later and the number among them likely to live for the minimum period of 14 days before they can transmit the parasite is extremely small. Hence, relatively large densities are consistent with the hypothesis of absence of transmission. As a corollary to this hypothesis, a plea is made for the collection of mosquitoes after 11 a.m. Such data have not been collected in our experiment. While in a future experiment the defect would be remedied, it may be noted that, if morning densities show a rise from day to day in sprayed villages at a rate more rapid than in unsprayed villages and very high densities are reached, it is permissible to assume that the age composition of the mosquito populations is not far different in the two groups. For, short-lived mosquito populations cannot contribute to a more rapid daily output than those with a more natural age composition. However, in future experiments a determination of the age composition of the mosquito populations in sprayed and unsprayed villages should be made in order to throw more light on this matter. As regards the second factor, it will be of application only when a nation-wide control is established; otherwise the movement of human populations makes the reservoir factor extremely variable and, in the epidemics in the Punjab and Sind, several workers have recorded extremely low gametocyte rates in the pre-epidemic periods. Hence, given favourable conditions for mosquito life, even a small initial factor of reservoir of infection is adequate to bring about considerable communal transmission.

As regards dosage of B.H.C., the data in Mokhada, where 11 mg. of gamma isomer per sq. foot was sprayed, can alone be taken for valid consideration. Even with this high dosage, there is a return of mosquito densities above the

critical threshold in 4 weeks. But more precise data on the significance of the densities of re-appearing anophelines more especially with regard to their age composition should be collected in a future experiment before the dosage and frequency of spraying could be precisely determined.

Finally, a definite smarting of the skin was felt when spraying B.H.C., but none whatever with D.D.T. The former has also a slight disagreeable odour but both these disadvantages are not beyond endurance.

SUMMARY.

(1) This paper records observations regarding Anopheles densities, spleen and parasite rates among children, infant parasite rates and malaria morbidity in villages sprayed with 11 mg. of B.H.C., and 56 mg. of D.D.T. per sq. foot respectively and in comparison villages. The sprayings were, however, carried out some weeks after transmission had commenced.

(2) In the above dosages, D.D.T. appears to lose its residual effect before the end of 8 weeks and B.H.C. before the end of 4 weeks, judging from densities of returning anophelines. The significance of densities of re-appearing anophelines with respect to their transmitting capacity as judged by longevity requires further study.

(3) Both insecticides have, in the dosage applied, considerably reduced transmission as judged by childhood parasite rates and infant parasite rates. Morbidity figures available only for one B.H.C.-treated village also confirm this conclusion.

(4) Dosage and frequency of application necessary for effective reduction of transmission require to be more precisely determined by further experiments.

ACKNOWLEDGMENTS.

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A STUDY OF THE EFFECTS OF MALARIA AND OF MALARIA CONTROL MEASURES ON POPULATION AND VITAL STATISTICS IN KANARA AND DHARWAR DISTRICTS AS COMPARED WITH THE REST OF THE PROVINCE OF BOMBAY.

BY

D. K. VISWANATHAN

(*Malaria Organization, Bombay Province*).

[May 14, 1949.]

INTRODUCTION.

VISWANATHAN AND RAO (1947, 1948) have described the first and second season's results of rural malaria control with the use of D.D.T. as an indoor residual spray in Dharwar and Kanara districts in the province of Bombay. The epidemiology of malaria in Kanara District has been described in detail by Jaswant Singh and Jacob (1944) and by Viswanathan and Rao, singly, conjointly and in collaboration with others in several papers. For the purpose of this paper it is enough to recall that malaria is (or rather was) hyperendemic in practically 100 per cent of the 6 forest-clad hilly taluks of Kanara District; the 5 coastal taluks of that district have about 20 per cent of their villages of the same degree of hyperendemicity (the revenue divisions not coinciding with the epidemiological divisions); and the rest are free from both malarial endemicity and epidemicity; about 2 taluks of Dharwar District have as much hyperendemicity as the hilly taluks of Kanara and the bulk of the remaining 11 taluks of that district have scattered patches of moderate endemicity and a liability to epidemic waves of malaria at ill-defined intervals. In this paper the growth of population, birth rates, death rates, natural increase rates, infantile mortality rates, malaria death rates and dysentery and diarrhœa death rates are studied in the two districts mentioned above as compared with the rest of the province.

CHANGES IN POPULATION.

Table I shows the census population in the hilly taluks of Kanara (KA), coastal taluks of Kanara (KB), Kanara as a whole (KT), Dharwar (D) and in the

rest of the province (R) from 1881 to 1941, figures for KA and KB not being separately available for 1881.

TABLE I.

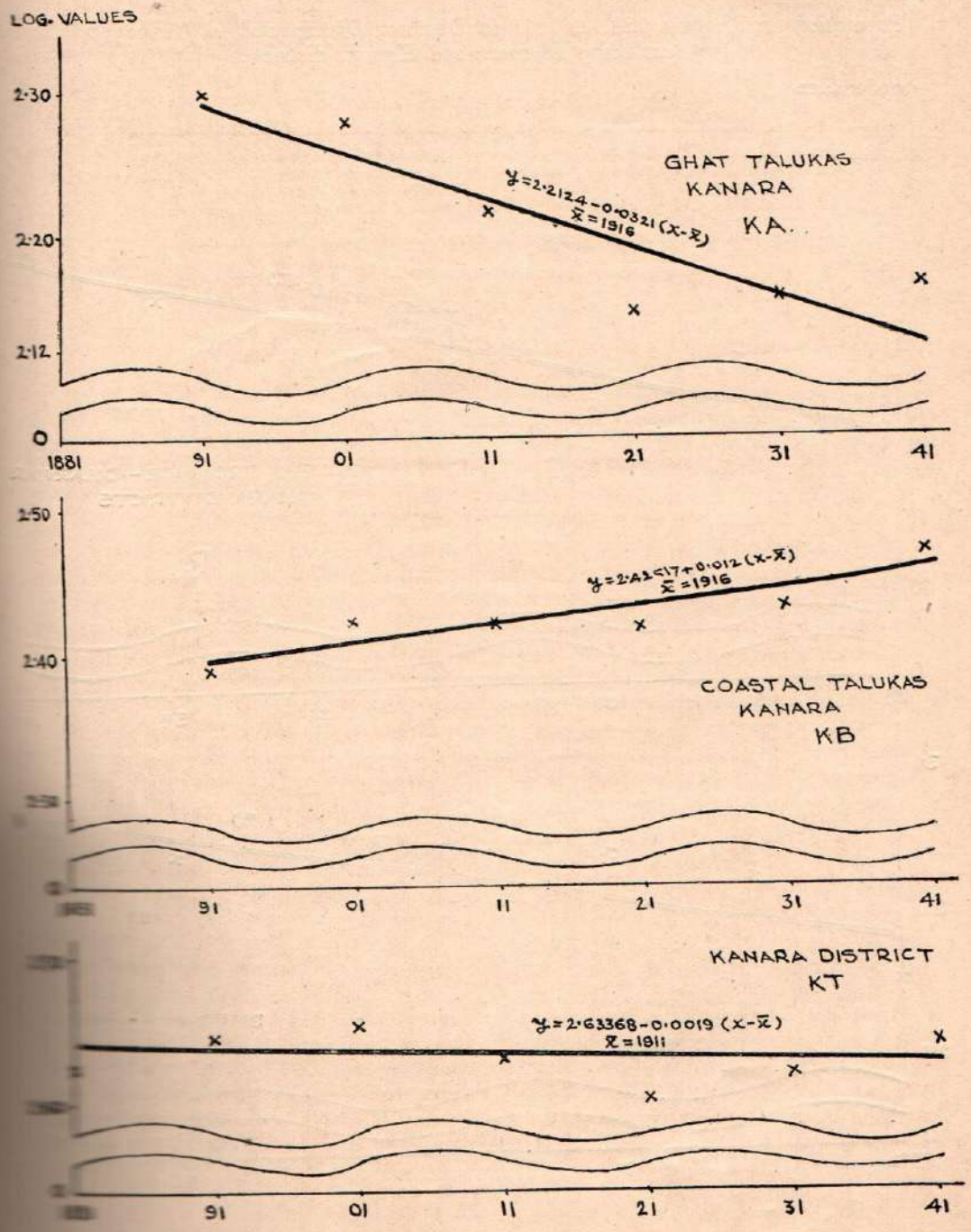
Census population in 1,000.

Year.	Kanara, KA.	Kanara, KB.	Kanara, KT.	Dharwar, D.	Rest of the province, R.
1881	...	...	421·9	893·8	12,747·3
1891	199·8	246·6	446·4	1,051·5	14,487·4
1901	190·3	264·2	454·5	1,113·6	13,751·3
1911	167·0	263·4	430·4	1,026·3	14,679·9
1921	140·4	261·3	401·7	1,036·9	14,573·7
1931	144·4	272·9	417·3	1,102·7	16,471·5
1941	146·5	294·7	441·2	1,201·0	19,207·7

A superficial inspection of the figures in Table I would show thati n the hilly taluks of Kanara, there is a steady decline, that in the rest of Kanara and in Dharwar, there has been some increase, and in the rest of the province a still larger increase from 1881 to 1941. Graph 1 shows the population in Kanara District and Graph 2 in Dharwar and rest of the province. A regression straight line is fitted to the observed data. Though the fit is not close except perhaps for the coastal taluks of Kanara, as it is not the purpose of this paper to estimate intercensal populations throughout the period, fitting curves with a multiplicity of constants, which would serve only as an algebraical expression without illustrating any particular law of population changes, have not been undertaken. Such a curve cannot also be used for estimating populations beyond 1941 by a process of extrapolation. Hence estimates of the population from 1936 to 1946 have later been made from the available population figures for 1931 and 1941, assuming that the rate of growth for a few years after 1941 has been of the same magnitude as from 1931 to 1941.

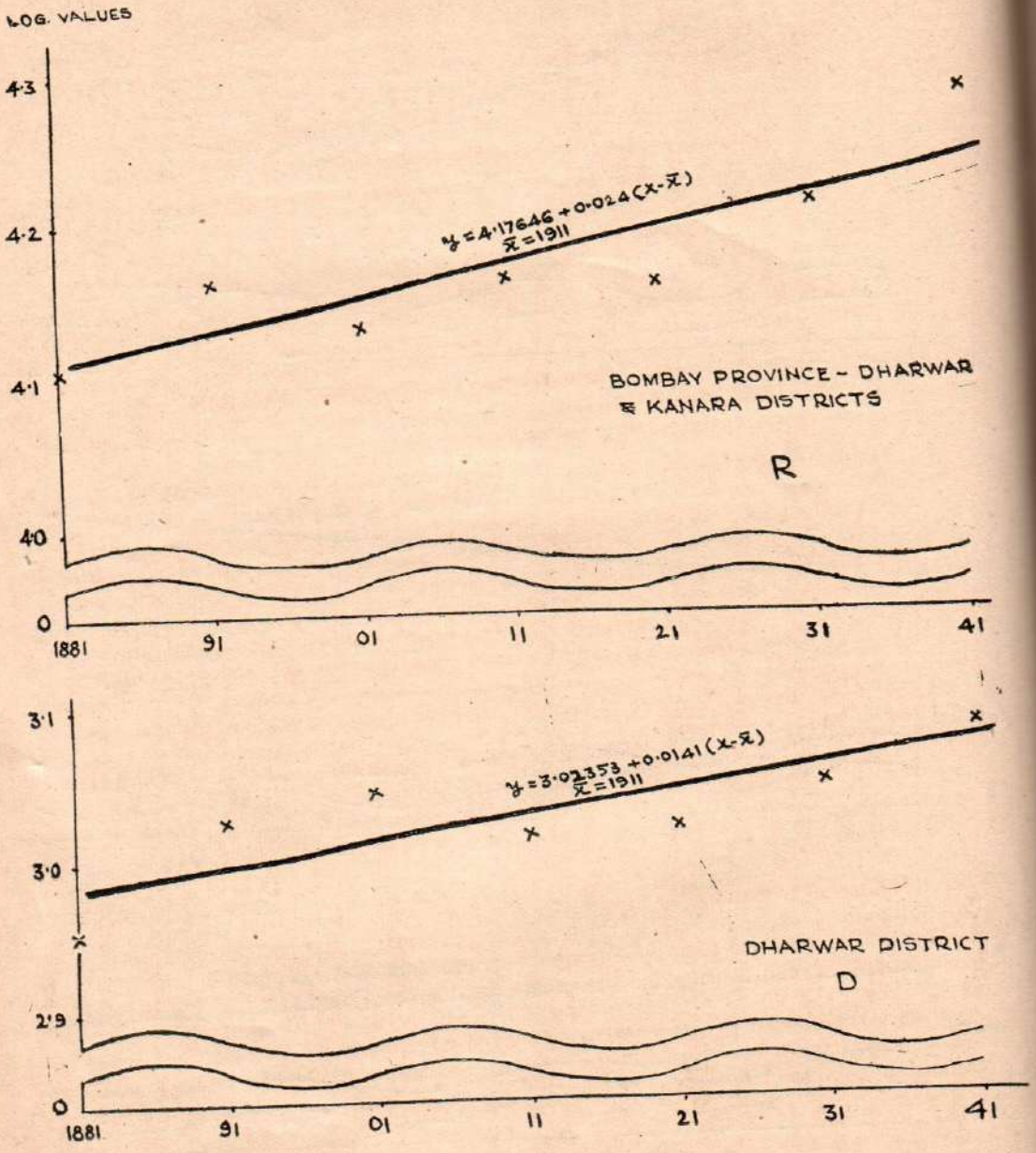
Graph 1.

Population growth from 1881 *to* 1941 *in the Coastal and Ghat talukas separately and the whole of Kanara District.*



GRAPH 2.

Population growth from 1881 *to* 1941 *in Dharwar District and in Bombay province excluding Dharwar and Kanara districts.*



Regression coefficients.—Appendix I shows the log values of the population, summation of log values, squares of the log values and the process of calculation of the regression coefficients and estimating if they are significantly different from O (Fisher, 1934). The equations for the straight lines are reproduced below :—

Kanara (A).

$y=2{\cdot}2124-0{\cdot}0321\ (x-\bar{x})$

$\bar{x}=1916$

Kanara (B).

$y=2{\cdot}42617+0{\cdot}012\ (x-\bar{x})$

$\bar{x}=1916$

Kanara (T).

$y=2{\cdot}63368-0{\cdot}0019\ (x-\bar{x})$

$\bar{x}=1911$

Dharwar (D).

$y=3{\cdot}02353+0{\cdot}0141\ (x-\bar{x}).$

$\bar{x}=1911$

Rest of the province (R).

$y=4{\cdot}17646+0{\cdot}024\ (x-\bar{x}).$

$\bar{x}=1911$

(*Note.*—y or population is expressed in logarithmic values.)

The details of analysis described in Appendix I show that the regression coefficient is negative in the hilly taluks of Kanara and Kanara District as a whole. But while the former is significantly different from O the latter is not. Thus there is a well-marked downward trend in the population of the hilly taluks but the population of the entire district has remained more or less stationary during the 60-year period 1881 to 1941.

In the coastal taluks of Kanara, in Dharwar and in the rest of the province there has been a significant increase in population during the 60-year period (the positive regression coefficient being significantly different from O—Appendix I). The values of the coefficients are 0·012, 0·014 and 0·024. In KB and D the values are very nearly equal and in R it is higher. Neither of the regression coefficients for KB and D is found to be significantly different from that for the rest of the province. If the observed difference is therefore not real but only due to sampling variations, it shows that the forces at play with regard to growth of population have had cumulatively the same effect in the three areas. If, however, the difference is real but not demonstrable, it shows that about 20 per cent of villages with hyperendemicity in KB and about 10 per cent with moderate endemicity and liability to epidemics in Dharwar District, have contributed to a smaller growth of population in these areas than in the rest of the province.

BIRTH RATES, DEATH RATES AND NATURAL INCREASE RATES.

Table II and Graphs 3, 4 and 5 show the birth and death rates for Dharwar and Kanara districts and the rest of the province separately from 1936 to 1948. The population for each year was estimated as stated earlier on the assumption of a geometric increase from the available population figures for 1931 and 1941. The mean rates and regression coefficients are all calculated for the pre-D.D.T. period 1936 to 1945. The observed rates in 1946, 1947 and 1948 are then compared with the rates which may be expected by extrapolation from the regression line in order to assess whether D.D.T. spray has had any effect in Dharwar and Kanara as compared with the rest of the province where no D.D.T. spray was carried out.

TABLE II.

Estimated population (in 1,000), total births, total deaths, birth rates and death rates for Dharwar (D), Kanara (KT) and the rest of the province (R).

Year.	KANARA (KT).					DHARWAR (D).					REST OF THE PROVINCE (R).				
	Population.	Births.	Deaths.	Birth rate, per mille.	Death rate, per mille.	Population.	Births.	Deaths.	Birth rate, per mille.	Death rate, per mille.	Population.	Births.	Deaths.	Birth rate, per mille.	Death rate, per mille.
1936	429	13,394	12,960	31·20	30·20	1,151	41,985	29,763	36·50	25·87	17,760	686,952	454,555	38·67	25·59
1937	432	14,185	11,716	32·86	27·14	1,161	43,783	26,765	37·73	23·06	18,037	671,508	418,727	39·65	22·74
1938	434	14,347	12,253	33·06	28·23	1,170	45,596	29,064	38·95	24·82	18,314	698,582	506,070	38·15	27·64
1939	436	14,127	11,653	32·37	26·71	1,181	45,703	27,335	38·71	23·16	18,594	715,715	455,899	38·47	24·49
1940	439	13,484	12,336	30·73	28·11	1,191	46,071	31,809	38·70	26·71	18,880	696,262	457,320	36·36	24·20
1941	441	13,223	11,518	29·97	26·10	1,200	42,994	38,774	35·80	32·28	19,179	706,895	489,445	36·84	25·51
1942	444	13,793	10,210	31·10	23·01	1,212	45,818	33,495	37·84	27·65	19,474	665,115	472,843	34·14	24·26
1943	446	14,262	10,371	31·98	23·24	1,222	42,784	38,441	35·01	31·47	19,772	656,594	453,713	33·18	22·93
1944	448	12,613	11,796	28·13	26·31	1,232	37,099	41,082	30·11	33·93	20,080	691,598	503,583	34·28	25·06
1945	451	13,289	10,465	29·45	23·20	1,243	40,084	31,027	32·27	24·98	20,386	693,022	543,400	33·95	26·63
Post-D.D.T. period. 1946	453	15,733	9,335	34·70	20·59	1,253	46,332	26,866	36·98	21·44	20,704	682,753	480,696	32·94	23·19
1947	456	15,474	10,583	33·94	23·2	1,264	46,014	29,500	36·40	23·33	21,020	685,576	527,961	32·58	25·18
1948	458	15,903	7,451	34·69	16·26	1,275	48,910	24,955	38·36	19·61	21,347	699,608	462,135	32·74	21·62

Birth rates (Graph 3).—The analysis of birth rates shown in Appendix II reveals that the mean birth rates in Dharwar (36·16) and the rest of the province (36·37) are significantly higher than in Kanara District (31·08). There has also been a steady and significant fall in the birth rates in all the areas, the negative regression coefficient for the rest of the province (—0·7116) being the highest, that for Dharwar (—0·7067) nearly as high and both of them being significantly larger than for Kanara (—0·3565). Neither the mean birth rates nor the negative regression coefficients are significantly different from each other in the case of Dharwar and the rest of the province.

Assuming that the birth rates continue to fall at the same rate during the next three years 1946 to 1948, the expected birth rates together with the observed rates are shown below :—

Year.	Kanara.		Dharwar.		Rest of the province.	
	T.	O.	T.	O.	T.	O.
1946	29·12	34·70	32·28	36·98	32·45	32·94
1947	28·76	33·94	31·58	36·40	31·74	32·58
1948	28·41	34·69	30·87	38·36	31·03	32·74

T.=*theoretical.* O.=*observed.*

It will be seen from the above data that while in the rest of the province the difference between the theoretical and observed rates is less than 1 in each of the 3 years, there has been a rise in birth rates in Kanara and Dharwar, varying from 5·18 to 6·28 in the former and 4·70 to 7·49 in the latter.

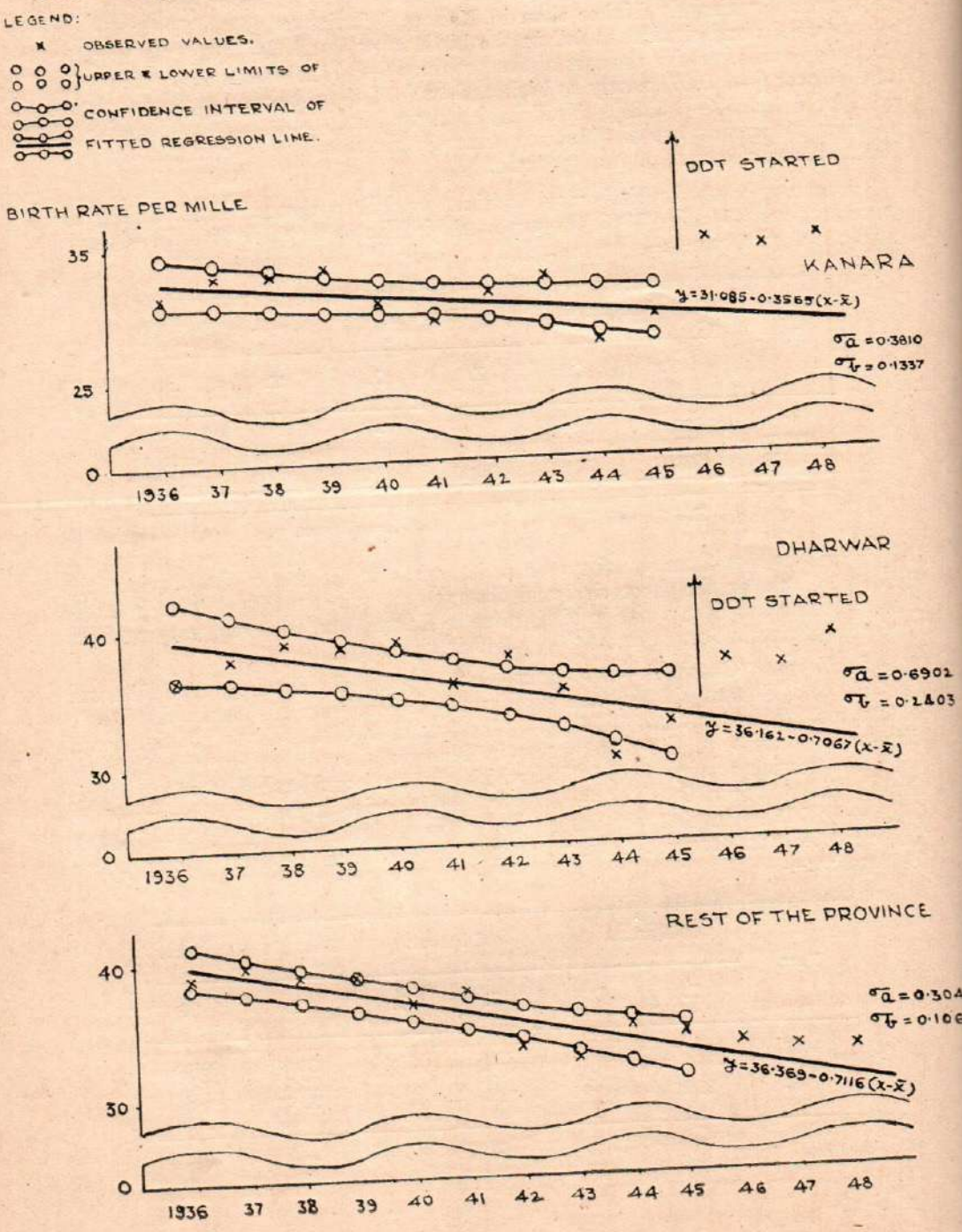
Death rates (Graph 4).—Appendix III shows that the death rates in Dharwar (27·393) and Kanara (26·225) are significantly higher than the mean death rate of the rest of the province (24·905). The former two rates have no significant difference. Although the death rates in Dharwar show a tendency to increase with a regression coefficient of 0·7295 which appears considerable, it is not significantly different from O. Nor of course is the slight rise in the rest of the province ($b = +0·0162$). In Kanara, death rates show a significant fall ($b = -0·6477$). The expected and observed death rates in 1946, 1947 and 1948 are shown below for 3 years :—

Year.	Kanara.		Dharwar.		Rest of the province.	
	T.	O.	T.	O.	T.	O.
1946	22·66	20·59	31·40	21·44	25·00	23·19
1947	22·01	23·20	32·13	23·33	25·00	25·18
1948	21·37	16·26	32·86	19·61	25·02	21·62

T.=*theoretical.* O.=*observed.*

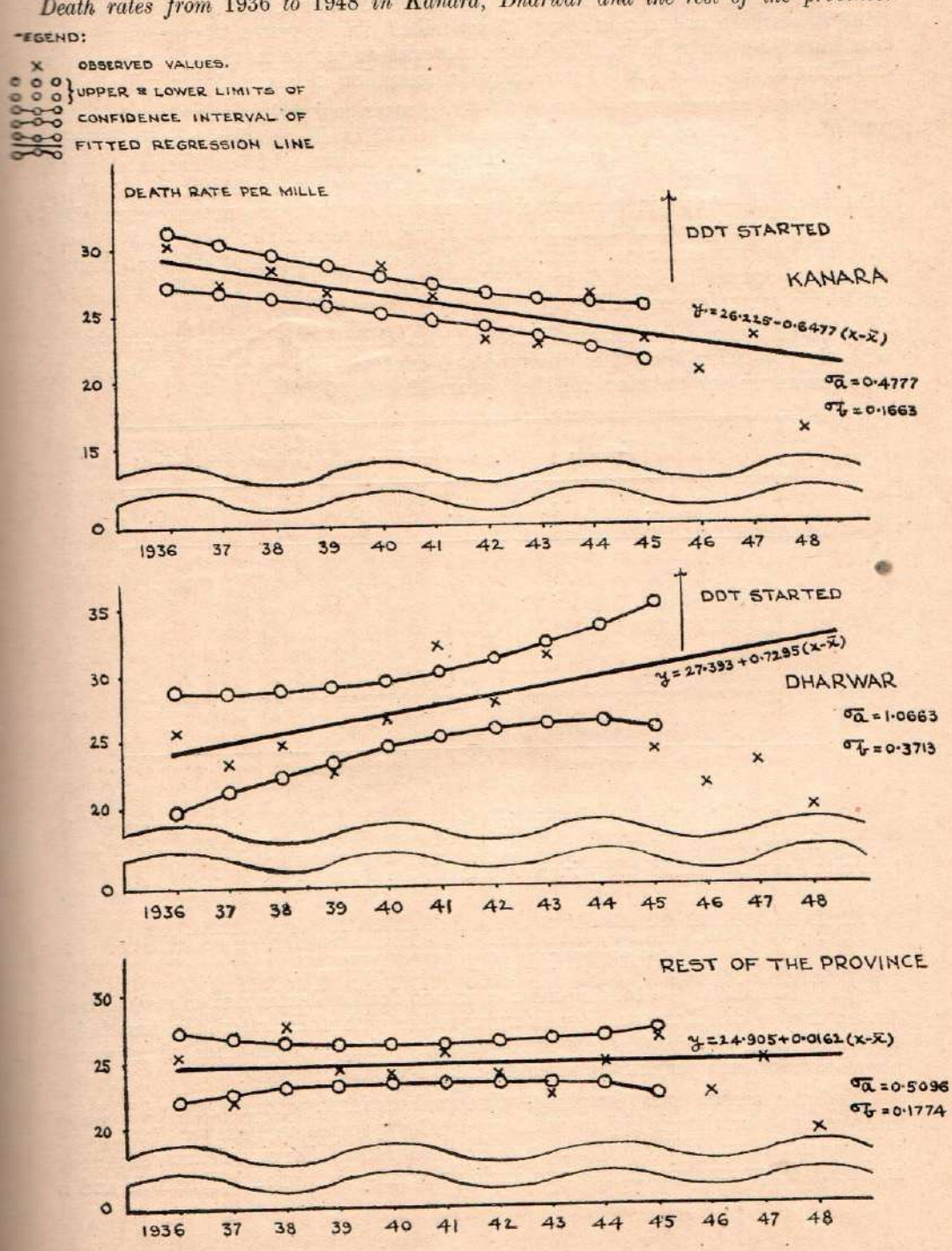
GRAPH 3.

Birth rates from 1936 to 1948 in Kanara, Dharwar and the rest of the province.



GRAPH 4.

Death rates from 1936 *to* 1948 *in Kanara, Dharwar and the rest of the province.*



The fall in death rate in Dharwar appears considerable in each year and in 1948 it has been as great as about 13 per mille. In the rest of the province there is a fall of 3·4 per mille in 1948 but no material difference in the other two years. In Kanara, only the 1948 figures show a reduction of about 5 per mille.

Natural increase rates (Graph 5).—The mean natural increase rates in the rest of the province (11·4664) and Dharwar (8·799) while not significantly differing from each other are significantly higher than in Kanara (4·36). The rates in the former two areas show a significant fall with a regression coefficient of —1·434 in Dharwar and —0·728 in the rest of the province, which are not however significantly different from each other. In Kanara, there is a rise in natural increase rate but the regression coefficient of 0·1396 is not significantly different from 0 though of course it is significantly different from the negative coefficients in the other two areas (Appendix IV).

Graph 5 shows the natural increase rates observed as well as in accordance with the regression line. The observed and expected natural increase rates in the three areas are shown below for the 3 years 1946 to 1948 :—

Year.	Kanara.		Dharwar.		Rest of the province.	
	T.	O.	T.	O.	T.	O.
1946	4·99	14·11	0·92	15·54	7·46	9·75
1947	5·13	10·74	—0·51	13·07	6·73	7·50
1948	5·27	18·43	—1·94	18·75	6·00	11·12

T.=*theoretical.* O.=*observed.*

Graph 5 clearly shows that while in the rest of the province there is no material change in the natural increase rate except in 1948, both in Dharwar and Kanara this rate has very considerably increased in all the three years.

INFANT MORTALITY, MALARIA DEATHS AND DEATHS DUE TO DIARRHŒA AND DYSENTERY.

Table III shows the total number of infant deaths, deaths due to malaria and deaths due to diarrhœa and dysentery together with their rates, the first being expressed in terms of 1,000 live births and the other two per mille of estimated population for each year in the three different areas under study.

Infant mortality rates (Graph 6).—The analysis in Appendix V shows that the mean infant mortality is highest in Kanara (176·5), lowest in Dharwar (146·1) and midway in the rest of the province and the mean rates differ significantly from one another. There is a rise in the rate during the ten-year period in Dharwar and a

GRAPH 5.

Natural increase rates from 1936 to 1948 in Kanara, Dharwar and the rest of the province.

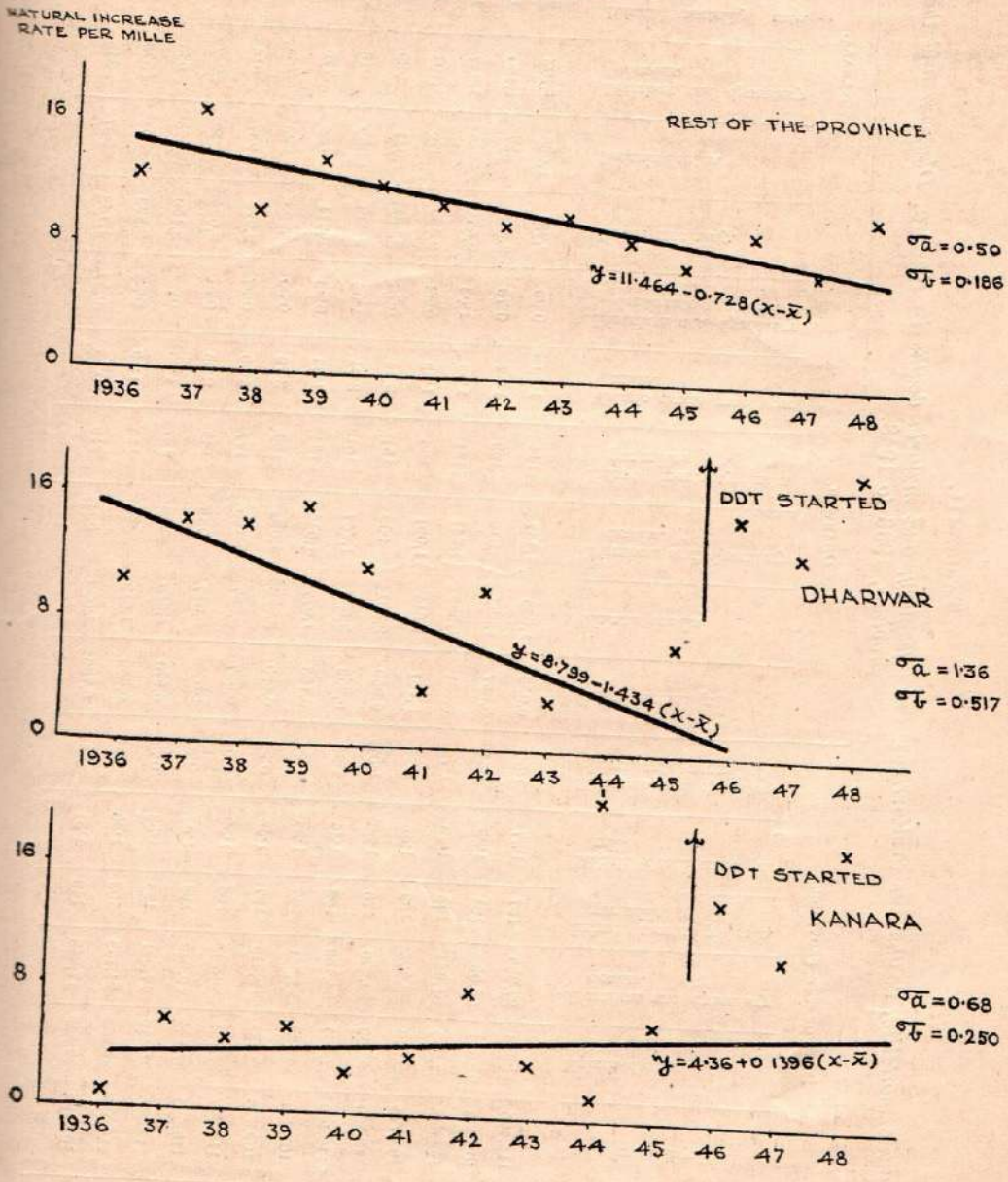


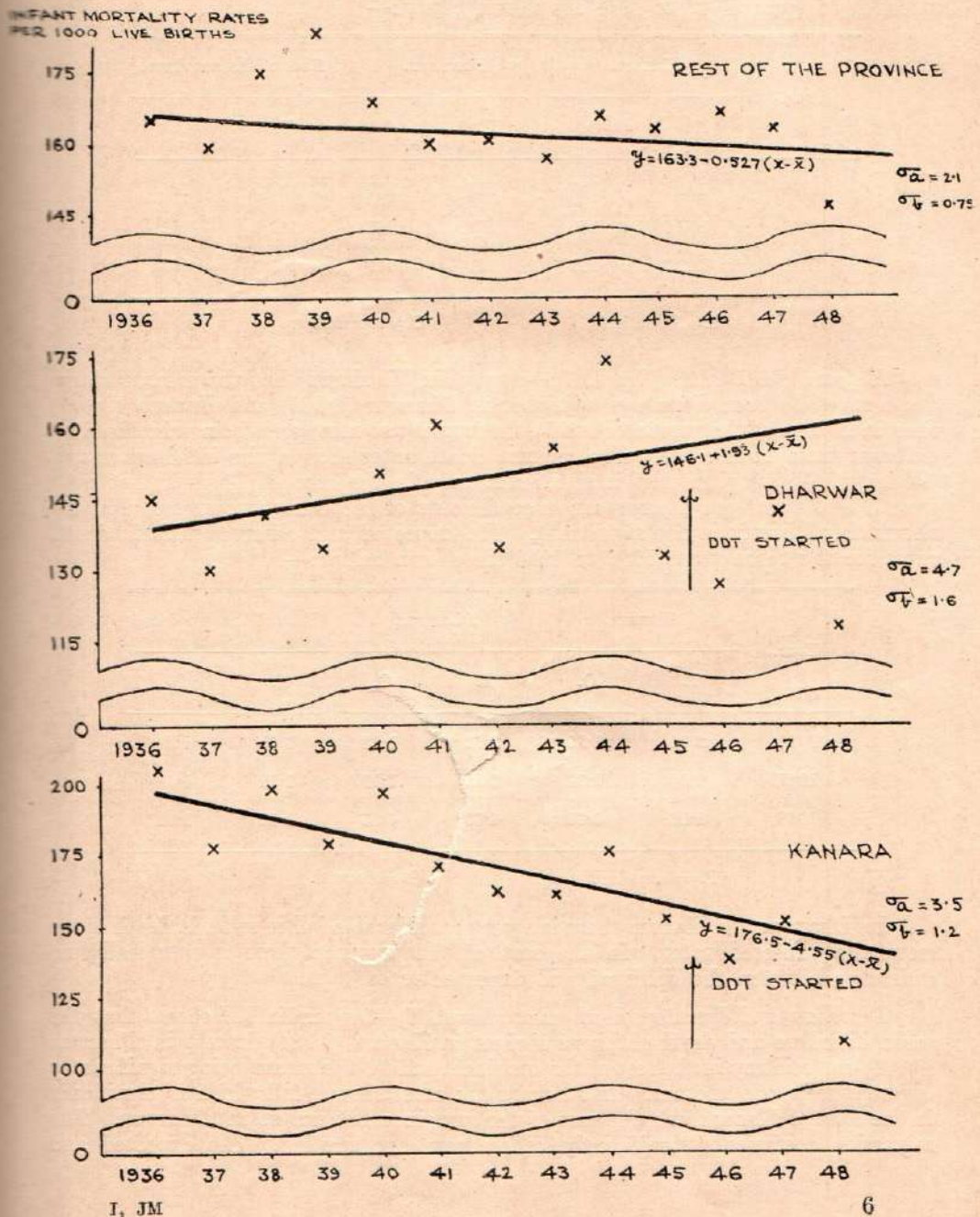
TABLE III.

Infantile mortality, deaths due to diarrhœa and dysentery, and malaria deaths in Kanara, Dharwar and the rest of the province, 1936 *to* 1948.

Year.	Rest of the province.						Dharwar.						Kanara.					
	Infant mortality.	Malaria deaths.	Diarrhœa and dysentery deaths.	Infant mortality rate, per mille.	Malaria death rate, per mille.	Diarrhœa and dysentery death rate, per mille.	Infant mortality.	Malaria deaths.	Diarrhœa and dysentery deaths.	Infant mortality rate, per mille.	Malaria death rate, per mille.	Diarrhœa and dysentery death rate, per mille.	Infant mortality.	Malaria deaths.	Diarrhœa and dysentery deaths.	Infant mortality rate, per mille.	Malaria death rate, per mille.	Diarrhœa and dysentery death rate, per mille.
1936	114,465	24,333	27,858	167	1·37	1·56	6,063	1,946	1,141	144	1·69	0·99	2,761	1,028	1,105	206	2·39	2·58
1937	109,083	22,624	26,062	161	1·25	1·44	5,657	1,882	912	129	1·62	0·79	2,518	867	1,120	178	2·01	2·60
1938	122,865	27,280	31,654	176	1·49	1·73	6,431	1,586	1,241	141	1·36	1·06	2,804	924	1,347	196	2·13	3·11
1939	109,774	26,313	23,375	153	1·32	1·26	6,161	1,644	1,091	135	1·39	0·92	2,490	876	1,101	176	2·01	2·53
1940	118,550	25,700	20,270	170	1·36	1·07	6,969	2,861	909	151	2·44	0·76	2,584	1,041	986	192	2·37	2·25
1941	112,149	27,697	24,166	159	1·44	1·26	6,906	3,533	1,267	161	2·94	1·05	2,243	869	916	170	1·97	2·08
1942	107,967	26,147	24,551	162	1·34	1·26	6,155	1,977	1,046	134	1·63	0·86	2,211	782	779	160	1·76	1·76
1943	103,378	28,583	19,643	157	1·44	0·99	6,690	3,328	1,094	156	2·72	0·90	2,255	908	785	158	2·04	1·76
1944	114,347	35,181	20,489	165	1·39	1·02	6,557	5,657	1,190	177	4·59	0·97	2,219	1,087	814	176	2·42	1·82
1945	112,987	37,234	19,849	163	1·82	0·97	5,322	3,517	758	133	2·83	0·61	2,034	880	607	153	1·95	1·35
1946	113,225	31,419	17,821	166	1·52	0·86	5,870	1,550	648	127	1·23	0·52	2,150	588	534	137	1·29	1·18
1947	112,062	39,282	24,162	163	1·48	1·15	6,556	1,513	420	143	1·19	0·33	2,333	604	876	151	1·33	1·92
1948	102,183	32,326	17,243	146	1·51	0·81	5,785	1,089	594	118	0·85	0·57	1,733	367	343	109	0·80	0·75

GRAPH 6.

Infant mortality rates from 1936 *to* 1948 *in Kanara, Dharwar and the rest of the province.*



fall in the rest of the province but these changes are not significant. There is however a significant fall in the rate in Kanara. The regression coefficients for the three areas differ significantly from one another. The observed and expected rates for the years 1946 to 1948 are shown below :—

Year.	Kanara.		Dharwar.		Rest of the province.	
	T.	O.	T.	O.	T.	O.
1946	151·5	137	157	127	160·5	166
1947	147·0	151	159	143	160·0	163
1948	142·0	109	161	118	159·0	146

T.=*theoretical.* O.=*observed.*

Malaria death rates (Graph 7).—Appendix VI shows that the mean malaria death rate has been significantly higher in Dharwar (2·321) and Kanara (2·105) than in the rest of the province (1·422), the former two means not being significantly different from each other. The malaria death rates show a rise in Dharwar and the rest of the province but is significant only in the case of Dharwar not only from O but also from the regression coefficients of the other two areas. Kanara shows a fall but it is not significant. The expected and observed malaria death rates for the three years 1946 to 1948 are shown below :—

Year.	Kanara.		Dharwar.		Rest of the province.	
	T.	O.	T.	O.	T.	O.
1946	2·02	1·29	3·62	1·23	1·59	1·52
1947	2·00	1·33	3·86	1·19	1·62	1·48
1948	1·98	0·80	4·09	0·85	1·65	1·51

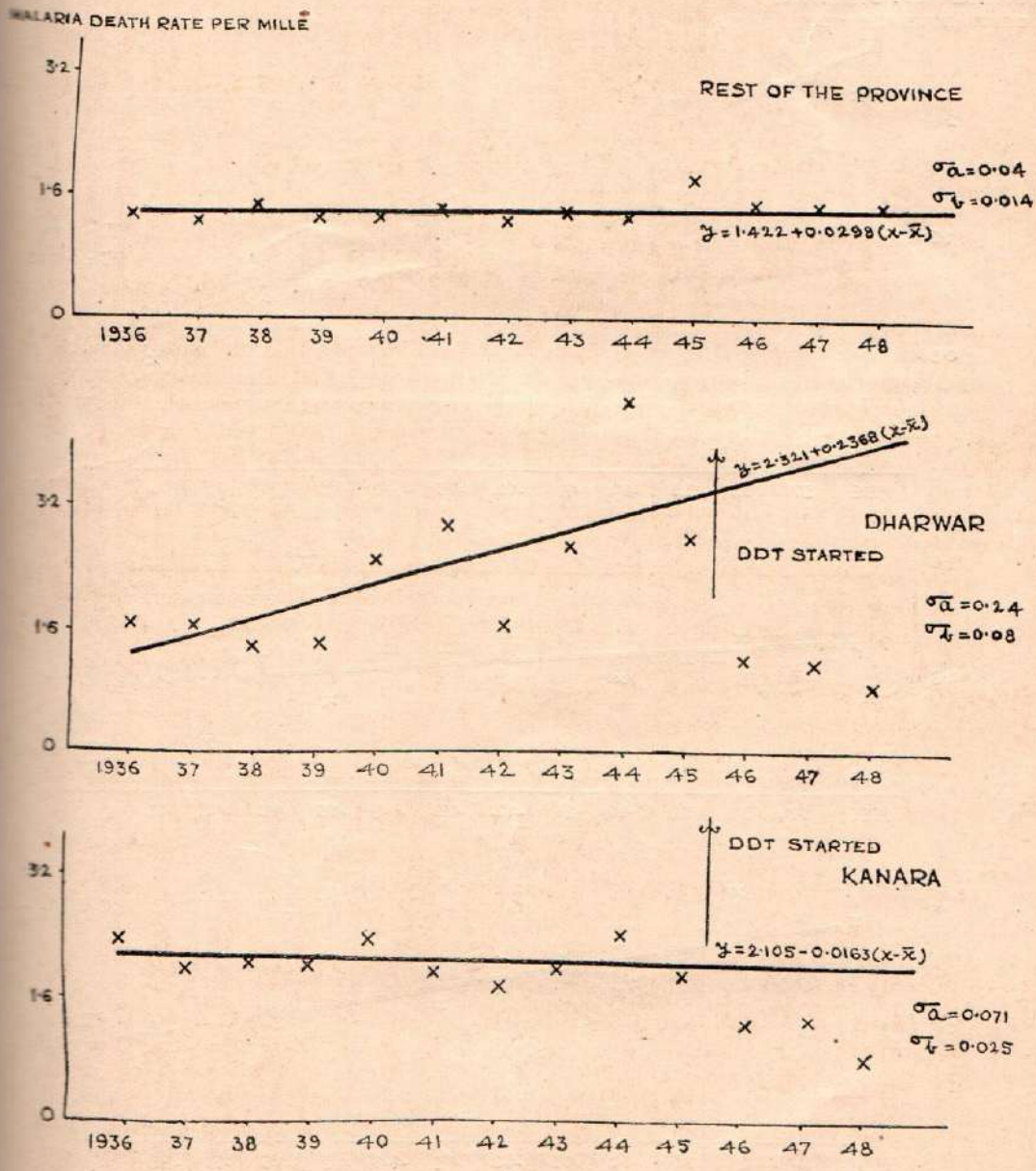
T.=*theoretical.* O.=*observed.*

In Dharwar and Kanara there is a very considerable fall in malaria death rates from the expected rates. In the rest of the province there is practically no difference between the observed and expected rates.

Diarrhœa and dysentery death rates (Graph 8)—.Appendix VII shows that the mean diarrhœa and death rate was highest in Kanara (2·184), lowest in Dharwar (0·891) and midway in the rest of the province (1·256), the means being significantly different from one another. In all areas the rates show a fall which is significant in the case of Kanara and the rest of the province but not so in Dharwar. The regression coefficients significantly differ from one another. The expected and

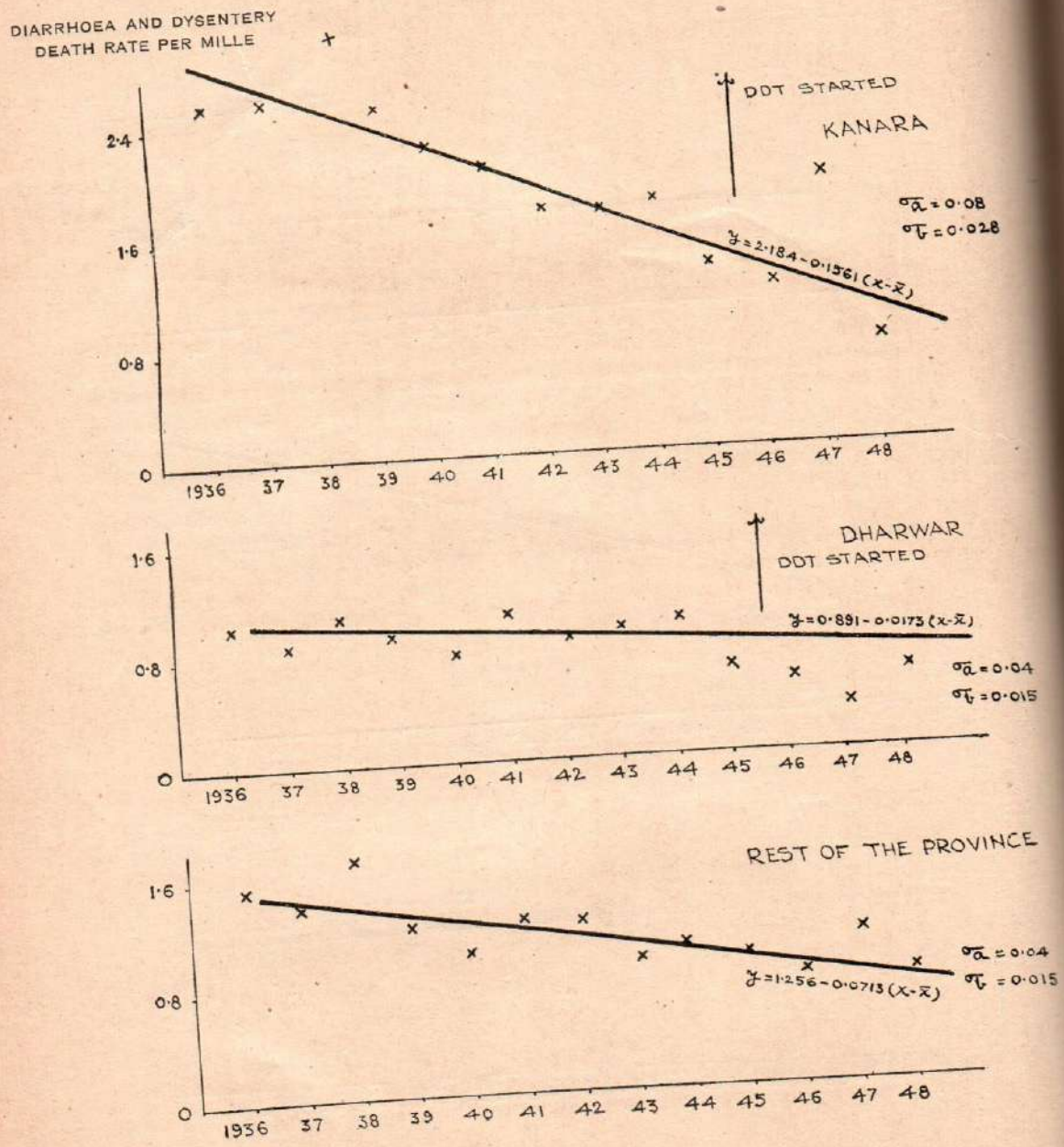
GRAPH 7.

Malaria death rates from 1936 to 1948 in Kanara, Dharwar and rest of the province.



GRAPH 8.

Diarrhœa and dysentery death rates in Kanara, Dharwar and the rest of the province.



observed diarrhœa and dysentery death rates in the three years 1946 to 1948 are shown below :—

Year.	Kanara.		Dharwar.		Rest of the province.	
	T.	O.	T.	O.	T.	O.
1946	1·33	1·18	0·80	0·52	0·86	0·86
1947	1·17	1·92	0·78	0·33	0·79	1·15
1948	1·01	0·75	0·77	0·57	0·72	0·81

T.=*theoretical.* O.=*observed.*

There is a reduction in diarrhœa and dysentery death rates in all the three years only in Dharwar. In Kanara, as in the rest of the province, it was higher than the expected rate in 1947.

Significance of rates in the post-D.D.T. period.—Before computing the theoretical rates in the post-D.D.T. period by extrapolation from the regression line, it will first be necessary to estimate if it gives a fair fit to the observed data. For this purpose it will be necessary to calculate their upper and lower limits of variation for each of the 10 years in accordance with the regression formula and at the level $P = \pm 0{\cdot}05$. The standard deviation of the rates as determined by the regression line at each year of observation is given by the formula—

$$s_{y \cdot x} = s_y \sqrt{\frac{1}{n} + \frac{(x - \bar{x})^2}{S(x - \bar{x})^2}}$$

The factor $(x - \bar{x})^2$ will be the same for $x = 1$ or 10, $x = 2$ or 9, $x = 3$ or 8, $x = 4$ or 7 and $x = 5$ or 6. Hence it would be enough for all rates to ascertain these 5 values and calculate the factor $\sqrt{\frac{1}{n} + \frac{(x - \bar{x})^2}{S(x - \bar{x})^2}}$ by which to multiply sy. These 5 values are calculated below :

x	$(x-\bar{x})^2$	$\frac{(x-\bar{x})^2}{S(x-\bar{x})^2}$	$\frac{1}{n} + \frac{(x-\bar{x})^2}{S(x-\bar{x})^2}$	Multiplying factor (sq. root of the previous column).
1	20·25	0·2545	0·3545	0·5954
2	12·25	0·1485	0·2485	0·4985
3	6·25	0·0682	0·1682	0·4101
4	2·25	0·0273	0·1273	0·3568
5	0·25	0·0030	0·1030	0·3209

The confidence interval is worked out below only for birth rates and death rates in the three areas.

BIRTH RATES.

Kanara.

Year.	Y	$^{s}y{\cdot}x$	$^{s}y{\cdot}x \times {}^{t}0{\cdot}05$	Confidence interval.	
				Upper limit.	Lower limit.
1936	32·69	0·72	1·66	34·35	31·03
1937	32·33	0·60	1·38	33·71	30·95
1938	31·97	0·50	1·15	33·12	30·82
1939	31·62	0·43	0·99	32·61	30·63
1940	31·26	0·40	0·92	32·18	30·34
1941	30·91	0·40	0·92	31·83	29·99
1942	30·55	0·43	0·99	31·54	29·56
1943	29·20	0·50	1·15	30·35	28·05
1944	28·84	0·60	1·38	30·22	27·46
1945	28·48	0·72	1·66	29·20	26·82

Dharwar.

1936	39·34	1·28	2·84	42·18	36·50
1937	38·63	1·07	2·46	41·09	36·17
1938	37·92	0·88	2·02	39·94	35·90
1939	37·22	0·76	1·75	38·97	35·47
1940	36·52	0·70	1·61	38·13	34·91
1941	35·81	0·70	1·61	37·42	34·20
1942	35·10	0·76	1·75	36·85	33·35
1943	33·40	0·88	2·02	35·42	31·38
1944	33·69	1·07	2·46	36·15	31·23
1945	32·98	1·28	2·84	35·82	30·14

Rest of the province.

1936	39·57	0·57	1·31	40·88	38·26
1937	38·86	0·48	1·10	39·96	37·76
1938	38·15	0·40	0·92	39·07	37·23
1939	37·44	0·34	0·78	38·22	36·66
1940	36·72	0·32	0·74	37·46	35·98
1941	36·01	0·32	0·74	36·75	35·27
1942	35·30	0·34	0·78	36·08	34·52
1943	34·59	0·40	0·92	35·51	33·67
1944	33·88	0·48	1·10	34·98	32·78
1945	33·17	0·57	1·31	34·48	31·86

DEATH RATES.

Kanara.

Year.	Y	$s_{y'x}$	$s_{y'x} \times t_{0.05}$	Confidence interval.	
				Upper limit.	Lower limit.
1936	29·14	0·90	1·97	31·11	27·17
1937	28·50	0·75	1·73	30·23	26·77
1938	27·85	0·62	1·43	29·28	26·42
1939	27·20	0·54	1·24	28·44	25·96
1940	26·55	0·50	1·15	27·70	25·40
1941	25·90	0·50	1·15	27·05	24·75
1942	25·25	0·54	1·24	26·49	24·01
1943	24·60	0·62	1·43	26·03	23·17
1944	23·95	0·75	1·73	25·68	22·22
1945	23·31	0·90	1·97	25·28	21·34
		Dharwar.			
1936	24·11	2·01	4·62	28·73	19·49
1937	24·84	1·68	3·86	28·70	20·98
1938	25·58	1·38	3·17	28·75	22·41
1939	26·30	1·20	2·76	29·06	24·54
1940	27·03	1·11	2·55	29·58	24·48
1941	22·76	1·11	2·55	30·31	25·21
1942	28·49	1·20	2·76	31·25	25·73
1943	29·21	1·38	3·17	32·38	26·04
1944	29·95	1·68	3·86	33·81	26·09
1945	30·68	2·01	4·62	35·30	26·06
		Rest of the province.			
1936	24·83	0·96	2·21	27·04	22·62
1937	24·85	0·81	1·86	26·71	22·99
1938	24·86	0·66	1·52	26·38	23·84
1939	24·88	0·58	1·33	26·21	23·55
1940	24·90	0·53	1·22	26·12	24·68
1941	24·91	0·53	1·22	26·13	24·69
1942	24·93	0·58	1·33	26·26	23·60
1943	24·95	0·66	1·52	26·47	23·43
1944	24·96	0·81	1·86	26·82	23·10
1945	24·98	0·96	2·21	27·19	22·77

The above confidence interval is shown in Graphs 3 and 4. The true regression line, of which the fitted line is only an approximation, lies somewhere in this confidence interval. As this is relatively narrow in the case of birth rates, it would seem permissible to extrapolate values from the fitted line and to assess the significance of the difference of observed rates in 1946, 1947 and 1948 from the extrapolated values. In the case of death rates the interval is narrow for Kanara and the rest of the province. But in Dharwar it is very wide and it can be seen that the true line may even have a downward slope instead of the upward slope exhibited by the fitted line. This however is already illustrated by the regression coefficient of the fitted line not significantly differing from O. However extrapolation of values for death rates in Dharwar beyond the period of fitting cannot be made with as much confidence as in the case of the other two areas. The same remarks also apply to natural increase rates, infant mortality rates and malaria mortality rates in Dharwar. This is in keeping with the known epidemiology of malaria in Dharwar with its tendency to prevalence of epidemics at ill-defined intervals. There would therefore be need to observe caution in interpretation of data and a much longer period is necessary for more precise assessment. Diarrhœa and dysentery rates in Dharwar District however do not exhibit wide variations. Hence extrapolation can be more validly resorted to.

The standard deviation of the difference between the observed and extrapolated values beyond the period of fitting is given by the formula which is shown below and which is slightly different from what has been used for defining the confidence interval in the period of fitting.

$$s_{y^1\,x^1 - Y^1\,x^1} = \sqrt{s^2_y \left[1 + \frac{1}{n} + \frac{(x^1 - \bar{x})^2}{S(x - \bar{x})^2}\right]}$$

Year.	x^1	$(x^1-\bar{x})^2$	$\frac{(x^1-\bar{x})^2}{S(x-\bar{x})^2}$	Multiplying factor for s^2_y.
1946	11	30·25	0·367	1·467
1947	12	42·25	0·512	1·612
1948	13	56·25	0·682	1·782

BIRTH RATES.

Kanara.

Year.	Standard deviation of difference between expected and observed.	Difference.	t.	Significant yes or no.
		$({}^{s}y^{1}\ x^{1} - Y^{1}\ x^{1})$		
1946				
1947	1·466	5·58	3·8	yes.
1948	1·536	5·18	3·3	yes.
	1·615	6·28	3·9	yes.
		Dharwar.		
1946				
1947	2·59	4·70	1·8	no.
1948	2·72	4·82	1·8	no.
	2·86	7·49	2·6	yes.
		Rest of the province.		
1946				
1947	1·17	0·49	0·4	no.
1948	1·22	0·84	0·7	no.
	1·29	1·71	1·3	no.

DEATH RATES.

Kanara.

1946				
1947	1·83	2·07	1·1	no.
1948	1·92	1·19	0·6	no.
	2·02	5·11	2·5	yes.
		Dharwar.		
1946				
1947	4·08	9·96	2·4	yes.
1948	4·28	8·80	2·0	no.
	4·50	13·25	2·9	yes.
		Rest of the province.		
1946				
1947	1·96	1·80	0·9	no.
1948	2·06	0·18	0·1	no.
	2·16	3·40	1·6	no.

NATURAL INCREASE RATES.

Kanara.

Year.	Standard deviation of difference between expected and observed.	Difference.	t.	Significant yes or no.
1946	2·80	9·12	3·3	yes.
1947	2·94	5·61	1·9	no.
1948	3·09	13·61	4·4	yes.
		Dharwar.		
1946	5·69	14·62	2·5	yes.
1947	5·97	13·58	2·3	no.
1948	6·27	20·69	3·3	yes.
		Rest of the province.		
1946	2·05	2·29	1·1	no.
1947	2·15	0·77	0·3	no.
1948	2·26	5·12	2·3	no.

INFANT MORTALITY RATE.

Kanara.

1946	13·4	14·5	1·1	no.
1947	14·1	4·0	0·3	no.
1948	14·8	33·0	2·2	no.
		Dharwar.		
1946	17·9	30	1·7	no.
1947	18·8	16	0·8	no.
1948	19·8	43	2·1	no.
		Rest of the province.		
1946	8·2	5·5	0·7	no.
1947	8·6	3·0	0·3	no.
1948	9·1	13·0	1·5	no.

MALARIA DEATH RATES.

Kanara.

Year.	Standard deviation of difference between expected and observed.	Difference.	t.	Significant yes or no.
1946	0·356	0·73	2·6	yes.
1947	0·343	0·67	2·3	yes.
1948	0·330	1·18	3·9	yes.
		Dharwar.		
1946	0·908	2·41	2·6	yes.
1947	0·952	2·67	2·8	yes.
1948	1·001	3·24	3·2	yes.
		Rest of the province.		
1946	0·157	0·07	0·4	no.
1947	0·163	0·14	0·8	no.
1948	0·173	0·14	0·8	no.

DIARRHŒA AND DYSENTERY DEATH RATES.

Kanara.

1946	0·31	0·15	0·5	no.
1947	0·32	0·75	2·4	yes (increase).
1948	0·34	0·26	0·8	no.
		Dharwar.		
1946	0·17	0·28	1·6	no.
1947	0·17	0·44	2·6	yes.
1948	0·19	0·20	1·0	no.
		Rest of the province.		
1946	0·17	0·00	0·0	no.
1947	0·18	0·36	2·0	no (increase).
1948	0·19	0·09	0·5	no.

The above analysis shows that in the post-D.D.T. period, there are in Kanara District a significant increase in birth rates and natural increase rates and a significant decrease in malaria death rates. There is no significant reduction in this district in total death rates or death rates due to diarrhœa and dysentery or in infant mortality. In the rest of the province there is no significant difference during 1946 to 1948 in any of the rates from the expected values in accordance with the trend from 1936 to 1945. Figures for Dharwar District cannot be assessed with any degree of confidence on account of their wide fluctuations except that there is an indication of increased birth rates in post-D.D.T. period and reduced death rates due to diarrhœa and dysentery. Further experience will show if the recorded lower death rates due to all causes and malaria are maintained, and the former liability of the district to epidemic prevalence of the disease at ill-defined intervals is held in check.

DISCUSSION.

Malaria has, through ages, contributed to the decline and fall of many empires and civilizations; facilitated the conquest of nations stricken with the malady and consequently weak; put a brake on the further conquest due to sickness amongst the invaders; stood as a barrier between human endeavour and accomplishment in many a scheme of exploitation of fertile land through irrigation and other agricultural pursuits and many large-scale engineering schemes of construction opening up highways of traffic through land or sea, and above all has always acted in a vicious circle by making the malarious poor and the poor malarious, more especially through its indirect effects even though the vector species of anophelines appears to be no respecter of social status or wealth.

In a series of illuminating papers, Sinton (1935*a*, 1935*b*, 1936) has estimated the enormous economic losses which occur in India due to this disease. In this paper the factual data over a relatively short period within human memory are presented and are statistically assessed which throw some light both on the effect of endemic malaria on the growth of population and on the results of malaria control measures with respect to some of the principal vital events.

In a fairly large portion of Kanara District, about 3,500 square miles in extent, the prevalence of hyperendemicity of malaria in practically all villages has contributed to a gradual decimation of human population. In the coastal area of this district which contains a far smaller number of villages with hyperendemic malaria and with practically no tendency to epidemic prevalence of the disease in other villages, there has been a steady growth of population from decade to decade but of a slightly smaller order than in the rest of the province which, however, is not statistically significant. In Dharwar District too, the growth of population has been of the same order as in the coastal taluks of Kanara District. If the district is taken as a whole, the number of hyperendemic villages in Dharwar District is slightly smaller than in the coastal taluks of Kanara District but there is a tendency for the prevalence of malaria in an epidemic form at ill-defined intervals in Dharwar District.

Gill (1928) has shown that during malarial epidemics in the Punjab, there is a very considerable reduction in the birth rate during the year following an

epidemic but there is a rapid return of the normal birth rates soon after, with even a slightly higher than normal birth rate in the second year after the epidemic. Likewise, he has also recorded an increase in death rates in the first and the last age groups during the epidemic year. Viswanathan (1936) has shown that the above phenomena are also manifest in malaria epidemics in the Deccan Plateau but these are liable to a more protracted duration with successive waves of increasing amplitude both in morbidity and mortality and that in the later phases of the epidemic even adult populations suffer a markedly higher mortality. Despite its tendency to epidemic prevalence of malaria, Dharwar District has exhibited a steady growth in its population. Likewise in coastal taluks of Kanara, there has been a steady growth despite a fifth of its area being hyperendemic for malaria. If the smaller growth rates in these areas are real though not significant, it would seem that the rest of the province has a smaller proportion of hyperendemic villages than in the coastal taluks of Kanara or a smaller liability to epidemic prevalence than in Dharwar District. These changes however do not apparently materially affect the census populations recorded at 10 yearly intervals so long as the epidemic year does not coincide with the period of census enumeration.

The hilly taluks of Kanara District showing decline in population are also the most fertile areas in the province and although the excess of deaths over births brought about by malaria due to its direct and indirect effects could not account in entirety for the decline in population, this ill health is the most dominant cause which has accounted for a considerable excess of emigration from the affected areas to other parts of the province. The population of Kanara District as a whole is not homogeneous. It consists of 2 groups, one under gradual decimation and the other showing normal increase. The two groups of population are equal in size but the density ratio is about 1 to 5. And it is the most fertile area that remains sparsely populated and hence inadequately exploited.

Birth rates.—It has not been possible to compile vital statistical data separately for the two broad divisions of Kanara District. Data are presented for Kanara and Dharwar districts as a whole and compared with those for the rest of the province. It would appear the mean birth rate of Kanara District during the period 1936 to 1945 is significantly lower than in Dharwar and in the rest of the province to the extent of about 5 per mille. There is no doubt that this smaller birth rate is mainly due to malaria as has been shown by Viswanathan (1945) in a study of the infant malaria parasite rates.

An interesting feature is the steady fall in birth rate throughout the province. The fall in the birth rate in Kanara District has been the least, presumably mainly because the mean birth rate is itself considerably lower and hence its capacity for reduction must be smaller.

It is apposite at this stage to state that the recording of vital statistics cannot be deemed to be complete anywhere in the province and there are no data to estimate the degree of completeness of reporting in different districts of the province. In a casual test check made by the author in Haliyal, a taluka headquarters station in Kanara District, in 1944, it was found that the completeness of recording of births is of the order of 85 per cent and of deaths 90 per cent, the bulk of the omissions regarding deaths being made up of infantile deaths. As much as 20 per

cent of infantile deaths failed to be recorded. The broad validity of conclusions arrived at in this analysis will not however be materially affected by the above lack of completeness of registration of vital events.

The recorded birth rates in Kanara and Dharwar are significantly higher during the post-D.D.T. period while they are more or less consistent with the theoretical rates in the rest of the province. Is this increase due to a rise in fertility rate or is it due to better recording ? Only a detailed house-to-house survey by trained workers would throw definite light on this question. Theoretically, it is possible that when any scheme of public utility is launched involving active contact with every householder, both the village officers who are in charge of recording the statistics and the people may show a better sense of responsibility and to that extent bring about better recording of the vital events. Whether the D.D.T. scheme which involves contact by the spraying team with the householder 3 times in a year at intervals of about two months would thus materially add to the public consciousness and a better sense of duty on the part of the village officers it is difficult to say. The very large reduction in malaria morbidity is however consistent with the hypothesis that the significant increase in the birth rates is partially at least due to an actual increase in fertility rates. Reduction in miscarriages, abortions, etc., due to reduction in malaria morbidity, may itself account for significantly larger birth rates.

In assessing the significance of the difference between the observed and theoretical rates during the post-D.D.T. period, the assumption is made that the rates would continue to show the same trend after 1945 as in the previous ten-year period. There are obvious limitations to such a trend. But in the short period of three years under study, the assumption may be deemed as valid especially if the same trend is maintained in the rest of the province. To give some indication of the confidence interval of the fitted line, the upper and lower limits are determined for birth rates and death rates. From these it could be seen that except for death rates in Dharwar (and by inspection malaria death rates, natural increase rates and infantile mortality rates) the other trends have a comparatively narrow interval and hence extrapolation from the fitted trend line for the period *immediately* after, can reasonably be resorted to. In any case due allowance is made for sampling variations in assessing the difference between the theoretical and observed rates.

Death rates.—The mean death rates in Dharwar and Kanara are significantly higher during the period 1936 to 1945 than in the rest of the province. The death rates in Dharwar show a tendency to rise during the period but they are very erratic. In Kanara, the death rates show a significant fall during this period. After the D.D.T. scheme, the fall in death rates in Dharwar appears to be significant in 1946 and 1948. On the whole there is no definite evidence available to show that the D.D.T. spraying operations have brought about any significant reduction in the total death rates in Kanara District. At all events if there is any reduction it is masked in the general trend for reduced total mortality rates.

Natural increase rates.—These rates are no doubt the cumulative effects of the previous two rates. But they provide an index of the probable growth of

population. These rates are significantly higher in Dharwar and in the rest of the province than in Kanara. They show a significant fall in the former two areas but in Kanara there is a small rise which however is insignificant. In 1946 and 1948, the natural increase rate is significantly higher in Dharwar and Kanara than expected. In the rest of the province, there is no significant change in the natural increase rate. On the whole, the D.D.T. operations seem to have brought about significant increase in the natural increase rates in the scheme area, in the hyper-endemic Kanara District principally through increased birth rates and in the epidemic district of Dharwar principally a decrease in death rates.

Infant mortality rates.—Although the recorded rates in 1946, 1947 and 1948 are slightly lower in Kanara and Dharwar, there is no demonstrable proof of their significance and further experience will alone show whether the reduction is maintained to a significant level.

Malaria death rates.—This index shows a most pronounced reduction in the scheme area as compared with the rest of the province. While the mean malaria death rate has been significantly higher in Kanara and Dharwar in 1936 to 1945 than in the rest of the province, after the D.D.T. operations were commenced they have shown a considerable fall, while in the rest of the province there has been practically no change. As the D.D.T. operations are mainly designed for malaria control, one would naturally expect the greatest significant reduction in malaria death rates, provided however the classification of causes of deaths may be considered of a reasonably high order. There is no warrant for this assumption. However the fact that the malaria death rate remained stationary in the rest of the province throughout the 13-year period from 1936 to 1948 and that it has shown a significant fall only in 1946 to 1948 in the scheme areas, may be taken as an adequate evidence of the effect of malaria control measures on the reduction in malaria death rates. This is not however due to any improved methods of treatment, but almost entirely due to a reduction in malaria morbidity itself as evinced by figures of malaria cases treated in the various dispensaries in the scheme areas. These figures show a reduction of more than 50,000 in 1948. As the dispensaries cater to only one-sixth of the population in the scheme area and as it can be reasonably assumed that malaria reduction has been fairly uniform, the total estimated reduction in malaria morbidity may be estimated as about 300,000 cases per year in the scheme area.

Diarrhœa and dysentery death rates.—These death rates show a fall in Dharwar District since 1946 but only the 1947 figures are significant. In Kanara and in the rest of the province, it is higher than the expected rate in 1947; 1948 has been an extremely healthy year throughout the province. It may be stated here that the D.D.T. spraying measures have been designed solely for malaria control through the destruction of vector species of anophelines. On account of the variations in the habits of the vector species in Dharwar and Kanara districts respectively, both human dwellings and cattlesheds are sprayed in Dharwar District but only the former in Kanara District, because in previous investigations it was found that there was only a very insignificant proportion of *fluviatilis*, the vector species in Kanara District resting in cattlesheds during day time. The question arises whether inclusion of cattlesheds in Dharwar District has contributed to a material reduction in fly population in addition to the mosquito population and consequently to a

reduction in diarrhœa and dysentery mortality through reduction in morbidity. This can only be precisely defined by estimates of fly densities in different parts of the scheme areas under different methods of treatment. The reduction in fly-borne diseases in Dharwar District, where cattlesheds are also sprayed, would seem to encourage the belief that the D.D.T. scheme with slight modifications is likely to yield collateral benefits other than malaria control by way of control of other insect-borne diseases as well. In fact, in the entire scheme area, there has not been a single case of human plague during the last three years although there have been a few cases of rat falls due to plague in all the years, and in 1948 in the adjoining Mysore State, there was a severe epidemic of plague.

From the foregoing observations two things are clear; first that in hyper-endemic malarious tracts there is a gradual decimation of population and as such tracts are also generally the most fertile, there is a progressive deterioration in the exploitation of land due to lack of man-power. In Kanara District, lack of man-power is partly due to a lower fertility rate brought about by malaria (decrease of about 5 per mille) and partly due to the excess of emigration on account of ill health. Compared with the rest of the province, there is only a slight excess in total death rates. This is a special feature of Kanara District and totally distinct from what obtains in other hyperendemic areas in the country of similar terrain but peopled by more backward communities. The economic condition of Kanara District is far better than in the latter areas. Again during the latter half of the ten-year period under study, economic conditions in Kanara were greatly improved on account of improved trade in betelnuts, better contracts for forest produce, better wages and better price for paddy, etc. Part at least of the significant down-ward trend in total mortality rates in Kanara may be due to these factors. The malaria death rate has however remained more or less stationary and it has been much higher than in the rest of the province (excluding Dharwar). The mean natural increase of about 4 per mille per annum in the whole district is more than offset by the excess of emigration with the result that man-power has remained stationary. And since only the more adventurous and more sturdy people would have resorted to emigration, the stationary population would be subject to a progressive deterioration both physically and mentally. If conditions have been not worse, it is because, as stated earlier, people are not backward and hence have managed to keep afloat in the past. The district contains the richest forests in the province, has great potentialities for harnessing energy out of its rivers, plenty of fertile cultivable lands and scope for location of many an industrial project depending on forest raw produce. In the past, malaria had been rightly advanced as the greatest barrier for an all-round development of the district.

The second clear lesson is that the above reproach is no longer valid. Nearly 90 per cent of the total population have been freed from the clutches of the disease. In this district during 1948, there have been a reduction in total deaths by 2,000 and an increase in total births by about 2,000, making a total rise of 4,000 in its population. Whatever the views of the students of demography on such a great rise in population with its bearing on food distribution, there is no doubt that in Kanara District, at all events, this is very desirable. For it will not be possible to colonize the area with people from far-off districts and only an increase in local stock would effectively conduce to development of agricultural land. It may be

a different matter in the case of industries with a promise of better wages, better housing and better all-round facilities. If the natural increase in 1948 is maintained in future, it would mean that every year there would be about 700 adults between 15 and 50 years saved from death (roughly 1/3rd of total deaths) and if it is assumed that for each death saved an expectation of life of 5 years would be added, there would be 3,500 adults more available every year from the fifth year onwards. From an increase in births of 2,000, not less than 1,200 more adults will be available for work from the 15th year onwards. Assuming that they will have a further expectation of life of 15 years, there will be about 18,000 people more adults available for work from the 30th year onwards. Thus to the existing 2 lakhs of adult population in the district there will be added from the 30th year onwards at least another 20,000 adults or about 10 per cent more.

To the above addition to man-power by saving deaths and increasing births, one has to add the increase in labour days and in all-round efficiency due to lowered incidence of sickness. In Kanara District, the existing dispensaries, which serve not more than one-sixth of the total population, show a reduction of 30,000 cases of malaria every year. On this basis the total reduction may be estimated at 180,000 every year. There is a reduction in malaria mortality rate of about 1·2 per mille or say about 500 deaths in 1948. Assuming the case mortality to be about 2 per cent (it is generally considered to be only one per cent), there should be a reduction of 250,000 cases of malaria in Kanara District. This is probably nearer the truth. Taking however only the dispensary figures, 180,000 cases of malaria saved every year would mean at least 1,080,000 labour days saved according to Sinton (1935*a*, 1935*b*) at the rate of 6 days lost due to every attack of malaria. Taking only the adult population into consideration this would mean 250,000 labour days saved. At the present lowest wage of Rs. 2 per day this involves a saving of Rs. 5,00,000 per year. Apart from that the saving of labour at the time of agricultural needs (sowing and harvesting) cannot be adequately priced. In plantation labour or in armies the health of labour or of soldiers can be maintained at a specified period by chemoprophylaxis. This is unavailing in civilian population. Hence the saving of labour for special needs of agriculture can never be adequately evaluated.

On the whole it has been shown that the D.D.T. scheme has helped to remove in a great measure the reproach that was hitherto associated with the development of Kanara District. More yet remains to be done. The smaller villages below 100 population have not yet been included in the scheme. It will cost more material, more supervision and of course more money. But if the sparse population of the district is to take a decisive upward trend in the near future and if a substantial proportion of the fallow lands is to be brought under cultivation, there would be need to include the smaller villages as well. This will lead to more certain development than attempts to colonize malaria-controlled areas in the district with immigrants from far-off districts and provinces. Before these smaller villages are included it would seem advisable to have an economic survey carried out in the malaria-controlled villages and in uncontrolled villages so that not only can a comparative idea of the economic conditions be obtained in the two groups but a more precise idea be obtained of the extent to which people in controlled villages have already started to cultivate lands which were hitherto fallow.

It would also seem advisable to include cattlesheds in D.D.T. spray and if possible to adapt it to general fly control so as to secure collateral benefits by way of control of other insect-borne diseases. Finally, a very strong plea is made that control of malaria in a district like Kanara, naturally fertile and peopled by not too backward a class of people, is far more likely to serve the objective of increased food production than in an equally fertile district but peopled with more backward classes such as primitive hill tribes.

SUMMARY.

(1) The effects of malaria on growth of population are discussed. In the hyperendemic portion of Kanara, there is a progressive decline from 1881 to 1941. In the coastal portion of this district and in Dharwar District, there is a progressive increase but of a smaller order than in the rest of the province but the difference is not significant.

(2) Hyperendemic malaria reduces birth rate by about 5 per mille, increases malaria death rate by about 0·75 to 1 per mille. Total death rates are only slightly raised presumably due to better economic conditions.

(3) Rural malaria control on an extensive basis has helped to reduce morbidity greatly, increase birth rates by about 5 per mille and only slightly, if at all, decrease total death rates.

(4) Malaria death rates are greatly reduced.

(5) In areas where cattlesheds are sprayed, deaths due to diarrhœa and dysentery are also reduced and in the entire area plague has been absent.

(6) It is estimated that in 30 years there will be 10 per cent more of adult population available for land development and every year at least 250,000 labour days saved from sickness.

(7) These changes make it desirable to consolidate still further by extending the benefits to smaller villages at additional expenditure which will however be more than repaid in increased food production to a far greater extent than in many similar areas but peopled with more backward classes.

(8) A plea is made for an economic survey of the villages under malaria control and without respectively so that further useful data may be made available on the economic advantages of malaria control.

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Appendix I.

Population growth (log values).

Kanara A.			Kanara B.			Kanara T.			Dharwar.			Rest of the province.		
Log value.	Summation.	Square of log value.					18·4358	6·8917	2·9513	21·1647	8·7102	4·1054	29·2352	16·8543
2·2998	13·2744	5·2891	2·3920	14·5570	5·7217	2·7649	15·8106	7·0209	3·0216	18·2134	9·1301	4·1610	28·1298	17·3139
2·2791	10·9746	5·1943	2·4219	12·1650	5·8656	2·6576	13·1609	7·0628	3·0455	15·1918	9·2751	4·1383	20·9688	17·1255
2·2227	8·6955	4·9404	2·4207	9·7431	5·8598	2·6339	10·5033	6·9374	3·0112	12·1463	9·0673	4·1668	16·8305	17·3622
2·1473	6·4728	4·6109	2·4171	7·3224	5·8424	2·6039	7·8694	6·7803	3·0132	9·1351	9·7094	4·1636	12·6637	17·3356
2·1596	4·3255	4·6639	2·4360	4·9053	5·9341	2·6209	5·2655	6·8691	3·0424	6·1219	9·2562	4·2166	8·5001	17·7797
2·1659	2·1659	4·6911	2·4693	2·4693	6·0974	2·6446	2·6446	6·9939	3·0795	3·0795	9·4833	4·2835	4·2835	18·3484
Total :— 13·2744	45·9087	29·3897	14·5570	51·1621	35·3210	18·4358	73·6901	48·5561	21·1647	85·0527	64·0016	29·2352	117·6116	122·1196
Mean :— 2·2124			2·42617			2·63368			3·02353			4·17646		
$\frac{n^1+1}{2}$	log value total 46·4604			50·9495			73·7432			84·6588			116·9408	
Sy(x—x)	—0·5617			0·2126			—0·0531			0·3939			0·6708	
S(x—x)²	17·5			17·5			28			28			28	
b	—0·032097			+0·012149			—0·001896			+0·014068			+0·023957	
b²S(x—x)²	0·018			0·0026			0·0001			0·0055			0·0161	
n^1Y^2	29·3683			35·31780			48·55389			63·99211			122·09974	
	29·3863			35·3204			48·5540			63·99761			122·1158	

$S(y-Y)^2$	0·0034	0·0006	0·0021	0·0040	0·0038
or ns^2					
n	4	4	5	5	5
s	0·029	0·0122	0·0205	0·0283	0·0276

$S(y-Y)^2$, KB 0·0006

$S(y-Y)^2$, R 0·0038

		$S(y-Y)^2$ D	0·004
		$S(y-Y)^2$ R	0·0038
			0·0078
ns^2	0·0044	n =	5+5 = 10
n =	4+5=9	s^2 =	0·00078
s^2 =	0·00049	$s^2/28$ =	0·000028
$s^2/28$ =	0·0000175	$s^2/28$ =	0·000028
$s^2/17·5$ =	0·000028		
Standard error of difference of b =	0·0067	Standard error of difference =	0·0075
		Diff/stand. error =	0·0099
			0·0075
t =	1·76	t =	1·3
P	lies between 0·2 and 0·1	P	lies between 0·3 and 0·2

Difference/standard error = 0·0118/0·0067

For testing significance of b from o

Calculate = $\frac{Sy}{\sqrt{S(x-\bar{x})^2}}$

and t = b divided by the above standard deviation.

For testing significance of means

Calculate = $\frac{Sy}{n^1}$

which gives the standard deviation of the mean.

Appendix II.

Birth rates.

Kanara.			Dharwar.			Rest of the province.		
y	Summation.	y^2	y	Summation.	y^2	y	Summation.	y^2
31·20	310·85	973·44	36·50	361·62	1332·25	38·67	383·69	1495·37
32·86	279·65	1079·78	37·73	325·12	1423·55	39·65	325·02	1572·12
33·06	246·79	1092·96	38·95	287·39	1517·10	38·15	285·37	1455·42
32·37	213·73	1047·82	38·71	248·44	1498·46	38·47	247·22	1479·94
30·73	181·36	944·33	38·70	209·73	1497·69	36·36	208·75	1322·05
29·97	150·63	898·20	35·80	171·03	1281·64	36·84	172·39	1357·19
31·10	120·66	967·21	37·84	135·23	1431·87	34·14	135·55	1165·84
31·98	89·56	1022·72	35·01	97·39	1225·70	33·18	101·41	1100·91
28·13	57·58	791·30	30·11	62·38	906·61	34·28	68·23	1175·12
29·45	29·45	867·30	32·27	32·27	1041·35	33·95	33·95	1152·60
Total :— 310·85	1680·26	9685·06	361·62	1930·60	13156·22	363·69	1941·58	13276·26
Mean :— 31·085			36·162			36·369		
	1709·675			1988·91			2000·29	
b	−0·3565			−0·7067			−0·7116	
s	1·21			2·18			0·965	
t	2·7			3·0			6·7	
P	lies between 0·02 and 0·05			0·02 and 0·01			less than 0·01	
Tests for significance of b								
	K and R						K and D	
Standard error of difference				0·155			0·270	
Difference				0·3551			0·3502	
t				2·3			1·3	
P				less than 0·05			between 0·1 and 0·2	
Means :—								
	K and R						K and D	
Standard error of difference				0·41			0·79	
Difference				5·28			5·077	
t				13			6·4	
P		less than 0·01					less than 0·01	

Appendix III.

Death rates.

Kanara.			Dharwar.			Rest of the province.		
y	Summation.	y^2	y	Summation.	y^2	y	Summation.	y^2
30·20	262·25	912·04	25·87	273·93	669·26	25·59	249·05	654·85
27·14	232·05	736·58	23·06	248·06	531·76	22·74	223·46	517·11
28·23	204·91	796·93	24·82	225·00	615·54	27·64	200·72	763·97
26·71	176·68	713·42	23·16	200·18	536·38	24·49	173·08	599·16
28·11	149·97	790·17	26·71	177·02	713·42	24·20	148·59	585·64
26·10	121·86	681·21	32·28	150·31	1042·00	25·51	124·39	650·76
23·01	95·76	529·46	27·65	118·03	764·52	24·26	98·88	588·55
23·24	72·75	540·10	31·47	90·38	990·36	22·93	74·62	525·78
26·31	49·51	692·22	33·93	58·91	1151·24	25·06	51·69	628·00
23·20	23·20	538·24	24·98	24·98	624·00	26·63	26·63	709·16
Total :— 262·25	1388·94	6930·37	273·93	1566·80	7638·48	249·05	1371·11	6223·58
Mean :— 26·225			27·393			24·905		
	1442·375			1506·615			1369·775	
b	−0·6477			+0·7295			+0·0162	
s	1·51			3·37			1·62	
t	3·9			1·97			0·09	
P	less than 0·01			less than 0·1 and greater than 0·05			greater than 0·09	
Tests for significance of b								
	K and R						D and R	
Standard error of difference 0·24							0·41	
difference		0·6639		0·7133				
t		2·77		1·74				
P		lies between 0·02 and 0·01		between 0·1 and 0·2				
Means :—								
Standard error of difference		0·7			1·18			
Difference		1·32			2·488			
t		1·9			2·11			
P		between 0·1 and 0·05			P is just above 0·05			

Appendix IV.

Natural increase rates.

Kanara.			Dharwar.			Rest of the province.		
y	Summation.	y^2	y	Summation.	y^2	y	Summation.	y^2
1·00	43·60	1·00	10·63	87·99	113·00	13·08	114·64	171·09
5·72	42·60	32·72	14·67	77·36	225·21	16·91	101·56	285·95
4·83	36·88	23·33	14·13	62·69	199·66	10·51	84·65	110·46
5·66	32·05	32·04	15·55	48·56	241·80	13·98	74·14	195·44
2·62	26·39	6·86	11·99	33·01	143·76	12·16	60·16	147·87
3·87	23·77	14·98	3·82	21·02	14·59	11·33	48·00	128·37
8·09	19·90	65·45	10·19	17·20	103·84	9·88	36·67	97·61
3·74	11·81	13·99	3·54	7·01	12·53	10·25	26·79	105·06
1·82	8·07	3·31	—3·82	3·47	14·59	9·22	16·54	85·01
6·25	6·25	39·06	7·29	7·29	53·14	7·32	7·32	53·58
Total :— 43·60	251·32	232·74	87·99	365·60	1122·12	114·64	570·47	1380·44
Mean :— 4·36			8·799			11·464		
	239·80			483·95			630·52	
b	0·1396			—1·4334			—0·728	
s	2·27			4·7			1·69	
t	0·5			2·8			3·9	
P	greater than 0·6			between 0·05 and 0·02			less than 0·01	
Significant tests of b	K and R			K and D			D and R	
Standard error of difference	0·31			0·57			0·55	
Difference	0·868			1·57			0·706	
t	2·8			2·8			1·28	
P	between 0·02 and 0·01			between 0·02 and 0·01			greater than 0·2	
Means :— Standard error of difference	0·893			1·66			1·58	
Difference	7·104			4·439			2·665	
t	about 8			2·7			1·7	
P	less than 0·01			between 0·02 and 0·01			between 0·1 and 0·2	

Appendix V.

Infantile mortality rates.

Kanara.			Dharwar.			Rest of the province.		
y	Summation.	y^2	y	Summation.	y^2	y	Summation.	y^2
206	1,765	42,436	144	1,461	20,736	167	1,633	27,889
178	1,559	31,684	129	1,317	16,641	161	1,466	25,921
196	1,381	38,416	141	1,188	19,881	176	1,305	30,976
176	1,185	30,976	135	1,047	18,225	153	1,129	23,409
192	1,009	36,864	151	912	22,801	170	976	28,900
170	817	28,900	161	761	25,921	159	806	25,281
160	647	25,600	134	600	17,956	162	647	26,233
158	487	24,964	156	466	24,336	157	485	24,649
176	329	30,976	177	310	31,329	165	328	27,225
153	153	23,409	133	133	17,689	163	163	26,569
Total :—								
1,765	9,332	314,225	1,461	8,195	215,515	1,633	8,938	267,063
Mean :—								
176·5			146·1			163·3		
	9707·5			8035·5			8981·5	

b	−4·55	+1·93	−0·527
s	11·1	14·81	6·8
t	3·7	1·2	0·7
P	less than 0·01	between 0·3 and 0·2	between 0·6 and 0·5
Significance of b	K and R	K and D	D and R
Standard error of difference	1·43	2·04	1·79
Difference	4·02	6·48	2·46
t	2·8	3·2	1·4
P	between 0·01 and 0·02	less than 0·01	between 0·2 and 0·1
Means :—			
Standard error of difference	4·12	5·88	5·16
Difference	13·2	30·4	17·2
t	3·2	5·2	3·3
P	less than 0·01	less than 0·01	less than 0·01

Appendix VI.

Malaria death rates.

Kanara.			Dharwar.			Rest of the province.		
y	Summation.	y^2	y	Summation.	y^2	y	Summation.	y^2
2·39	21·05	5·71	1·69	23·21	2·86	1·37	14·22	1·88
2·01	18·66	4·04	1·62	21·52	2·62	1·25	12·85	1·56
2·13	16·65	4·54	1·36	19·90	1·85	1·49	11·60	2·22
2·01	14·52	4·04	1·39	18·54	1·93	1·32	10·11	1·74
2·37	12·51	5·62	2·44	17·15	5·95	1·36	8·79	1·85
1·97	10·14	3·88	2·94	14·71	8·64	1·44	7·43	2·07
1·76	8·17	3·10	1·63	11·77	2·66	1·34	5·99	1·80
2·04	6·41	4·16	2·72	10·14	7·40	1·44	4·65	2·07
2·42	4·37	5·86	4·59	7·42	21·07	1·39	3·21	1·93
1·95	1·95	3·80	2·83	2·83	8·01	1·82	1·82	3·31
Total :— 21·05	114·43	44·75	23·21	147·19	62·99	14·22	80·67	20·43
Mean :— 2·105			2·321			1·422		
	115·775			127·655			78·21	
b	−0·0163			+0·2368			+0·0298	
s	0·2272			0·75			0·13	
t	0·65			2·8			2·08	
P	between 0·5 and 0·6			between 0·05 and 0·02			between 0·05 and 0·1	
Significance of b	K and R			K and D			D and R	
Standard error of difference	0·029			0·087			0·084	
Difference	0·0135			0·253			0·207	
t	0·5			2·9			2·5	
P	between 0·6 and 0·7			between 0·02 and 0·01			between 0·05 and 0·02	
Means :— Standard error of difference	0·08			0·245			0·240	
Difference	0·683			0·216			0·899	
t	8·5			0·88			3·7	
P	less than 0·01			between 0·5 and 0·4			less than 0·01	

Appendix VII.

Diarrhœa and dysentery death rates.

Kanara.			Dharwar.			Rest of the province.		
y	Summation.	y^2	y	Summation.	y^2	y	Summation.	y^2
2·58	21·84	6·66	0·99	8·91	0·980	1·56	12·56	2·43
2·60	19·26	6·76	0·79	7·92	0·624	1·44	11·00	2·07
3·11	16·66	9·67	1·06	7·13	1·124	1·73	9·56	2·99
2·53	13·55	6·40	0·92	6·07	0·846	1·26	7·83	1·59
2·25	11·02	5·06	0·76	5·15	0·578	1·07	6·57	1·14
2·08	8·77	4·33	1·05	4·39	1·102	1·26	5·50	1·59
1·76	6·69	3·10	0·86	3·34	0·739	1·26	4·24	1·59
1·76	4·93	3·10	0·90	2·48	0·810	0·99	2·98	0·98
1·82	3·17	3·31	0·97	1·58	0·941	1·02	1·99	1·04
1·35	1·35	1·82	0·61	0·61	0·372	0·97	0·97	0·94
Total :—								
21·84	107·24	50·21	8·91	47·58	8·116	12·56	63·20	16·36
Mean :—								
2·184			0·891			1·256		
	120·12			49·005			69·08	
b	−0·1561			−0·0173			−0·0713	
s	0·253			0·138			0·141	
t	6·1			1·1			4·5	
P	less than 0·01			between 0·3 and 0·4			less than 0·01	
Significance of b	K and R			K and D			D and R	
Standard error of difference	0·031			0·0316			0·0219	
Difference	0·0848			0·139			0·054	
t	2·7			4·4			2·5	
P	between 0·02 and 0·01			less than 0·01			between 0·05 and 0·02	
Means :—								
Standard error of difference	0·089			0·091			0·063	
Difference	0·928			1·293			0·365	
t	10·4			14·2			5·8	
P	less than 0·01			less than 0·01			less than 0·01	

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A NOTE ON A NATURAL OCCURRENCE OF SPOROZOITES OF PLASMODIUM IN *ANOPHELES TURKHUDI* LISTON.

BY

H. R. BHATT, L.C.P.S.

(*Medical Officer, Malaria Squad, Nasik, Malaria Organization, Bombay Province.*)

[May 11, 1949.]

DURING the course of routine dissections of anophelines in connection with a malaria survey of Kalwan and Dindori talukas of Nasik District, a specimen of *Anopheles turkhudi* was found naturally infected with malaria plasmodia. As there are no published records of a natural infection in this species, its details are presented in the short note in advance of the fuller findings of the survey to be reported at a later date. Covell's recent compilation (1944) does not include *turkhudi* among the species which are either recorded to have been found naturally infected or have been suspected to be vectors on epidemiological grounds. Christophers (1933) states that it has been experimentally infected with *P. falciparum* to the zygote stage. Russell, Rozeboom and Stone (1943) simply state regarding the relation of this species to malaria transmission that the data are inadequate.

A. culicifacies has been determined to be the principal human malaria vector in this area—4 out of 3,675 specimens dissected from October 1947 to March 1948 having been found infected (all gland infections). A good number of *A. fluviatilis* and *stephensi* have also been dissected but with negative results.

Out of 417 specimens of *A. turkhudi* dissected during the same months, one specimen showed sporozoites in the salivary glands. This specimen was collected on November 17, 1947, in a mixed dwelling in Kalwan and dissected on November 18, 1947. As the sporozoites appeared to be a little shorter and stouter than usual, they were measured. The average length of 7 sporozoites was 8·8 microns (largest 9·8 and smallest 8·4) as against an average length of 12·3 microns (largest 13·0 and smallest 11·2) of sporozoites found in a specimen of *A. culicifacies* from the same area. The sporozoites found in *turkhudi* do not obviously belong to any of the human plasmodium because of their short length and are probably of avian origin.

It is usual to presume that the natural infections found in anophelines from highly malarious areas are of human plasmodia. In this particular case it is shown that the sporozoites could not have been of the human species and therefore it stresses the need for caution in determining the status of a species as a malaria vector on the basis of only one or two infected specimens. The necessity of making of the permanent preparations and the measurements of the parasites in all the infections found in nature, are also emphasized.

A. turkhudi is fairly abundant in Kalwan and Dindori talukas of Nasik District, being next only to *culicifacies* in the order of abundance.

SUMMARY.

A natural occurrence of sporozoites of plasmodium probably of avian origin is recorded in a specimen of *Anopheles turkhudi* from Nasik District.

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INSECTICIDAL PROPERTIES OF HEXACHLOROCYCLOHEXANES, D.D.T. AND RELATED COMPOUNDS.

BY

(Miss) S. B. KULKARNI.

(*Indian Institute of Science, Bangalore.*)

[April 6, 1949.]

The insecticidal properties of gammexane and dichloro-diphenyl-trichloroethane (D.D.T.) have formed the subject of numerous investigations. The tests leading to the toxicity of the various isomers of hexachlorocyclohexanes and of D.D.T. and its related compounds have been reviewed by Slade (1945), Haller (1947) and Deonier and Jones (1946). The toxicity of these compounds as measured by percentage mortality is not at all proportional to time or concentration. The relative concentrations required to cause equal mortality are different at different mortality levels. Thus in the case of D.D.T., at concentration 0·005 (P.P.M.) the mortality is 83 per cent after 24 hours, and 97 per cent after 48 hours, while, at half the concentration, the mortality is 55 per cent after 24 hours and 82 per cent after 48 hours. Hence a large number of tests with various concentrations had to be carried out to find the minimum concentration for 100 per cent mortality. In spite of the patient work carried out, it was not possible to come to any conclusion as to the quantitative measure of the toxicity.

Dosage of toxic materials sufficient to ensure complete mortality of insects could not be determined very precisely because of the S-shaped curve between the dosage and the mortality.

Number of estimates of relative toxicity were made by applying various equations like $K_x^n = \frac{y}{100-y}$ (Bliss, 1935), $X = K + k \times \log \frac{y}{100-y}$ (Shepard, 1934), where x and y are the dosage and mortality and K, k, n, etc., same constants ; the calculated values were still distributed in a sigmoid manner.

Bliss (1934) has suggested the Probit method in which the mortality in Probits (probability integral of mortality) is plotted against logarithm of the concentration of the drug and a linear graph is obtained. This method of Probit analysis has been developed and applied by Finney (1942–1947) to large number of organisms.

Way and Sygne (1948) have applied this method in their study on the effect of D.D.T. and hexachlorocyclohexane on honey-bees. The Probit method involves introduction of the arbitrary constants and does not give any quantitative idea about the relative toxicity. The arbitrary probability units, Probits, are based

upon Pearson's original tables for statisticians and biometricians by shifting the origin, i.e. 0·01 per cent kill equal to 0·00 on arbitrary scale, and 5·0 for 50 per cent kill and 10·00 for 99·99 per cent kill and calculating the intermediate values in a symmetrical manner. This method is only of use in calculating the concentration of the drug for 100 per cent kill.

In the present paper the author has applied the law of unimolecular action $Kc = \frac{2\cdot3}{t} \log \frac{100}{100-x}$, where x is the percentage of insects killed in time t, and Kc is the mortality coefficient of the various toxicants at concentration C, Kc being directly proportional to concentration. The insects are regarded as the mass of molecules and the number of insects is small compared with the number of molecules of the insecticide. These assumptions have been justified by the constancy of the values of K calculated for various concentrations and times.

The constant K gives an idea not only of the toxicity of the compound but also of the relative order of toxicity in the case of various toxicants like gammexane, D.D.T., pyrethrins, etc. After establishing the fact that there is a certain toxicity constant for a particular toxicant, for the purpose of examining a number of compounds in the laboratory, it is not necessary to carry out so many tests which are so difficult and tedious at different concentrations and periods, unless the data is required for field work. One or two careful measurements of percentage mortality in given time for a given concentration should give a quantitative basis of the relative value of the toxicity.

The results of such calculations are given in the following tables :—

TABLE I.

Toxicity of hexachlorocyclohexane isomers to fourth instar larvæ of Anopheles quadrimaculatus.*

Isomer.	Dosage P.P.M. C.	Percentage mortality.		Mean K × 10^2 hour per unit conc.
		24 hr.	48 hr.	
α	2·5	88	92	28
		Kc(0·088)	(0·053)	
β	100	22	40	0·1
		Kc(0·013)	(0·016)	
γ	0·01	88	100	6700
		Kc(0·067)	...	
δ	2·5	40	62	8
		Kc(0·021)	(0·20)	

* Data from Orlando Fla Laboratory (Haller, 1947).

Table I shows the toxicity of the four isomers of hexachlorocyclohexanes to mosquito larvæ. Original data quoted by Haller (*loc. cit.*) is given in columns 2, 3 and 4. Immediately under the percentage mortality the values of mortality coefficients given by $Kc = \frac{2{\cdot}3}{t} \log \frac{100}{100-x}$ are shown. Kc is nearly constant irrespective of time, e.g. the values of Kc after 24 and 48 hours are 0·021, 0·020 for δ and 0·013, 0·016 for β isomer. Column 5 shows the mean mortality coefficient per hour per unit concentration, from which it can be concluded that γ is the most effective insecticide of the four isomers, being 240 times as effective as α and 840 times as effective as δ isomer, β having no insecticidal value.

TABLE II.

*Toxicity of hexachlorocyclohexanes to rats.**

Isomer.	Dosage per kg. weight.	Mortality in 7 days, per cent.	Kc.	$K \times 10^2$ per day per unit conc.
α	1·7	50	0·099	5·8
β		No animals were killed.		
γ	1·19	50	0·099	52
δ	1·0	50	0·099	9·9

* H. Taylor (Slade, 1945).

Table II shows the toxicity of hexachlorocyclohexanes to rats. Original data from H. Taylor is given in columns 2 and 3, the column 3 giving minimum concentration per kilogram body weight to give 50 per cent kill in 7 days. Column 5 gives the mortality coefficient K per day per unit concentration. It is evident that γ is the efficient toxicant, being in the case of rats 10 times as toxic as α and 5 times as toxic as δ isomer.

TABLE III.

*Relative toxicity of hexachlorocyclohexane isomers to grain weevils.**

Isomer.	Dosages, relative amount by weight.	Mortality in 5 days.	Kc per day.	$K \times 10^5$ per unit conc. per day.
α	900	50	0·138	15·3
β		Almost non-toxic.		
γ	1	50	0·138	13800
δ	5500	50	0·138	2·5
D.D.T.	15	50	0·138	920

* H. S. S. Bovingdon (Slade, 1945).

Table III gives the relative toxicity of hexachlorocyclohexane isomers and of p,p¹-D.D.T. to grain weevils. The original data by H. S. S. Bovingdon is shown in columns 2 and 3. The mortality coefficient Kc per day is given in column 4. From the mean mortality coefficient K (column 5), it will be seen that the γ isomer is 15 times as toxic as D.D.T. and 900 times as toxic as α isomer to grain weevils.

TABLE IV.

Toxicity of various insecticides to the larvæ of the yellow fever mosquito (Ædes ægypti).*

Insecticide.	lbs./acre C.	Percentage of larvæ dead after 1 day.	2 days.	3 days.	Mean K per day per unit conc.
Crude mix. of hexachloro-cyclohexane	0·5	0	23	93	1·0
	Kc	...	**0·13**	0·88	
	0·06	0	0	20	1·2
	Kc	...	...	0·074	
γ Isomer ...	0·5	0	97	100	3·5
	Kc	...	1·75	...	
	0·06	0	33	80	3·3
	Kc	...	0·20	**0·53**	
D.D.T. ...	0·5	0	43	97	2·3
	Kc	...	**0·23**	1·17	
	0·06	0	23	47	2·2
	Kc	...	0·13	**0·21**	
Cuprous cyanide	0·5	47	73	87	1·2
	Kc	0·63	0·48	0·70	
	0·06	30	40	65	5·8
	Kc	0·35	**0·25**	0·34	

* F. J. D. Thomas (Slade, 1945).
Abnormal values of Kc are shown in heavy type.

Table IV gives the relative toxicity of the various insecticides to mosquito larvæ. The original data is given in columns 2, 3, 4 and 5. The abnormal values of Kc are indicated in heavy type. From the mean mortality coefficient K (column 6), it will be seen that γ is 1·5 times as effective as D.D.T. Cuprous

cyanide appears to be an exception and is more effective than even the γ isomer in dilute solutions.

TABLE V.

Toxicity of D.D.T. and its isomers to fourth instar larvæ of Anopheles quadrimaculatus.

Isomer.	Dosage, P.P.M.	Mortality percentage after		Mean K.	$\mu \times 10^{13}$.	log K.
		24 hrs.	48 hrs.			
p,p¹-D.D.T.* ...	0·005	83	97	14·6	1·10	1·16
	Kc	0·073	0·074			
	0·0025	55	82	14·6		
	Kc	0·033	0·036			
m,p¹-D.D.T.† ...	0·05	100	100	2·6	1·50	0·42
	Kc	...	...			
	0·02	73	98			
	Kc	0·054	0·082			
	0·01	33	77			
	Kc	0·015	0·021			
o,p¹-D.D.T.† ...	0·025	100	100	1·6	1·90	0·20
	Kc	...	...			
	0·010	35	45			
	Kc	0·018	0·015			
o,o¹-D.D.T.‡ ...	5·0	...	17	0·0008	...	...
	Kc	...	0·004			

* Deonier and Jones (1946).
† Deonier, Jones and Haller (1946).
‡ Cristol and Haller (1946).

The toxicity of D.D.T. and isomeric compounds to mosquito larvæ is given in Table V. Original data on the toxicity tests by Deonier and Jones (*loc. cit.*) is given in columns 2, 3 and 4. Mortality coefficient Kc in the case of p,p¹-D.D.T. is proportional to the concentration C and is independent of the time, e.g. at concentration C = 0·005, Kc = 0·073 and at C = 0·0025, Kc is 0·033 which is

roughly half the value; from the values of K = Kc/C, the mortality coefficient per unit concentration per unit time, it will be obvious that p,p^1-D.D.T. is 7 and 10 times as toxic as m,p^1 and o,p^1 isomers.

TABLE VI.

*Toxicity of D.D.T. and pyrethrins to house-flies.**

Compound.	Conc. mg./c.c.	Mean mortality in one day.	Kc.	Mean K.
p,p^1-D.D.T. ...	1·00	93	...	1·7
	0·50	57	0·8	
Pyrethrins ...	1·18	40	0·5	0·51
	2·36	74	1·34	
	1·40	50	0·69	

* Gersdorff (1946).

Comparative toxicities of gammexane, p,p^1-D.D.T. and pyrethrins to house-flies have been determined by Gersdorff and McGovran (1945) at different concentrations and it has been found from graph that for 50 per cent mortality the γ isomer is 9 times as toxic as p,p^1-D.D.T. and 18 times as toxic as pyrethrins. The data on toxicity of p,p^1-D.D.T. and pyrethrins to house-flies by Gersdorff (1946) are given in Table VI. The mean mortality coefficients (K) for p,p^1-D.D.T. and pyrethrins are 1·7 and 0·51 from which it can be concluded that p,p^1-D.D.T. is 3 times as toxic as pyrethrins to house-flies.

CAUSE OF PHARMACOLOGICAL ACTIVITY OF p,p^1-D.D.T. AND GAMMEXANE.

The mode of action of gammexane is not yet very clear; the toxicity of gammexane has been attributed to the similarity in spatial structure of γ isomer with i-inositol, the activity is supposed to be due to the interference of gammexane with i-inositol content of the cells (Kirkwood and Phillips, 1946). The toxicity of p,p^1-D.D.T. has been ascribed to the HCl evolved on decomposition of p,p^1-D.D.T. According to Martin and Wain (1944), the toxicity of D.D.T. is due to its chemi-sorption at vital centres, by interference with essential system. As diphenyl-trichloroethane also readily loses HCl, but is non-toxic to insects, the toxicity of D.D.T. has been attributed to the lipoid soluble chlorophenyl group (Martin and Wain, *loc. cit.*).

ELECTRIC MOMENT AND THE TOXICITY OF THE INSECTICIDES.

TABLE VII.*

Insecticide.	$\mu \times 10^{18}$. (a)	$K \times 10^{2}$. (b)
α	1·7	2·8
β	0·0	0·01
γ	2·55	670·0
δ	0·0	0·8
p,p^1-D.D.T. ...	1·10	1460·0
o,p^1-D.D.T. ...	1·50	260·0
m,p^1-D.D.T. ...	1·90	160·0

* Author's Ph.D. Thesis submitted to the Bombay University, 1948.

(a) Kulkarni (1949). (b) Jatkar and Kulkarni (1949).

In Table VII are given the values of dipolemoments (μ) and mortality coefficients (K) of hexachlorocyclohexanes and of D.D.T. and isomeric compounds. In the case of hexachlorocyclohexanes, the comparison between the electric moment and the toxicity reveals the fact that the logarithm of mortality coefficient varies linearly with the dipolemoment of the isomers and that the γ isomer which possesses outstanding insecticidal properties has highest dipolemoment. As, however, the ϵ isomer, which is reported to be non-toxic, is likely to have a moment of 3·6 D, it appears that the insecticidal property requires a certain optimum electric moment as in the case of dissociation constant of sulpha compounds. The study of dipolemoment of o,p^1- m,p^1- and p,p^1-D.D.T. reveals the fact that in this series the logarithm of the mortality coefficient decreases with the increase of the dipolemoment, p,p^1-D.D.T. with lowest dipolemoment (1.1 D) being the most toxic compound.

SUMMARY.

The law of unimolecular reaction $Kc = \frac{2{\cdot}3}{t} \log \frac{100}{100 - x}$ is employed to calculate the mortality coefficients of the various insecticides and it has been shown that the mortality coefficient $K = Kc/C$ is independent of concentration and time. Gammexane (m.p. 112·5°C.) is the most toxic compound, being 240 times as effective as α and 840 times as effective as δ isomer towards mosquito larvæ. Of the D.D.T. isomers p,p^1-D.D.T. is 7 to 10 times as toxic as m,p^1- and o,p^1-isomers. (The compound TDE possesses equal toxicity as p,p^1-D.D.T.) Comparison of the toxicity of gammexane, p,p^1-D.D.T. and cuprous cyanide to mosquito larvæ of yellow fever, shows that gammexane is 1·5 times as effective as p,p^1-D.D.T., cuprous cyanide being nearly twice as effective as gammexane in dilute solutions. The correlation between

the electric moments and the mortality coefficient indicates that in hexachlorocyclohexane isomers the toxicity (log K) increases linearly with the moment which is the other way round in D.D.T. isomers.

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INVESTIGATIONS ON D.D.T. BARRIER SPRAY IN *A. LETIFER** AREAS.

BY

MAJOR C. P. NAIR.

(*Malaria Institute of India, Delhi.*)

[May 2, 1949.]

INTRODUCTION.

OUTDOOR spraying of insecticides to check insect nuisance has already been tried by a number of workers. Ginsburgh (1936) first used a diluted pyrethrum spray to protect audience from mosquito bites at outdoor gatherings in New Jersey. During the last war, D.D.T. preparations were used by the army as a barrier spray to combat malaria and other insect-borne diseases. Probably as a result of these experiments, Russell *et al.* (1946) and Afridi (1947) advocate the use of D.D.T. as a barrier spray to protect bivouacs, gun emplacements, observation posts, outdoor theatres and camps occupied for short periods by parties engaged on road and railway construction. Viswanathan and Parikh (1946) used a 5 per cent D.D.T. solution in light diesel oil as a barrier spray and came to the conclusion, on the basis of a single experiment, that while mosquitoes with half-digested blood can cross the sprayed area, freshly fed specimens which are more sluggish in their movements, may not be able to break the barrier. Puri and Pal (1947) conducted a single experiment on the effect of barrier spray with D.D.T. oil solution using $1\frac{1}{2}$ gallons per acre in *A. culicifacies* area and found that it did not affect the mosquito population resting inside huts within the barrier. Puri and Krishnaswami (1947), however, obtained encouraging results with D.D.T.-kerosene solution and D.D.T./M.K.E. emulsion using 1 to 3 gallons per acre in *A. minimus* area in some tea estates in Bengal Terai.

While serving in the army in Malaya in 1946, the author carried out a few experiments on the effect of D.D.T. barrier spraying in two villages, lying in the coastal plains of Klang District, Selangor. The results obtained have been discussed in this paper.

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* *A. letifer* of Malayan authors differs from *A. umbrosus* in the absence of propleural setæ and in possessing a group of sclerotised papillæ on the posterior hard palate of the pharynx, between the pigmented area and the region above the ventral papillæ [Sandosham, A. A. (2604 Japanese year)].

One village formed a part of Kampong Jalan Kebun and the other Kampong Sijankong lay on Klang-Morib Road. In both the villages malaria was hyper-endemic. In the preliminary survey carried out in March 1946, 71 to 75 per cent of children had enlarged spleens and 22 to 30 per cent showed malaria parasites in their peripheral blood. The species distribution was approximately 12 per cent *P. vivax*, 16 per cent *P. falciparum* and 72 per cent *P. malariæ* and the vector species was found to be *A. letifer*.

PLAN OF THE EXPERIMENT.

The experiments were meant to determine the residual effect of barrier spray against *A. letifer* group of mosquitoes using D.D.T. solution in grade III kerosene at 5 per cent and 2·5 per cent strength.

A 2·5 per cent D.D.T. solution, with a small amount of solvent groundnut oil added to it, was also tried.

In each village, four groups of more or less similar houses were selected. After making observations on the prevalence of mosquitoes in these houses, a fifty yards belt around the buildings was sprayed with varying strengths of D.D.T. solutions in two groups in the first village (sites 1 and 2) and in one in the second village (site 3). 5 and 2·5 per cent solutions respectively were sprayed at the rate of five gallons per acre in the sites 1 and 2. Site 3 was sprayed with 2·5 per cent solution to which groundnut oil had been added. In another group of houses in the second village (site 4), kerosene grade III unadulterated was sprayed. The remaining two groups of houses in each village were left untreated for purposes of comparison. The distance of the untreated groups of houses from those treated with D.D.T. solutions varied from 100 to 400 yards.

Sites 1 and 2 received four treatments, site 3 two, and site 4 only one treatment during the whole period of the experiment.

TECHNIQUE.

In each site, a perimeter line was drawn to include all houses or sheds and a belt of 50 yards wide was demarcated all along this perimeter either by fencing in or by driving pegs into the ground. The total area thus in each case varied from 3·2 to 9·2 acres.

The spraying squad of 6 to 12 men was made to stand in a line so that each man was separated from his neighbour by a distance equal to that of his extended arms. They walked in a row from a central place to the periphery of the fifty yards belt spraying uniformly on either side. In most cases MISH sprayers with their nozzle aperture sufficiently wide, to give a coarse spray, were used. In some cases knapsack sprayers and stirrup pumps were also employed. Reaching the peripheral limit, the men turned at right angles and marched along the fence to an unsprayed area. They then took up new positions and walked to the centre of the area, spraying as before. This process was repeated till every inch of the area (vegetation and all other features) inside the belt was sprayed as uniformly as possible. All vegetation was sprayed only up to a height of about 4 feet.

Mosquitoes were collected during night from 6 p.m. to 6 a.m. from buildings all lying within the sprayed area. The collections were made by a squad of 3 insect collectors, each man collecting in turn twice for two hours during the night. To ensure uniformity, as far as possible the same squad and the same persons in the squad were detailed for the collection from each site.

Pre-spray mosquito catches were made four times (on consecutive days) from the experimental and twice from comparison sites, and only the mean of these readings has been taken for the analysis of the data. After spraying, collections were made mostly on alternate nights but occasionally every night. Re-spraying was done in all sites only when the night catches included anopheline mosquitoes.

The data collected are given in a simplified form in Table I. Detailed records of collections from the different sites are given as an Appendix to this paper.

An analysis of the data recorded shows that no mosquito was found for about 9 days in the area sprayed with 5 per cent solution, and for 5 days in that sprayed with 2·5 per cent solution, and the density gradually increased, reaching a figure 10 per cent of the catches in the comparison site, in about 10 days in the area treated with 5 per cent solution and 7 days in the other two areas sprayed with 2·5 per cent solution. But to reach a figure 10 per cent of the pre-spray catch, it took 11 and 7 days in the areas sprayed with 5 per cent and 2·5 per cent solutions respectively. Anophelines appeared after 12, 13 and 9 days in areas sprayed with 5 per cent, 2·5 per cent, and 2·5 per cent (plus groundnut oil) solution in kerosene, respectively. In area sprayed with plain kerosene grade III, mosquito catches were almost as high as the density during the pre-spray period, on the very day of spraying (within 12 hours).

DISCUSSION.

Out of three types of equipment used, MISH sprayers, though laborious, were found to be very satisfactory for applying a uniform spray over the whole area. Knapsack sprayers, though very convenient to manipulate, were not fitted with suitable nozzle for uniformly applying the limited dosage of five gallons per acre. Stirrup pump, which is a 3-man unit, was unsuited, because of the various obstructions in the 50 yards belt such as thick bush and vegetation.

Although the comparison areas for experimental sites 1 and 2 were located about 100 and 250 yards away respectively, yet it was observed that as a result of spraying the experimental sites, there was a marked reduction of mosquito density in the corresponding comparison sites as well, 73 per cent of the pre-spray catch in site 1 and 57 per cent in site 2. But this reduction was practically nil in comparison site 3 which was located about 400 yards away from its corresponding experimental site 3.

It seems evident that the effect of barrier spray operates to a certain extent beyond the sprayed belt up to a distance of nearly 400 yards and hence the area used for comparison should be at least 400 yards away from the area sprayed.

An analysis of the data shows that the effect of the 5 per cent and 2·5 per cent solutions in respect of anopheline mosquitoes was almost the same. For culicine mosquitoes, however, the comparative effect is much less for the weaker solution.

TABLE I.

Results of spraying based on mosquito collection and expressed in average number of days after spraying.

Particulars of spraying solution used.	NUMBER OF DAYS AFTER WHICH THE NUMBER OF MOSQUITOES WENT UP.				NUMBER OF DAYS AFTER WHICH			
	To 10 PER CENT OF THE CATCH IN THE COMPARISON SITE.		To 10 PER CENT OF THE INITIAL CATCH FROM THAT AREA.		MOSQUITOES REAPPEARED.		ANOPHELINES REAPPEARED.*	
	Range.	Average.	Range.	Average.	Range.	Average.	Range.	Average.
5 per cent D.D.T. in kerosene grade III (site 1).	7 to 12	10	9 to 12	11	6 to 12	9	11 to 14	12
2·5 per cent D.D.T. in kerosene grade III (site 2).	...	7	...	7	3 to 6	5	11 to 14	13
2·5 per cent D.D.T. in kerosene grade III containing 2 per cent groundnut oil (site 3).	...	7	...	7	...	5	7 to 10	9
Plain kerosene grade III (site 4) ...	Less than 12 hours.				Less than 12 hours.			

* *A. letifer* was the first anopheline to appear in most cases.

The addition of 2 per cent groundnut oil to the 2·5 per cent D.D.T. solution in kerosene did not in any way affect the residual toxicity of the solution. In order to see whether the solvent had any appreciable effect, site 4 was sprayed with plain kerosene. Its effect on the mosquitoes did not last even for 12 hours.

CONCLUSION AND SUMMARY.

(1) Investigations to find out the residual effect of barrier spray in areas where *A. letifer*, an outdoor resting species, is the malaria vector, were carried out in two hyperendemic villages in Klang District, Selangor, Malaya, between May and June 1946.

(2) Four experimental sites were selected, out of which first and second were sprayed with 5 per cent and 2·5 per cent solution of D.D.T. in kerosene grade III respectively; third site with 2·5 per cent D.D.T. solution in kerosene grade III added with 2 per cent groundnut oil; and the fourth site was sprayed with plain kerosene grade III. The dosage applied in respect of each site was 5 gallons per acre.

(3) The technique of spraying is described.

(4) MISH sprayers, with their nozzle apertures sufficiently wide to give a coarse spray, were found to be most suitable for barrier spraying.

(5) The data collected have shown that the sites used for comparison should be at least 400 yards away from the area sprayed.

(6) Mosquito density was kept low to an extent of 10 per cent of the pre-spray and comparison site catches for 10 to 11 days with 5 per cent solution, and for 7 days, when 2·5 per cent solution was used.

(7) The area sprayed with 5 per cent D.D.T. solution remained completely free from mosquitoes for 9 days while those sprayed with 2·5 per cent D.D.T. solution were free for 5 days.

(8) Anopheline mosquitoes were absent for an average of 12 and 11 days from the areas sprayed with 5 per cent and 2·5 per cent solutions respectively.

(9) For anopheline control, 2·5 per cent D.D.T. spray seems to be almost as effective as a 5 per cent spray but for culicine mosquitoes, 5 per cent solution proved definitely superior to 2·5 per cent solution.

(10) Incorporation of 2 per cent groundnut oil in the 2·5 per cent D.D.T.-kerosene spray did not show any increase in the duration of the residual effect.

(11) Kerosene by itself, if used as a barrier spray, had no effect.

ACKNOWLEDGMENT.

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APPENDIX.

Details of mosquito catch from the experimental and comparison sites.

Experimental site number.	Date of spraying.	MOSQUITO COLLECTION FROM EXPERIMENTAL SITE. Anopheline.	Culicine.	MOSQUITO COLLECTION FROM COMPARISON SITE. Anopheline.	Culicine.	Number of days after spraying.
(1) (Sprayed with 5 per cent D.D.T. in kerosene grade III.)	Pre-spray*	3·5†	21·25	6†	18·5	...
	May 4, 1946	0	0	1	6	3
		0	0	1	12	5
		0	0	1	16	7
		0	0	1	18	10
		1	2	1	15	12
	May 16, 1946	0	0	1	14	4
		0	1	1	14	6
		0	4	0	21	9
		1	3	1	10	11
	May 28, 1946	0	0	...	...	5
		0	2	0	7	7
		0	0	0	14	9
		2	5	0	11	11
	June 11, 1946	0	0	...	...	5
		0	0	0	10	7
		0	0	0	7	9
		0	0	...	...	11
		0	3	2	8	12
		1	3	0	7	14

* Average of four days' collection in the experimental, and two days' collection in the comparison sites.

† Mostly *A. letifer*.

APPENDIX—contd.

Experimental site number.	Date of spraying.	MOSQUITO COLLECTION FROM				Number of days after spraying.
		EXPERIMENTAL SITE.		COMPARISON SITE.		
		Anopheline.	Culicine.	Anopheline.	Culicine.	
(2) (Sprayed with 2·5 per cent D.D.T. in kerosene grade III.)	Pre-spray*	7†	20	7·5†	20·5	...
	May 2, 1946	0	0	0	12	2
		0	0	1	6	5
		0	2	1	12	7
		0	3	1	16	9
		0	3	...	...	10
		2	10	1	18	12
	May 15, 1946	0	0	1	15	1
		0	1	0	37	3
		0	7	1	14	7
		0	4	0	21	9
		0	4	...	...	10
		1	4	1	10	13
	May 28, 1946	0	0	...	...	3
		0	2	...	...	5
		0	5	...	...	7
		0	6	...	...	9
		0	5	...	...	10
		0	5	0	11	11
		2	7	0	11	14
	June 11, 1946	0	0	...	...	3
		0	0	...	...	5
		0	2	0	10	7
		0	4	0	7	9
		0	4	...	...	11
		0	6	2	8	12
		1	6	0	7	14

* Average of four days' collection in the experimental, and two days' collection in the comparison sites.

† Mostly *A. letifer*.

APPENDIX—concld.

Experimental site number.	Date of spraying.	MOSQUITO COLLECTION FROM				Number of days after spraying.
		EXPERIMENTAL SITE.		COMPARISON SITE.		
		Anopheline.	Culicine.	Anopheline.	Culicine.	
(3) (Sprayed with 2·5 per cent D.D.T. in kerosene grade III containing 2 per cent groundnut oil.)	Pre-spray*	5†	74	5†	13	...
	May 30, 1946	0	0	6	20	3
		0	2	...	...	5
		0	8	...	...	7
		0	8	...	...	8
		1	9	2	22	10
	June 11, 1946	0	0	...	...	3
		0	4	...	...	5
		1	9	8	57	7
		4	15	1	60	9
(4) (Sprayed with plain kerosene grade III.)	Pre-spray*	2 †	33	...	...	...
	May 31, 1946	4	20	5	13	Same day.
		2	36	...	...	4
		3	33	...	...	6
		2	47	2	22	9

* Average of four days' collection in the experimental, and two days' collection in the comparison sites.

† Mostly *A. letifer*.

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CONTROL OF RURAL MALARIA WITH D.D.T. INDOOR RESIDUAL SPRAYING IN DELHI PROVINCE DURING THE YEAR 1948.

BY

LIEUT.-COLONEL JASWANT SINGH, M.B., Ch.B. (Edin.), D.P.H. (Eng.), D.T.M. & H. (Edin.),

AND

CAPTAIN DALIP SINGH, EX-I.A.M.C.

(*Malaria Institute of India.*)

[February 21, 1949.]

AN account of area, population, number of villages, incidence of malaria, selection of control measures, organization and details of spraying was given in detail by Afridi and Dalip Singh (1947). In this paper the results of second year of malaria control with D.D.T. are presented.

The scheme for malaria control with D.D.T. indoor residual spraying in Delhi Province was planned in May and put into operation from the first week of July 1947. The abnormal conditions prevailing during the malaria season of that year were, little rains during July and August, heavy rains and floods and communal riots and influx of refugees during September and October. The D.D.T. spraying operations and parasitological and entomological investigations had to be suspended as a result of communal tension and riots and important data could not therefore be collected.

During the year 1948, the rainfall was excessive and evenly distributed during July, August and September. The total rainfall during that period was 30·93 inches against an average of about 22 inches for this period. The first heavy shower of rain fell on July 9 and since then there had been showers of rain at intervals of 3 to 7 days.

The entire riverain area was flooded due to the abnormal rise in the River Yamuna which was sustained for a long period during August and

September. The relative humidity was also favourable for the transmission of malaria.

The rainfall and floods in River Yamuna during the malaria season of 1948 were comparable to those during the corresponding period in 1942, an epidemic year throughout the Indo-Gangetic Plains.

The rainfall and flood level figures for the years 1942 and 1948 are given in Tables I and II respectively and the data about flood levels in the river is also shown in Chart 1.

TABLE I.

Rainfall in inches during 1942 *and* 1948.

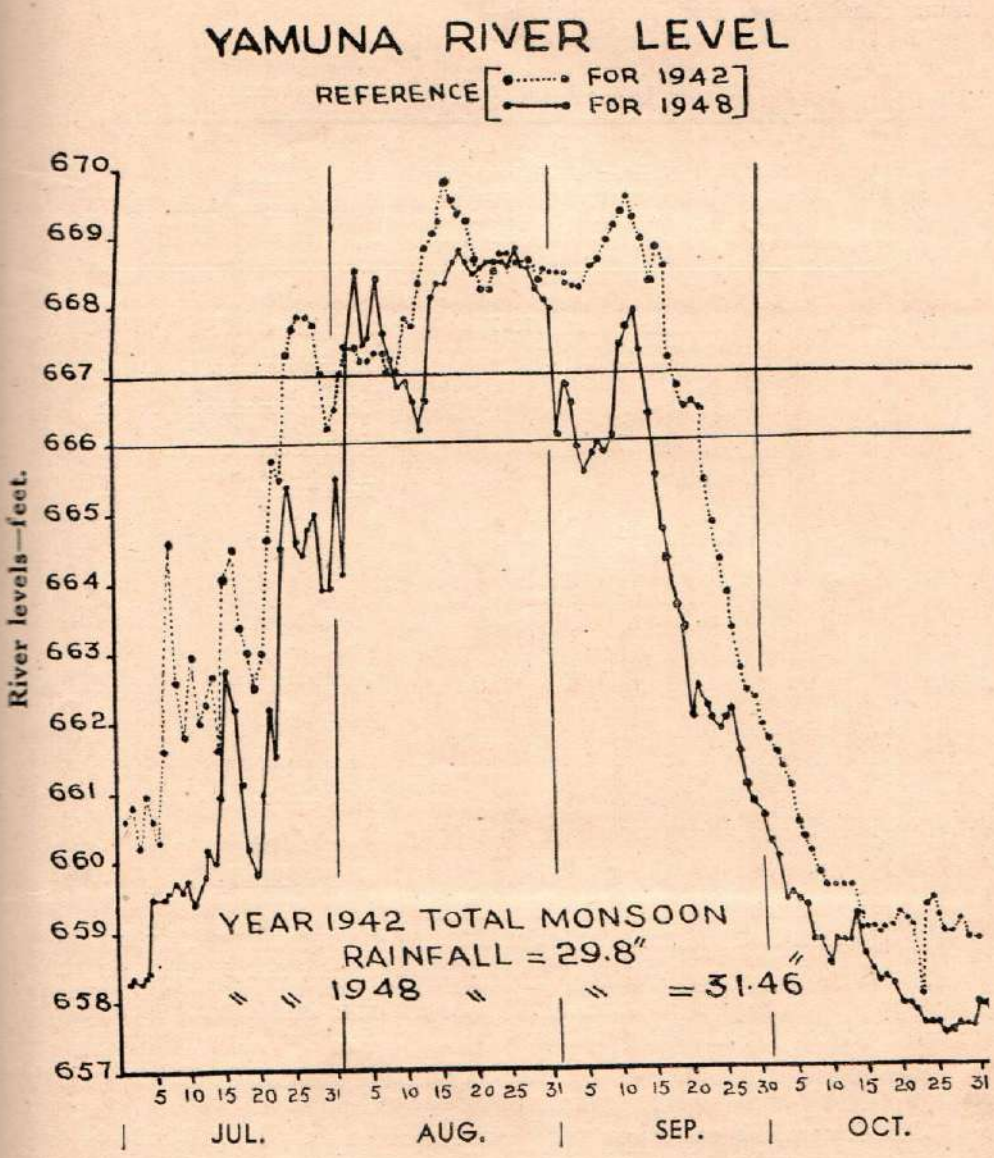
Month.	1942.	1948.
July	13·93	6·98
August	7·60	14·67
September	5·38	9·28
TOTAL ...	26·91	30·93

TABLE II.

Flood levels of River Yamuna during 1942 *and* 1948, *above mean sea level, recorded at the Electric Power House, Delhi, in feet.*

Month.	1942.		1948.	
	Highest.	Lowest.	Highest.	Lowest.
July	667·9	661·1	665·4	658·3
August	669·8	666·5	668·8	665·1
September ...	669·5	661·9	667·8	661·0

CHART 1.



ORGANIZATION.

The regular staff sanctioned in the scheme (Afridi and Dalip Singh, 1947) remained as before but the temporary labour was cut down to meet the situation arising out of application of enhanced scales of dearness allowance for labour. As

such there was a radical change in the organizational set up of the malaria spraying teams, details of which are given below :—

Details of personnel of malaria spraying teams.

Zone.	Number of spraying teams.	Number of stirrup pumps working at a time in one team.	Personnel of one spraying team.
1947			
Narela ...	2	9	Malaria inspector ... 1 Driver or mechanic ... 1 Superior field workers 4 Field workers ... 22
Nangloi ...	2	9	Do.
Badarpur ...	2	9	Do.
1948			
Narela ...	2	8	Malaria inspector ... 1 Driver or mechanic ... 1 Superior field workers 2 Field workers ... 19
Nangloi ...	2	8	Do.
Badarpur ...	2	6	Malaria inspector ... 1 Driver or mechanic ... 1 Superior field workers 2 Field workers ... 15

D.D.T. spraying operations were commenced on July 12 and brought to a finish on September 30. Eighty-nine villages showing spleen rates between 5 and 20 per cent received only one spray during the season while those with spleen rates above 20 per cent (159) received two sprays during the season and those below 5 per cent (47) were not sprayed. This included all the villages situated in the 3-mile belt area which surround Delhi urban area. In the 3-mile belt, villages showing spleen rates less than 5 per cent were also sprayed.

Villages earmarked to be sprayed twice received the first round of spraying from July 12 to August 12 and the second round from September 1 to September 30. During the intervening period from August 13 to August 30, only villages requiring one treatment were sprayed.

As a result of reduction in the number of superior field workers and field workers in Narela and Nangloi zones, one superior field worker had to supervise the working of four stirrup pumps. The programme of spraying was prolonged for 15 extra days, i.e. up to September 30, 1948, which afforded adequate protection from malaria. The transmission season commences in July and ends in November.

SPRAYING MATERIAL USED.

The formulation of D.D.T. spray used was D.D.T.-M.K.E. soap emulsion. The stock emulsion was prepared at the Malaria Institute of India under the supervision of a malaria inspector. The following formula was used :—

D.D.T. technical	...	...	110 lbs.
M.K.E.	...	...	36 gallons.
Soap solution, 20 per cent		...	4 gallons.

Approximately 44 gallons of stock emulsion (25 per cent) resulted from the above ingredients. The actual technique employed in preparing the 25 per cent D.D.T. emulsion concentrate was as follows :—

One barrel (44 to 48 gallons capacity) with open top was buried in the ground so that its upper edge was about 6 inches above ground level to avoid ground dust falling into it. The required quantity of D.D.T. (110 lbs.) was put in the barrel and lumps were broken by a wooden rod. Then about 4 gallons of M.K.E. was mixed with D.D.T. making a thin paste. More M.K.E. was added gradually while shaking the contents vigorously till all the M.K.E. was poured. Four gallons of 20 per cent soap solution, prepared separately, was added and thoroughly mixed. The resulting 25 per cent D.D.T.-M.K.E. soap emulsion was taken out by buckets and poured into empty barrel for transportation to the spraying teams. The whole process lasted about two hours.

The D.D.T. emulsion in concentrate form was supplied to the malaria spraying units in 44-gallon barrels. The spraying teams before going out for spraying were supplied with 4-gallon tins of this emulsion after vigorously shaking the bulk so that the consistency remained homogeneous. On the work spot one part of stock emulsion was diluted with 9 parts of water. Thus 4 gallons of stock emulsion was put into a 44-gallon barrel and water drawn from a local well added gradually bringing the contents up to 40 gallons. The final strength of the emulsion was 2·5 per cent.

Part of the D.D.T. was dissolved in aromax, a substitute of M.K.E., supplied by Messrs. Burmah-Shell Oil Storage & Distributing Co. of India Ltd. This solvent gave a better emulsion.

TECHNIQUE OF SPRAYING AND DOSAGE.

The equipment used for spraying was double-barrel stirrup pumps with release valve and spray head nozzle of 1/32 inch aperture supplied by the Modern

Technical Industries, Delhi. The average output of these pumps was about 20 oz. of fluid per minute.

The dosage of D.D.T. applied was 50 mg. per square foot, i.e. 2 c.c. of 2·5 per cent D.D.T. emulsion per square foot of surface area.

The method utilized for application of this dose was by regulating the up and down movement of the stirrup pump lance by making the field worker to count one—two at a specified rhythm. This method was practised for a week before the start of the spraying operations. A measured surface, say 600 square feet, was marked and 1,200 c.c. of spraying fluid supplied. The field workers were asked to spray that surface evenly regulating the up and down movements of stirrup pump lance.

SPRAYING MATERIALS CONSUMED.

The amounts of D.D.T., M.K.E. and soap used are given below :—

D.D.T.	...	...	...	10,380 lbs.
M.K.E.	...	...	...	3,888 gallons.
Soap	...	...	...	984 lbs.
Malariol	...	...	...	1,616 gallons.

STRUCTURES SPRAYED.

All human, animal, mixed dwellings and verandahs were sprayed from inside only. All the walls, ceilings up to 10 feet height (most of the ceilings in the villages of Delhi Province are about 10 feet high) or as far as the field worker's fully extended arm could reach, were sprayed. All the resting places of mosquitoes, i.e. furniture, household articles, hanging clothes, firewood and stocks of cowdung cakes, shelves, etc., were sprayed. The only articles not sprayed were cooking utensils and foodstuff.

TRANSPORT AND SPRAYING PROGRAMME.

The mechanical transport supplied to the spraying units was one truck per unit and this was found adequate for the execution of spraying operations. The additional reserve trucks at the Institute were used for supplying the insecticide and transporting the checking staff and barrels to and fro.

It may be stated here that the mechanical transport at the disposal of the organization played a very important rôle in the successful execution of spraying operations.

ANTILARVAL MEASURES.

In addition to D.D.T. residual indoor spraying, antilarval measures were carried out in villages situated within the 3-mile belt and the towns of Narela, Najafgarh, Mehrauli and Shahdara.

FINANCIAL EFFECT.

Actual expenditure during the year, i.e. from January 1 to December 31, 1948, is given as below :—

	Rs.	A.	P.
Pay of establishment	20,492	9	0
Allowances including dearness and travelling	12,187	9	0
Contingencies :			
Pay and allowances of labour	22,393	8	0
Maintenance of mechanical transport and petrol ...	6,250	6	9
D.D.T., etc.	47,957	5	0
Miscellaneous	3,867	11	6
TOTAL ...	1,13,149	1	3

The *per capita* cost works out at Re. 0-5-10 per head per year. The present population of Delhi rural area is about 3,10,000.

RESULTS.

In Table III and Chart 2 are given the data collected to assess the effect of D.D.T. residual spraying on densities of anopheline mosquitoes in a sprayed and an unsprayed village.

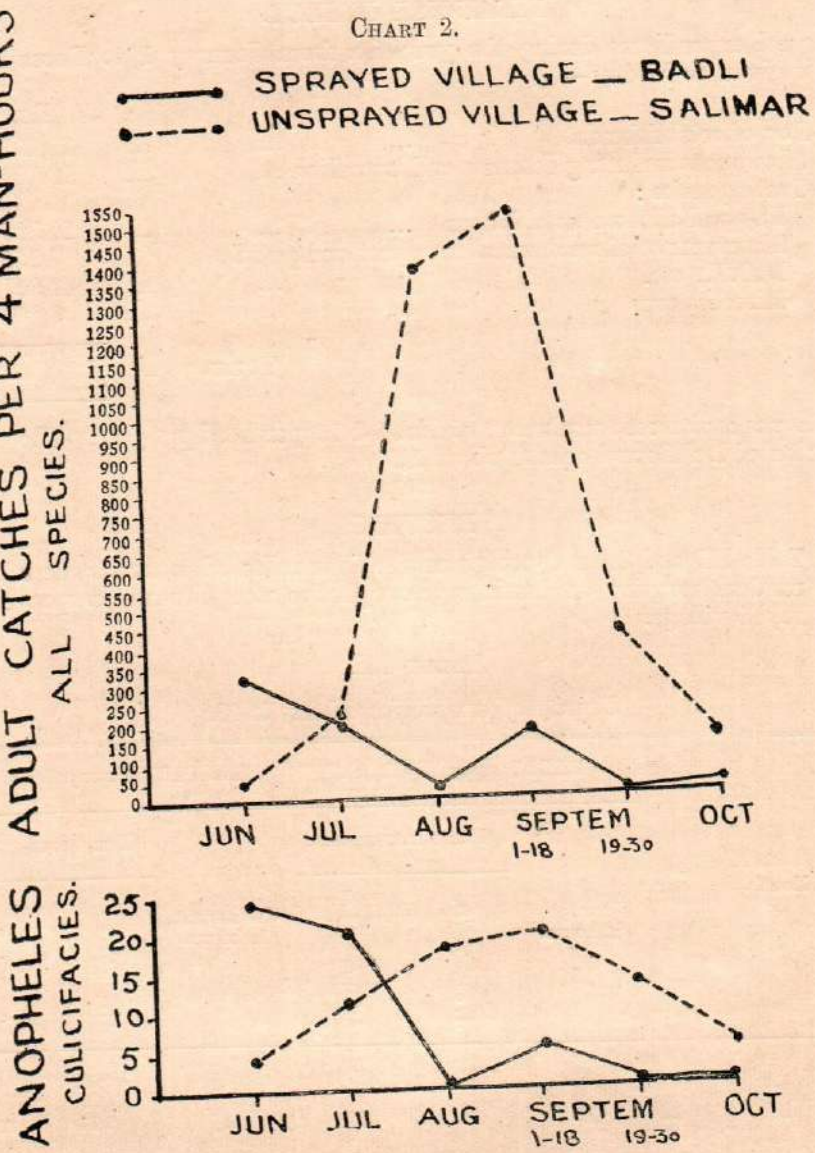
TABLE III.

Density of anopheline adults, 1948.

Month.	BADLI (sprayed village).			SALIMAR (unsprayed village).		
	Time spent in hours.	PER 4 MAN-HOURS.		Time spent in hours.	PER 4 MAN-HOURS.	
		A. culicifacies.	All anophelines.		*A. culicifacies.*	All anophelines.
Jan.	3½	24	320	3½	4·57	65·14
Jul.	12	21	209	13½	7·11	233·1
Aug.	12½	0·32	22·08	10½	17·5	1,361·8
Sep. 1 to 18 ...	7½	5·86	177·06	7	20·0	1,515·4
Sep. 19 to 30 ...	4½	0	0	5	12·8	403·2
Oct.	3½	0	6·85	5	4·8	152·8

Note.—Badli was sprayed on July 31 and again on September 18.

CHART 2.



Both these villages are situated in the tract irrigated by Western Yamuna Canal and are half a mile apart from each other.

It is quite evident from this data that *A. culicifacies* and the other anopheline catches were higher in the sprayed village than in the unsprayed village before the application of spray. After the application of D.D.T. in Badli (sprayed village),

the *culicifacies* density was reduced to 98·5 per cent for a month whereas the culicifacies and all anopheline density in the unsprayed village kept on rising.

The second spray of D.D.T. applied as late as September 18 was effective for reduction of density of mosquitoes for a much longer period and the effects were more pronounced than the first spray applied on July 31.

EFFECT ON SPLEEN AND PARASITE RATES.

Comparative spleen rates for the years 1947 and 1948 are given in Table IV and Charts 3 and 4.

TABLE IV.

Spleen rates in certain selected villages during 1947 *and* 1948, *per cent.*

Villages.	1947.	1948.	
	March-April.	March-April.	November.
Barari	32·3	33·3	20·0
Palla	37·8	20·6	6·9
Mandauli	37·3	11·5	6·8
Bawana	22·3	13·9	15·0
Shahabad Daulatpur	40·0	14·8	6·6
Kanjaula	30·6	26·6	17·5
Bahlolpur Dehri	71·1	42·1	21·6
Basi Daryapur	33·3	17·1	13·0
Fatehpur Beri	25·6	16·0	15·3
Palam	20·0	10·0	13·9
Tughlakabad	31·3	5·0	3·4
Badarpur	64·5	21·9	26·9
Madanpur Khadar	50·0	15·6	12·1
Tikhand	65·9	50·0	45·4
Badli	38·8	19·1	20·6
Salimar	50·0	40·5	52·1 (Not sprayed. Comparison village).

All these villages were treated with D.D.T. residual indoor spray during the malaria seasons of 1947 and 1948 except Salimar which was not sprayed during 1948.

CHART 3.

SPLEEN RATE OF 14 SELECTED VILLAGES – DELHI PROV:

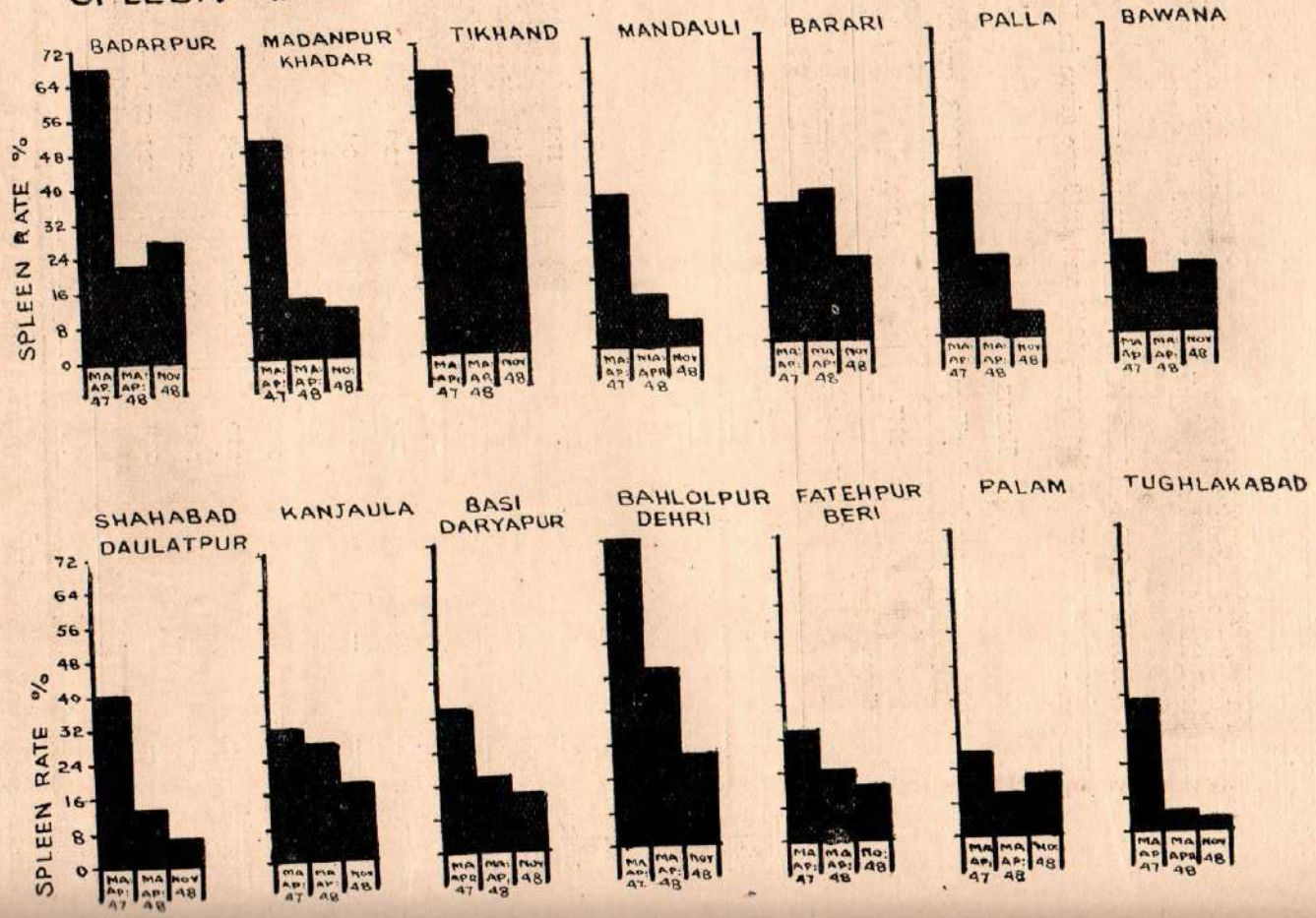
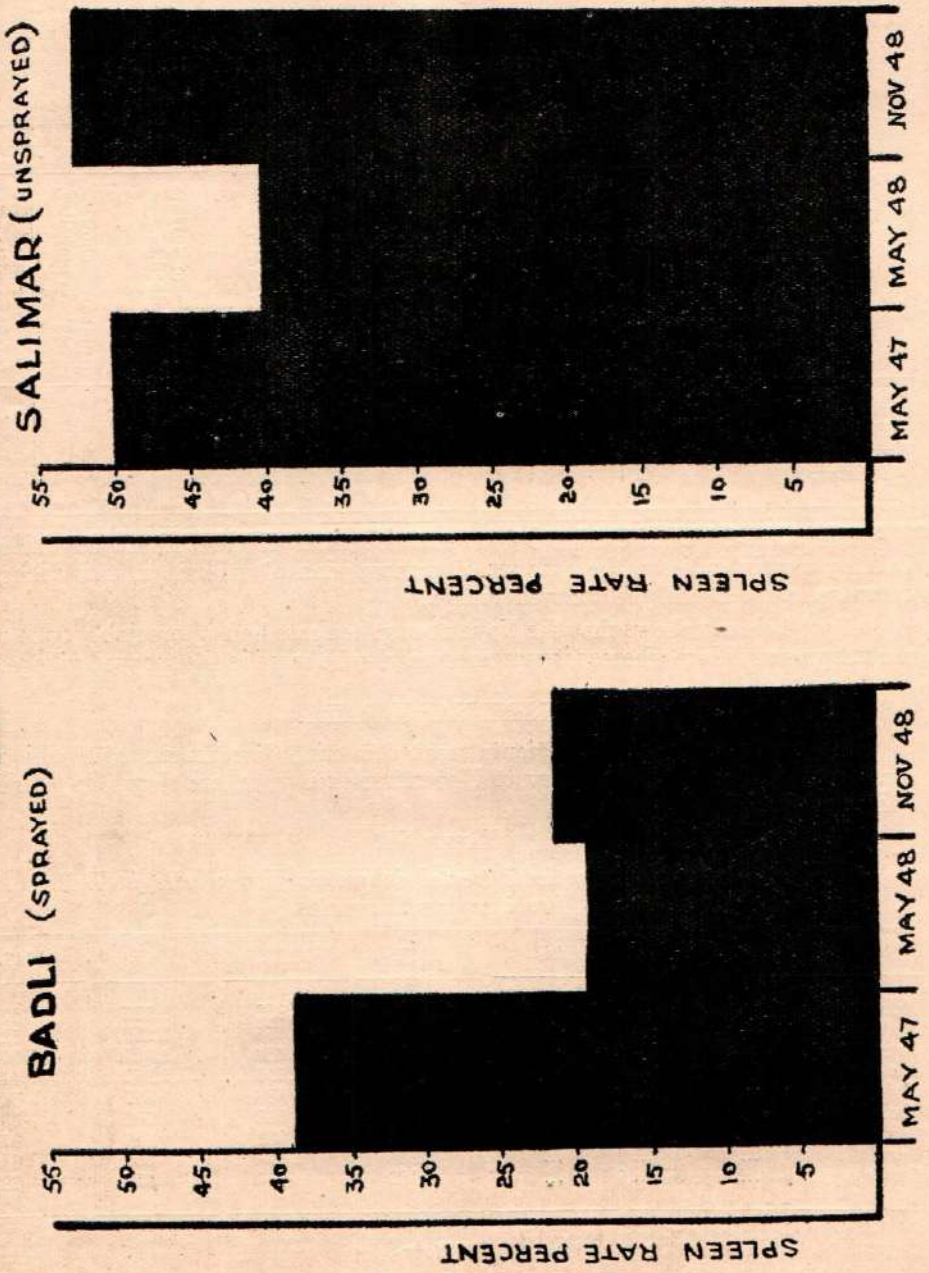


CHART 4.


SPLEEN RATES
BADLI (SPRAYED)
SALIMAR (UNSPRAYED)
SPLEEN RATE PERCENT
55
50
45
40
35
30
25
20
15
10
5
MAY 47
MAY 48
NOV 48

In Table V is given the effect of D.D.T. indoor residual spraying on spleen rate, parasite rate and infant parasite rate in a sprayed and unsprayed village.

TABLE V.

Spleen, parasite and infant parasite rate in a sprayed and an unsprayed village.

Village.	Spleen rate.			Parasite rate.			Infant parasite rate.		
	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined, blood slides.	Number showing malaria parasites.	Parasite rate, per cent.	Number of infants examined.	Number showing malaria parasites.	Infant parasite rate, per cent.
Sprayed village—Badli.	63	13	20·6	63	2	3·1	26	0	0
Unsprayed village—Salimar.	23	12	52·1	23	4	17·3	10	2	20

The spleen, parasite and infant parasite rates in the unsprayed village of Salimar clearly indicate the amount of transmission of malaria in this village as compared with Badli, the sprayed village.

Chart 4 clearly shows that the November spleen rate had appreciably gone up in the unsprayed village whereas it remained nearly the same in the sprayed village.

DISPENSARY DATA.

The number of malaria patients treated in nine dispensaries of Delhi rural area from 1944 to 1948, January to November each year, are shown in Table VI and Chart 5.

TABLE VI.

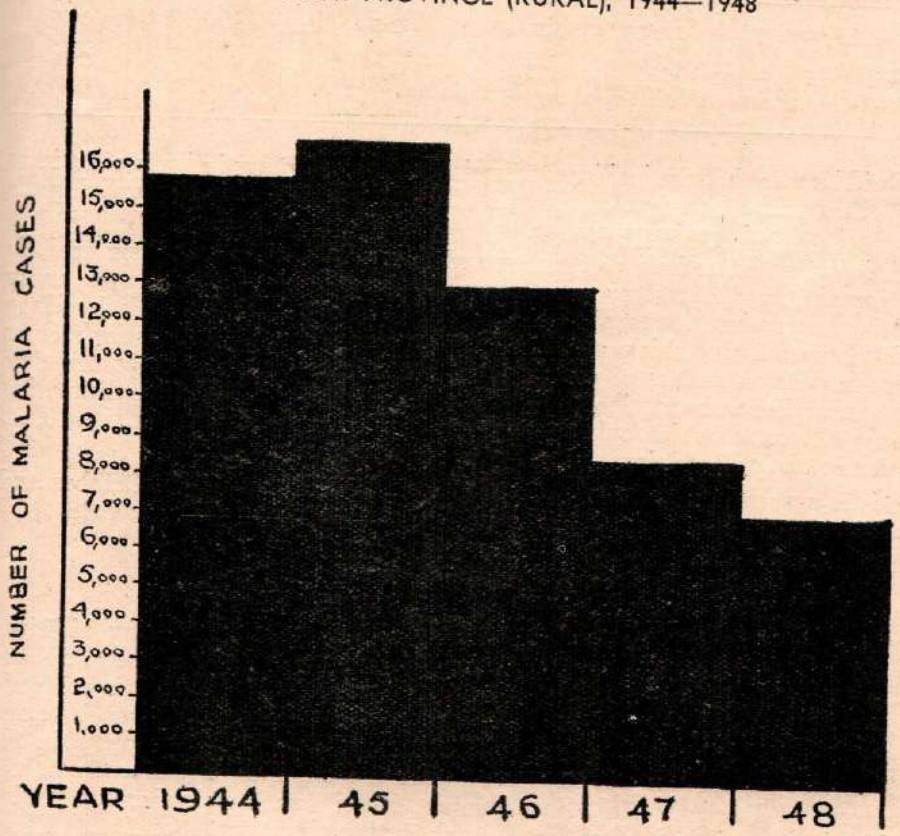
Number of malaria patients treated in public dispensaries in Delhi Province (rural areas).

Dispensary.	1944.	1945.	1946.	1947.	1948.
Mehrauli	2,976	2,330	1,863	915	477
Badarpur	...	159	1,555	1,292	528
Jung Pura	1,958	1,634	1,459	1,007	1,004
Shahdara	6,147	5,775	3,808	2,957	2,444
Najafgarh	1,902	750	583	379	547
Nangloi	2,213	1,993	887	705	309
Ojwah	696	743	231	181	376
Narela	...	2,304	1,664	866	649
Bawana	...	1,189	950	512	688
TOTAL ...	15,892	16,877	13,000	8,814	7,022

A large number of malaria cases from adjoining districts of the East Punjab and U. P. are also included in figures from Narela, Ojwah, Badarpur and Shahdara dispensaries.

CHART 5.

TOTAL MALARIA CASES TREATED IN 9 DISPENSARIES OF DELHI PROVINCE (RURAL), 1944—1948



The data collected regarding fever cases in experimental and comparison villages of Badli and Salimar respectively is given in Table VII.

TABLE VII.

Malaria cases in a sprayed and an unsprayed village from July to November 10, 1948.

Village.	Population.	Number of fever cases.	Percentage of fever cases in relation to population.	Number of blood smears showing malaria parasites.	Percentage of positive malaria cases in relation to population.
Sprayed village—Badli	2,400	35	1·5	4	0·16
Unsprayed village—Salimar.	300	33	11·0	8	2·66

It will be seen that the incidence of malaria in the unsprayed village of Salimar was 16 times more than that of sprayed village Badli, although the malariogenic factors for both villages were similar.

During the latter half of October and first half of November, six malaria inspectors and four laboratory assistants were detailed to visit each and every village in the province for mepacrine distribution in malaria cases. Each village was visited once during this period and house-to-house enquiries about fever cases were made. Blood smears of patients actually suffering from fever at the time of visit were taken and examined. Total number of slides taken during this period were only 173, out of which only 34 were positive for malaria parasites, i.e. 10, 22 and 2 for *P. vivax*, *P. falciparum* and *P. malariæ* respectively.

This is a surprisingly low figure for the whole province during October and November, the notorious months for malaria prevalence in Delhi Province.

DISCUSSION.

The excessive rains during July, August and September, flooding of River Yamuna sustained for a long period during August and September and the economic condition of the population were all favourable for a malaria epidemic in Delhi Province this year. The data regarding flooding of River Yamuna (Chart 1) compares well with that in 1942, an epidemic year for malaria in the Indo-Gangetic Plains. As was customary with the old Punjab Government, the Department of Public Health, East Punjab, issued forecast circular forecasting an epidemic of malaria in the East Punjab during the autumn of 1948. These forecasts have always proved true and malaria took an epidemic form in the East as well as West Punjab. It is evident from press reports, extracts from which are quoted below :—

The Indian News Chronicle, October 4, 1948.—'Amritsar, Oct. 4—The municipal health authorities have taken drastic steps to prevent further spread

of malaria in the city . . . . According to a medical practitioner, there is on an average one malaria case in every house.'

The Statesman, December 3, 1948.—'As a result of heavy floods in the province during the last monsoon, West Punjab has been in the grip of a malaria epidemic, but the Government despite the inadequate resources at its disposal has done all in its power not only to bring relief to the citizens but also to control the outbreak. A vigorous antimalaria campaign is being carried on and the Government hopes to have the situation well under control soon.'

Whenever there is an epidemic of malaria in the Punjab, Delhi Province is always affected. Malaria has been reported in an epidemic form in the neighbouring district of Gurgaon. In Ballabgarh Tehsil of Gurgaon District, which is just on the border of Delhi Province, the malaria incidence during October this year was about 12 times more than during the same period last year. The malaria figures from three dispensaries of Gurgaon District are given in Table VIII.

TABLE VIII.

Number of malaria cases treated in dispensaries in East Punjab situated at the border of Delhi Province.

Dispensary.	Month.	1946.	1947.	1948.
Ballabgarh	Aug.	178	46	391
	Sept.	178	46	391
	Oct.	124	63	766
Punahanan	Aug.	91	62	113
	Sept.	198	72	600
	Oct.	181	152	872
Nuh	Aug.	40	16	224
	Sept.	79	39	274
	Oct.	54	77	1,208

On the other hand, the incidence of malaria this year in Delhi Province was lower than the previous years (Table VI).

The spleen rate, particularly in November, is usually higher in an epidemic year but it mostly came down this year (Chart 3). The results obtained were due to the thoroughness with which D.D.T. was applied and keen interest taken by the Delhi Province Malaria Organization in the execution of its duties. Delhi villagers, who at first viewed the D.D.T. spraying machines with scepticism, are now firm believers in the miraculous qualities of this insecticide.

The effects of reduced malaria prevalence and undisturbed rest at night for human beings and their cattle due to reduction in the population of mosquitoes

and other insects have been very much appreciated by the rural population and as a result numerous letters appreciating the work done by the spraying teams and requesting for more sprays were received in from all parts of the province.

The villagers also realized the collateral benefits of a D.D.T. spray, i.e. increase in farm production and its lethal action on flies, bed bugs and ticks, the latter two being better appreciated.

The *per capita* cost at Re. 0-5-10 per annum is quite low considering, in addition to malaria control, the other collateral benefits derived from D.D.T. indoor residual spraying.

SUMMARY.

Results of second year of rural malaria control in Delhi Province with D.D.T. indoor residual spraying together with epidemiological features, organizational changes, technique of spraying and cost are described.

The *per capita* cost works out at Re. 0-5-10 only.

The formulation of D.D.T. used was D.D.T.-M.K.E. soap emulsion and the dose of D.D.T. sprayed was 50 mg. per square foot.

Reduction of 98·5 per cent in *A. culicifacies* in the sprayed structures was noticed.

Infant parasite rate was nil in the sprayed village and 20 per cent in the unsprayed village.

Incidence of malaria in the unsprayed village was 16 times the sprayed village.

The climatic conditions during the malaria season of 1948 were favourable for an epidemic of malaria in Delhi, but the incidence of malaria was found to be lower this year than the preceding four years.

A pile of letters from village headmen appreciating the effectiveness of D.D.T. sprays has been received.

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TRANSMISSION EXPERIMENTS WITH *P. KNOWLESI.*

BY

LIEUT.-COLONEL JASWANT SINGH, M.B., CH.B. (Edin.), D.P.H. (Eng.), D.T.M. & H. (Edin.),

MAJOR A. P. RAY, M.B., B.S.,

AND

MAJOR C. P. NAIR.

(*Malaria Institute of India.*)

[April 25, 1949.]

OBJECT.

1. To establish the complete life cycle of the parasite in North Indian *rhesus* monkeys, *Macaca mulatta ;*
2. Exaltation of the virulence of the parasite which during the last year had shown some attenuation ; and
3. To test drugs against sporozoite induced infections.

INTRODUCTION.

IN assessing the merits of antimalarial drugs, it is essential that it should be tested against both blood and sporozoite induced infections. While lately this has been the standard procedure in respect of human and avian malaria, the same could not be implemented in simian malaria. This is mainly due to the paucity of the existing knowledge regarding the transmitting agent—the vector species of mosquitoes of *P. knowlesi,* against which drugs are usually tested on account of its virulence in certain species of monkeys. With the main object of establishing a suitable vector for *P. knowlesi* and also for exaltation of the virulence of the strain of *P. knowlesi* maintained in these laboratories, the present series of experiments were planned in 1947 when it was observed that the parasite was gradually losing its former virulence. The attenuation may have been caused by repeated passages through monkeys over a period of several years.

In India, various attempts had been made in the past for finding a vector species of *P. knowlesi*, but without success. Mulligan and Knowles (Russell, 1942) in 400 attempts with various species of mosquitoes obtained only one *A. stephensi* with oöcysts. Russell and Mohan (Russell *et al.*, 1946) found sporozoites in a single specimen of *Aedes* (Diceromya) *reginæ*, a tree-hole breeder of rare species, fed on a monkey infected with *P. knowlesi*. Further attempts to procure more of the same species to repeat the experiment had failed. Subsequent investigations by Mohan (private communication) show that three specimens of *Armigeres obturbans* out of a batch of 28 could be experimentally infected and sporozoites detected in the salivary glands. Saline suspension of the infected glands of one mosquito inoculated intravenously to a monkey had produced infection after an incubation period of 16 days. The same was repeated with the other two mosquitoes in two different monkeys without any result. Sub-inoculation from the first monkey proved successful. Up to 1943, Russell and Mohan (private communication) had dissected 6,672 mosquitoes. These included Anopheles, Culex, Aedes and Armigeres. Out of the anophelines, the total dissections of *A. stephensi* (type), *A. annularis* and *A. culicifacies* were 1,318, 102 and 774 respectively with negative results. No anopheline vector had been established by them.

The present series of experiments conducted at the Malaria Institute of India were started early in 1948. *A. stephensi*, *A. annularis*, *A. subpictus* and *Culex bitæniorrhynchus* were used.

CONDUCT OF THE EXPERIMENT.

Feeding.—Initially the method of feeding was as follows :—The cage containing the monkey was kept inside a larger cage ($3' \times 3' \times 3'$) covered with mosquito netting on all sides excepting the bottom. The cage was placed on a detachable wooden board. The mosquitoes were released inside the cage and were allowed to feed both by day and at night. During the day the cage was kept covered with a dark cloth. Although each time a large number of mosquitoes fed on the monkey, the number recovered alive were very few, as most of the mosquitoes were killed by the monkey either during or immediately after feeding. The dead mosquitoes could be detected on the wooden board. In view of this, the mode of feeding had to be revised and the method used subsequently consisted of a wooden cross to which the monkey was tied and the mosquitoes fed from a glass chimney two ends of which were covered with mosquito netting.

Parasites.—In all cases, total gametocyte count was carried out. Exflagellation test was also conducted in a large number though not in all cases. The parasites were counted against W.B.C.

Preservation of the mosquitoes.—Initially, considerable difficulty was experienced to keep even a few mosquitoes alive for period more than a few days only. This was mainly due to high temperature and low humidity. To adjust the temperature and humidity, the mosquitoes were kept on the top shelf of an ice chest in Barraud's cages. Some measure of success was achieved in this manner. Desiccators containing various percentages of sulphuric acid producing the required degree of humidity were also used to preserve the mosquitoes. The desiccators were kept in cooler atmosphere during the summer. This method was of little avail as most of the mosquitoes died within 72 hours perhaps due to the fume of the

sulphuric acid used. During early winter larger number of mosquitoes were available for dissection as also from February 1949 onwards. The mosquitoes were kept in the insectarium where temperature and humidity were controlled to some extent but again a large number of mosquitoes died within a few days.

Temperature and humidity.—Temperature in the insectarium varied between 68 and 80°F. and the relative humidity from 66 to 75 per cent.

Dissections.—The results of dissection are shown in Tables I and II.

Infected glands from mosquitoes dissected during November and early December were preserved for study. Details of the morphology of the sporozoites are :—

Length (average)	...	...	8·8/μ.
Breadth ,,	...	...	0·7/μ.
Size of the chromatin		...	2·0/μ.

Majority of the sporozoites were slender and pointed at both ends. A few, however, were blunted at one end only, these characters resembling those of *P. falciparum* to a great extent.

The chromatin was either centrally located or as in the majority of the parasites, it began near the centre but was drawn towards one of the poles. Some of the nuclei were constricted in the middle and appeared dumb-bell shaped.

On February 26, one monkey was inoculated with the sporozoites obtained from one infected mosquito (Table I), another monkey on March 4 had similar inoculation while the third on March 18, 1949. The fourth monkey received inoculation of infected salivary glands from two mosquitoes on March 23 and another injection on March 25 from one mosquito. The fifth monkey received the inoculation on April 11, 1949. The first received the inoculation by the intravenous route, the second by intravenous, intraperitoneal and subcutaneous routes while the third and fourth by intravenous and intraperitoneal routes. Before dissection the infected mosquitoes were first fed on the monkeys. The second monkey receiving the injection on March 4 showed demonstrable parasites for the first time after 20 days on March 24 while the fourth monkey receiving the injection on March 23 became positive only 7 days after. Sub-inoculation from the first monkey becoming positive was carried out on two clean monkeys both of which became positive within 5 to 6 days. The second monkey which became positive showed very very scanty infection for a day or two and then became negative for a period of 3 to 4 days and then again showed transient parasitæmia at intervals. While the infection in the first monkey increased up to the state of ' scanty ' * infection and then gradually subsided to ' very very scanty ' † stage, the results of sub-inoculation were found to be more encouraging as the infection progressively increased to ' scanty '. Further observations are in progress.

In the absence of a larger series of cases it is not possible to arrive at a definite conclusion at this stage. Contrary to expectations, after passage through an insect

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* Scanty.—One parasite detected in every alternate microscopic field of the thin film.

† Very very scanty.—One parasite detected in every 5 to 7 microscopic fields of the thin film.

host, the parasite has undergone still further attenuations and one is driven to the following speculations :—

1. It may be necessary to have perhaps several more passages than one through the insect host.

2. *A. stephensi* and *A. annularis* are not the natural hosts and passage through them has produced further attenuation.

3. The present source of the monkeys supplied (Sansi near Hathras) is different from those which were being obtained up to about two years ago (near Mathura). Although these animals appear to be similar to those from Mathura area, there may be a slight difference at least in regard to the biological response to the parasites as individual variations to the same stimuli even in human beings are not unknown. Alternatively the present lot may be different from the previous ones.

Whatever be the reason, this experiment has thrown open a considerable field of study not only on parasitology but on immunological aspects as well.

SUMMARY.

(*i*) *A. stephensi* and *A. annularis* were experimentally infected with *P. knowlesi.*

(*ii*) Inoculation of infected glands to clean monkeys produced infection in two out of four animals.

(*iii*) Sub-inoculation results were successful.

(*iv*) *P. knowlesi* infection produced experimentally in healthy monkeys after one passage through insect host did not increase the virulence of the parasites which appeared to be even less virulent than before.

ACKNOWLEDGMENTS.

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TABLE I.

Observations up to March 23, 1949.

Species of mosquitoes.	Total number fed.	Total number dissected.	Total positive. Gut.	Total positive. Gland.	Percentage. Gut.	Percentage. Gland.	Details of those positive. Gut.	Details of those positive. Gland.	Dates.	Parasitological observations in host monkeys at the time of feeding of mosquitoes. Parasite count per c.mm.	Total gametocyte count per c.mm.	Ratio of male to female.	Remarks.
									1948				
							...	1	Dec. 3 ...	5,850	1,040	1 : 3	Fed on monkey No. 1290. Preserved for study.
									1949				
							...	1	Mar. 18 ...	1,860	350	1 : 1	Inoculated to monkey No. 1305 on Mar. 18.
									Mar. 29	20,000	2,000	1 : 2	3 feedings on Mar. 4, 7 and 9.
A. annularis ...	708	123	2	5	1·652	4·06	1	...		18,590	3,646	1 : 6	
										480	80	1 : 1	
									Apr. 4	480	80	1 : 1	Preserved.
							...	1		6,720	84	...	
										2,304	384	1 : 5	
							1	1	Apr. 11 ...	9,200	800	1 : 3	Inoculated to monkey No. 1308 on Apr. 11.
							...	1	Apr. 22 ...	...	...	...	Inoculated to monkey No. 1392.

Table II.

Observations up to April 23, 1949.

Species of mosquitoes.	Total number fed.	Total number dissected.	Total positive.		Percentage.		Details of those positive			Parasitological observations in host monkeys at the time of feeding of mosquitoes.			Remarks.
			Gut.	Gland.	Gut.	Gland.	Gut.	Gland.	Dates.	Parasite count per c.mm.	Total gametocyte count per c.mm.	Ratio of male to female.	
									1948				
							...	1	Nov. 18 ...	40,320	15,360	5 : 7	Fed on monkey No. 1290. Preserved for study.
							3	...	Dec. 29 ...	30,300	300	1 : 2	Fed on monkey No. 1300.
									1949				
							...	1	Feb. 26 ...	1,638	252	1 : 1	Fed on monkey No. 1273; inoculated to monkey No. 1304 on Feb. 26, 1949.
										1,500	500	2 : 3	Inoculated to monkey No. 1306 on Mar. 4, 1949 (two feedings, one on Feb. 12 and the other on Feb. 14, 1949).
A. stephensi ...	1,194	317	7	7	2·2	2·2	...	1	Mar. 4	4,720	800	1 : 2	
							1	...	Mar. 23	9,390	102	1 : 1	Inoculated to monkey No. 1355 on Mar. 29, 1949. (Three feedings on Mar. 3, 5 and 8, 1949.)
							1	...	Mar. 26	8,540	2,060	1 : 5	
							2	...	Mar. 27	3,220	276	1 : 2	
							...	2	Mar. 29				
										18,590	3,640	1 : 6	Inoculated to monkey No. 1355 on Mar. 31, 1949. (Three feedings on Mar. 7, 9 and 11, 1949.)
							...	1	Mar. 31	480	80	1 : 1	
										6,720	84	...	
							...	1	Apr. 22 ...	...	...	...	Inoculated subcutaneously (only) to monkey No. 1392.
A. subpictus ...	436	6	...	...	...	...	...	...	...	...	...	...	
C. bitæniorrhynchus	155	31	...	...	...	...	...	...	...	...	...	...	

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MALARIA IN ORISSA.

BY

V. VENKAT RAO, F.R.S.T.M. & H., M.R.San.I.

[May 11, 1949.]

WHEN Orissa was constituted as a separate Governor's province in the year 1936, it consisted of only six districts with a total extent of 32,198 sq. miles and a population of 8,728,544. Four of the six districts, viz. Ganjam, Puri, Cuttack and Balasore, are situated along the coast of the Bay of Bengal, while the other two, Sambalpur and Koraput, are situated inland mostly in the hill tracts of the Eastern Ghats. The area comprising the province was not all contiguous, owing to the presence of several feudatory states between some of these districts.

Owing to the political changes brought about after the declaration of Indian Independence, a number of the neighbouring Feudatory States were integrated with the province of Orissa and are likely to be fully merged into it in the near future. Most of the area covered by these states and the entire district of Koraput are situated in the hill tracts, while the coastal districts and a large portion of Sambalpur District are situated in the plains. The population of the plains is largely made up of Oriyas, who are the descendants of the Aryan invaders of early times, whereas the peoples of the hill tracts are aboriginal tribes who are the natives of the area, being therefore called the 'Adibasis', though at present these tracts are also inhabited by a number of Oriya settlers. Owing to the virtual merger of the states, the present area of what is sometimes described as United Orissa is 59,869 sq. miles and its population is 13,774,668 according to the 1941 census.

From the Public Health point of view, Orissa is more backward than some other provinces of India. Malaria, filariasis, leprosy and cholera are endemic over a greater part of the plains, while the hill tracts are the home of the most virulent malaria in Peninsular India.

Though the hill tracts may be presumed to have always been highly malarious, there is evidence to show that the plains districts were free from malaria till recently. Hunter (1872) did not find malaria prevailing generally in the coastal districts which he visited in the year 1870. He refers to the prevalence of malaria only 'in Puri Town and its environs' and in the deltaic areas of Cuttack District. The area around the Chilka Lake and the district of Balasore, which are now intensely malarious, were practically free from malaria at that time, though filariasis was prevalent already in Balasore and parts of Cuttack and Puri districts.

The first investigation into malaria of the plains was carried out by Fry (1912). He found an intense degree of malarial infection in Puri and around the Chilka Lake with spleen rates indicating hyperendemicity, showing that conditions altered very much for the worse since 1870. He was followed by Sarathy (1932), who carried out investigation in parts of Balasore and Puri districts, including the Chilka area. Though he failed to locate the vector of the Chilka coast, he found the important fact that *A. annularis*, a highly zoophilic insect of no malariogenic importance elsewhere, was the vector of malaria in the plains portions of Balasore and Puri districts.

Between 1931 and 1936, no further investigations were carried out, though the Bengal Nagpur Railway started control operations which were directed only against *A. annularis* on the basis of Sarathy's work at Bhadrak (Balasore District) and Khurda Road (Puri District). As a result of severe epidemics which broke out during the winter of 1936-37 at a number of railway stations on the Chilka coast, investigation was started which revealed the presence of *A. sundaicus* (Senior White, 1937*a*).

This important discovery was followed by intensive studies during 1937-38 by Senior White and Adhikari (1939) and later by Covell and Pritam Singh (1942). These studies proved that *A. sundaicus* was the only vector of the area and that this mosquito was present in large numbers, not only in the Chilka area, but also further north in Puri District.

Meanwhile, malaria in Puri Town was studied by Panigrahi (1942). He found that epidemics, which occurred at somewhat irregular intervals, were caused by *A. sundaicus* with *A. annularis* playing a secondary part in the transmission. Further unpublished work done by the Orissa Public Health Department shows that *A. sundaicus* is present as far north as Chandipur in Balasore District and that *A. annularis* is the main, if not the only vector all over the plains.

An intensive study of the plains districts along the Bengal-Nagpur Railway was also made simultaneously by Senior White *et al.* (1943) which confirmed the findings of the previous workers that, in spite of the occasional presence of infections in certain other species like *A. aconitus*, the main carrier was *A. annularis*.

In 1943, *A. sundaicus* invaded certain areas south of the Chilka and caused widespread epidemics in the southernmost areas of the province and in the adjoining areas of the North Madras coast.

Coming now to the hill tracts, the first investigation of malaria in the Koraput District was made by Perry (1914), who incriminated mosquitoes of the *fluviatilis* group (adopted to modern nomenclature) as the more important vectors and *A. maculatus* as a vector of secondary importance.

After the opening of the Raipur-Vizianagram section of the Bengal-Nagpur Railway, Senior White (1937*b* and 1938) carried out exhaustive studies of malaria between the railway stations of Rayaghada and Lanjigarh Road in what he described as the Jeypore Hills, and arrived at the following conclusions :—

(*a*) *A. fluviatilis*, *A. varuna* and *A. minimus*, sometimes described as the *fluviatilis* group, were the only vectors of importance ;

(*b*) *A. culicifacies* and *A. jeyporiensis*, in which gut infections but no gland infections were occasionally seen, were of no practical importance ; and

(*c*) The other anophelines present in the area were absolutely harmless.

No work has been done in the hill tracts of Puri, Cuttack and Balasore districts or in most of the areas comprising the integrated states which, as stated above, are also situated mostly in the hills. There is, however, little reason to assume that things are different there. North-west of this area lies the Singhbhum District of Chota Nagpur, where malaria is hyperendemic and where the *fluviatilis* group has been proved to be the only vector, while *A. culicifacies* and *A. jeyporiensis* were found to play no part in malaria transmission (Senior White and Das, 1938). The work done in the states of Kalahandi and Bolangir-Patna confirms this view.

The position may thus be summed up as follows :—

(*a*) In the hills, *A. fluviatilis*, *A. varuna* and *A. minimus* are the vectors ;

(*b*) Over the plains, *A. annularis* is the main, if not the only vector ; and

(*c*) Along the coast, *A. sundaicus* is the only vector, though in and around Puri *A. annularis* plays a secondary part in the transmission.

In the hills, hyperendemic malaria is the rule and transmission takes place almost throughout the year with an autumnal peak in October-November and a vernal peak in March-April, the former being always higher than the latter.

Annual outbreaks are practically unknown in large areas of the plains districts, though, in certain localities, the spleen rates indicate endemic, and even hyperendemic conditions. Epidemics, which occur at irregular intervals of four to six years, constitute the rule and transmission is confined to the autumnal months.

Along the coast, where *A. sundaicus* is the vector, endemic conditions are established. Some places like Kespur near Khallikoba railway station, are hyperendemic and act as centres from which malaria radiates in all directions (Covell and Pritam Singh, *loc. cit.*). Severe epidemics are found to superimpose even these highly endemic conditions at periodical intervals of about five years. In these areas, transmission in years of high vector prevalence is perennial and occurs throughout the year, except possibly in June (Senior White *et al.*, 1947). On the other hand, in areas recently invaded by *A. sundaicus*, there is no evidence of malaria transmission for some years after the previous epidemic, the vector having almost disappeared from the scene and spleen rates having been reduced gradually but definitely to the healthy level.

This is the malaria picture of Orissa. Malariologically, Orissa is not dissimilar to the Malaya Peninsula. There, malaria in the hills is propagated by *A. maculatus*, which breeds mostly in jungle streams open to sunshine ; plains malaria is caused by *A. umbrosus* breeding in deeply shaded waters characteristic of the Malaya plains and coastal malaria is caused by *A. sundaicus* breeding in the brackish waters of the coast. In Orissa, the malaria vectors of the hills breed in clear running waters and seepages, the vector of the plains breeds in weed-choked tanks and ponds and in certain conditions, in ricefields, and the vector of coastal malaria which is common to both Malaya and Orissa, breeds, as is shown later, in both fresh and saline waters in association with certain forms of aquatic vegetation.

The coastal area, where *A. sundaicus* is the undoubted vector, is considered first. The original home of this mosquito is supposed to be the eastern oriental region comprising the Indian Archipelago of Java, Sumatra, Borneo, Malaya and other smaller islands in the vicinity. It is also found in the Andamans, in the west coast of Burma and in French Indo-China. In these areas, it breeds almost wholly in brackish

waters of varying degrees of salinity, the minimum being 200 and the maximum 1,500 parts per 1,00,000 parts of water. The infection rates vary between 2 and 6 per cent.

In India, *A. sundaicus*, then known as *A. ludlowi*, was located in the marshy areas of the Sunderbans of Bengal in 1912. Later, it invaded Lower Bengal gradually till almost the whole of its coastal area up to a maximum depth of 50 miles was found infested by 1931. Here also, the bulk of its breeding was found in brackish waters, the optimum salinity having been estimated to be between 150 and 250 per 1,00,000 parts of water (Iyengar, 1931). The infection rates ranged between 2 and 6 per cent.

The discovery of *A. sundaicus* in Orissa by Senior White (1937*a*) constitutes a landmark in the study of malaria in this province. Fry (*loc. cit.*) found highly endemic conditions without being able to locate the vector. Sarathy (*loc. cit.*) suspected the presence of *A. sundaicus* and specially looked for it but not having found it, came to the conclusion that it did not exist there. This naturally raises the question whether it invaded the area for the first time after 1931 but before 1937, and the question was discussed in some detail by Senior White and Adhikari (*loc. cit.*). The fact that Hunter (*loc. cit.*) did not refer to malaria around the Chilka Lake indicates absence of malaria and, therefore, absence of *A. sundaicus* but his reference to malaria in ' Puri Town and its environs ' shows that the insect was present there at the time. Its absence in the Chilka Lake might be due to the fact that the lake had still access to the sea and its water was too brackish for *sundaicus* breeding. The complete choking of the outlet and the resultant freshening of the lake water and growth of fresh water flora might have facilitated the rapid extension of *A. sundaicus* into this area. The extension might have taken place either from Ganjam Town in the south or Puri in the north. Ganjam was a flourishing port and local head-quarters of the East India Company with a population of 35,000 till the year 1815, when a very severe outbreak of, which must be, malaria occurred. The British residents who were the worst sufferers soon abandoned the port, but the town, which rapidly resumed its insignificance, still exhibits through its temples and the fort, its former splendour. This epidemic clearly points to the first invasion of this part of the country by *A. sundaicus* (Senior White and Venkat Rao, 1946). In spite of malaria being present in and around the town, Puri long enjoyed an enviable reputation as a sea-side health resort. This must be due to the fact that Puri is not endemic for malaria but, as shown by Panigrahi (*loc. cit.*), is subject to epidemics occurring at intervals of four to six years and that the long inter-epidemic periods being marked by freedom from malaria attracted and enabled visitors to enjoy the benefits of a sea-side climate without the risk of exposure to malarial infection. From whichever direction *A. sundaicus* invaded the Chilka area, it established there more firmly than elsewhere, rendering any attempt at dislodgement extremely difficult.

North of Puri, *A. sundaicus* has been found at Gop near Konarak (Puri District) and at Chandipur in Balasore District. Whether it exists all along the coast between these two places and, if so, how much malaria is present, is unknown as the area is very inaccessible and has not so far been surveyed.

South of the Chilka Lake, the insect existed, as already shown, at Ganjam and also maybe at Humma for a long time. Its extension south of the Rishikulya River, however, appears to be a recent event. The first epidemic of malaria at Chatrapur

occurred in 1929, while Gopalpur, another well-known sea-side health resort about ten miles south of Chatrapur, appears to have been invaded later.

So far, *A. sundaicus* was known to exist only along the sea coast and in the immediate vicinity of other large bodies of brackish water like the Chilka Lake. Even in Bengal, where it exists up to fifty miles from the sea, it is confined to waters well within tidal influence. Sarathy (*loc. cit.*) found spleen rates falling abruptly as he moved away from the shore of the lake. His spleen rates are as follows :—

On shore	53	per cent
¼ mile from shore	49	,,
½ mile from shore	35	,,
1 mile from shore	25	,,
2 miles from shore	3	,,

Earlier, Fry (*loc. cit.*) also found similar conditions, which were as follows :—

Lakeside villages	87	per cent
Villages partly on shore and partly in land ...	50	,,
Inland villages	3	,,

Evidently, conditions did not vary very much during the twenty years which elapsed between the two investigations. But in 1942, several inland villages situated up to six miles from the shore, suffered malaria outbreaks (Covell and Pritam Singh, *loc. cit.*). These authors found adults of *A. sundaicus* in these villages but as the villages were well beyond salt water influence and as breeding of the mosquito could not then be located nearby, concluded that the infestation was due to infiltration of adults from the shore. Breeding was, however, found subsequently in some of these villages (unpublished), indicating that irrespective of whether long-range infiltration took place or not, *A. sundaicus* was breeding locally in fresh waters.

Clearer proof was forthcoming in 1943, when the town of Berhampur experienced a severe epidemic simultaneously with other places in the adjoining Vizagapatam District. This town is about ten miles from the sea and at least five miles from the brackish water of Gangi Thampara referred to by Covell and Pritam Singh (*loc. cit.*) and is thus beyond all saline water influence. The breeding was found locally in overgrown fresh water tanks. Subsequently, larvæ and adults of *A. sundaicus* were found at Boddamohiri (twelve miles west of Berhampur and nineteen miles from the sea) and at Bouginabodi (nine miles west of Berhampur and eighteen miles from the sea). Severe outbreaks occurred in both these villages.

This extension into purely fresh water areas demanded the attention of malariologists working in the area. Dissections of mosquitoes yielded somewhat striking results. At Berhampur, the sporozoite rate was as high as 10 per cent. In the adjoining areas of the Vizagapatam District, where also breeding of *A. sundaicus* was confined to fresh waters, the sporozoite rates varied between 7 and 10 per cent (Senior White *et al.*, 1947). As against this, the sporozoite rates of *A. sundaicus* from the Chilka area dissected by Senior White and Adhikari (*loc. cit.*) and Covell and Pritam Singh (*loc. cit.*) over a period of four years comprising both epidemic and non-epidemic years was only 0·3 per cent.

Further work showed that larvæ of *A. sundaicus* in this area were found more often in fresh than in saline waters, though the output from saline waters was much

higher. This suggests the existence of two races or varieties, one breeding in fresh and the other in saline waters. Indeed, the possibility of two races was already advanced by Taylor (1943) referring to a statement by Dr. Gater that there was sometimes a lack of direct correlation between *A. sundaicus* prevalence and malarial incidence in Singapore. If this possibility proves to be correct in the area under study, the fresh water form should be a very potent carrier on account of its high infection rates whereas the low infection rates obtained in the Chilka area should be due to a small number of the dangerous fresh water form being dissected along with a large number of the relatively or entirely harmless salt water form.

It may be stated here that control of both forms of *A. sundaicus* by antilarval methods is an almost hopeless proposition owing to the vast extent of salt water bodies. The Chilka Lake itself is about 500 sq. miles in extent. Deweeding or chemical treatment of even a two-mile belt from the shore would be prohibitively costly. Raising the salinity of the lake beyond the tolerance limit of this mosquito by the introduction of sea water is impracticable owing to the rapid silting up of the outlet and the lake water at present irrigating a large extent of paddy land. Even if the lake is dealt with in this manner, there are large numbers of inshore saline water tanks which keep up optimum salinity by the influx of lake water at high levels. If, on the other hand, the salt water form is left out and only the fresh water form controlled, *sundaicus*-caused malaria is likely to become an economically feasible proposition. Fresh water bodies, even in this area, are far less in number and smaller in extent than saline waters.

On this hypothesis, a large amount of work has been done by Senior White *et al.* (1947). At all the railway stations along the Chilka coast, the fresh water form was completely eliminated by very careful deweeding and regular paris-green dusting. The saline waters were left untouched. Dissections of *A. sundaicus*, which should be of the salt water form, were made in large numbers but no infections were encountered. Every station where this method of malaria control was in vogue kept remarkably healthy for a period of nearly three years. That the healthy conditions were not due to the absence of transmission was ascertained by the unhealthy condition prevailing in a ' Control ' station in the same area where infections in *A. sundaicus* were found to the same extent as before (Senior White *et al.*, 1947).

As *A. sundaicus* is normally a saline water breeder throughout the remaining area of its prevalence, the fresh water form found in this area may be a new mutant race adopted to breeding in fresh waters or it may represent the primitive type, which is supposed to be a fresh water species present in the marginal zones of its distribution (Senior White, 1948). In the alternative, the two forms may be morphologically valid sub-species owing to the differences in the structures of the leaflets of their phallosomes observed by Venkat Rao and Ramakrishna (unpublished).

Thus, the problem of *sundaicus*-caused malaria may not be as difficult to solve as it first appears to be because :—

firstly, the bulk of transmission occurs in the immediate vicinity of large bodies of saline waters ;

secondly, though it is shown that *A. sundaicus* extends inland up to a maximum of twenty miles from saline water influence, there is no evidence that it is firmly established there and becomes responsible for endemic conditions. Perhaps this is due to the individuals which have migrated beyond

their normal habitat not being able to survive either by adopting slightly different habits or by colonizing areas outside their range permanently, which is shown in other species to be a possibility by Huxley (1942). It may also be that the mosquito will reappear later and will then be better able to colonize as is shown to have been the case in Lower Bengal by Senior White (1948), a danger to be always kept in view ;

thirdly, the vast output of *A. sundaicus* from saline waters is observed to be harmless ; and

lastly, only those mosquitoes which emerge from fresh waters are dangerous.

Therefore, malaria control by antilarval methods in such areas should consist of paris-green dusting, which is a costly method, or deweeding measures, which are very economical in the long run and which are described in detail by Venkat Rao and Ramakrishna (1947), attention being paid to fresh waters only in either case.

Control of *A. sundaicus* by anti-adult methods is also a feasible method. In the endemic areas D.D.T. spraying of all houses and cowsheds once during the autumn and again in the spring at 100 mg. per sq. ft. would go a long way in solving the problem. This method is, however, likely to be costlier (but more effective) than antilarval measures owing to climatic and meteorological conditions being always favourable for transmission.

As it is difficult to forecast epidemics with certainty, epidemics can only be dealt with by D.D.T. after they have broken out and before much damage has been done. This presupposes the existence of a technical organization to watch the beginning of an outbreak and then to start D.D.T. spraying of any locality with the least possible delay.

The problem of plains malaria transmitted by *A. annularis* is now considered. This species, though present in numbers, is not of any importance in the Ganjam District or south of Khurda Road in Puri District. In Puri Town, as already observed, it plays only a secondary part in transmission. Its importance as a vector is felt only in the area between Khurda Road and Rupsa, excluding the sea-coast and hill tracts but including parts of Dhenkanal, Talcher and a few other states in the vicinity which are integrated with Orissa. North of Rupsa, *A. annularis* again pales into virtual insignificance owing to the presence of a more potent vector, *A. philippinensis*.

It is necessary at this stage to consider why *A. annularis*, a harmless insect everywhere else, has come to be the main vector in this area comprising the districts of Balasore, Cuttack and Puri. These three districts are not only the most important in the province but they had always been the centre of Orissa's culture and civilization. Cuttack, the capital of Orissa for several centuries, is situated in this area. All the beautiful temples and other works of art are also found here, admired and praised by pilgrims and conquerors alike.

Orissa enjoyed peace and prosperity under the Hindu Rajahs up to the beginning of the thirteenth century. The wars before 1200 A.D. were mostly fought outside Orissa proper by victorious Rajahs trying to extend their kingdom. During the thirteenth century, the king was able to raise a militia of 3,60,000 men (Hunter, *loc. cit.*), indicating a total vigorous population of at least three millions. The troubles in Orissa began in 1205 A.D. when the first Mohammedan invasion

took place. Successive wars were fought, in which the Rajahs were mostly successful. But, in the long run, they could not withstand the might and superiority of Mohammedan forces. The last Hindu king Mukunda Deva was slain in battle in 1568, leaving the country to be ruled by the Afghans.

The Afghans ruled the country for only a few years. In 1612, they were vanquished by the Moghul armies who annexed Orissa to the Moghul Empire. Orissa remained as a province of the Moghul Empire till 1751, when it passed on to the Mahrattas. The Mahrattas held it till 1803, when it was conquered by the British.

The country was thus torn and ravaged by continuous wars for nearly six centuries. The conquerors did not care for the well-being of the inhabitants. Under the Moghul Emperors, Governors were sent from Bengal to administer the country who were sufficiently out of reach to do more or less as they liked. Also, internal troubles which affected the Moghul Empire in the latter part of the period rendered good administration impossible even had it been desired (Cousins, 1933).

If the administration of the country under Muslim rulers was bad, it was far worse under the Mahrattas. The administration of the Mahrattas was fatal to the welfare of the people and the prosperity of the country and exhibits a picture of misrule, anarchy, weakness, rapacity and violence combined which makes one wonder how society could have kept together under so calamitous a tyranny (Stirling quoted by Cousins, *loc. cit.*).

Things did not improve with the advent of the British in 1803. Orissa was attached to Bengal and administered from Calcutta. The interests of the people were neglected. Whole estates were sold 'for a mere song' in public auctions in Calcutta in favour of absentee landlords. When peace prevailed and settled government was restored, the population increased from 13 lakhs in 1822 (a third of what it was in the twelfth century) to 40 lakhs in 1931, an increase of 311 per cent in the first hundred and thirty years of British rule, but it is a poorer physical type that has come into being (Senior White and Venkat Rao, 1946).

Another peculiar feature helped this process of deterioration. The decline in the power and martial spirit of the people is intimately connected with Chaitanya's residence and teachings in Orissa. His influence over King Prataparudra was very great. The King wholeheartedly accepted Chaitanya's cult of *Bhakti*, which teaches love, equality *and sublime meekness*, which is fatal to the martial spirit of people in an age when strife was perennial and the sword often decided the fate of nations. The people flattered the king by full imitation. The cult of Chaitanya thus brought in its train a false faith in man and thereby destroyed the structure of society and government (Banerjee, 1938).

Several factors have thus contributed to the disintegration of the country and the deterioration of the people. War, famine, misrule, plunder and religion have all combined to complete the process. The type of people who survived the calamities are physically poor, unable to withstand the ravages of any disease, communicable or otherwise.

The problem of *A. annularis* has to be viewed against this historical background. In this part of the country, no major anopheline vector exists which explains its remarkable freedom from malaria till recent times. As long as there was good government, the people, under an organized village administration, were conscious

of their civic duties to keep their tanks and ponds free from vegetation. Flood water, in the absence of embankments, spreads all over the country and inundates a large part of it, including ricefields and tanks and restricts the extent and growth of aquatic vegetation. Thus *A. annularis* prevalence was kept down well below the level at which it can take effective part in malaria transmission.

Village government was naturally one of the first casualties of prolonged anarchy and misrule. Whole villages were either abandoned or their population decimated, with the result that the tanks became choked with weeds and provided a perpetual nidus for this mosquito, enabling it to breed in enormous numbers. To add to this, a new condition was introduced, which favoured the extension of *A. annularis* into the vast paddy lands. Embankments of rivers were constructed during the British period to secure protection against inundation by flood. These embankments, by confining the spread of the water, raised the level of river beds, necessitating longer and stronger embankments to resist the floods; these new embankments in their turn again raised the level of the water, silting up the river bed in the process and thus led to the addition of more embankments, so that their construction was steadily progressive (Cousins, *loc. cit.*).

Thus, ricefields are deprived of flood water and are irrigated only with rain water; when this water is conserved in the fields, as it must be in the absence of other sources, and also owing to defective agriculture, breeding of *A. annularis* on a large scale is introduced.

It is the vast scope for the immense breeding of *A. annularis* and the steep fall in the resisting powers of the general population that have made this mosquito a menace to public health in this area. In the absence of either condition, perhaps malaria would not have been so prevalent.

It has been stated already that *annularis*-caused malaria is confined to the autumnal months. This may be due to the enormous output of the vector during this period from tanks and ricefields.

The sporozoite rate of this mosquito being as low as 0·08 per cent (Senior White *et al.*, 1943), effective transmission would not be possible except when the mosquito is present in large numbers. Venkat Rao (1947) has observed a peculiar condition in this mosquito which favours its becoming a vector during the autumn. During this period a certain proportion of the *annularis* population enters into a condition described as 'Gonotrophic discordance' whereby they are temporarily freed from the obligations of reproduction and establish greater contact with man by being fixed in houses for prolonged periods. This condition also appears to depend on the mosquito being present in large numbers.

Control of *A. annularis* should be carried out on the following lines :—

Antilarval measures.—Chemical treatment of the vast extent of paddy land is impracticable owing to its high cost. Naturalistic measures may be more advantageous, not because they are much more efficient, but because of their beneficial effect on the rice crop. One such method is flooding the fields with silty flood water. It has been observed that flood years are marked by absence of malaria in the flooded areas. Another method is heavy manuring of the fields during the dry season with such organic substances as compost or crude sullage. It has been found that periodical flooding of the fields with crude sullage during the dry season reduces the breeding

of *A. annularis* to a marked extent and increases the rice crop by about 20 per cent (Venkat Rao, 1942).

Deweeding of tanks and ponds is carried out in the same manner as in the case of *A. sundaicus.*

Anti-adult measures.—An easier and more effective method is offered by D.D.T. spraying of all houses and cowsheds. As the malaria season in the plains is limited to August–November, one D.D.T. spray in July or August at 100 mg. per sq. ft. should be sufficient to control malaria in all endemic areas. In epidemic areas, similar spraying is indicated in the epidemic years only.

The hill tracts comprise the whole district of Koraput and a considerable part of Sambalpur. Parts of the coastal districts and most of the states which are integrated with Orissa Province are also situated in this area. In fact, hill tracts cover a larger part of Orissa than the plains and coastal area combined.

Malaria is hyperendemic in the hills with spleen rates ranging between 70 and 90 per cent. Blackwater fever is present over a wide area in the hills.

The vectors of malaria here are, as already stated, *A. fluviatilis, A. varuna* and *A. minimus.* Owing to the similarity of their breeding and feeding habits and vectorial capacity, they are commonly called the *fluviatilis* group, though evidence is accumulating which suggests that, among them, *A. varuna* may be a complex of more than one biological race and may not be a vector everywhere. The sporozoite rate of this group ranges between 2 and 5 per cent and its anthropophilic index, specially of *A. fluviatilis* and *A. minimus*, is usually as high as 90 per cent. Viswanathan (1946) has calculated that a density of 0·4 per man-hour of *A. fluviatilis* is the level at which it can carry on effective transmission and Senior White *et al.* (1945) held that this is true of *A. fluviatilis* and other members of the group generally in the hill tracts of East Central India, including Orissa.

The breeding places of these mosquitoes are nullahs, irrigation channels and seepages, including what are known as ' seeping ricefields ', of which there are many in the hills, specially in the monsoon and winter months.

The extent of these breeding places is so great and their chemical antilarval treatment will be so difficult and costly that antilarval measures may be brushed aside as impracticable. Large-scale bonification measures on the lines of the Pontine marshes of Italy are also inapplicable to Indian conditions owing to their prohibitive initial cost.

It may, at this stage, be asked whether large-scale antimalaria operations are at all necessary in the hill tracts as the area is poorly inhibited by aboriginal tribes, who have already acquired a high degree of immunity against the disease. In answer to this, it should be stated that the tribal people have acquired the immunity at a heavy cost, incurred by way of high infantile mortality and heavy morbidity in the surviving population. Also, this area is rich in natural resources, which cannot be properly developed unless malaria is kept absolutely under control. The land is at present poorly and badly cultivated. If agriculture is developed, there would be an enormous yield of food crops, rendering import from other countries unnecessary and paving the way for solution of the displaced persons' problems.

Therefore, proper antimalaria operations are necessary and should be carried out. As antilarval and bonification measures are impracticable, anti-adult measures should be decided upon.

Anti-adult measures by pyrethrum spraying, so successful against *A. culicifacies* (Russell and Knipe, 1941), are not effective against the *fluviatilis* group except when the costly method of daily spraying is adopted (Senior White *et al.*, 1945).

The only effective and economical anti-adult measure is D.D.T. spraying of all houses once annually at the rate of 200 mg. per sq. ft. or twice annually at half this dosage. Owing to the small size of houses in this area, the *per capita* cost of D.D.T. spraying does not exceed six to eight annas annually. A very high degree of protection from malaria is obtained in this manner and the whole area can be colonized and developed in every direction.

Another aspect of malaria has to be considered at this stage. Vast irrigation schemes are either being constructed or under consideration in Orissa as in other provinces. The Hirakud Project is one of these. While all irrigation schemes are to be welcomed the fact that in India malaria and irrigation have come hand in hand in the past, should not be lost sight of. All the three major irrigation schemes which have been constructed in this country in recent times, viz. the Lloyd Barrage Scheme in Sind, the Mettur Project in South India and Irwin Canal Scheme in Mysore, have resulted in malaria stabilizing itself in endemic form in their respective areas. It cannot, of course, be said that irrigation *per se* is responsible for the malaria. Such schemes are essential for a country like India where the pressure of population on land is heavy and food shortage is chronic. But, an increase in malaria is not a necessary concomitant of irrigation. It is that irrigation which is wrongfully applied or improperly carried out that causes the whole trouble.

Faulty and uncontrolled irrigation often leads to complex situations in respect of subsoil water level, tanks and valleys, the system of crop rotation and subsidiary local industries (Rao, 1945).

In irrigated areas, the subsoil water level rises to practically ground level as a result of which houses become damp, unhealthy and almost uninhabitable. Lack of proper drainage results in tanks remaining always full, the seepages arising out of them create conditions favourable to the breeding of the more dangerous anophelines, besides rendering the fields constantly damp and preventing aeration of the soil. Aquatic vegetation grows profusely in tanks and the valleys become shallow and water-logged. Wet crops, which are more paying than dry crops, are grown everywhere and cultivation is extended right up to the edge of the villages. Subsidiary industries like sheep-rearing and making of dairy produce directly suffer for want of pasture land and, in some cases, by introduction of disease among sheep and cattle (Rao, *loc. cit.*).

In addition, there are certain defects, not in the design and layout of the schemes but in the actual construction which are also responsible for a large part of the trouble. Defective sluice gates, seeping canal banks, unfilled borrowpits,

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Note.—Single dose of 200 mg. of D.D.T. has been applied more extensively by the World Health Organization Malaria Control Demonstration teams in different parts of India. The results are not yet available.—EDITOR.

insufficient bridges, and above all, absence of drainage may be quoted as examples. There are also the ' small things ' like a missing pipe or a leaking joint, which, by sheer carelessness of the maintenance staff, may promote malaria to an appreciable extent (Knipe and Russell, 1942).

It is thus quite possible to forestall the defects and prevent them. It is equally possible to ignore the warning and court disaster. The activities of Tennessee Valley Authority, particularly as regards the inclusion of antimalaria precautions at every stage of planning and construction, provide an outstanding example of the happy results which may be obtained by the maintenance of harmonious relations between health staff, including sanitary engineers, biologists and entomologists, and the various other departments engaged (Covell, 1946). It is expected that a similar consummation would result in connection with the irrigation schemes now on hand so that timely steps may be taken to prevent increase of malaria even in this highly endemic province.

' One of the main tasks of any Indian Government will be to rid their country of this disease. This will be a much more efficient measure of national defence than making aerodromes and warships ' (Haldane, 1949).

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A NOTE ON THE PRELIMINARY TRIALS WITH D.D.T. FOR THE CONTROL OF MOSQUITO BREEDING IN PADDY FIELDS.

BY

S. NAGENDRA, B.SC.,

AND

K. S. NARAYANA MURTHY.

(Station Sanitary Organization, Agram, Bangalore.)

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INTRODUCTION.

CONTROL of mosquito breeding, particularly anophelines, in paddy fields has been carried out by workers with different insecticides in the powder form and in the form of emulsions diluted to various strengths.

The object of the present trials was to test the effects of D.D.T. as a larvicide in the emulsion and powder forms in paddy fields in strengths of 2·5, 1·25, 5·0 and 2·5 per cent, respectively. Comparisons of larval catches were made in untreated and paris green treated fields.

EXPERIMENT.

Measured portions of sites under paddy cultivation were selected in different parts of the town. The actual plot for a particular insecticide was so selected that it was separated with an interval of at least two untreated plots from the next one under treatment; the idea was to minimize the chances of interconnection of waters (and therefore of insecticide) between different plots under treatment.

As the stage of growth of plant and also the water levels in fields are known to affect breeding, regular measurements were made of these factors (a number of readings being taken before arriving at the average figures for each plot).

Paddy fields were selected in the following extensions of the station:—

Vannarpet, Kodihalli, Chellagatta, Binnamangala and Bypanahalli.

All field observations pertaining to larval catches, condition of plants, depth of water levels in fields, etc., were made personally by the junior author in whose presence it was also ensured that dosages of insecticides were evenly applied in the different plots under treatment. D.D.T. powder and paris green, diluted with soapstone, were applied by hand and D.D.T.-toluene-turpentine emulsion, diluted with water, was sprayed by means of a hand-sprayer (MISH pattern), the approximate dosages of insecticides applied per acre being two gallons of D.D.T. emulsion in water or 24 oz. of D.D.T. powder in soapstone or 6 oz. of paris green with soapstone, of specified strengths. The number of dips for larval collection (in ladles) was restricted to fifteen for each plot.

PADDY CULTIVATION.

It may be of interest to make a brief note on the method of irrigation as practised here. Prior to the commencement of the monsoon (south-west), paddy grains are sown in a carefully prepared plot of land which is maintained in a wet condition with over half a foot of standing water. The plants come up and are allowed to grow for three to five weeks by which time they are about 8 to 10 inches in height. The plants are next taken out, four to five of them tied together and transplanted with an interspacing of half a foot between them, into the regular paddy fields. The latter are situated below the tanks, which are bounded to conserve the rain waters.

The tank water is led through irrigation channels into the paddy fields which also derive water directly from the monsoon showers. Plenty of standing water in field is necessary for the growth of paddy, especially in the early stages. Thus, a good monsoon ensures satisfactory produce while a defective monsoon results in bad outturn of crop. The flowering sets in during October, about three months after transplanting and the grain set a month later (November) and the crop is ready for harvest by December. The yield per acre in the region varies from 2,000 lb. to 3,000 lb.

RESULTS.

The results of larval collections and other relevant data are presented in Tables I to V. In Table VI are given mean monthly figures of maximum and minimum temperatures, relative humidity and rainfall for the period September to December 1948. It is seen from Table I (Vannarpet area) that in the plots under D.D.T. emulsion of strengths of 2·5 and 1·25 per cent, breeding was lower than in the other two. From Table II (Kodihalli area) similar results were obtained, breeding in plots under D.D.T. emulsion of strengths of 2·5 and 1·25 per cent being lower than in the other plots treated with paris green (2·5 per cent), D.D.T. powder (2·5 per cent) and the untreated plot. Results of larval collections are not convincing in the Chellagatta area (Table III), breeding observed being lower in the plot under D.D.T. emulsion (1·25 per cent) than in the other three cases. It was curious that breeding was less in the untreated plot than in those under D.D.T. powder (5 per cent) or D.D.T. emulsion (2·5 per cent) or paris green (5 per cent). It is seen from observations in Binnamangala area (Table IV) that larval collections were less in the plots under D.D.T. emulsion of strengths of 2·5 and 1·25 per cent than in the paris green treated (5 per cent) and untreated plots,

respectively. In the Bypanahalli area (Table V) also, better results were obtained with D.D.T. emulsion (1·25 per cent) than with D.D.T. powder (5 per cent) or paris green (5 per cent) and in the untreated comparison plots.

DISCUSSION.

The common method of irrigating paddy fields situated in the deltas of important rivers is the one known as inundation irrigation by which the lands under crop are flooded with water taken from canals and the water allowed to stand continuously for months. Accordingly, it was thought that the plots selected for observations would be inundated for a period of at least three months with water derived from the tanks, situated at higher levels and also aided directly by the monsoon showers. (This view was actually corroborated by the owners of paddy fields whose plots were chosen for experiment.) While selecting fields it was, therefore, not anticipated that plots either completely dry or partially dry, would be met with, frequently in the later stages. And to assess the effectiveness of a mosquito larvicide, it is essential that fields are maintained wet over long periods.

It is obvious that the water level in fields is one of the most important factors which would affect mosquito breeding. Thus, if a system of irrigation, with a dry day once in five or six days, could be practised by the cultivators, then the necessity for the use of insecticides for the control of mosquito breeding in paddy fields might be altogether obviated.

In the course of routine observations of heights of paddy plants it was noticed that top portions in certain plots were sometimes cut or trimmed. It was explained that the cultivators would do this purposely (*i*) to reduce an unnecessarily high growth of plant which would begin to sag after flowering, (*ii*) to stimulate better growth of paddy, and (*iii*) to use the cut portions of plants as cattle feed.

As mentioned earlier, larval collections were restricted to only fifteen dips for each set of plots under experiment, and this number was maintained throughout, for regularity. It is, however, felt that from the relatively smaller numbers of larvæ collected especially *vide* Tables IV and V, the number of dips may be increased to fifty, which might give a sufficiently greater number of larval catches for comparative study.

The larval collections included *A. subpictus*, *A. annularis* and *C. fatigans*. Identification of all larvæ collected for each set of plots was not carried out. Possibly, such a study might yield interesting results. No adverse effects in the quality or quantity of paddy were reported from any of the fields under experiment. The cultivators, who were kind enough to let us carry out the observations and trials, stated that the produce for the year was almost the same as for any previous years.

CONCLUSIONS.

1. From a study of Tables I, II, IV and V it is seen that D.D.T. emulsion applied in strengths of 2·5 and 1·25 per cent at the rate of two gallons per acre has good larvicidal action.

2. Results with D.D.T. powder of strengths of 2·5 and 5 per cent did not compare favourably with paris green of similar strengths, applied approximately at a rate of 24 oz. per acre.

3. In the course of the experiment, paddy fields either partially dry or completely dry were frequently met with (contrary to expectations) affecting the results of larval catches. It is thus obvious that to assess the efficacy of larvicides, fields must be maintained wet with standing water over sufficiently long periods.

4. No damage of any kind either in quality or quantity of paddy was reported from any plot under experiment.

TABLE I.

Vannarpet area.

Date, 1948.	D.D.T. EMULSION, 2·5 PER CENT. AREA UNDER EXPERIMENT, 1,100 SQ. YARDS.						
	Larval catches.			Average plant height, inches.	Average water level, inches.	Larvicide used, gallons.	REMARKS.
	A	C	P				
Sep.							
10	40	...	20	22·0	1·0	0·75	Before experiment.
17	...	6	2	25·0	0·2	0·5	
24	...	...	...	26·5	0·2	0·5	
Oct.							
1	...	17	7	28·8	0·3	0·4	Moderately dry.
8	1	42	...	32·3	1·6	0·5	
15	...	...	...	36·4	7·4	0·5	
22	...	...	...	44·6	2·0	0·5	
29	...	...	...	50·5	2·5	0·5	
Nov.							
5	...	...	...	57·0	1·2	0·5	
12	...	...	...	57·3	1·0	0·5	
19	...	...	...	61·1	2·6	0·5	
26	...	...	...	62·0	1·2	0·5	
Dec.							
3	...	...	...	63·4	...	...	Ready for harvest; completely dry.
Total catches after commencing experiment.	1	65	9				

A = Anopheline. C = Culicine. P = Pupæ.

TABLE I—*contd.*

Date, 1948.	D.D.T. EMULSION, 1·25 PER CENT. AREA UNDER EXPERIMENT, 1,300 SQ. YARDS.						
	Larval catches.			Average plant height, inches.	Average water level, inches.	Larvicide used, gallons.	REMARKS.
	A	C	P				
Sep.							
10	...	...	...	...	...	...	
17	25	35	...	21·0	1·0	0·5	Before experiment.
24	...	...	...	23·5	0·5	0·5	Moderately dry.
Oct.							
1	1	...	...	23·7	2·6	0·5	
8	...	30	20	24·1	0·7	0·5	Slightly dry.
15	...	1	...	28·9	2·1	0·5	
22	...	...	...	30·7	0·8	0·5	Slightly dry.
29	...	...	...	36·0	0·3	0·5	Moderately dry.
Nov.							
5	...	20	...	43·4	1·3	0·5	Slightly dry.
12	...	4	...	51·0	1·0	0·5	Moderately dry.
19	...	...	...	52·1	1·0	0·5	Slightly dry.
26	...	...	...	51·8	0·7	0·5	Moderately dry.
Dec.							
3	...	...	...	61·0	...	...	Ready for harvest; completely dry.
Total catches after commencing experiment.	1	55	20				

A = Anopheline. C = Culicine. P = Pupæ.

TABLE I—*contd.*

Date, 1948.	PARIS GREEN, 2·5 PER CENT. AREA UNDER EXPERIMENT, 1,350 SQ. YARDS.						
	Larval catches.			Average plant height, inches.	Average water level, inches.	Larvicide used, oz.	REMARKS.
	A	C	P				
Sep.							
10	...	...	...	...	...	...	
17	13	1	...	16·0	1·5	7	Before experiment.
24	20	80	...	21·3	0·2	3	Moderately dry.
Oct.							
1	...	...	...	22·0	...	...	Completely dry.
8	...	1	...	24·2	2·0	6	
15	1	...	...	26·6	2·2	6	
22	...	9	1	31·2	1·3	6	
29	...	...	...	40·5	...	...	Completely dry.
Nov.							
5	...	...	...	42·6	...	...	Completely dry.
12	...	...	...	50·0	...	...	,,
19	...	10	...	49·1	...	2	Mostly dry.
26	20	48	...	46·9	0·5	6	Moderately dry.
Dec.							
3	...	...	...	50·2	...	...	Completely dry; ready for harvest.
Total catches after commencing experiment.	41	148	1				

A = Anopheline. C = Culicine. P = Pupæ.

TABLE I—*concld.*

Date, 1948.	COMPARISON (UNTREATED). AREA UNDER EXPERIMENT, 1,300 SQ. YARDS.					
	Larval catches.			Average plant height, inches.	Average water level, inches.	REMARKS.
	A	C	P			
Sep.						
10	...	...	...	...	...	
17	9	4	...	21·5	1·0	Before experiment.
24	18	130	1	28·0	0·2	Moderately dry.
Oct.						
1	...	...	...	32·0	...	Completely dry.
8	...	...	...	26·2	2·1	
15	...	...	...	33·5	2·3	
22	5	6	...	33·6	1·0	Slightly dry.
29	...	...	...	42·1	...	Completely dry.
Nov.						
5	...	...	...	45·8	...	,,
12	...	...	...	49·4	...	,,
19	...	1	...	50·9	0·8	Slightly dry.
26	...	...	...	46·9	...	Completely dry.
Dec.						
3	...	...	...	50·4	...	Completely dry; ready for harvest.
Total catches after commencing experiment.	23	137	1			

A = Anopheline. C = Culicine. P = Pupæ.

TABLE II.

Kodihalli area.

Date, 1948.	D.D.T. EMULSION, 2·5 PER CENT. AREA UNDER EXPERIMENT, 1,260 SQ. YARDS.						
	A	C	P	Average plant height, inches.	Average water level, inches.	Larvicide used, gallons.	REMARKS.
Sep.							
13	1	...	...	20·0	2·0	1·0	Before experiment.
20	2	...	...	23·8	1·5	0·5	
27	...	...	...	25·5	1·0	0·5	
Oct.							
4	...	...	...	31·0	1·0	0·5	
11	...	...	...	40·0	2·1	0·5	
18	...	...	...	50·3	1·4	0·5	
25	...	...	...	41·3	1·5	0·5	
Nov.							
2	1	...	...	40·0	0·8	0·5	Slightly dry.
8	...	...	...	53·5	1·0	0·5	
15	...	3	...	51·0	1·8	0·5	
22	...	...	...	53·1	1·3	0·5	
29	...	...	...	57·1	...	...	Completely dry.
Dec.							
6	...	...	...	...	...	...	Ready for harvest.
Total catches after commencing experiment.	3	3	...				

A = Anopheline. C = Culicine. P = Pupæ.

TABLE II—*contd.*

Date, 1948.	D.D.T. EMULSION, 1·25 PER CENT. AREA UNDER EXPERIMENT, 1,250 SQ. YARDS.						
	A	C	P	Average plant height, inches.	Average water level, inches.	Larvicide used, gallons.	REMARKS.
Sep.							
13	...	...	...	...	...	...	
20	2	...	...	19·0	0·75	0·5	Before experiment.
27	1	...	...	21·0	0·8	0·5	Moderately dry.
Oct.							
4	...	...	...	28·4	1·0	0·5	
11	...	...	...	25·4	2·0	0·5	
18	...	...	...	34·5	1·1	0·5	
25	...	...	...	37·5	1·1	0·5	
Nov.							
2	...	...	...	39·0	1·0	0·5	
8	...	...	...	46·8	1·3	0·5	
15	1	...	...	43·7	1·3	0·5	
22	1	...	...	44·6	1·1	0·5	
29	...	...	...	48·0	0·3	0·5	Mostly dry.
Dec.							
6	...	...	...	...	...	...	Ready for harvest.
Total catches after commencing experiment.	3	...	...				

A = Anopheline. C = Culicine. P = Pupæ.

TABLE II—*contd.*

Date, 1948.	D.D.T. POWDER, 2·5 PER CENT. AREA UNDER EXPERIMENT, 1,350 SQ. YARDS.						
	A	C	P	Average plant height, inches.	Average water level, inches.	Larvicide used, oz.	REMARKS.
Sep.							
13	...	...	...	...	...	...	
20	...	...	...	20·8	1·3	6	Before experiment.
27	6	6	...	26·0	0·5	6	Moderately dry.
Oct.							
4	3	8	...	26·2	0·9	6	
11	2	3	...	29·5	1·3	6	
18	...	...	...	42·0	1·3	6	
25	...	...	...	41·3	0·8	6	Slightly dry.
Nov.							
2	6	2	...	45·0	0·6	6	Slightly dry.
8	2	4	...	51·3	0·9	6	
15	10	...	...	46·5	1·5	6	
22	...	...	...	47·8	0·2	2	Mostly dry.
29	...	...	...	49·0	...	...	Completely dry.
Dec.							
6	...	...	...	...	...	...	Ready for harvest.
Total catches after commencing experiment.	29	23	...				

A = Anopheline. C = Culicine. P = Pupæ.

TABLE II—*contd.*

Date, 1948.	PARIS GREEN, 2·5 PER CENT. AREA UNDER EXPERIMENT, 1,200 SQ. YARDS.						
	A	C	P	Average plant height, inches.	Average water level, inches.	Larvicide used, oz.	REMARKS.
Sep.							
13	...	...	...	...	...	...	
20	...	...	...	18·5	1·0	6	Before experiment.
27	8	...	...	24·0	1·0	6	Slightly dry.
Oct.							
4	1	2	...	27·0	1·0	6	,,
11	3	4	...	30·7	1·0	6	,,
18	1	4	...	43·8	0·6	6	,,
25	...	...	...	44·0	0·5	6	Moderately dry.
Nov.							
2	6	9	...	50·4	0·6	6	,,
8	1	...	...	51·7	0·8	6	Slightly dry.
15	...	2	...	49·1	1·4	6	
22	...	...	...	49·1	...	...	Completely dry.
29	...	...	...	50·5	...	...	,,
Dec.							
6	...	...	...	...	...	...	Ready for harvest.
Total catches after commencing experiment.	20	21	...				

A = Anopheline. C = Culicine. P = Pupæ.

TABLE II—*concld.*

Date, 1948.	COMPARISON (UNTREATED). AREA UNDER EXPERIMENT, 1,100 SQ. YARDS.					
	A	C	P	Average plant height, inches.	Average water level, inches.	REMARKS.
Sep.						
13	...	1	...	22·0	1·4	Before experiment.
20						
27	6	2	...	26·0	0·5	Moderately dry.
Oct.						
4	2	7	...	32·3	1·0	
11	5	...	...	32·3	1·0	
18	...	1	...	44·4	1·0	
25	3	...	...	42·7	0·5	Slightly dry.
Nov.						
2	5	2	...	51·4	0·6	Slightly dry.
8	2	4	1	53·0	1·2	
15	2	3	...	51·2	1·7	
22	...	...	...	48·8	...	Completely dry.
29	...	...	...	53·4	...	,,
Dec.						
6	...	...	...	...	...	Ready for harvest.
Total catches after commencing experiment.	25	19	1			

A = Anopheline. C = Culicine. P = Pupæ.

TABLE III.

Chellagatta area.

Date, 1948.	D.D.T. EMULSION, 2·5 PER CENT. AREA UNDER EXPERIMENT, 1,000 SQ. YARDS.						
	A	C	P	Average plant height, inches.	Average water level, inches.	Larvicide used, gallons.	REMARKS.
Sep.							
16	49	15	...	16·0	3·0	1·0	Before experiment.
23	3	5	...	21·5	0·8	0·3	Slightly dry.
30	...	4	...	22·8	2·5	0·4	
Oct.							
7	...	21	1	27·0	4·0	0·5	
14	1	1	4	25·0	0·7	0·5	Slightly dry.
21	...	...	...	29·5	0·9	0·5	,,
28	...	...	...	32·0	0·8	0·5	,,
Nov.							
4	...	...	...	36·0	...	...	Completely dry.
11	...	...	...	41·3	...	...	,,
25	...	...	...	59·5	0·2	0·5	Mostly dry.
Dec.							
2	...	...	...	59·7	1·4	0·5	
9	...	...	...	...	...	...	Completely dry; ready for harvest.
Total catches after commencing experiment.	4	31	5				

A = Anopheline. C = Culicine. P = Pupæ.

TABLE III—*contd.*

Date, 1948.	D.D.T. EMULSION, 1·25 PER CENT. AREA UNDER EXPERIMENT, 900 SQ. YARDS.						
	A	C	P	Average plant height, inches.	Average water level, inches.	Larvicide used, gallons.	REMARKS.
Sep.							
16	...	10	...	24·0	2·0	1·0	Before experiment.
23	...	...	...	25·8	0·8	0·3	Slightly dry.
30	2	14	...	23·5	0·8	0·4	,,
Oct.							
7	...	2	...	25·8	2·4	0·5	
14	...	...	...	31·1	...	...	Completely dry.
21	...	...	...	30·0	...	...	,,
28	...	...	...	42·3	...	...	,,
Nov.							
4	...	...	...	45·1	...	...	Completely dry.
11	...	...	...	50·3	0·9	0·5	Slightly dry.
25	4	...	...	59·7	0·5	0·5	Mostly dry.
Dec.							
2	...	...	...	61·0	...	...	Completely dry.
9	...	...	...	...	...	...	Completely dry; ready for harvest.
Total catches after commencing experiment.	6	16	...				

A = Anopheline. C = Culicine. P = Pupæ.

TABLE III—*contd.*

Date, 1948.	D.D.T. POWDER, 5 PER CENT. AREA UNDER EXPERIMENT, 950 SQ. YARDS.						
	A	C	P	Average plant height, inches.	Average water level, inches.	Larvicide used, oz.	REMARKS.
Sep.							
16	16	18	3	17·0	2·5	8	Before experiment.
23	160	10	...	23·5	1·0	6	
30	1	2	...	21·0	3·4	6	
Oct.							
7	...	...	2	24·6	3·3	6	
14	...	...	1	26·2	1·0	6	
21	...	...	...	27·2	0·8	6	
28	11	2	...	19·4	0·5	6	Moderately dry.
Nov.							
4	...	...	...	37·5	...	...	Completely dry.
11	...	64	...	42·8	2·0	6	
25	...	...	...	58·3	0·5	6	Moderately dry.
Dec.							
2	...	...	...	61·2	0·2	6	Mostly dry.
9	...	...	...	...	...	...	Completely dry; ready for harvest.
Total catches after commencing experiment.	172	78	3				

A = Anopheline. C = Culicine. P = Pupæ.

TABLE III—*contd.*

Date, 1948.	PARIS GREEN, 5 PER CENT. AREA UNDER EXPERIMENT, 950 SQ. YARDS.						
	A	C	P	Average plant height, inches.	Average water level, inches.	Larvicide used, oz.	REMARKS.
Sep.							
16	14	8	...	19·5	3·0	8	Before experiment.
23	1	4	7	20·8	0·8	6	Slightly dry.
30	19	25	7	22·3	1·3	6	
Oct.							
7	3	1	...	27·0	3·0	6	
14	2	1	...	29·3	0·8	6	Slightly dry.
21	6	5	...	32·6	1·5	6	
28	2	...	...	36·0	0·5	6	Slightly dry.
Nov.							
4	...	...	...	31·5	...	...	Completely dry.
11	...	...	...	37·5	...	...	,,
25	...	...	...	58·8	...	...	,,
Dec.							
2	...	...	...	55·8	0·3	6	Mostly dry.
9	...	...	...	...	...	...	Completely dry; ready for harvest.
Total catches after commencing experiment.	33	36	14				

A = Anopheline. C = Culicine. P = Pupæ.

TABLE III—*concld.*

Date, 1948.	COMPARISON (UNTREATED). AREA UNDER EXPERIMENT, 900 SQ. YARDS.					
	A	C	P	Average plant height, inches.	Average water level, inches.	REMARKS.
Sep.						
16	5	15	...	24·0	1·3	Before experiment.
23	...	...	...	25·0	0·5	Slightly dry.
30	3	30	...	26·5	0·6	,,
Oct.						
7	...	2	...	28·7	1·8	
14	...	...	...	31·3	0·7	Slightly dry.
21	...	...	...	35·3	...	Completely dry.
28	...	...	...	31·5	...	,,
Nov.						
4	...	...	...	43·0	...	Completely dry.
11	...	...	...	47·1	...	,,
25	...	...	...	58·5	0·8	Slightly dry.
Dec.						
2	...	...	...	57·5	...	Completely dry.
9	...	...	...	...	...	Completely dry; ready for harvest.
Total catches after commencing experiment.	3	32	...			

A = Anopheline. C = Culicine. P = Pupæ.

TABLE IV.

Binnâmangala area.

Date, 1948.	D.D.T. EMULSION, 2·5 PER CENT. AREA UNDER EXPERIMENT, 950 SQ. YARDS.						
	A	C	P	Average plant height, inches.	Average water level, inches.	Larvicide used, gallons.	REMARKS.
Sep.							
14	14	2	2	10·0	4·5	1·0	Before experiment.
21	...	...	...	17·0	3·8	0·3	
28	...	...	...	16·3	0·8	0·3	Slightly dry.
Oct.							
5	...	1	...	16·8	1·3	0·3	
19	...	...	...	19·6	0·6	0·4	Slightly dry.
26	...	...	...	22·6	0·5	0·4	Moderately dry.
Nov.							
2	...	...	...	24·6	0·5	0·5	,,
9	...	...	...	28·0	0·8	0·5	Slightly dry.
23	...	...	...	40·4	1·1	0·5	
30	...	...	...	39·6	1·9	0·5	
Dec.							
7	...	...	...	...	...	...	Completely dry; ready for harvest.
Total catches after commencing experiment.	...	1	...				

A = Anopheline. C = Culicine. P = Pupæ.

TABLE IV—*contd.*

Date, 1948.	D.D.T. EMULSION, 1·25 PER CENT. AREA UNDER EXPERIMENT, 960 SQ. YARDS.						
	A	C	P	Average plant height, inches.	Average water level, inches.	Larvicide used, gallons.	REMARKS.
Sep.							
14	...	3	...	12·0	1·5	1·0	Before experiment.
21	...	...	...	15·0	3·0	0·3	
28	...	...	...	19·0	0·5	0·25	Moderately dry.
Oct.							
5	1	...	...	19·0	0·8	0·4	Slightly dry.
19	...	...	...	22·8	0·9	0·4	,,
26	...	...	...	28·0	1·0	0·4	
Nov.							
2	...	...	...	31·0	2·5	0·5	
9	...	...	...	35·5	1·4	0·5	
23	...	...	...	39·7	2·1	0·5	
30	...	...	...	39·8	0·7	0·5	Slightly dry.
Dec.							
7	...	...	...	...	...	...	Completely dry; ready for harvest.
Total catches after commencing experiment.	1	...	...				

A = Anopheline. C = Culicine. P = Pupæ.

TABLE IV—*contd.*

Date, 1948.	PARIS GREEN, 5 PER CENT. AREA UNDER EXPERIMENT, 1,000 SQ. YARDS.						
	A	C	P	Average plant height, inches.	Average water level, inches.	Larvicide used, oz.	REMARKS.
Sep.							
14	3	2	...	14·0	2·0	7	Before experiment.
21	...	...	...	16·0	2·5	6	
28	...	3	...	16·5	0·4	6	Moderately dry.
Oct.							
5	...	...	...	18·0	0·2	2	Mostly dry.
19	...	...	...	22·5	0·5	4	Moderately dry.
26	...	...	...	25·5	...	...	Completely dry.
Nov.							
2	1	4	...	30·7	0·5	6	Moderately dry.
9	4	1	...	34·3	1·9	6	
23	4	6	...	41·3	0·6	6	Slightly dry.
30	1	3	...	39·3	0·5	6	Moderately dry.
Dec.							
7	...	...	...	...	...	...	Completely dry; ready for harvest.
Total catches after commencing experiment.	10	17	...				

A = Anopheline. C = Culicine. P = Pupæ.

TABLE IV—*concld.*

Date, 1948.	COMPARISON (UNTREATED). AREA UNDER EXPERIMENT, 975 SQ. YARDS.					
	A	C	P	Average plant height, inches.	Average water level, inches.	REMARKS.
Sep.						
14	3	7	...	14·0	2·0	Before experiment.
21	...	...	...	16·5	3·0	
28	2	8	...	19·0	3·0	Moderately dry.
Oct.						
5	4	4	...	18·2	0·3	Mostly dry.
19	...	...	...	21·4	0·3	Moderately dry.
26	...	...	...	25·5	...	Completely dry.
Nov.						
2	2	5	...	27·0	2·0	
9	10	2	...	31·0	1·2	
23	2	3	...	40·9	0·5	Moderately dry.
30	4	2	...	38·4	...	Mostly dry. Only small pool of water.
Dec.						
7	...	...	...	...	...	Completely dry; ready for harvest.
Total catches after commencing experiment.	24	24	...			

A = Anopheline. C = Culicine. P = Pupæ.

TABLE V.

Bypanahalli area.

Date, 1948.	D.D.T. EMULSION, 1·25 PER CENT. AREA UNDER EXPERIMENT, 1,150 SQ. YARDS.						
	A	C	P	Average plant height, inches.	Average water level, inches.	Larvicide used, gallons.	REMARKS.
Sep.							
21	...	3	...	16·0	2·5	0·3	Before experiment.
28	3	...	...	20·0	0·7.	0·3	Slightly dry.
Oct.							
5	2	...	...	21·0.	0·5	0·5	Slightly dry.
19	2	...	...	24·2	0·3	0·5	Moderately dry.
26	...	...	...	29·2	2·0	0·5	
Nov.							
2	...	...	...	30·5	0·7	0·5	Slightly dry.
9	...	...	...	32·4	0·5	0·5	Moderately dry.
23	...	4	...	50·8	0·3	0·5	Mostly dry.
30	...	...	...	...	...	...	Completely dry; ready for harvest.
Total catches after commencing experiment.	7	4	...				

A = Anopheline. C = Culicine. P = Pupæ.

TABLE V—*contd.*

Date, 1948.	D.D.T. POWDER, 5 PER CENT. AREA UNDER EXPERIMENT, 1,150 SQ. YARDS.						
	A	C	P	Average plant height, inches.	Average water level, inches.	Larvicide used, oz.	REMARKS.
Sep.							
21	...	2	...	15·0	5·3	5	Before experiment.
28	14	...	...	20·7	0·1	2	Mostly dry.
Oct.							
5	...	...	...	21·3	...	...	Completely dry.
19	...	...	...	23·2	...	...	,,
26	...	...	...	30·0	0·1	1	Mostly dry.
Nov.							
2	...	...	...	35·6	...	...	Completely dry.
9	8	4	...	34·8	...	2	Mostly dry. Pools of stagnant water.
23	...	...	...	36·1	...	...	Completely dry.
30	...	...	...	...	...	...	Completely dry; ready for harvest.
Total catches after commencing experiment.	22	4	...				

A = Anopheline. C = Culicine. P = Pupæ.

TABLE V—*contd.*

Date, 1948.	PARIS GREEN, 5 PER CENT. AREA UNDER EXPERIMENT, 1,150 SQ. YARDS.						
	A	C	P	Average plant height, inches.	Average water level, inches.	Larvicide used, oz.	REMARKS.
Sep.							
21	1	4	...	19·5	5·3	5	Before experiment.
28	...	...	1	20·3	1·5	6	
Oct.							
5	...	2	...	24·3	1·8	6	
19	...	...	...	24·7	0·5	6	Slightly dry.
26	4	...	...	32·7	2·3	6	
Nov.							
2	3	16	...	36·6	1·0	6	
9	...	...	...	37·4	...	...	Completely dry.
23	6	31	...	44·3	0·5	6	Slightly dry.
30	...	...	...	...	...	...	Completely dry; ready for harvest.
Total catches after commencing experiment.	13	49	1				

A = Anopheline. C = Culicine. P = Pupæ.

TABLE V—*concld.*

Date, 1948.	COMPARISON (UNTREATED). AREA UNDER EXPERIMENT, 1,100 SQ. YARDS.					
	A	C	P	Average plant height, inches.	Average water level, inches.	REMARKS.
Sep.						
21	2	7	...	17·0	2·5	Before experiment.
28	2	4	...	21·5	2·0	
Oct.						
5	4	...	...	26·7	0·3	Moderately dry.
19	...	2	...	32·6	1·5	
26	5	...	...	46·0	3·3	
Nov.						
2	8	20	...	36·5	0·6	Slightly dry.
9	...	3	...	34·6	0·2	Mostly dry.
23	3	22	...	40·0	0·5	Slightly dry.
30	...	...	...	...	...	Completely dry; ready for harvest.
Total catches after commencing experiment.	22	51	...			

A = Anopheline. C = Culicine. P = Pupæ.

TABLE VI.

Temperature, humidity and rainfall recorded at Bangalore during the period September to December 1948.

Months.	Mean maximum temperature, °F.	Mean minimum temperature, °F.	Relative humidity, per cent.	Rainfall, inches.
1948.				
Sep. ...	81·4	65·9	87·9	3·23
Oct. ...	82·3	65·9	85·8	5·37
Nov. ...	80·2	66·7	87·7	1·49
Dec. ...	77·7	59·1	82·6	0·20

Indian Journal of Malariology, **3**, *2 and 3, June/September 1949.*

MALARIA CONTROL IN COORG.

BY

LIEUT.-COLONEL JASWANT SINGH, M.B., CH.B. (Edin.), D.P.H. (Eng.), D.T.M. & H. (Edin.)

(Director, Malaria Institute of India, Delhi.)

AND

MAJOR C. B. KARIAPA, M.B., B.S.

(Malaria Officer, Coorg, Mercara.)

[May 31, 1949.]

COORG, the smallest centrally administered province, lies in Southern India on the top of the Western Ghats. Its sixteen hundred square miles of mountain and forest merge on the north and east with the upland plateau of the Mysore State while towards the Indian Ocean, at the foot of the Ghats, stretch the lowland districts of Malabar and South Kanara. The climate is humid and temperate, rainfall is intense, vegetation profuse, and wild animals, birds and insects abound in the forests. Seen from the heights round Mercara, the vista is that of a green sea stretching away to the far horizon; hills and ridges form the waves, crests and sides of which are thickly clad with forests in whose shade grow the coffee bushes; in the troughs of the waves lie patches of the rice cultivation which yield the staple food crop of Coorg. In the lower tracts round Fraserpet in the Cauvery Valley, where the country is more open and less hilly, and rainfall not as high, the views are those of open grassy glades between avenues of trees and hedgerows.

Malaria is prevalent throughout the province and in such intensity that Coorg has been classed as a hyperendemic area. There is no reason to believe that this is a recent development. In 1870, Rev. Richter, in the Gazetteer of Coorg, refers to 'the dreaded Coorg fever which appears at its worse in summer'. Some figures

put forward by the Coorg Malaria Committee of 1926 indicate the extent of malaria in Coorg in a progressive scale (Table I).

TABLE I.

Malaria and other cases treated in dispensaries and hospitals.

Year.	Number of patients treated.	Number of malaria cases.
1918 ...	106,794	31,865
1923 ...	152,695	64,269
1924 ...	187,447	90,598
1935 ...	302,430	122,560
1940 ...	358,340	144,243
1945 ...	297,201	130,599
1948 ...	261,417	64,127

The vital statistics of Coorg are not sufficiently accurate to afford true evidence for a discussion of the conceptions of malaria in Coorg but the census returns (Table II), if examined critically, do appear to throw some light.

TABLE II.

Census returns.

Census year.	Total population.
1871 ...	168,312
1881 ...	178,302
1891 ...	173,055
1901 ...	180,607
1911 ...	174,976
1921 ...	163,838
1931 ...	163,327
1941 ...	168,726

At first sight these figures seem to support the view that some depopulating influence has been at work and there is a definite lack of natural expected increase.

The above figures include immigrant labour to coffee estates from South Kanara and Mysore, but if only the permanent population is taken into consideration there has been a notable decrease since about 50 years.

TABLE III.

Statement of births and deaths from 1944 *to* 1948.

Year.	Births.	Deaths.
1944 ...	3,173	3,459
1945 ...	3,116	3,148
1946 ...	3,220	3,646
1947 ...	3,063	2,545
1948 ...	3,237	2,119

The above figures show that till 1946 death-rates are higher than birth-rates.

Of late years the state of malaria in Coorg reached an alarming pitch so much so that the economic conditions of Coorg were rapidly deteriorating. Village after village was being deserted. Coffee plantations were not fully functioning for want of labour. Fifty years ago, a Coorg could grow 100 battees (1 battee = 80 seers) of paddy in 3 acres of land which in 1940 came down to an average of 40 battees. A European planter bought for farming about 200 acres of land where twenty years ago there were three villages. When this farm was examined in 1946 there were 72 fever cases among 136 labourers.

That the whole province was highly malarious and the conditions were getting worse every year, was realized by Major-General Sir Gordon Covell who put up a strong recommendation in 1946 to the Government of India to start a Malaria Unit in Coorg. Accordingly a sum of Rs. 1,73,000 was sanctioned out of which Rs. 50,000 was allocated for building a laboratory and office, and the unit consisting of the following staff started functioning in the beginning of 1947 :—

1 Malaria Officer.
1 Malaria Assistant (not appointed).
1 Assistant Entomologist.
1 Overseer (not appointed).
2 Laboratory Assistants.
2 Clerks.
8 Malaria Inspectors.
5 Insect Collectors.
40 Field Workers.
3 D.D.T. Grinders.
2 Drivers.
1 Peon.

PLAN OF WORK.

The province was divided into 8 circles, each under an inspector with adequate number of field workers.

Though it is not possible to describe in detail the work carried out throughout the province, an account of the work in Mercara which is typical in all respects of other parts of Coorg, except a small portion round about Fraserpet which is in conformation identical with the Mysore plateau, is given here.

Mercara is the administrative capital of Coorg. It is a picturesque, green, well-wooded rural town of about 7,000 inhabitants situated at an elevation of 3,800 ft. above sea level (Map 1).

It has a rainfall of 120 to 130 inches per annum, which starts about the middle of June. Torrential rainfall is experienced during the months of July and August and it slackens in September and October. From December to March it is usually dry. April has an average rainfall of two to three inches. The average mean maximum temperature is 85·8°F. in the hottest months, April-May. During the monsoon, i.e. in July and August, it falls down to 64·2°F. The average minimum temperature is 62·2°F., minimum being 56·7°F. in December. The humidity is usually at all times high. For about nine months in a year the average relative humidity is about 80 to 85 per cent. It reaches saturation point during monsoon, i.e. July and August, and only in dry months—February, March and April—it falls down to 65 per cent.

The town itself is situated on a series of ridges. On the main ridge stands the fort. Between the ridges most of the inhabitants of the town live amidst streams and seepages. A large stream called the fish stream receives all the water from the small streams and seepages and takes a course towards the west of the town. Houses have been built all along this stream.

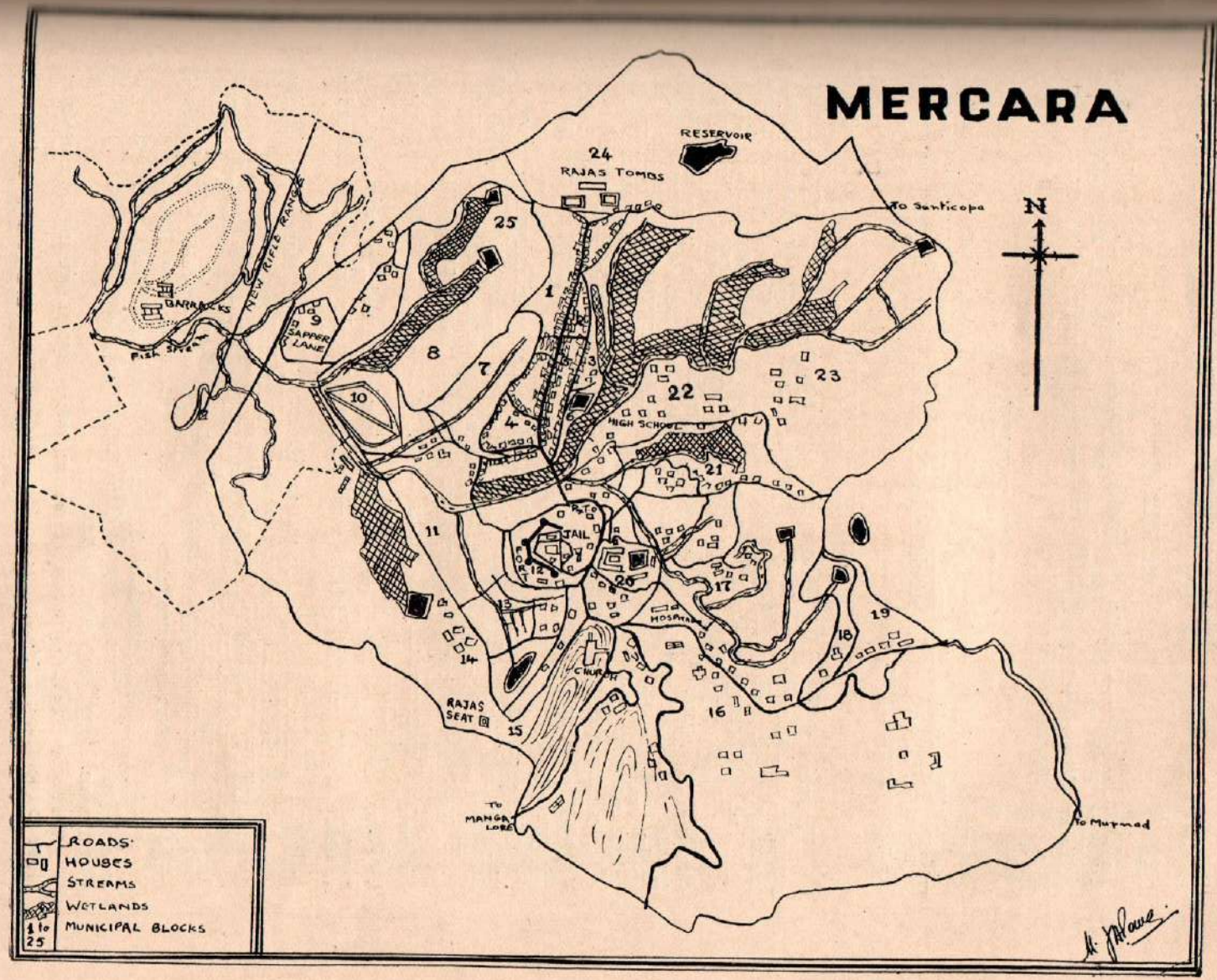
ANOPHELINE FAUNA.

In 1919, Hasel Wright found anopheline larvæ breeding in these streams and he recommended that all the streams should be drained. Unfortunately, the municipal authorities built open stone-pitched drains with unfortunate results as breeding of mosquitoes increased in the crevices and malaria incidence naturally increased.

Larvæ of *A. fluviatilis* were found in large numbers in all the stone-pitched drains in the month of February 1947. This was the most numerous species in the inhabited area. *A. splendidus* was another common species. Besides these two, the following other species were also collected :—

A. maculatus.
A. vagus.
A. hyrcanus.
A. karwari.
A. jeyporiensis.
A. philippinensis.

The common breeding places of these non-vectors were tanks, up-valley streams, wells, stagnant water collections in stone quarries, etc.


MERCARA
RESERVOIR
24
RAJAS TOMBS
To Santicopa
N
25
1
BARRACKS
NEW RIFLE RANGE
9
SAPPER LANE
FIRE STATION
8
7
3
10
4
22
23
HIGH SCHOOL
21
11
JAIL
12
20
17
HOSPITAL
19
18
14
CHURCH
RAJAS SEAT
15
16
To MANGALORE
To Murmad
ROADS
HOUSES
STREAMS
WETLANDS
1 to 25 MUNICIPAL BLOCKS

SPLEEN RATE.

Hasel Wright (1919) found the spleen rate to be 54 per cent. McCombie Young and Baily (1927) recorded a spleen rate of 55·4 per cent which in the beginning of 1947 was 58·2 per cent showing that Mercara was as malarious as before. This is true for the rest of Coorg.

ADULT CATCHES.

House searches for adult mosquitoes were rather disappointing. Even though *A. fluviatilis* were breeding prolifically all round the dwelling houses, only 6 specimens per man-hour could be caught while the catches in other species range from 15 to 20 per man-hour.

ANTIMALARIA MEASURES.

The senior author visited Mercara in the beginning of 1947 and suggested that as the transmission was about to begin, residual D.D.T. spraying programme should be initiated. Accordingly D.D.T. indoor spraying was started in the beginning of March 1947 using for the first round a 5 per cent D.D.T.-kerosene solution at the rate of 2·5 c.c. per square foot. Mercara has in all about 1,756 dwelling houses and 259 out-houses. Three batches using three stirrup pumps each completed the indoor residual spraying of all the dwelling houses and out-houses in three weeks. Insect Collectors with the Assistant Entomologist went round all the catching stations daily. For about ten days dead mosquitoes of all species were collected and afterwards, for eight weeks, no mosquitoes were encountered.

The following formula of D.D.T. suspension was used in the subsequent sprays :—

Five grammes of flake gelatine and ten grammes of powdered gum acacia were dissolved in 480 c.c. of hot water and cooled before grinding with 600 grammes of D.D.T. powder in a stone mortar into a fine homogeneous paste. This process took about half an hour. The paste was diluted with water as follows :—

For a 5 per cent D.D.T. spray, thoroughly mix up with water to make $2\frac{1}{2}$ gallons.

For a 2·5 per cent D.D.T. spray, thoroughly mix with water to make 5 gallons.

For a 1·25 per cent D.D.T. spray, thoroughly mix with water to make 10 gallons.

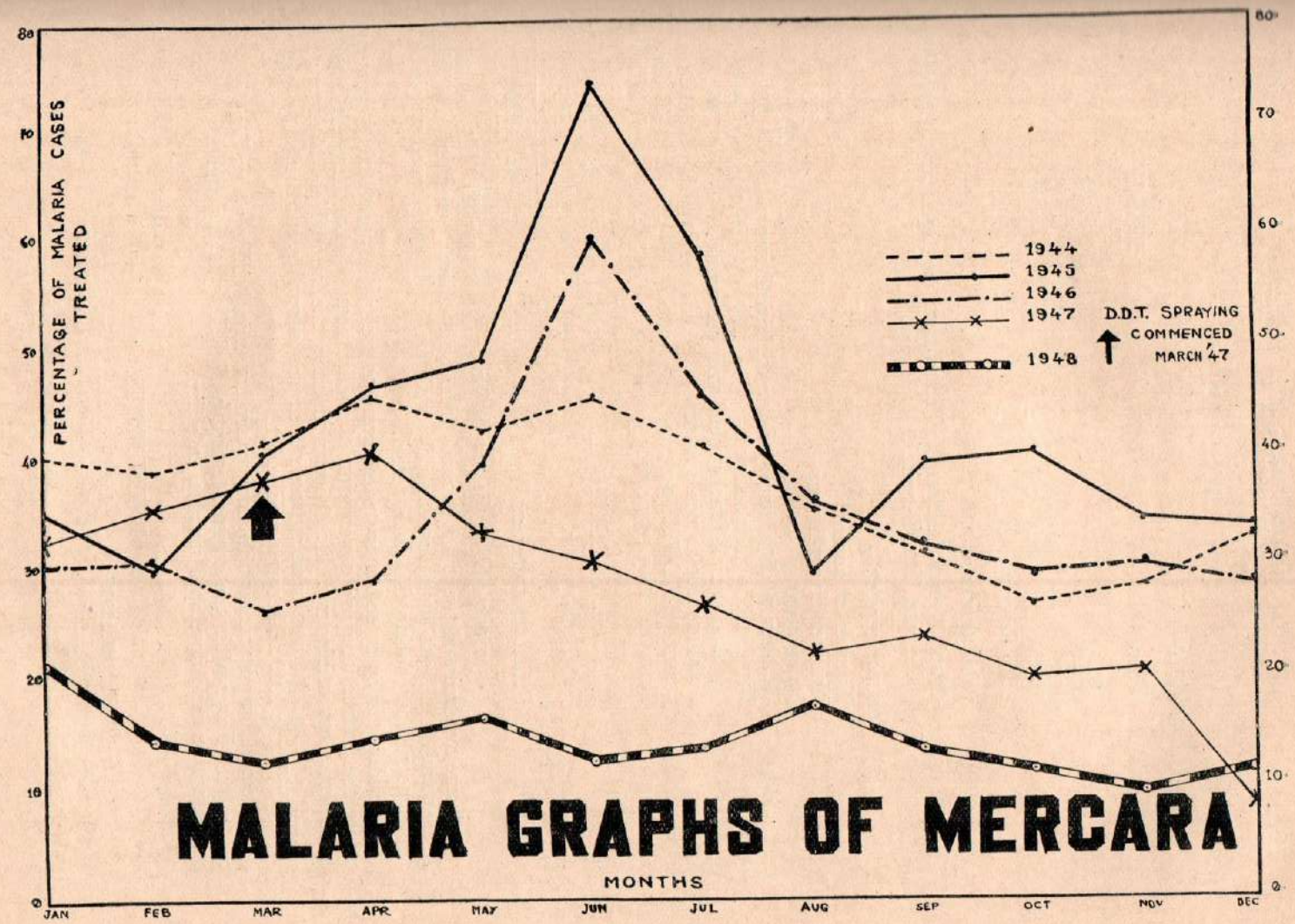
While spraying, the suspension in the container was stirred occasionally otherwise the particles of D.D.T. tended to settle at the bottom.

Ten pounds of D.D.T. were ground at a time in one hour or a little more. For the second round which commenced in the beginning of May 1947, 2·5 per cent of this suspension was used at the rate of 2·5 to 3 c.c. per square foot. Another round of the same suspension was sprayed in the month of October 1947.

During 1948, three rounds of spraying were carried out at an interval of eight weeks commencing from January. One more round was given in the month of October, i.e. immediately after monsoon.

Three rounds of 2·5 per cent suspension were sprayed beginning from January 1949 to the end of May.

Graph 1.


PERCENTAGE OF MALARIA CASES TREATED
1944
1945
1946
1947
1948
D.D.T. SPRAYING COMMENCED MARCH '47
MONTHS
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
MALARIA GRAPHS OF MERCARA

RESULTS.

(*i*) *Spleen rate* :—

Year.	Spleen rate, per cent.
1919	54·00
1927	55·40
1946	58·20
1949 January	10·00

(*ii*) *Hospital figures* :—

Graph 1 gives the figures collected from the dispensary attached to Mercara Civil Hospital.

(*iii*) *Cost* per capita :—

Number of houses	1,756
Number of out-houses	259
Population	7,102
Number of days employed for one round spray	16
Number of rounds sprayed	4
Number of field workers engaged per round ...	96
Total number of men worked	384
Quantity of D.D.T. per round	175 lbs.
Total quantity consumed	700 lbs.
Total cost of D.D.T.	Rs. 3,920
Total salary, etc.	,, 816
Total amount spent	Rs. 4,736

Per capita cost on the population = Re. 0-10-8 per year.

Three hundred and twenty-eight rural towns and villages consisting of 22,744 houses and 11,121 out-houses covering a population of 123,401, were sprayed in the same manner during the same period.

Besides this, every coffee estate management took up regular D.D.T. spraying under the instructions and supervision of the authors. Everywhere the results have been most satisfactory. To quote an instance, the farm which was examined in 1946 and had 72 fever cases among 136 coolies, showed a surplus cooly labour strength during 1949 all keeping good health.

A careful watch was kept all along to see how many children were born in the controlled villages after the commencement of D.D.T. spraying and happily not one infant or child developed malarial infection.

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A NEW METHOD OF DIAGNOSIS OF KALA-AZAR.

BY

N. G. S. RAGHAVAN.

(*Malaria Institute of India.*)

[June 17, 1949.]

In the laboratory diagnosis of kala-azar, the 'aldehyde test' of Napier and the 'urea stibamine test' of Chopra have been widely used. As Napier (1928) put it, 'the aldehyde and antimony test with diluted sera are tests of almost equal and very considerable practical value in the diagnosis of kala-azar but antimony tests with undiluted serum fell short of both'. The former method consisted in adding a drop of 40 per cent formalin solution (commercial formalin) to about 1 c.c. of plasma or serum, and shaking gently. The basis of this test was on the report of Spackman (1921). Various grades of positives have been described depending on the time taken to form a formal 'leucogel' and the consistency and opacity of the same (Napier, 1922).

Chopra's test consists in running a few drops of 4 per cent solution of urea stibamine down the side of a test tube containing serum or plasma diluted in saline in the proportion of 1 in 10. Flocculation at the junction of the fluids is diagnostic of kala-azar (Chopra *et al.*, 1927, 1927*a*; Chopra and Choudhury, 1928; Chopra and De, 1929).

Bose *et al.* (1928) found that the optimum strengths of the reagent and serum to be used in conducting Chopra test were (*a*) four per cent solution of urea stibamine and (*b*) 1 in 10 dilution of serum. They also used a tube of 55 mm. length and 3 mm. diameter.

Lloyd and Paul (1928) conducted studies in 'serum changes in kala-azar' and showed that only ammonium salts interfere with the 'leucogel' formation.

Chopra and Mukherjee (1930) described a finger-prick method for diagnosing kala-azar. A drop or two of blood from a pricked finger were received in a small test tube having an internal diameter of about three-eighths of an inch containing 0·25 c.c. of a two per cent potassium oxalate solution, and after standing for some time the supernatant fluid was withdrawn into a smaller tube. A solution of four per cent urea stibamine was then added to it and results noted. This method had obvious advantages in that vein puncture was avoided and the quantity of blood taken was small, but involved transfer of supernatant fluid from one tube to another.

Boyd and Roy (1928) described that when 1 in 40,000 solution of methylene blue was allowed to stand in contact with the serum of kala-azar cases, there was a marked decolorization and motile Gram-negative bacilli were seen in 72–96 hours. They concluded that the mixture formed a suitable medium for the growth of micro-organism which bleached the dye.

PRESENT WORK.

Studies were undertaken to determine how far blood taken straight in a capillary tube, 1 mm. in diameter and 9 cm. long, containing a small amount of 2 per cent potassium citrate solution could be used with advantage in the field and the changes that might occur by addition of diluted dyes in the entire solution.

Work carried out can be divided into two parts : (1) during a rapid survey in Central Nepal Terai in February 1949, where malaria and kala-azar were present in a hyperendemic state ; and (2) work at the Malaria Institute of India, in April-May 1949.

First series of test were carried out during a routine malaria survey in Central Nepal Terai in February 1949. Blood exuding from a pricked finger was taken for blood films for examination of malaria parasites, as also in separate capillary tubes containing 2 per cent potassium citrate solution for kala-azar test. The latter was drawn to about one-fourth of the length of the tube and the blood was drawn till the resultant column reached about two-thirds of the length of the tube. The capillaries were placed vertically stuck in plasticine on which the same serial numbers as of the source of blood were indicated. Enlargement of liver and spleen and consistency of same were also noted.

After about an hour all the sera had well separated. The lower portion of capillary tubes containing a deposit of cells and a plug of plasticine was ' filed off ' at the junction of the serum and cell layers. The broken end was then dipped in 40 per cent formalin so that the resultant column of fluid in the tube nearly reached three-quarters of its length. The positive cases gave a 'leucogel' in varying periods of time.

In Napier test it was noted that the gel formation was not uniform in some of the capillary tubes. But when the capillaries were rolled between the fingers the whole column gave a uniform thick gel in positive cases. To obviate such differences and to enable easier detection, diffusable dyes not interfering with the reaction were tried. It was found that eosin diffused very quickly through the colourless column of formalin and serum thus carrying the formalin through affording it a better chance for diffusion. Colour changes in positive cases became perceptible readily. Methylene blue solution (Jaswant Singh and Bhattacharji, 1944, Stain No. 1) did not diffuse as rapidly as eosin and the colour changes were not as perceptible as in eosin.

Solutions of J.S.B. 1 (Jaswant Singh and Bhattacharji, *loc. cit.*) from undiluted to 1 : 40,000 were kept in contact with sera from negative and positive kala-azar cases. In positive sera the solutions were decolorized in 18 hours but with eosin no such change was noticed even after 96 hours.

Blood from vein of positive kala-azar cases and from others not suffering from kala-azar together with their history and examination results were supplied by

the Medical Officer of Health, Nepal Government, which help the author gratefully acknowledges. From these persons blood drawn into capillaries was also obtained.

Napier test was carried out in blood drawn from vein and by finger-prick capillary method. On comparison it was found that the results were almost identical (Table I).

Blood from these sources was taken to the Malaria Institute of India for further work.

TABLE I.

Napier test of a few cases carried out in Nepal Terai in blood drawn from vein and by finger-prick capillary method.

Number.	TIME (MINUTES) TAKEN FOR A POSITIVE FORMOLGEL IN BLOOD FROM		NATURE OF REACTION OBTAINED IN BLOOD FROM		REMARKS.
	Vein.	Finger-prick capillary method.	Vein.	Finger-prick capillary method.	
38	5	4½	+++	+++	Degree of positive according to Napier (1927).
405	30	25	+	+	
9	10	11	++	++	Time indicated is for complete jellification and development of opacity.
92	7	6	++	++	
103	8	6	+++	+++	
14	11	10	++	++	
60	—	—	—	—	
70	4	4	+++	+++	

— = negative.

RESULTS OF MODIFIED NAPIER AND CHOPRA TESTS CARRIED OUT AT THE MALARIA INSTITUTE OF INDIA.

When eosin was added to the serum with formalin, the colour started changing rapidly from the fluorescent red to a light rose colour. With jellification setting in completely, this light rose was kept unbroken even after shaking. In normal cases the eosin colour in the serum formalin column was unchanged and was liquid throughout the column.

Chopra test.—In carrying out Chopra tests, the two per cent solution of potassium citrate was taken in capillaries to about two-thirds of its length. Blood

from the pricked finger was drawn till the resultant column was,about three-fourths of its length. This meant a dilution of the serum of about 1 : 8. The other steps taken were the same as in Napier test except that 4 per cent urea stibamine was used in place of formalin. Results were recorded as per standards stated by Chopra and De (*loc. cit.*).

In carrying out the above, it was noted that eosin when added to column of positive kala-azar serum and 4 per cent urea stibamine did not diffuse throughout the length of fluid, even after standing vertically for hours after being inverted. Eosin when added to serum before addition of urea stibamine diffused well. This indicates difficulty of diffusal through the column of ' floccules '.

The immediate nature of formation of floccule does not allow passage of eosin, whereas in Napier test, on account of the comparatively longer time taken for jellification, the eosin diffused rapidly. With eosin, flocculation was made out easily.

Napier and Chopra tests were carried out with sera and citrated blood from patients suffering from established cases of kala-azar before, during and after treatment, as also from blood and sera brought from Nepal.

Normal monkey and human blood and blood from monkeys infected with simian malaria were tested. Blood from patients suffering from pulmonary tuberculosis and others suffering from non-specific fevers were also tested. The results of the above are set in Table II.

SUMMARY AND CONCLUSIONS.

1. A finger prick capillary blood test method for the diagnosis of kala-azar by applying Napier and Chopra tests is described.

2. The advantages of above are :—

   (*a*) Vein puncture is avoided.

   (*b*) Quantity of blood to be tested is small.

   (*c*) The method is simple requiring little equipment and is easy to carry out in rapid surveys.

3. Modification of Napier and Chopra tests by addition of eosin brings out the reactions more perceptible and differences between positives and negatives are easily distinguishable.

4. The failure of eosin to diffuse through a column of ' floccules ' formed in a positive 1 in 10 saline diluted kala-azar serum and 4 per cent urea stibamine might be taken as ancillary in correlating Chopra tests.

5. The results set out here are perforce of a preliminary nature on account of limited facilities available, especially in the matter of intermediate grade cases of kala-azar being not available.

ACKNOWLEDGMENTS.

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TABLE II.

Napier and Chopra tests carried out at the Malaria Institute of India.

Number of sera tested.	Source of sera.	NATURE OF REACTION AND RESULTS.				Remarks.
		Original.		Modified.		
		Napier test.	Chopra test diluted serum.	Napier.	Chopra test diluted serum.	
137	Non-kala-azar cases (human).	—	—*	—	—	Apparently healthy individuals (staff of the Malaria Institute of India, Delhi). * One case gave a doubtful reaction. His sedimentation rate was high.
143	Monkeys (infected with simian malaria).	—	—	—	—*	* One serum gave a ± reaction. Examination of blood revealed many malarial parasites in every microscopical field.
27	Tuberculous patients	—	—	—	—	

TABLE II—*concld.*

Number of sera tested.	Source of sera.	NATURE OF REACTION AND RESULTS.				REMARKS.
		ORIGINAL.		MODIFIED.		
		Napier test.	Chopra test diluted serum.	Napier.	Chopra test diluted serum.	
19	Kala-azar cases from Nepal Terai.	++ to +++	+ to +++	++ to +++	+ to +++	Liver and spleen palpable. Living in highly endemic areas of kala-azar. Differential counts suggestive of kala-azar. No sternal, tibial or spleen punctures made. No malarial parasites noted. Malaria co-existent in the area in a hyperendemic form.
7	Non-kala-azar cases from Nepal Terai.	—	—	—	—	Spleen palpable and hard. Malarial parasites found in 3 cases.
3	Cases from Calcutta Tropical School.	++ +++	+* +++	++* +++	+* +++	* Serum from a case after treatment for kala-azar, others are sera from cases before and during treatment.

sera from kala-azar cases and the Superintendent, Irwin Hospital, New Delhi, for facilities afforded in examining blood from tuberculosis and non-specific fever cases.

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A PRELIMINARY NOTE ON NAPIER AND CHOPRA TESTS CARRIED OUT IN 'RECONSTITUTED SERA' OF KALA-AZAR CASES.

BY

MAJOR N. G. S. RAGHAVAN,

AND

CAPTAIN SATYA PRAKASH.

(*Malaria Institute of India, Delhi.*)

[June 24, 1949.]

IN carrying out Napier (1922) and Chopra *et al.* (1927) tests, blood has to be obtained by vein puncture. Chopra and Mukherjee (1930) described a finger-prick blood test for kala-azar. Chopra and De (1928) suggested 1 in 10 saline diluted serum from kala-azar cases to be tested with 4 per cent urea stibamine.

Napier (1928) stated that the aldehyde test in undiluted and 4 per cent urea stibamine test in 1 in 10 saline diluted serum for kala-azar were equally efficacious in the diagnosis of the same.

Raghavan (1949) suggested the finger-prick capillary method with modification of Napier and Chopra tests in the diagnosis of kala-azar.

The present work was carried out under the Director, Malaria Institute of India, early in 1949 to determine the efficacy of Napier and Chopra tests in 'reconstituted sera' from kala-azar and other cases.

PLAN OF WORK.

In all cases examined for enlargement of liver and spleen, drops of blood exuding from finger were taken on two separate filter papers in each case and also to make a blood smear for examination of parasites. One set of filter paper was kept in the refrigerator and the other at room temperature. Drops from citrated blood and sera obtained from cases known to be suffering from kala-azar in Nepal

(high endemic area) and that from positive cases supplied by the Director, School of Tropical Medicine, Calcutta, were also taken on filter paper and treated as above.

Blood from persons suffering from pulmonary tuberculosis, non-specific fevers and healthy individuals and blood from normal monkeys and those suffering from malarial infections were treated in the same manner.

METHOD OF RECONSTITUTING SERUM.

Pieces of filter paper containing blood were cut out and put into small tubes each containing 2 c.c. of normal saline and allowed to stand for an hour (Kolmer and Boerner, 1945). After this the reconstituted serum was drawn into a capillary tube. The lower end of the tube was dipped in formalin or urea stibamine (of various strengths) and reactions noted. The results of the tests given in Table I show that in cases where positive diagnosis had been established by Napier and Chopra's tests in undiluted and 1 in 10 diluted sera respectively, neither jellification nor haziness developed with any strength of formalin. With urea stibamine, however, a positive reaction was obtained in the 2 per cent solution and with 4 per cent solution. The non-kala-azar cases did not give a positive reaction.

SUMMARY.

A method for testing 'reconstituted sera' from kala-azar cases is suggested. Advantage of this lies in the fact that it is easily applicable in the field.

As the dilution of the serum becomes nearly 1 in 1,000, it appears that the urea stibamine test in 2 per cent solution can be used with advantage. Napier test appears to be inapplicable to 'reconstituted sera' from kala-azar cases.

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Table I.

Showing results of Napier and Chopra tests (varying dilutions of formalin and urea stibamine) in 'reconstituted sera' from various sources.

Source and nature of serum and numbers tested (in brackets).		Napier test. Strength of formalin (per cent). 40	20	10	5	2	1	Chopra test. Strength of urea stibamine (per cent). 4	2	1	0·4	0·2	0·1	Remarks.
Human : from positive kala-azar cases (24).	(a)	+++ to +	+++ to +	++ to ±	+ to ±	−	−	+++ to +	+++ to +	++ to +	+ to ±	+ to ±	+ to ±	2 per cent urea stibamine appeared to give better results than 4 per cent urea stibamine.
	(b)	−	−	−	−	−	−	+++ to +	+++ to +	++ to +	+ to ±	+ to ±	+ to ±	
Human : from positive malaria cases (6).	(a)	−	−	−	−	−	−	−	−	−	−	−	−	
	(b)	−	−	−	−	−	−	−	−	−	−	−	+ in − case	
Human : from normal cases (49).	(a)	−	−	−	−	−	−	−	−	−	−	−	−	1 case gave a positive result in 2 per cent urea stibamine.
	(b)	−	−	−	−	−	−	−(!)	−	−	−	−	−	
Human : from tuberculous patients (27).	(a)	−	−	−	−	−	−	−	−	−	−	−	−	
	(b)	−	−	−	−	−	−	−	−	−	−	−	−	

Notes.— 1. (a) denotes serum from blood, (b) denotes 'reconstituted serum'.
2. All degrees of positive in Napier and Chopra tests are according to standards laid by them [Napier, L. E. (1922) and Chopra, R. N., and De, N. N. (1928)].
3. Results of Chopra tests were read after five and before twenty minutes.
4. No difference was noted in 'reconstituted sera' from dried blood kept on filter paper either in the refrigerator or at laboratory temperature.

TABLE I—*concld.*

Source and nature of serum and numbers tested (in brackets).		NAPIER TEST.						CHOPRA TEST.						REMARKS.
		Strength of formalin (per cent).						Strength of urea stibamine (per cent).						
		40	20	10	5	2	1	4	2	1	0·4	0·2	0·1	
Human : from persons suffering from non-specific diseases (79).	(a)	—	—	—	—	—	—	—	—	—	—	—	—	
	(b)	—	—	—	—	—	—	—	—	—	—	—	—	
Monkey : with malaria (121).	(a)	—	—	—	—	—	—	—	—	—	—	—	—	1 case gave a ± with 2 and 4 per cent urea stibamine. Blood of this monkey was showing malarial parasites in every microscopic field.
	(b)	—	—	—	—	—	—	—(1)	—(1)	—	—	—	—	
Monkey : non-malarial (6).	(a)	—	—	—	—	—	—	—	—	—	—	—	—	
	(b)	—	—	—	—	—	—	—	—	—	—	—	—	

Notes.—1. (a) denotes serum from blood, (b) denotes ' reconstituted serum '.
2. All degrees of positive in Napier and Chopra tests are according to standards laid by them [Napier, L. E. (1922) and Chopra, R. N., and De, N. N. (1928)].
3. Results of Chopra tests were read after five and before twenty minutes.
4. No difference was noted in ' reconstituted sera ' from dried blood kept on filter paper either in the refrigerator or at laboratory temperature.

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OBSERVATIONS ON THE INFECTIVITY OF TISSUES OF *MACACA MULATTA* DURING THE INCUBATION PERIOD FOLLOWING EXPOSURE TO INFECTION WITH SPOROZOITES OF *PLASMODIUM CYNOMOLGI*.*

BY

H. W. MULLIGAN, M.D., D.Sc.,

T. SOMMERVILLE, M.B., Ch.B.,

AND

O. C. LLOYD, M.D.

(*Mammalian Malaria Enquiry, Central Research Institute, Kasauli.*)

[July 7, 1949.]

INTRODUCTION.

THE experiments which form the subject of this communication were carried out at the Central Research Institute, Kasauli, as part of the work of the Mammalian Malaria Enquiry, the primary object of which was to elucidate the fate of sporozoites in the interval between their introduction into a susceptible mammalian host and the appearance of erythrocytic forms of the parasite. It had already been established that, in avian malaria such as *P. gallinaceum* in chickens, sporozoites undergo a pre-erythrocytic cycle of development (James and Tate, 1938 ; Huff and Coulston, 1944 ; and others). There was good reason to believe that a somewhat similar cycle of development might occur in mammalian malaria (Fairley, 1945 ; Davey, 1946). Before the enquiry had been brought to a successful conclusion, this problem, which had baffled many workers, was solved by Shortt and his colleagues (Shortt *et al.*, 1948*a*, 1948*b* ; Shortt and Garnham, 1948*a*, 1948*b*) in a series of classical researches carried out at the London School of Hygiene

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and Tropical Medicine. There seems to be little doubt that the successful outcome of the latter experiments was accountable mainly to the excessively large dosage of sporozoites used. Reports from this enquiry had referred to ' overwhelming ' doses of sporozoites when upwards of 100 infected mosquitoes were used, yet re-examination of our histological material in the light of the findings reported by Shortt and his colleagues failed to reveal the presence of exo-erythrocytic forms of the parasite in sections of liver or other tissues.

In the work of the Mammalian Malaria Enquiry it was the constant aim so to narrow the field of investigation as to be able to concentrate the histological investigations on a limited variety of tissues taken from monkeys at a stage of the incubation period when the hypothetical pre-erythrocytic forms would be most prevalent and most easily recognized. With this end in view, and on the assumption that such forms, if present, would be infective on sub-inoculation to susceptible animals (as had been shown to be the case in avian malaria) the series of experiments to be described below was undertaken.

MATERIALS AND METHODS.

In all of the experiments to be described the infection studied was *Plasmodium cynomolgi* in *Macaca mulatta* (*Silenus rhesus*).

Strain of plasmodium.—The strain of *P. cynomolgi* employed was that originally isolated by the senior authors from a specimen of *Macacus irus* purchased in Calcutta in 1932 and believed to have been imported from Malaya. A detailed description of this parasite has been given by Mulligan (1935). This is undoubtedly the same strain as that used by Shortt *et al.* (*loc. cit.*). *P. cynomolgi* was selected on account of its close resemblance to *P. vivax* and because of the relative ease with which it can be transmitted through anopheline mosquitoes.

Source of monkeys.—All of the monkeys were captured from the wild state in the Ambala District of the Punjab. Of the many hundreds examined from this locality none has ever been found to harbour a natural plasmodial infection. It was not possible accurately to determine the age of the monkeys used but it may be stated in general terms that all were young adolescents.

Mosquito vectors.—As no ' colonized ' anopheline vector was available in India at the time, all mosquitoes used were bred from larvæ taken in the field. The species chiefly used were *A. annularis* and *A. subpictus* which were the most prevalent species at the seasons (spring and autumn, respectively) when temperatures were favourable for the development of the sporogony cycle. No controlled temperature insectarium was available in the field and transmission experiments were done only at those seasons when natural temperatures were favourable. Relative humidity was maintained at 80 per cent or over by artificial means. Mosquitoes were bred, fed and maintained by routine methods.

Gametocyte carriers.—The majority of the monkeys used to infect mosquitoes had been infected by blood inoculation but in some cases sporozoite-infected animals were used. Experience showed the difficulty of determining by gametocyte counts or other means whether a monkey was a ' good infector ' and the routine eventually adopted was to allow a batch of mosquitoes to feed repeatedly on the monkey throughout the course of the primary attack.

Infected mosquitoes.—The percentage of mosquitoes which became infected varied considerably in different batches. On this account an attempt was made to 'standardize' the dosage of sporozoites by multiplying the actual number of mosquitoes used by the index of experimental infection of the batch as determined by sample dissections of approximately 5 per cent of the batch. Thus, if 100 mosquitoes were fed on an experimental animal and if the index of infection were 0·75 (i.e. 75 per cent of the batch infected), the number of *infected* mosquitoes used has been recorded in the protocols as 75. This does not, however, take account of the intensity of infection in individual mosquitoes. Before using a batch to produce infection an interval of 2 to 3 days was allowed to elapse after sporozoites appeared in the salivary glands so as to ensure maximum prevalence of mature sporozoites.

Technique of infecting monkeys.—Two methods of infecting monkeys with sporozoites were employed :—

(*a*) *By mosquito bite.*—Infected mosquitoes were allowed to feed on monkeys in the natural way. When the batch to be fed consisted of less than about 100 mosquitoes they were allowed to feed through one side of a Barraud's cage tightly stretched over the shaven abdomen of the monkey. For batches consisting of more than 100 mosquitoes, the monkey, suitably restrained, was placed in a large cage containing the infected mosquitoes. The mosquitoes which fed were subsequently collected, counted and identified.

(*b*) *By sporozoite inoculation.*—Mosquito tissues containing sporozoites were prepared for inoculation in a mixture of equal parts of normal monkey serum and physiological saline solution. The time interval between the manipulation of the mosquito tissues and the injection of the preparation containing sporozoites into the monkey host was reduced to a minimum so as to ensure the viability of the sporozoites. When only a small number of mosquitoes was used the salivary glands were dissected out, and were drawn up, together with the dissecting fluid, into the bore of a 27-gauge needle. The needle was then attached to the barrel of a tuberculin syringe containing 0·1 ml. serum-saline mixture which served to ensure the evacuation of the contents of the needle at the time of injection. Sporozoite inoculation by this method was usually completed within 10 minutes of the commencement of dissection. This technique proved to be too slow when large numbers of infected mosquitoes were used and was replaced or supplemented by a cruder method in which suspensions of the whole thoracic contents (and sometimes also the gut) were ground into a fine suspension using serum-saline mixture as a diluent. The wings and legs of all the mosquitoes in the batch were first removed followed by the removal of the head and abdomen of each mosquito. Care was taken when severing the head not to remove the salivary glands from the thorax. A batch of about 50 infected mosquitoes could be prepared for injection in this way within half an hour of the commencement of the first manipulation. Suspensions of sporozoites were injected by the intradermal, subcutaneous, intramuscular, intravenous or intraperitoneal route as shown in the protocols.

The reliability of the above methods for the preparation and inoculation of sporozoites was proved by successful transmission of the infection to monkeys on many occasions.

Technique for the sub-inoculation of blood and other tissues.—Blood for sub-inoculation was obtained as a routine from the donor monkey by cardiac puncture, the syringe being previously charged with a quantity of citrate-saline solution sufficient to prevent clotting. When the animal is lightly anæsthetized, withdrawal of the required quantity of blood can be easily and quickly effected.

For tissues other than blood the method of sub-inoculation was either by direct transplant (spleen) or by injection of a suspension of the tissue in physiological saline solution. In the case of the spleen the desired amount of tissue was obtained either by biopsy or by complete extirpation. In the case of other tissues such as liver, kidney, lung, brain, pituitary, etc., the donor was sacrificed and the material obtained at autopsy under aseptic conditions. Spleen tissue, suitably divided, was introduced into the peritoneal cavity through an abdominal incision, the pieces of spleen being wrapped in the greater omentum. Suspensions of spleen and other tissues were prepared by grinding in a mortar with physiological saline solution. Details of the quantity of tissue sub-inoculated and of the route of injection are given in the protocols (Tables I–IV).

RESULTS.

SUB-INOCULATION OF BLOOD TAKEN FROM MONKEYS WITHIN 16 HOURS OF INFECTION WITH SPOROZOITES.

Blood from monkeys infected with sporozoites in various ways was withdrawn at spaced intervals after the injection of sporozoites and inoculated into clean monkeys by various routes. Details of these experiments and of the results observed are summarized in Table I.

Donor monkeys were infected by mosquito bite (3 experiments) or by the injection of suspensions of sporozoites administered by the subcutaneous (14 experiments) or intradermal (3 experiments) route. The number of mosquitoes used to infect the donors varied from 8 to 114 (average 48). All the donor monkeys, except two which died during the incubation period, ultimately developed patent malarial infections. The two which died have been recorded as positive, since other monkeys bitten at the same time by mosquitoes from the same batch developed malaria.

The time interval elapsing between sporozoite injection and withdrawal of blood for sub-inoculation varied from less than 1 minute up to 16 hours.

Eighteen of the sub-inoculated monkeys failed to develop malaria during an observation period varying from 34 to 98 days and despite the fact that eight of them were splenectomized as a test for latent infection. Two of the sub-inoculated monkeys developed patent infections, one of these (E-392) received 20 ml. of blood intravenously from a donor (E-391) 20 minutes after the subcutaneous injection of a suspension of the thoraces and guts of 114 infected mosquitoes. The other (E-385) received 15 ml. of blood intravenously from a donor (E-386) 45 minutes after the subcutaneous injection of a suspension of thoraces and guts from 100 infected mosquitoes.

These experiments provide clear evidence of the infectivity of the blood up to 45 minutes following the injection of large numbers of sporozoites by the

subcutaneous route, and may be presumed to be due to the presence of viable sporozoites in the blood stream.

SUB-INOCULATION OF BLOOD TAKEN FROM MONKEYS AT DAILY INTERVALS AFTER THE INJECTION OF SPOROZOITES.

These experiments were an extension of those described above and summarized in Table I. Blood was taken from monkeys at daily intervals (two or more observations for each day up to 10 days) after the injection of sporozoites. Details of the experiments and of the results obtained are summarized in Table II.

In all, 35 sub-inoculation experiments were made. The donors received sporozoites by mosquito bite (19 experiments) or by the injection of sporozoite suspensions by the subcutaneous (11 experiments), intravenous (3 experiments) and intrasplenic (2 experiments) routes. The number of infected mosquitoes used varied widely but was in excess of 50 in 17 of the experiments. All of the 31 donors which survived long enough developed patent malarial infections; of the remaining 4, three were sacrificed and one died during the incubation period. These four animals have been recorded as positive since other monkeys infected from the same batch of mosquitoes at the same time developed patent malaria.

Quantities of blood varying from 7 ml. to 20 ml. were sub-inoculated into clean monkeys by the intraperitoneal (26 experiments), intravenous (8 experiments), and the intramuscular (1 experiment) routes.

In no case was the blood of monkeys taken at intervals varying from 1 to 8 days found to be infective when sub-inoculated to clean monkeys. The latter were observed for periods ranging from 31 to 112 days; in one case (E-459) which received blood from a donor injected with sporozoites 8 days previously no evidence of malaria was observed after splenectomy.

In every one of the 10 experiments in which blood for sub-inoculation was taken after a lapse of more than 8 (9 to 14) days following the injection of sporozoites, the recipients developed patent malarial infections.

Conclusions from blood sub-inoculation experiments.—From the experiments summarized in Tables I and II, it seems reasonable to conclude that, in *M. mulatta* infected with sporozoites of *P. cynomolgi*, viable sporozoites may be present in the blood stream for a relatively short time after the subcutaneous injection of large numbers of sporozoites, the maximum observed period being 45 minutes. During the next 8 days, that is, during the remainder of the incubation period, the blood is consistently non-infective when sub-inoculated into clean monkeys. From the 9th day onwards the blood is consistently infective when sub-inoculated to clean monkeys. In some cases the blood may be infective on sub-inoculation from 1 to 3 days before erythrocytic forms of the parasite are detectable by routine thick-film examination.

SUB-INOCULATION OF SPLEEN TISSUE FROM MONKEYS PREVIOUSLY INJECTED WITH SPOROZOITES.

In these experiments, spleen tissue was removed from monkeys at intervals varying from 2 to 14 days following the injection of sporozoites and sub-inoculated into clean monkeys. Details of the experiments and the results observed are given in Table III.

The twelve donor monkeys were infected by mosquito bite (7 experiments) or by the injection of suspensions of sporozoites given intravenously (3 experiments) or implanted directly into the spleen (2 experiments). All but three of these monkeys developed patent malarial infections, the three exceptions being animals sacrificed during the incubation period. These have been recorded as positive in the protocols, since other monkeys bitten by the same batch of mosquitoes at the same time contracted the infection.

Spleen tissue varying in amount from the whole spleen to a fraction of approximately one-sixth of the spleen was implanted intraperitoneally either by direct transplantation at open operation or by the injection of a suspension of spleen tissue prepared by the method already described.

Immediately prior to removal of spleen tissue from the donor by biopsy or splenectomy, blood from the donor was inoculated into a clean monkey as a control of the infectivity of the blood (' blood control ').

None of the 9 monkeys which were sub-inoculated with spleen tissue removed from the donors at intervals varying from 2 to 10 days following the introduction of sporozoites developed malaria within observation periods varying from 30 to 63 days. On the other hand all of the three monkeys which were sub-inoculated with spleen tissue removed from monkeys between 12 and 14 days following the introduction of sporozoites developed patent infections.

Sub-inoculation of a small fraction one-sixth of the spleen of one monkey (E-147) failed to produce infection in the recipient (E-99) when blood removed at the same time produced infection in another monkey. The probable explanation of this apparent discrepancy is that the dosage of blood administered was many times greater than the dosage of spleen tissue.

An observation of interest was that transplantation of the whole spleen from monkeys E-282 and E-264 at intervals of 2 and 4 days respectively following intra-splenic injection of large numbers of sporozoites failed to produce infection in the sub-inoculated animals.

Conclusion from spleen sub-inoculation experiments.—From the experiments summarized in Table III it may reasonably be concluded that, in *M. mulatta* infected with sporozoites of *P. cynomolgi*, spleen tissue is non-infective to sub-inoculated animals after the lapse of 2 days from the time of exposure to infection and thereafter throughout the whole of the incubation period.

SUB-INOCULATION OF OTHER TISSUES TAKEN FROM MONKEYS FOLLOWING EXPOSURE TO INFECTION WITH SPOROZOITES.

In these experiments suspensions of various tissues taken from monkeys at intervals during the incubation period following the injection of sporozoites were sub-inoculated to clean monkeys. The tissues sub-inoculated included blood, liver, bone marrow, brain, lung, kidney, suprarenal, pituitary and striped muscle. Details of these experiments and of the results observed are summarized in Table IV.

Three donor monkeys (E-310, E-256 and E-116) were infected by the bites of 65, 60 and 5 infected mosquitoes, respectively. Monkey E-310 was sacrificed on the 5th day, E-256 on the 7th day and E-116 on the 8th day following exposure to infection and their tissues, removed under aseptic conditions and suspended in physiological saline solution, were injected intraperitoneally into clean monkeys.

a separate animal being used for the sub-inoculation of each tissue. None of the sub-inoculated monkeys developed malaria within observation period varying from 35 to 116 days. As the three donor monkeys were sacrificed during the incubation period, there is no absolute proof that they had been successfully infected, but they have been presumed to be positive and recorded as such in the protocols, since other animals bitten at the same time by mosquitoes of the same batch developed malaria.

Similar experiments were carried out with monkeys E-133 (blood, liver, bone marrow, brain, lung, kidney and muscle) on 13th day and monkey E-134 (blood and brain) on 14th day following exposure to infection. All of the tissues from these two donors produced malaria in the sub-inoculated monkeys except the bone marrow of E-133.

Conclusion from tissue sub-inoculation experiments.—The results of these experiments indicate that in *M. mulatta* exposed to infections with sporozoites of *P. cynomolgi* neither the blood nor any other tissues sub-inoculated to clean monkeys is infective at the stages at which observations were made, namely the 5th, 7th and 8th days of the incubation period. Once the blood becomes infective, other tissues are also infective, presumably by reason of the parasitized erythrocytes contained in them.

DISCUSSION.

The experiments described above failed to achieve the object for which they were designed, namely, to provide an indication as to the probable location of hypothetical pre-erythrocytic forms of *P. cynomolgi*. They are, however, of considerable interest in relation to the results obtained from similar work in avian malaria and particularly in regard to the recent demonstration of pre-erythrocytic forms of *P. cynomolgi* in monkey liver following the injection of massive doses of sporozoites (Shortt *et al.*, *loc. cit.*).

Re-examination of histological material taken by the authors from monkeys at all stages of the incubation period following injections of large doses of sporozoites has failed to reveal the presence of pre-erythrocytic schizogony in the liver or elsewhere. There is, therefore, no absolute proof that the tissues used for the sub-inoculation experiments described above actually contained pre-erythrocytic forms. Failure to detect these forms at microscopical examination does not, of course, mean that there were none present ; it is a common experience to find that blood is infective on sub-inoculation when the presence of parasites cannot be detected by routine thick-film examinations. When it is remembered that the doses of sporozoites given were very considerable (often more than 50 infected mosquitoes) and the quantities of various tissues used for sub-inoculation were relatively enormous, it seems reasonable to assume that pre-erythrocytic forms were, in fact, present in at least some of the tissues used in these experiments. Up to the present, pre-erythrocytic forms of *P. cynomolgi* have been observed only in the liver but there is as yet no proof that they do occur elsewhere albeit in insufficient numbers to be readily detected at histological examination.

The consistent failure to produce infection in sub-inoculated monkeys by the injection of tissues from animals within 8 days of exposure to infection with sporozoites (except the blood up to 45 minutes) appears to warrant the conclusion that pre-erythrocytic forms of *P. cynomolgi* are non-infective to animals

susceptible to infection with either sporozoites or trophozoites. This conclusion receives the strongest support from the observation of Shortt *et al.* (1948*a*) that liver tissue known to contain pre-erythrocytic forms of *P. cynomolgi* failed to produce infection in a sub-inoculated animal.

The finding that tissues are consistently non-infective on sub-inoculation during the incubation period in mammalian malaria is of special interest in relation to previous findings in avian malaria. Numerous authors have reported that, in avian malaria, various tissues are infective during the incubation period following sporozoite injection and at a time when the blood is non-infective (Warren and Coggeshall, 1937; Kikuth and Mudrow, 1938, 1939; de Court and Schneider, 1938; Coulston *et al.*, 1945). Tissues which have been shown to be infective during this phase include spleen, liver, kidney, brain, ovary, lymph nodes and thymus. Coulston *et al.* (*loc. cit.*), in studies on *P. gallinaceum*, demonstrated that, in infections produced by the bites of infected mosquitoes, the only tissues which were infective during the 36 hours following exposure to infection were the skin at the site of biting and the spleen, whereas following intravenous injection of sporozoites, many other tissues including heart, brain, thymus, intestine and bone marrow were infective during the same period. These findings in avian malaria are in marked contrast with what is at present known of mammalian malaria and require elucidation.

As regards the infectivity of the blood following exposure to infection with sporozoites, there is a closer parallel between avian, simian and human malaria. In infections with *P. gallinaceum*, Coulston *et al.* (*loc. cit.*) found that the blood was infective during the first 20 minutes following sporozoite injection and again 36 hours later. This latter period of infectivity coincided with the liberation of merozoites from cells of the lymphoid-macrophage systems. In our experiments with *P. cynomolgi* the blood was proved to be infective on sub-inoculation at intervals of 20 minutes and 45 minutes following subcutaneous injection of large numbers of sporozoites but was subsequently negative during the remainder of the incubation period. It is reasonable to suppose that the infectivity of the blood at this early stage is due to the presence of viable sporozoites. These findings are very similar to those reported by Fairley (*loc. cit.*) who observed that in human volunteers infected with *P. vivax* and *P. falciparum* the blood was infective for about half an hour after injection of sporozoites and then remained negative throughout the rest of the incubation period.

The duration of the incubation period as estimated by the infectivity of the blood on sub-inoculation was found by Fairley (*loc. cit.*) to be 6 days for *P. falciparum* and 8 days for *P. vivax*. The duration of the incubation period of *P. cynomolgi* in *M. mulatta* has been found to be 9 days as estimated by the sub-inoculation experiments described above. This coincides exactly with the direct observations on pre-erythrocytic schizogony of this species of plasmodium reported by Shortt and Garnham (*loc. cit.*).

SUMMARY AND CONCLUSIONS.

A series of experiments has been described in which blood and other tissues of *M. mulatta*, exposed to infection with sporozoites of *P. cynomolgi*, were sub-inoculated into clean monkeys of the same species at intervals following sporozoite

injection. These experiments failed to achieve their primary objective which was to obtain information as to the location of hypothetical pre-erythrocytic stages of *P. cynomolgi*.

The blood of two monkeys was found to be infective on sub-inoculation at intervals of 20 and 45 minutes, respectively, following the subcutaneous injection of large numbers of sporozoites. The infectivity of the blood at this early stage is presumably due to the presence of viable sporozoites in the blood stream.

Thereafter, the blood and all other tissues used for sub-inoculation (spleen, liver, bone marrow, lung, kidney, pituitary, brain and suprarenal) were found to be non-infective throughout the remainder of the incubation period, that is, for 9 days following exposure to infection with sporozoites.

From the 9th day onwards the blood was found to be infective on sub-inoculation although, in some cases, the presence of erythrocytic forms of the parasite could not be detected by microscopical examination until 1 to 3 days later.

Once the blood has become infective, other tissues are also infective, presumably by reason of the presence of infected erythrocytes in the capillaries.

The significance of these findings has been discussed in relation to the recent demonstration by Shortt *et al.* (*loc. cit.*) of the pre-erythrocytic development of *P. cynomolgi* and it has been concluded that there is strong evidence to support the belief that pre-erythrocytic forms of *P. cynomolgi* are non-infective when sub-inoculated to susceptible monkeys.

These findings are at variance with those reported in avian malaria by other workers.

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TABLE I.

Protocols of experiments in which blood was sub-inoculated within 16 hours of sporozoite inoculation.

Recipient monkey number.	Donor monkey number.	Time interval between sporozoite injection and withdrawal of blood for sub-inoculation.	Method of administration of sporozoites to donor.*	Number of infected mosquitoes used to infect donor.†	Amount of blood in millilitres sub-inoculated intravenously (iv), intramuscularly (im), or intraperitoneally (ip).	Results (+ malaria : − no malaria).	
						Donor.	Recipient.‡
E–415	E–414	Immediate	Subcutaneous (thoraces and gut).	26	20 (iv)	+	− (86) Spl.
E–427	E–426	Do.	Do.	29	20 (iv)	+	− (85) Spl.
E–440	E–439	1½ minutes	Do.	39	12 (iv)	+	− (81) Spl.
E–418	E–417	2 ,,	Do.	60	20 (im)	Died §	− (86) Spl.
E–384	E–386	5 ,,	Do.	100	13 (im)	+	− (98) Spl.
E–434	E–433	5 ,,	Do.	30	20 (iv)	+	− (81) Spl.

* The mosquito tissue in parenthesis indicates the source from which the sporozoites were obtained, thus (thoraces and gut) signifies the entire contents of the thorax including salivary glands together with the midgut.

† The number of *infected* mosquitoes stated was calculated by multiplying the number actually used by the index of infection for the batch. In all cases, therefore, the number actually used was equal to, or greater than, the numbers given in the protocols.

‡ The number in parenthesis indicates the period in days during which the recipient monkey was observed.

§ Donor died during incubation period but control bitten by mosquitoes from same batch developed malaria.

Spl. indicates that malaria did not develop after splenectomy.

TABLE I—*contd.*

Recipient monkey number.	Donor monkey number.	Time interval between sporozoite injection and withdrawal of blood for sub-inoculation.	Method of administration of sporozoites to donor.*	Number of infected mosquitoes used to infect donor.†	Amount of blood in millilitres sub-inoculated intravenously (iv), intramuscularly (im), or intraperitoneally (ip).	Results (+ malaria : — no malaria).	
						Donor.	Recipient.‡
E–160	E–159	5 minutes	Mosquito bite	11	10 (ip)	+	— (36)
E–436	E–435	8 ,,	Subcutaneous (thoraces and gut).	25	20 (iv)	+	— (81) Spl.
E–171	E–170	10 ,,	Intradermal (salivary glands).	8	9·5 (ip)	+	— (35)
E–281	E–270	10 ,,	Intradermal (thoraces).	80	20 (ip)	+	— (63)
E–392	E–391	20 ,,	Subcutaneous (thoraces and gut).	114	20 (iv)	+	+
E–161	E–159	25 ,,	Mosquito bite	11	6 (ip)	+	— (36)
E–393	E–391	30 ,,	Subcutaneous (thoraces and gut).	114	3 (iv)	+	— (44)

* The mosquito tissue in parenthesis indicates the source from which the sporozoites were obtained, thus (thoraces and gut) signifies the entire contents of the thorax including salivary glands together with the midgut.

† The number of *infected* mosquitoes stated was calculated by multiplying the number actually used by the index of infection for the batch. In all cases, therefore, the number actually used was equal to, or greater than, the numbers given in the protocols.

‡ The number in parenthesis indicates the period in days during which the recipient monkey was observed.

Spl. indicates that malaria did not develop after splenectomy.

TABLE I—*concld.*

Recipient monkey number.	Donor monkey number.	Time interval between sporozoite injection and withdrawal of blood for sub-inoculation.	Method of administration of sporozoites to donor.*	Number of infected mosquitoes used to infect donor.†	Amount of blood in millilitres sub-inoculated intravenously (iv), intramuscularly (im), or intraperitoneally (ip).	Results (+ malaria : — no malaria).	
						Donor.	Recipient.‡
E–451	E–450	30 minutes	Subcutaneous (thoraces and gut).	58	20 (iv)	Died §	— (80) Spl.
E–172	E–170	37 ,,	Intradermal (salivary glands).	8	10·5 (ip)	+	— (35)
E–385	E–386	45 ,,	Subcutaneous (thoraces and gut).	100	15 (iv)	+	+
E–163	E–159	72 ,,	Mosquito bite	11	12 (ip)	+	— (36)
E–442	E–435	4 hours	Subcutaneous (thoraces and gut).	25	20 (iv)	+	— (35)
E–446	E–433	8 ,,	Do.	30	20 (iv)	+	— (35)
E–448	E–439	16 ,,	Do.	78	15 (iv)	+	— (34)

* The mosquito tissue in parenthesis indicates the source from which the sporozoites were obtained, thus (thoraces and gut) signifies the entire contents of the thorax including salivary glands together with the midgut.

† The number of *infected* mosquitoes stated was calculated by multiplying the number actually used by the index of infection for the batch. In all cases, therefore, the number actually used was equal to, or greater than, the numbers given in the protocols.

‡ The number in parenthesis indicates the period in days during which the recipient monkey was observed.

§ Donor died during incubation period but control bitten by mosquitoes from same batch developed malaria.

Spl. indicates that malaria did not develop after splenectomy.

TABLE II.

Protocols of experiments in which blood was sub-inoculated at daily intervals following sporozoite injection.

Recipient monkey number.	Donor monkey number.	Time interval in days between sporozoite injection and removal of tissue for sub-inoculation.	Method of administration of sporozoites to donor monkey.*	Number of infected mosquitoes used to infect donor monkey.†	Amount of blood in millilitres sub-inoculated intravenously (iv), intramuscularly (im), or intraperitoneally (ip).	Results (+ malaria: —no malaria).	
						Donor.	Recipient.‡
E–303	E–300	1	Mosquito bite	36	15 (ip)	+	— (56)
E–387	E–386	1	Subcutaneous (thoraces and gut).	100	20 (im)	+	— (49)
E–431	E–426	1	Do.	118	10 (iv)	+	— (36)
E–307	E–301	2	Mosquito bite	35	15 (ip)	+	— (55)
E–293	E–282	2	Intrasplenic (thoraces).	79	20 (ip)	+	— (61)
E–456	E–450	2	Subcutaneous (thoraces and gut).	58	20 (iv)	Died §	— (31)
E–309	E–300	3	Mosquito bite	36	15 (ip)	+	— (54)
E–432	E–426	3	Subcutaneous (thoraces and gut).	118	20 (iv)	+	— (35)

* The mosquito tissue in parenthesis indicates the source from which the sporozoites were obtained, thus (salivary glands) signifies dissected glands (thoraces), the entire thoracic contents including glands (gut) and the dissected midgut.

† The number of *infected* mosquitoes was calculated by multiplying the actual number used by the index of experimental infection for the batch.

‡ The figure in parenthesis indicates the number of days for which the recipient monkey was under observation for malaria.

§ These monkeys were sacrificed or died of other causes during the incubation period but have been recorded as positive since other monkeys on which the same batch of mosquitoes fed at the same time developed malaria.

TABLE II—*contd.*

Recipient monkey number.	Donor monkey number.	Time interval in days between sporozoite injection and removal of tissue for sub-inoculation.	Method of administration of sporozoites to donor monkey.*	Number of infected mosquitoes used to infect donor monkey.†	Amount of blood in millilitres sub-inoculated intravenously (iv), intramuscularly (im), or intraperitoneally (ip).	Results (+ malaria : —no malaria).	
						Donor.	Recipient.‡
E–323	E–301	4	Mosquito bite	35	20 (ip)	+	— (53)
E–295	E–264	4	Intrasplenic (thoraces).	64	15 (ip)	+	— (61)
E–438	E–426	4	Subcutaneous (thoraces and gut).	118	20 (iv)	+	— (34)
E–324	E–300	5	Mosquito bite	36	20 (ip)	+	— (52)
E–330	E–310	5	Do.	65	20 (ip)	+ §	— (48)
E–449	E–426	5	Subcutaneous (thoraces and gut).	118	20 (iv)	+	— (33)
E–326	E–301	6	Mosquito bite	35	20 (ip)	+	— (51)
E–104	E–95	6	Intravenous (salivary glands).	1	7 (ip)	+	— (112)
E–268	E–257	6	Mosquito bite	100	20 (ip)	+	— (63)

* The mosquito tissue in parenthesis indicates the source from which the sporozoites were obtained, thus (salivary glands) signifies dissected glands (thoraces), the entire thoracic contents including glands (gut) and the dissected midgut.

† The number of *infected* mosquitoes was calculated by multiplying the actual number used by the index of experimental infection for the batch.

‡ The figure in parenthesis indicates the number of days for which the recipient monkey was under observation for malaria.

§ These monkeys were sacrificed or died of other causes during the incubation period but have been recorded as positive since other monkeys on which the same batch of mosquitoes fed at the same time developed malaria.

TABLE II—*contd.*

Recipient monkey number.	Donor monkey number.	Time interval in days between sporozoite injection and removal of tissue for sub-inoculation.	Method of administration of sporozoites to donor monkey.*	Number of infected mosquitoes used to infect donor monkey.†	Amount of blood in millilitres sub-inoculated intravenously (iv), intramuscularly (im), or intraperitoneally (ip).	Results (+ malaria : — no malaria).	
						Donor.	Recipient.‡
E–454	E–426	6	Subcutaneous (thoraces and gut).	118	20 (iv)	+	— (32)
E–327	E–300	7	Mosquito bite	36	15 (ip)	+	— (50)
E–267	E–253	7	Do.	79	20 (ip)	+	— (63)
E–283	E–256	7	Do.	60	17 (ip)	+ §	— (62)
E–455	E–426	7	Subcutaneous (thoraces and gut).	118	20 (iv)	+	— (78)
E–329	E–301	8	Mosquito bite	35	20 (ip)	+	— (49)
E–137	E–116	8	Do.	5	15 (ip)	+ §	— (32)
E–459	E–426	8	Subcutaneous (thoraces and gut).	118	20 (ip)	+	— (77) Spl.
E–338	E–300	9	Mosquito bite	36	15 (ip)	+	+

* The mosquito tissue in parenthesis indicates the source from which the sporozoites were obtained, thus (salivary glands) signifies dissected glands (thoraces), the entire thoracic contents including glands (gut) and the dissected midgut.

† The number of *infected* mosquitoes was calculated by multiplying the actual number used by the index of experimental infection for the batch.

‡ The figure in parenthesis indicates the number of days for which the recipient monkey was under observation for malaria.

§ These monkeys were sacrificed or died of other causes during the incubation period but have been recorded as positive since other monkeys on which the same batch of mosquitoes fed at the same time developed malaria.

Spl. indicates that malaria did not develop following splenectomy.

TABLE II—*concld.*

Recipient monkey number.	Donor monkey number.	Time interval in days between sporozoite injection and removal of tissue for sub-inoculation.	Method of administration of sporozoites to donor monkey.*	Number of infected mosquitoes used to infect donor monkey.†	Amount of blood in millilitres sub-inoculated intravenously (iv), intramuscularly (im), or intraperitoneally (ip).	Results (+ malaria : − no malaria).	
						Donor.	Recipient.‡
E-460	E-426	9	Subcutaneous (thoraces and gut).	118	20 (ip)	+	+
E-340	E-301	10	Mosquito bite	35	18 (ip)	+	+
E-81	E-147	10	Do.	4	12 (ip)	+	+
E-461	E-426	10	Subcutaneous (thoraces and gut).	118	17 (iv)	+	+
E-341	E-300	11	Mosquito bite	36	18 (ip)	+	+
E-342	E-301	12	Do.	35	18 (ip)	+	+
E-94	E-144	12	Intravenous (salivary glands).	4	10 (ip)	+	+
E-152	E-133	13	Do.	2	12 (ip)	+	+
E-167	E-134	14	Mosquito bite	13	12 (ip)	+	+

* The mosquito tissue in parenthesis indicates the source from which the sporozoites were obtained, thus (salivary glands) signifies dissected glands (thoraces), the entire thoracic contents including glands (gut) and the dissected midgut.

† The number of *infected* mosquitoes was calculated by multiplying the actual number used by the index of experimental infection for the batch.

‡ The figure in parenthesis indicates the number of days for which the recipient monkey was under observation for malaria.

TABLE III.

Protocols of experiments in which spleen tissue was sub-inoculated at intervals of 2 to 14 days following sporozoite injection.

Recipient monkey number.	Donor monkey number.	Time interval in days between sporozoite injection and removal of spleen for sub-inoculation.	Method of administration of sporozoites to donor.*	Number of infected mosquitoes used to infect donor.†	Method of intraperitoneal sub-inoculation of spleen.‡	Fraction of whole spleen sub-inoculated.	Results (+ malaria : − no malaria). Donor.	Blood control.§	Recipient.‖
E–294	E–282	2	Intrasplenic (thoraces).	79	Transplant	1	+	−	− (61)
E–296	E–264	4	Do.	64	Do.	1	+	−	− (61)
E–331	E–310	5	Mosquito bite	65	Suspension	½	+¶	−	− (48)
E–105	E–95	6	Intravenous (gut)	1	Transplant	1/6	+	−	− (43)
E–269	E–257	6	Mosquito bite	100	Do.	1	+	−	− (63)
E–266	E–253	7	Do.	79	Do.	1	+¶	−	− (63)
E–284	E–256	7	Do.	60	Suspension	¾	+¶	−	− (62)

* The mosquito tissue in parenthesis indicates the source from which the sporozoites were obtained, thus (salivary glands) signifies dissected glands while (thoraces) represents the entire thoracic contents including glands and (gut) means dissected midgut.

† The number of *infected* mosquitoes was calculated by multiplying the actual number used by the index of experimental infection of the batch. The words intrasplenic and intravenous signify that the sporozoites were injected into the substance of the spleen and intravenously respectively.

‡ The term transplant signifies that the splenic tissue was sub-inoculated by transplantation and the word suspension means that a suspension of spleen tissue was prepared and sub-inoculated by injection intraperitoneally.

§ The blood control signifies a monkey which was sub-inoculated with blood removed immediately preceding the excision of the spleen tissue from the donor.

‖ The number in parenthesis indicates the period in days during which the recipient monkey was under observation for malaria.

¶ These monkeys were sacrificed during the incubation period but as other monkeys on which the same batch of mosquitoes fed at the same time developed overt malaria they are recorded as positive.

TABLE III—*concld.*

Recipient monkey number.	Donor monkey number.	Time interval in days between sporozoite injection and removal of spleen for sub-inoculation.	Method of administration of sporozoites to donor.*	Number of infected mosquitoes used to infect donor.†	Method of intraperitoneal sub-inoculation of spleen.‡	Fraction of whole spleen sub-inoculated.	Results (+ malaria : − no malaria).		
							Donor.	Blood control.§	Recipient.‖
E–139	E–116	8	Mosquito bite	5	Suspension	1/6	+¶	−	− (31)
E–99	E–147	10	Do.	4	Do.	1/6	+	+	− (30)
E–96	E–144	12	Intravenous (salivary glands).	4	Do.	1/6	+	+	+
E–152	E–133	13	Do.	2	Do.	½	+	+	+
E–169	E–134	14	Mosquito bite	13	Do.	½	+	+	+

* The mosquito tissue in parenthesis indicates the source from which the sporozoites were obtained, thus (salivary glands) signifies dissected glands while (thoraces) represents the entire thoracic contents including glands and (gut) means dissected midgut.

† The number of *infected* mosquitoes was calculated by multiplying the actual number used by the index of experimental infection of the batch. The words intrasplenic and intravenous signify that the sporozoites were injected into the substance of the spleen and intravenously respectively.

‡ The term transplant signifies that the splenic tissue was sub-inoculated by transplantation and the word suspension means that a suspension of spleen tissue was prepared and sub-inoculated by injection intraperitoneally.

§ The blood control signifies a monkey which was sub-inoculated with blood removed immediately preceding the excision of the spleen tissue from the donor.

‖ The number in parenthesis indicates the period in days during which the recipient monkey was under observation for malaria.

¶ These monkeys were sacrificed during the incubation period, but as other monkeys on which the same batch of mosquitoes fed at the same time developed overt malaria they are recorded as positive.

TABLE IV.

Protocols of experiments in which tissues other than spleen were sub-inoculated at intervals of 5 to 14 days following sporozoite injection.

Recipient monkey number.	Donor monkey number.	Time interval in days between sporozoite injection and removal of tissue for sub-inoculation.	Method of administration of sporozoites to donor.*	Number of infected mosquitoes used to infect donor.†	Tissue used for sub-inoculation.	Quantity of tissue used in sub-inoculation.‡	Results (+ malaria : — no malaria).		
							Donor.	Blood control.§	Recipient.‖
E–333	E–310	5	Mosquito bite	65	Liver	Slice measuring $3 \times 2 \times \frac{1}{2}$ inches.	+¶	— (35)	— (35)
E–337	E–310	5	Do.	65	Bone marrow	Marrow from right femur.	+	— (35)	— (35)
E–336	E–310	5	Do.	65	Brain	$\frac{3}{4}$ of right frontal lobe	+	— (35)	— (35)
E–334	E–310	5	Do.	65	Lung	Lower lobe of right lung.	+	— (35)	— (35)
E–335	E–310	5	Do.	65	Kidney	1/3 of right kidney (cortex and medulla).	+	— (35)	— (35)

* The mosquito tissue in parenthesis indicates the source from which the sporozoites were obtained, thus (salivary glands) signifies dissected glands.

† The number of infected mosquitoes was calculated by multiplying the actual number used by the index of experimental infection of the batch.

‡ A suspension in saline was prepared for sub-inoculation in all cases.

§ The blood control signifies a monkey which was sub-inoculated with blood removed immediately preceding the removal of the tissues from the donor.

‖ The number in parenthesis indicates the period in days during which the recipient monkeys and blood control were under observation for malaria.

¶ These monkeys were sacrificed during the incubation period but as other monkeys on which the same batch of mosquitoes fed at the same time developed overt malaria they are recorded as positive.

TABLE IV—*contd.*

Recipient monkey number.	Donor monkey number.	Time interval in days between sporozoite injection and removal of tissue for sub-inoculation.	Method of administration of sporozoites to donor.*	Number of infected mosquitoes used to infect donor.†	Tissue used for sub-inoculation.	Quantity of tissue used in sub-inoculation.‡	Results (+ malaria : — no malaria).		
							Donor.	Blood control.§	Recipient.‖
E–332	E–310	5	Mosquito bite	65	Suprarenal	Left suprarenal (complete).	+	— (35)	— (35)
E–285	E–256	7	Do.	60	Liver	Slice measuring 3 × 2 × ½ inches.	+¶	— (50)	— (50)
E–289	E–256	7	Do.	60	Bone marrow	Marrow from right femur.	+	— (50)	— (50)
E–291	E–256	7	Do.	60	Brain	¾ of left frontal lobe	+	— (50)	— (50)
E–287	E–256	7	Do.	60	Lung	Lower lobe of left lung	+	— (50)	— (50)

* The mosquito tissue in parenthesis indicates the source from which the sporozoites were obtained, thus (salivary glands) signifies dissected glands.

† The number of infected mosquitoes was calculated by multiplying the actual number used by the index of experimental infection of the batch.

‡ A suspension in saline was prepared for sub-inoculation in all cases.

§ The blood control signifies a monkey which was sub-inoculated with blood removed immediately preceding the removal of the tissues from the donor.

‖ The number in parenthesis indicates the period in days during which the recipient monkeys and blood control were under observation for malaria.

¶ These monkeys were sacrificed during the incubation period but as other monkeys on which the same batch of mosquitoes fed at the same time developed overt malaria they are recorded as positive.

TABLE IV—*contd.*

Recipient monkey number.	Donor monkey number.	Time interval in days between sporozoite injection and removal of tissue for sub-inoculation.	Method of administration of sporozoites to donor.*	Number of infected mosquitoes used to infect donor.†	Tissue used for sub-inoculation.	Quantity of tissue used in sub-inoculation.‡	Results (+ malaria : — no malaria).		
							Donor.	Blood control.§	Recipient.‖
E–288	E–256	7	Mosquito bite	60	Kidney	1/3 of right kidney (cortex and medulla).	+	— (50)	— (50)
E–286	E–256	7	Do.	60	Suprarenal	Left suprarenal (complete).	+	— (50)	— (50)
E–290	E–256	7	Do.	60	Pituitary	Pituitary (complete)	+	— (50)	— (50)
E–141	E–116	8	Do.	5	Liver	Slice measuring $1\frac{1}{2} \times \frac{1}{2} \times \frac{1}{4}$ inches.	+¶	— (116)	— (116)
E–140	E–116	8	Do.	5	Bone marrow	All marrow from left femur.	+	— (116)	— (116)

* The mosquito tissue in parenthesis indicates the source from which the sporozoites were obtained, thus (salivary glands) signifies dissected glands.

† The number of infected mosquitoes was calculated by multiplying the actual number used by the index of experimental infection of the batch.

‡ A suspension in saline was prepared for sub-inoculation in all cases.

§ The blood control signifies a monkey which was sub-inoculated with blood removed immediately preceding the removal of the tissues from the donor.

‖ The number in parenthesis indicates the period in days during which the recipient monkeys and blood control were under observation for malaria.

¶ These monkeys were sacrificed during the incubation period but as other monkeys on which the same batch of mosquitoes fed at the same time developed overt malaria they are recorded as positive.

TABLE IV—*contd.*

Recipient monkey number.	Donor monkey number.	Time interval in days between sporozoite injection and removal of tissue for sub-inoculation.	Method of administration of sporozoites to donor.*	Number of infected mosquitoes used to infect donor.†	Tissue used for sub-inoculation.	Quantity of tissue used in sub-inoculation.‡	Results (+ malaria : — no malaria).		
							Donor.	Blood control.§	Recipient.‖
E–138	E–116	8	Mosquito bite	5	Brain	Slice measuring $1\frac{1}{2} \times 1 \times \frac{1}{4}$ inches from frontal lobe.	+	— (116)	— (116)
E–142	E–116	8	Do.	5	Lung	Slice measuring $1 \times 1 \times \frac{1}{2}$ inches.	+	— (116)	— (116)
E–143	E–116	8	Do.	5	Kidney	Slice measuring $1 \times \frac{1}{2} \times \frac{1}{4}$ inches (cortex and medulla).	+	— (116)	— (116)
E–154	E–133	13	Intravenous (salivary glands).	2	Liver	Slice measuring $1\frac{1}{2} \times \frac{1}{2} \times \frac{1}{4}$ inches.	+	+	+
E–157	E–133	13	Do.	2	Bone marrow	All marrow from left femur.	+	+	— (221)

* The mosquito tissue in parenthesis indicates the source from which the sporozoites were obtained, thus (salivary glands) signifies dissected glands.

† The number of infected mosquitoes was calculated by multiplying the actual number used by the index of experimental infection of the batch.

‡ A suspension in saline was prepared for sub-inoculation in all cases.

§ The blood control signifies a monkey which was sub-inoculated with blood removed immediately preceding the removal of the tissues from the donor.

‖ The number in parenthesis indicates the period in days during which the recipient monkeys and blood control were under observation for malaria.

TABLE IV—*concld.*

Recipient monkey number.	Donor monkey number.	Time interval in days between sporozoite injection and removal of tissue for sub-inoculation.	Method of administration of sporozoites to donor.*	Number of infected mosquitoes used to infect donor.†	Tissue used for sub-inoculation.	Quantity of tissue used in sub-inoculation.‡	Results (+ malaria : — no malaria).		
							Donor.	Blood control.§	Recipient.‖
E-156	E-133	13	Intravenous (salivary glands).	2	Brain	Slice measuring $1\frac{1}{2} \times 1 \times \frac{1}{4}$ inches from frontal lobe.	+	+	+
E-153	E-133	13	Do.	2	Lung	Slice measuring $1 \times 1 \times \frac{1}{4}$ inches.	+	+	+
E-155	E-133	13	Do.	2	Kidney	Slice measuring $1 \times \frac{1}{2} \times \frac{1}{4}$ inches (cortex and medulla).	+	+	+
E-158	E-133	13	Do.	2	Muscle	Slice measuring $1 \times \frac{1}{2} \times \frac{1}{4}$ inches.	+	+	+
E-168	E-134	14	Mosquito bite	13	Brain	Slice measuring $1 \times 1 \times \frac{1}{2}$ inches from frontal lobe.	+¶	+	+

* The mosquito tissue in parenthesis indicates the source from which the sporozoites were obtained, thus (salivary glands) signifies dissected glands.

† The number of infected mosquitoes was calculated by multiplying the actual number used by the index of experimental infection of the batch.

‡ A suspension in saline was prepared for sub-inoculation in all cases.

§ The blood control signifies a monkey which was sub-inoculated with blood removed immediately preceding the removal of the tissues from the donor.

‖ The number in parenthesis indicates the period in days during which the recipient monkeys and blood control were under observation for malaria.

¶ These monkeys were sacrificed during the incubation period but as other monkeys on which the same batch of mosquitoes fed at the same time developed overt malaria they are recorded as positive.

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A MICRO-HYGROMETER.

BY

SONTI DAKSHINAMURTY, M.B., B.S., B.S.Sc. (Madras),
D.P.H., D.T.M. & H., Ph.D. (London),
(*Deputy Director, Malaria Institute of India.*)

AND

M. I. D. SHARMA, M.B., B.S.
(*Malaria Institute of India.*)

[July 13, 1949.]

INTRODUCTION.

'CLIMATE has a great influence on the incidence and dispersion of malaria because the disease is transmitted by an insect highly susceptible to climatic factors. Considerable research is needed in the climatology of malaria, too little being known about the effect of environmentals in the epidemiology of the disease. It is needful, therefore, to improve methods of collecting information about climate in connection with surveys and control work' (Russell *et al.*, 1946).

Climate in relation to insects is quite different from that in relation to man because insects live in their nitches which have their own microclimate. For example, the mosquito rests in a variety of dark humid shelters undisturbed by winds such as near the roof in the thatch of a hut, or in the crevices of walls, or in a tiny tree hole or within bushes by the side of a stream or under culverts. While the importance of the measurement of microhumidity for correctly understanding the bionomics of insects is fully recognized, the methods have not yet been perfected for use in the field where insects breed and rest. The climatic conditions in such situations cannot be the same as in a Stevenson's Screen, from where the meteorologist collects his data. Entomologists in general and malariologists in particular are interested in the measurement of microclimatic conditions in the environments where insects actually breed and rest (Buxton, 1932). There are many lacunæ in the present-day knowledge of microclimate in relation to insects which are mainly due to paucity of instruments for the measurement of microhumidity.

The microclimate of insect resting places is mainly the resultant of both temperature and humidity in these nitches; the former presents little difficulty because there are a great variety of instruments for its measurement. The measurement of microhumidity is difficult as we can neither use the wet and dry bulb thermometer

nor the sling psychrometer in such places, as the former for accurate readings requires adequate ventilation, while the latter due to whirling will disturb the microclimate, if at all it can be used in such nitches. Hair hygrometer (Buxton, 1931) and paper hygrometer (Mellanby, 1933 ; Buxton and Mellanby, 1934) involve the use of a torsion balance to obtain quick and accurate readings, which is certainly an advantage in the laboratory but cannot be used as conveniently in the field.

Instruments like the dew-point hygrometer and chemical hygrometer where air is sucked in may be used for the measurement of microhumidity with some precautions against dust and the possibility of extra air entering into the chamber. One great disadvantage of chemical hygrometer is that it is made of glass, mercury and sulphuric acid, it is fragile and disagreeable, if broken (Buxton, 1931). All these are bulky and delicate instruments which require skilled and practised handling in the field.

A simple, cheap and fairly accurate instrument capable of being handled and read by an insect collector in the field is called for, so that the worker may leave the instrument in the insect nitch where he collects his insects daily and picks it up again the following day for reading. It is hoped that this micro-hygrometer may answer the purpose for the measurement of humidity under special conditions.

METHOD AND TECHNIQUE.

1. Capillary tubes 10 cm. long and 1 mm. in diameter of uniform bore are used. These should be absolutely clean. They may be boiled first in weak soap solution and then immersed in strong sulphuric acid for 4 to 5 days, after which they are washed thoroughly in water and dried in a desiccator over strong sulphuric acid.

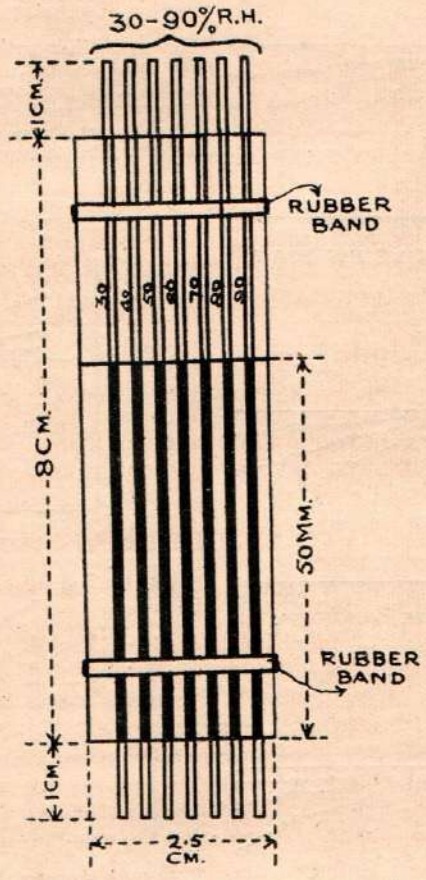
2. As the humidity range of insects' resting places is generally from 30 to 90 per cent, these tubes are filled in with relative humidity mixtures of sulphuric acid and water of varying strengths—30, 40, 50, 60, 70, 80 and 90 per cent (Buxton and Mellanby, *loc. cit.*). In practice, the columns are made by dipping them in these mixtures a little longer than the 50 mm. mark, and the extra fluid length is readily removed on a piece of filter paper to bring it to the exact desired length of 50 mm.

3. The set of seven capillary tubes containing relative humidity mixtures from 30 to 90 per cent is adjusted side by side in order, and mounted on an ordinary glass slide, and are held in position by two rubber bands. The capillary tubes are 10 cm. long, while the slide is 8 cm. long, so that these tubes project a cm. on either side to aid further manipulation. On the underneath of the slide a strip of graph paper graduated in mm. is pasted to enable ready reckoning of the lengths of the columns. The apparatus is now ready for use (Diagram 1).

USE OF THE INSTRUMENT.

For use, the apparatus is left in a horizontal position at the microclimate for eight hours or more, depending on the type of the nitch, convenience of the field or laboratory worker, the length of columns of the mixtures taken initially, and the bore of the tubes. The increase or decrease in the length of the columns for a given humidity will depend on the initial length of the columns, the bore of the capillary tubes, and the period of exposure to attain equilibrium in the microclimate. After leaving the hygrometer for a certain period the lengths of columns

DIAGRAM 1.



of liquid in different tubes are compared. Length of all columns *below* the relative humidity of the unknown space would *increase*, while those *above* it, will all *decrease* and there will be *no change* in the length of column which is in equilibrium with that particular atmosphere which thereby denotes the relative humidity of the unknown space. In case *no* particular column is unchanged, the required humidity of the microclimate can be interpolated from the lengths of the columns in the two adjacent capillary tubes, where there is an increase in length in one and decrease in the other. In practice, one need not use all the seven capillary tubes, if, say, the expected relative humidity of the space is 40 per cent, one need only use three capillary tubes of 30, 40 and 50 per cent relative humidity mixtures. Again, if more accurate readings are needed, one may step up the gradations by 5 per cent instead of 10 per cent, when one may expect a reading correct to 2·5 per cent relative humidity. Diagram 2 and Table I show changes in the lengths of columns when exposed to atmospheres of different relative humidities from 30 to 90 per cent for 8, 24 and 48 hours.

DIAGRAM 2. (Data from Table I.)

THE BEHAVIOUR OF THE MICRO-HYGROMETER SHOWING CHANGES IN LENGTH OF COLUMNS WHEN KEPT IN DIFFERENT RELATIVE HUMIDITY ATMOSPHERES.

Seven capillary tubes are used with 50 mm. lengths of columns. Graph indicates changes in length when hygrometer was kept in different atmospheres ranging from 30 to 90 per cent relative humidity for three different periods—8 hours, 24 hours and 48 hours.

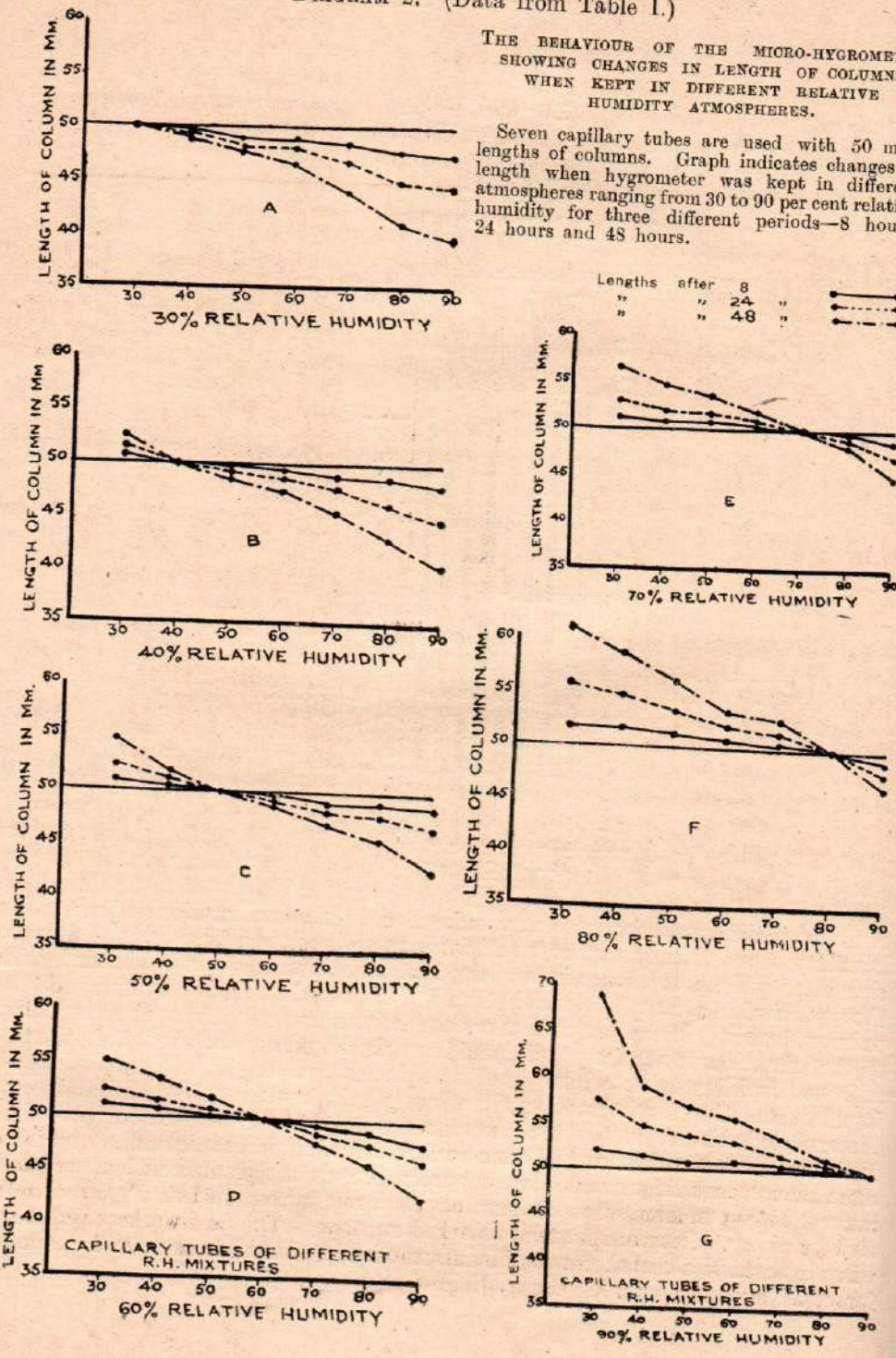


TABLE I.

Length of columns in capillary tubes containing various relative humidity mixtures (horizontal) kept in atmospheres of different relative humidity (vertical).

Relative humidity of the atmospheres in which the micro-hygrometer is kept.	Capillary tubes containing mixtures of relative humidity, per cent.							Readings taken after hours.
	30 R. H.	40 R. H.	50 R. H.	60 R. H.	70 R. H.	80 R. H.	90 R. H.	
30 per cent ...	50·0	49·8	49·0	49·0	48·5	47·8	47·5	8 hrs.
	50·0	49·5	48·2	48·0	46·8	45·0	44·5	24 ,,
	50·0	49·2	48·0	46·8	44·0	41·0	39·8	48 ,,
40 per cent ...	50·8	50·0	49·8	49·5	49·0	48·8	48·0	8 hrs.
	51·5	50·0	49·5	48·8	47·8	46·2	44·8	24 ,,
	52·5	50·0	48·8	47·5	45·5	43·0	40·5	48 ,,
50 per cent ...	51·0	50·2	50·0	49·8	49·0	49·0	48·8	8 hrs.
	52·5	51·0	50·0	49·2	48·2	48·0	46·8	24 ,,
	54·8	51·8	50·0	49·0	47·0	45·8	43·0	48 ,,
60 per cent ...	51·0	50·8	50·2	50·0	49·5	49·0	48·0	8 hrs.
	52·5	51·5	50·8	50·0	48·8	47·8	46·5	24 ,,
	55·0	53·5	51·8	50·0	48·0	46·0	43·0	48 ,,
70 per cent ...	51·2	50·8	50·8	50·2	50·0	49·8	48·8	8 hrs.
	53·0	52·0	51·8	51·0	50·0	49·0	47·2	24 ,,
	56·5	54·8	53·8	52·0	50·0	48·5	45·0	48 ,,
80 per cent ...	52·0	51·8	51·2	50·8	50·5	50·0	49·2	8 hrs.
	55·8	54·8	53·5	52·0	51·5	50·0	48·0	24 ,,
	60·8	58·8	56·2	53·5	52·8	50·0	46·8	48 ,,
90 per cent ...	52·0	51·5	51·0	51·0	50·8	50·2	50·0	8 hrs.
	57·5	54·8	53·8	53·2	51·8	51·0	50·0	24 ,,
	68·8	59·0	56·8	55·8	53·8	51·5	50·0	48 ,,

R. H. = Relative humidity.

The capillary tubes may be filled with mixtures up to exactly 50 mm. in some mechanical way in the laboratory, and the two ends of the tubes sealed, and these may be stored indefinitely. These tubes can then be supplied to field workers in batches with the relative humidity marked on them. The field workers before use can break the two sealed ends and mount them on a glass slide as desired. A hand lens may be used for taking the readings if necessary.

DISCUSSION.

Unlike the wet and dry bulb thermometer, this micro-hygrometer does not require ventilation, and in this way does not disturb the atmosphere of the microclimate.

In all ordinary hygrometers, the humidity at any one instant is recorded. If there are diurnal or other variations in the relative humidity of the microclimate, this instrument will give a mean microhumidity for the period recorded. For purposes of eliciting the behaviour of insects it is as necessary to measure the humidity at any time for *choice*, as the mean humidity over a certain period for *resting*, as the insects are not likely to be affected by any variations for a short period for purposes of resting. At the same time, however, it should be borne in mind that diurnal variations of humidity in nature are not likely to disturb the microclimate to any large extent.

This hygrometer is slow in attaining equilibrium with the microclimate, and equally slow in losing that equilibrium. The paper and hair hygrometers are also equilibrium hygrometers but they attain equilibrium quickly and lose it much more quickly, which make them eminently suitable for laboratory work where the weighings can be finished on the torsion balance in a few seconds. But this is a disadvantage for field work as these bits of paper or hair cannot be carried to the laboratory for weighing within a few seconds, nor the torsion balance carried conveniently to the field to enable weighing to be done at the spot.

The use of paper hygrometer in the laboratory is easy enough, but in the field it is rather difficult to hang a bit of paper in a nitch without being soiled by dust or moisture. These take only 12 minutes to get into equilibrium with air and do not change in weight appreciably in 10 seconds within which time it is possible to weigh them on a torsion balance (Buxton and Mellanby, *loc. cit.*). This is quite suitable in the laboratory but not practicable in the field.

The final record of humidity by this method may take considerable time, but it can very quickly demonstrate that the mixture in any tube is not in equilibrium with the water in the air of the microclimate. It is therefore a quick method for showing the upper and lower limits if they are sufficiently wide apart. By choosing the right two tubes at the start, one might be able to show that the humidity in a microclimate is, say, more than 60 per cent and less than 80 per cent. One may then with two more tubes show that it is between 60 per cent and 70 per cent.

SUMMARY.

A cheap, simple, portable and at the same time fairly accurate instrument which can be manipulated without much skill by any field worker, has been described.

There are two kinds of hygrometers, those which need ventilation and those that do not. The former are ruled out for use in the microclimate which interest an entomologist, the latter belong to the class of hygrometers which may be called 'equilibrium' hygrometers. To this class belong the paper and hair hygrometers which require weighing on a torsion balance within a few seconds after they are removed from the microclimate. This is an advantage and is quite practicable in

the laboratory, but there are several disadvantages in the field where a delicate torsion balance cannot be carried.

The paper and hair hygrometers are 'equilibrium' hygrometers, but they attain their equilibrium or lose their equilibrium in such a short time that they register the humidity for the short period before they are removed from the microclimate. This micro-hygrometer takes some hours to attain equilibrium and will register a mean microhumidity for that period, say 8 hours or more, which is what is required for the study of the microclimate of insect resting places.

Instruments like the dew-point hygrometer and chemical hygrometer for the measurement of microhumidity which involve the sucking in of a small sample of air are difficult to use in the field without special care and technique.

It is suggested that in all future records of malaria surveys and control operations, the mean microhumidity and micro-temperature be recorded in the feeding, breeding and resting places of mosquitoes in order to elucidate their bionomics and the effect of control. These methods may replace the present practice of furnishing meteorological data from the nearest meteorological station or even data recorded at the site of the local malaria station from a Stevenson's Screen.

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The authors wish to acknowledge the helpful suggestions received from Prof. P. A. Buxton and Mr. P. R. Pisharoty, and the help rendered by Mr. V. Ramakrishna, a colleague of the authors, who was associated with them during the early part of this work.

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A PRELIMINARY NOTE ON EXPERIMENTAL INFECTIONS OF AVIAN MALARIA AND SAUROPSIDAL FILARIASIS IN *C. FATIGANS* WEID., 1828.

BY

N. G. S. RAGHAVAN,

AND

B. G. MISRA.

(*Malaria Institute of India, Delhi.*)

[July 15, 1949.]

Ross (1898) carried out successful experimental infections of *Plasmodium praecox* in *C. fatigans* which led to the famous discovery of malaria transmission by mosquitoes. Daniels (1899) confirmed this. Neuman, Sergent Ed. and Et. have also reported positive experimental infections (as quoted by Wenyon, 1926).

Wenyon (1926) states that *Plasmodium praecox* of sparrows is transmitted in nature by *Culex fatigans*.

Successful experimental infection of *C. guindiensis* to their infective stage in *C. fatigans* has been reported by Pandit *et al.* (1929).

No combined infection of both in the same mosquito has so far been reported.

PRESENT WORK.

This note sets out results of combined infections (experimental) to their respective infective stages, in *C. fatigans* Weid., 1828, of avian malaria *P. relictum* Grassi and Felleti, 1891, in sparrows, and reptilian filaria *C. guindiensis* in *Calotes versicolor* or garden lizard or blood sucker carried out under the Director, Malaria Institute of India early in 1949.

PLAN OF WORK.

Laboratory bred *C. fatigans* of same broods were kept starved for about 48 hours and used in these experiments.

Sparrows showing ' very scanty ' infections of *P. praecox* synonym *P. relictum* Grassi and Felleti, 1891 (Russell *et al.*, 1946) were used in these infection experiments. (The term ' very scanty ' infection signifies presence of parasites in every fifth microscope field.)

Starved mosquitoes were first fed on the infected sparrows overnight. In the morning fully fed mosquitoes were separated and kept fed on glucose. After twenty-four hours of such feeding, they were divided into two lots. One lot was kept as a control and the other, after a preliminary period of 48 hours of starvation, was fed overnight, on an infected calotes. The number of circulating microfilariæ in the *Calotes versicolor* was determined by examining three serial slides containing 5 c.mm. of blood.

After the second 'infective' feed, fully fed mosquitoes were separated and were subsequently fed on glucose. Every morning and evening the cages were examined and freshly dead or moribund mosquitoes were dissected and results noted. The remaining mosquitoes were killed and examined between the twelfth to eighteenth day of original infective feeds and results recorded.

A lot of *C. fatigans* fed only on the same infected *Calotes versicolor* was kept as a control.

The order of infective feeds was reversed in another set of mosquitoes but all other steps taken were identical.

RESULTS.

It will be noticed from Tables I to IV that in *C. fatigans* with combined infections, or in controls with either infection, the results of individual infections were identical. The various infective stages, i.e. sporozoites in salivary glands and/or infective larvæ in head/proboscis, appeared at about the same time.

TABLE I.

Results of infection of P. relictum *Grassi and Felleti*, 1891, *in* C. fatigans.

Number of mosquitoes dissected.	Number of days after first infective feed.	Positive plasmodial infection in: Gut (oöcyst).	Positive plasmodial infection in: Salivary glands (sporozoites).	Remarks.
		Batch I.		
3	6	−	...	Small.
2	7	+	...	Medium sized.
1	8	++	...	
2	11	++	+	Big sized. Some oöcysts had ruptured and very few sporozoites were noted.
4	14	−	++	Sporozoites seen.
21	16	−	++	Plenty of sporozoites seen.
		Batch II.		
2	3	−	−	
4	5	++	...	Small sized.
3	6	++	...	Medium sized.
1	9	++	...	Large sized.
2	11	−	+	
4	13	−	++	
6	15	−	++	
1	18	−	++	

Note.—1. All the infection experiments were carried out under the following conditions of temperature and humidity :—

Batch number.	Month (1949).	Temperature range, °F.	Relative humidity range, per cent.
I	March	80–70	52–84
II	May	90–74	48–85

2. Microfilarial counts of infected lizards used in the experiments :—
(Average count of three serial slides of 5 c.mm. each.)

Batch I = 235. Batch II = 95.

TABLE II.

Results of infection of reptilian filaria (C. guindiensis) *in* C. fatigans.

Number of mosquitoes dissected.	Number of days after first infective feed.	Positive for filarial infection in			Remarks.
		Abdomen.	Thorax.	Head/Proboscis.	
			Batch I.		
2	4	—	++	—	Sausage forms.
3	5	—	++	—	Do.
1	9	—	++	...	Infective larvæ.
3	11	—	++	—	Do.
2	13	—	++	+	Infective larvæ in thorax and head.
3	14	—	+	++	Infective larvæ in thorax and head/proboscis.
9	15	—	—	++	Infective larvæ in head.
4	16	+	—	++	Infective larvæ in head and in abdomen.
			Batch II.		
3	2	—	+	...	
5	4	—	++	...	
4	8	—	++	...	Infective larvæ and sausage forms.
2	10	—	++	...	More infective larvæ.
3	11	—	+	+	Infective larvæ in head.
4	12	—	+	++	Infective larvæ in head and proboscis.
5	15	—	—	++	Infective larvæ in proboscis.
2	18	+	—	++	Infective larvæ in abdomen and proboscis.

TABLE III.

Results of combined infection of avian malaria and reptilian filaria in the same C. fatigans (*first fed on avian malaria and then on reptilian filaria*).

Number of mosquitoes fed.	Number of days after first infective feed.	Positive for filaria in			Positive for plasmodiæ in		Remarks.
		Abdomen.	Thorax.	Head/ Proboscis.	Gut (oöcysts).	Glands (sporozoites).	
				Batch I.			
1	9	−	+	+	+	−	Infective larvæ in thorax.
4	15	−	−	+	−	+	
				Batch II.			
1	11	−	−	+	+	+	Infective larvæ in head and proboscis; sporozoites in salivary gland.
6	14	−	−	++	−	++	

TABLE IV.

Results of combined infection in the same C. fatigans (*order of feed reversed, i.e. first fed on infected calotes and then on infected bird*).

Number of mosquitoes dissected.	Number of days after first feed.	Positive for filaria in			Positive for plasmodiæ in		Remarks.
		Abdomen.	Thorax.	Head/ Proboscis.	Gut (oöcysts).	Gland (sporozoites).	
				Batch II.			
1	8	−	+	−	+	−	Small oöcysts and infective larvæ and sausage forms in thorax.
3	10	−	++	−	+	−	Large size oöcysts and infective larvæ in thorax and head.
2	12	−	+	++	++	−	Large size oöcysts. Infective larvæ in thorax, head and proboscis.
3	15	−	−	++	+	+	
1	18	+	−	+	−	++	

In the two series mentioned, density of microfilarial infection in *Calotes versicolor* did not alter the rate of development of infection in the mosquitoes. Initial mortality on account of effects of hyperfilariation in mosquitoes however was evident.

CONCLUSIONS.

There is no antagonism between avian malaria and reptilian filaria infections developing in the same specimens of *C. fatigans* Weid., 1828.

ACKNOWLEDGMENT.

The authors gratefully acknowledge help from the Director, King Institute, Guindy, Madras, for supplying infected *Calotes versicolor*.

Note.—The infections in mosquitoes are being transmitted to 'clean sparrows' and results will be published later.

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A NOTE ON EXPERIMENTAL INFECTIONS OF *MF. MALAYI* BRUG IN *C. FATIGANS* AND *A. STEPHENSI* (TYPE).

BY

N. G. S. RAGHAVAN,

AND

K. S. KRISHNAN.

(*Malaria Institute of India.*)

[July 15, 1949.]

THESE investigations were carried out under the Director, Malaria Institute of India, Delhi, in 1948, in an area in South India, Sri Harikotta Island, Nellore District, Madras Presidency, where both malaria and filariasis existed (Raghavan and Krishnan, 1949).

A review of the literature shows that while experimental infections of *Mf. bancrofti* were found to be highly positive in *C. fatigans* Weid. (the vector in nature) and in *A. stephensi* (Rao and Iyengar, 1932), the former was highly refractory to experimental infections of *Mf. malayi* (Lichtenstein, 1927, quoted by Brug, 1927; Iyengar, 1932, 1938). *M. annulifera*, the vector of *Mf. malayi* in nature, has been reported to be highly susceptible to experimental infections. So far no mention has been made of experimental infections of *Mf. malayi* in *A. stephensi*.

This note sets out the negative results of experimental infections of *Mf. malayi* in *A. stephensi* (type) and *C. fatigans*.

PLAN OF WORK.

Suitable volunteers were obtained while carrying out routine filariasis surveys. 100 c.mm. of peripheral blood was drawn from each volunteer before feeding mosquitoes and, for the purpose of determining average microfilarial counts, 5 smears were made on blood slides each containing 20 c.mm. of blood.

Actual feeding of mosquitoes of the same brood was usually arranged between 8-30 p.m. and 11-0 p.m. After 48 hours of starvation, about forty of the mosquitoes were put into bamboo rings covered with bobbinet on both sides. The bamboo rings containing *C. fatigans* and *A. stephensi* were then applied separately to similar parts of the body of each volunteer, care being taken to see that the bobbinet on one side of the ring was kept in close contact with the skin. In all these experiments, the rings were kept applied to the volunteer for exactly one hour.

After this, the mosquitoes were released into a larger cage when the fully fed ones were separated and transferred to smaller cages and subsequently fed on glucose and water.

All the cages were examined daily both in the mornings and evenings, and during the first 8 days any specimens found dead or in a moribund state were dissected. The survivors were dissected between eighth and twelfth day.

As the results of the experimental infections in the first two batches of *A. stephensi* (type) turned out to be as refractory as in *C. fatigans* fed on the same volunteers, further series were taken, using different volunteers in whom clinically no lesion could be noted.

By now the microfilaria of the local people (volunteers were from the same source) was morphologically made out to be *Mf. malayi* of which vectors in nature were *Mansonioides annulifera* and *Mansonioides uniformis* (Raghavan and Krishnan, *loc. cit.*).

In addition to the above, experimental infections of *Mf. malayi* and *M. annulifera* were carried out as a control. The method of feeding, aftercare, conditions of humidity and temperature were identical in all these experiments.

DISCUSSION.

An analysis of the results set in Table I shows that, in experimental infections of *Mf. malayi*, *C. fatigans* was absolutely refractory even though volunteers with varying numbers of microfilariæ were used in the experiments.

Experimental infections in *A. stephensi* were carried out on the same volunteers and conditions of feeding, aftercare, humidity and temperature were identical to the experimental infections in *C. fatigans*. Results were nearly identical, exceptions being specimens in each of three batches where degenerative thoracic forms were noted at a time, by which in *M. annulifera* used as controls, the experimental infections had gone to the infective larvæ in head/proboscis stage. Thus the results of experimental infections of *Mf. malayi* in *A. stephensi* could be taken to be as refractory as in *C. fatigans*.

This might be used as a diagnostic test in the detection of the nature of infection, provided optimum climatic infection and aftercare conditions are made available to the *A. stephensi*.

SUMMARY AND CONCLUSIONS.

1. Experimental infections of *Mf. malayi* in *Culex fatigans* Weid., 1828, are characteristically negative. This corroborates the findings of Lichtenstein (*loc. cit.*) in Borneo, and Iyengar (1932) in N. Travancore.

2. In *M. annulifera* used in the experimental infections of *Mf. malayi*, the infections underwent the normal course of development to their infective stage, i.e. infective larvæ in head/proboscis.

3. Experimental infections of *Mf. malayi* in *A. stephensi* (type) were equally refractory as in *C. fatigans*.

4. It is suggested that the refractoriness of experimental *Mf. malayi* infection in *A. stephensi* (type) might be used as a diagnostic feature of the infection.

ACKNOWLEDGMENT.

The authors wish to record their thanks to Mr. S. Cornelius, Laboratory Assistant, Malaria Institute of India, for the help rendered by him.

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TABLE I.

Experimental infections of Mf. malayi (*Brug*, 1927) *in* C. fatigans, A. stephensi (*type*) *and* M. annulifera (*various workers*).

Species of mosquito.	Number infected.	Number positive.	Microfilarial rate.	REMARKS.
C. fatigans ...	300 (mostly *C. fatigans*).	...	Not stated	Lichtenstein (1927).
C. fatigans ...	800	...	Not stated	Iyengar (1932).
	About 400	...	Not stated	*Idem* (1938). One specimen showed a few young larvæ poorly developed in the thoracic muscles at a time by which in control *M. annulifera* normal development was noted.
C. fatigans ...	21	...	19 in 10 c.mm.	Present work.
	36	...	17 in 10 c.mm.	
	11	...	67 in 20 c.mm.	
	14	...	31 in 20 c.mm.	
	10	...	25 in 20 c.mm.	
	18	...	90 in 20 c.mm.	
	46	...	87 in 20 c.mm.	
	6	...	167 in 20 c.mm.	
A. stephensi (type).	38	...	* 87 in 20 c.mm.	*, †, ‡ denote one specimen from each of these batches which showed degenerative thoracic forms on 10th, 13th and 15th day respectively, by which time in *M. annulifera* used as controls the infection had gone to the head/proboscis stage.
	22	...	† 67 in 20 c.mm.	
	10	...	25 in 20 c.mm.	
	12	...	‡ 31 in 20 c.mm.	
	19	...	19 in 20 c.mm.	
	2	...	167 in 20 c.mm.	
M. annulifera	Not stated	++	Not stated	Iyengar (1938).
M. annulifera	10	7	87 in 20 c.mm.	Developed in $9\frac{1}{2}$–$10\frac{1}{2}$ days.
	9	6	67 in 20 c.mm.	Present work.
	7	4	31 in 20 c.mm.	10–11 days.

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COMMENTS ON MAN-MADE MALARIA IN INDIA.*

BY

JOHN M. HENDERSON, C.E.

(*Professor of Sanitary Science, Columbia University School of Public Health.*)

[July 21, 1949.]

As the name indicates, man-made malaria is created by man's actions. In its broadest sense, it includes malaria arising from war, changes in social and agricultural practices, population migrations, and from the selection of unsuitable housing sites for permanent or temporary settlement. While it is important that these aspects are not overlooked in order that unnecessary disease may be avoided where feasible, the term *man-made malaria* is more commonly applied to that arising from the creation of breeding places of malaria carrying mosquitoes.

The number of types of such breeding places is innumerable due to the large number of species of anopheline mosquitoes that transmit malaria in India, and the diversity of aquatic situations in which they breed. In some localities, for example, the malaria vectors may include species which breed in small wells, blocked-up roof gutters, small water-holding containers such as tins, vases and pitchers, and borrowpits produced during the construction of commercial and industrial buildings in the centre of a city.

However, in spite of the diversity of the problem, a majority of the breeding places created by man are related to irrigation systems, borrowpits along railroads and highways, and obstructions to natural drainage. Malariologists estimate that about 25,000,000 cases of malaria are caused annually by such situations. This constitutes one-quarter of all the malaria in India and results in socio-economic losses totalling many crores of rupees. The object of this article is to review the problem of the borrowpit and the irrigation system from the standpoint of a civil engineer engaged in malaria control and to present some solutions.

Consideration of the physical problem must be preceded by brief consideration of the biology of the malaria mosquito. Of the 9 species of anopheline mosquitoes which

* Presented at the 19th Annual Meeting, Research Committee, Central Board of Irrigation, Government of India, Simla, July 19 1949.

are important vectors of malaria in India, only one is usually found to transmit in different parts of the country ; the occurrence of all others in sufficient numbers to transmit malaria being regional. Since types of breeding places differ by species, geographic location in consequence has bearing on what constitutes a *man-made malaria* situation. But this factor is not as important as it might at first appear. Some species are rare in certain areas because few suitable breeding places exist. These can and do become important vectors when new favourable breeding places give rise to increased prevalence of the species. Moreover, anopheline species are not uncommonly imported. When local conditions are adverse the new species fails to become established. When favourable, as when new suitable breeding places are created, the imported species may become permanently established. Hence, in constructing long-enduring public works such as dams and irrigation canals, measures to permanently exclude the creation of breeding places for malaria mosquitoes should be designed not only against the locally prevalent species but also against the nearby species which may likely be imported. However, steps against the locally indigenous species should be prosecuted to the fullest extent since these species may give rise to a certain and prompt rise in malaria. Measures against other species may be precautionary and more limited, less intensive and less costly.

As regards character of breeding places it is not possible to definitively classify them by mosquito species. The picture is rather one of each species breeding in a range of situations, but demonstrating marked preference for a few types of places within that range. This is often so pronounced that breeding in sufficient abundance to constitute an important malaria hazard takes place only in the more favoured situations. Without attempting to enumerate the breeding places of each species, it may be said that, in so far as most engineering constructions are involved, the outstanding factors are that the more widespread and important species prefer shallow water to deep water, little waters to large waters, and temporary or semi-permanent to permanent bodies of water. This highlights the peculiar significance of the borrowpit and of seepages from irrigation systems in the malaria control picture.

SURFACE IRRIGATION SYSTEMS.

From the standpoint of malaria control, surface irrigation systems may be broadly subdivided into : (1) open water channels, (2) water utilization in the fields, and (3) drainage to remove spent water. Important malaria problems exist in the first two places but water channels in the form of canals, branches and distributary systems are reported as usually more important. This is because seepages from water channels give rise to more prolific breeding than do the wet fields themselves, and because the fields tend to be localized. Moreover, the total length of channels in a single irrigation system may be over a thousand miles. These affect not only people within mosquito flight range of the fields, but many others as well. Shallow still water within the channel section, which occurs when it is under repair or not in use, also provides prolific breeding and at times augments the malarial importance of the channel.

The history of surface irrigation in India dates back many centuries. Until very recently, construction methods appear to have shown no change over this

period. The same head-carry methods, practised during the days of the Moghul emperors, have prevailed into the twentieth century, and are still widely used even in current constructions. This method of construction appears to be the greatest single physical reason for the extensive malaria caused by irrigation channels and by the borrowpits in irrigation, highway and railway constructions.

With head-carry, earth must be borrowed at or close to the base of the embankment, and continuously along it. Since embankments are generally necessary only in low land, this tends to ensure the presence of water in borrowpits and renders many pits undrainable. The presence of many small pits establishes the ' small waters ' condition favoured by the malaria carrying mosquito and also brings more human population within flight range. The tendency towards shallow cuts due to hand labour excavation at times is beneficial, but also results in shallow temporary water favoured by the malaria carrying mosquito. The location of pits adjoining the embankments also tends to ensure the impoundment of leakage from the water channel. Earth measurement practices associated with head-carry and hand excavation encourage barrier strips between pits which prevent drainage.

The evils of head-carry are not only limited to the creation of water-holding borrowpits. Top soils tend to be utilized for embankments instead of more impermeable subsoils. Designed soil mixes are generally not possible, since only the types of soil at the embankment base are procurable. Lack of mechanical equipment on head-carry projects prevents proper compaction of the fill. Thus, the total effect on irrigation systems is not only to create shallow pools, but to provide them with a source of water.

Malaria lconditions on such channel embankments may be bad enough following construction but on older projects at least tend to become worse with passage of time. The absence of siltation tanks at the headworks, their improper hydraulic management, or lack of capacity, have caused extensive siltation in many channels, principally in the main canals. As this proceeds, embankment walls are raised by maintenance forces, with consequent increase in borrowpits. Leakage tends to increase with each successive lifting of the channel flow section above adjoining ground levels.

BORROWPITS.

The borrowpit in relation to irrigation channels has already been dealt with. When borrowpits for highway and railway embankments are considered separately from those related to irrigation, the most significant factor is their intimate relationship to human population. Villages, towns and cities are located and expand along routes of transportation and communication and vice versa. This tends to make certain that wet borrowpits along highways and railroads will cause a maximum amount of malaria. It is common knowledge that many roads and railways are bordered by a succession of walled borrowpits extending for hundreds of miles, and for which no drainage has been provided. This condition is even more extensive than with irrigation systems because fill is needed for local drainage of the road surface in elevated flat land as well as for low land embankments. The problem is greatly aggravated by road maintenance practices, which give rise to the creation of thousands of new undrained pits each year.

THE SOLUTION.

Irrigation systems.—Although irrigation malaria poses a far more complex problem than does that of highway and railway borrowpits, there is evidence that more progress is being made toward its solution.

As regards irrigation channels, the fundamental solution is not cured by drainage, but the application of preventive measures against the leakage itself. This not only avoids economic loss from water wastage, but provides more certain protection and requires less maintenance. In many cases the topography is too flat for effective or economical drainage. In fertile soils, growth of vegetation in wet ditches may obstruct drainage and may be too rapid for effective maintenance. Where drainage is the only alternative, as with some existing systems, suitable grades should be selected. Small ditches for residual drainage which carry shallow flows have a poor hydraulic radius and a high coefficient of roughness, unless lined. The minimum slope of such ditches should be 1 : 1,000 and the preferred slope 2 or 3 ft. per thousand. Drainage is also often essential to dispose of water downstream from the fields.

During his visits to various irrigation projects under construction, the author noted many signs of intelligent effort towards providing tight channels as well as the impact of engineering economics in providing indirect solutions. These may suffice, but it should be recognized that the solution extends deeply into the domain of agricultural economics. From the standpoint of the designing engineer, the investment that can be justified in preventing water leakage is largely determined by water revenues, and in turn on water rates. Improved practices by the cultivator would permit him to pay higher prices for water and this would make possible more durable and costly construction.

In one of the technical proceedings (C-56) for the nineteenth annual meeting (1949) of the Research Committee of Central Board of Irrigation, the following statement appears : 'In almost all the new projects all canals are being lined from the very start, because it has been found that the extra cost of constructing lined canals is insignificant compared to the advantages which would accrue.' If such lining practices were universal, and if they could be extended to include all branches and distributaries, there would be little need for discussion on malaria hazards arising from the channels themselves. Under the circumstances however, further comment is indicated.

The most important indirect solution is the gradual trend taking place in India towards the mechanization of earth moving practices. This not only shortens the period of construction and lowers costs, but does away with the evils of head-carry methods, as described previously. The engineer is free to reach out for his earth, use designed soil mixes, obtain proper compaction, borrow earth at greater depths, largely avoid adverse locations for borrowpits, and reduce the number of individual pits. It is trusted that no long period of evolution will be required for the construction engineer to fully utilize these ancillary advantages of mechanization.

In spite of its merits, the use of heavy machinery for earth moving is subject to limitation of application. Its rôle is in building main and branch canals. It may be remarked that the change now occurring in India from head-carry to the heavy tractor and carry-all represents a bridging of several centuries of evolution in earth-handling practices. It by-passes the wheel-barrow and all animal-powered

methods, including the wagon, the drag pan and the mule-drawn wheeled scraper with snatch team, as well as dump cars on tracks. During the war, the author used derricks and drag pans powered by bullocks with results that were far from satisfactory but yet superior to head-carry or even wheel-barrows. It is worth considering whether these methods deserve more consideration in constructing embankments for distributaries or for even larger channels where mechanical equipment is not available.

As for more specific methods of controlling water leakage, the author has noted the developing interest at project sites in lining irrigation channels with portland cement concrete, surkhi concrete and soil-cements. A substantial part of the added cost is being recovered by savings in excavation and fill due to smoother flow sections.

In yet other projects with a suitable topography, the leakage problem is being largely overcome by careful layout to place nearly all channels in cut. Drainage-ways are crossed by viaduct or siphon.

Another point warrants emphasis. It is the great value of the applied research work being sponsored, co-ordinated and undertaken by this Research Committee of the Central Board of Irrigation. The relationship between soil research and malaria control is particularly great in the direction of low-cost linings and improved soil mixes, but research in general water utilization, including agronomy, is equally fundamental.

As regards distributaries, field systems and field practices in India, progress in three directions deserves comment. One is intermittent perennial irrigation, i.e., providing water 4, 5 or 6 days in the week instead of 7 days. This method has been practised successfully to curb malaria mosquitoes produced within the field systems without injury to crop yield, but is not feasible in all areas or in all seasons, due to differences in rainfall, topography and crop varieties.

A second method is being adopted on a project where the land area exceeds the supply of water to be made available. Twenty-five per cent of the land area is to be placed under perennial irrigation. These tracts will be located beyond the flight range of malaria carrying mosquito to existing or proposed villages. The remaining 75 per cent of the land area will be irrigated for dry crop cultivation.

A third method has significant, but more limited, application. Field systems down to the individual plot may be constructed as a part of the project, instead of by the cultivator, to minimize water losses and malaria and the cultivator to pay for the cost of construction.

Borrowpits.—The borrowpit problem is simpler than that of irrigation malaria, but the present prospects for its solution are less favourable. It is receiving attention on some irrigation and dam construction projects, but even here it is not always handled adequately. The most flagrant abuses however are in highway construction and maintenance, where thousands of new undrained borrowpits are being constantly created in disregard of the public interest. Many of these could be drained merely by breaking through the thin walls separating the individual pits.

The regrettable thing about this blot on the engineering profession and this social blight is that every engineer the author talked with in India, is familiar with the malarial significance of the water-holding borrowpit. Moreover, the drainage of most borrowpits does not require engineering design or supervision, but can be accomplished under the direction of mates whenever instructions are issued and enforced.

Under these circumstances, there is only one apparent solution. Assuming that instructions have been issued by superior authorities requiring the drainage of water-holding borrowpits where practicable, the next logical action is to summarily discharge for gross negligence the immediate supervisor responsible. Where water-holding borrowpits continue to persist over a general area, it can be due only to wilful disregard of instructions at higher levels in which case similar action is indicated.

If a serious train wreck were caused by the direct wilful negligence of a railroad employee, those directly responsible would surely be discharged without question. In view of the far greater loss of life and disability caused by millions of cases of 'borrowpit malaria', there is no good reason why similar action should not be taken.

Let it be frankly recognized however that the water-holding borrowpit cannot be condemned out of hand, and that there are a few exceptional situations where it may be sanctioned. The most apparent case is where no human population is resident, as with some pioneering transportation routes passing through virgin areas. But this practice is warranted only where the land is unfit for settlement, as with vast expanses of marsh and swamp, for people otherwise move in rapidly. In some coastal plain areas, permanently water-holding borrowpits could be sanctioned to avoid hauling fill from many miles away at huge expense. These may involve situations where the added breeding from borrowpits will be unimportant in relation to existing anopheline production, or where deep pits with clean vertical edges may provide unsuitable habitats for species that are indigenous or likely to be imported. Decision on such matters requires intelligent malariological advice and approval. Such exceptions however should not divert attention from the fact that most borrowpit nuisances reflect careless, untidy practices, have no economic justification, and are frequently correctable at trivial expense.

REMARKS.

There is one more point that applies to civil engineering in general. Malaria not only causes a greater toll in India than in any other country, but the number of cases and deaths exceeds that of any continent outside Asia. The civil engineering profession has such a major potential rôle to play in preventing and abating malaria in this country that a thorough grounding in the rudiments of malaria control engineering is an essential part of the undergraduate training of every civil engineer, regardless of specialized interest. It is essential that such training be provided by a qualified resident faculty member at every college of engineering in India. This requires, first, agreement and positive decision by the faculties and administrative heads of the institutions, and secondly, provision for training teachers in malaria control engineering. Such training requires substantial

study and exposure to malaria control activities, probably between regular school sessions. The short courses in malaria control provided for engineers by the Malaria Institute of India are invaluable, but do not supplement the need for undergraduate training in the engineering colleges themselves. The ultimate rôle of the Institute in this respect should be in advanced training.

Progress in malaria control and interest in the subject by the general civil engineering profession also can be furthered by the training, attraction and utilization of malaria control engineers within the health services themselves as an active and responsible component of the medical-engineer-entomologist team.

Indian Journal of Malariology, **3**, 2 and 3, June/September 1949.

ABSTRACT.

MALARIA IN TIRUMALAI VILLAGE, CHITTOOR DISTRICT, MADRAS PRESIDENCY*.

BY

H. SUBRAMANIAM.

AND

K. N. CHETTY.

(*Tirumalai Tirupathi Devasthanam.*)

[April 24, 1948.]

TIRUMALAI village, a famous place of all-India pilgrimage, is situated on the top of a hill at an altitude of 2,820 ft. in Chittoor District about 100 miles from Madras City. It is surrounded by forests and the nearest inhabited villages are situated at the foot of the hill at a distance of about 7 miles. In 1941, its population was 3,357.

It has always had a reputation for being unhealthy, many pilgrims visiting the shrine suffering from malaria either at the place itself or on their return home. But a survey carried out in 1928 revealed almost nil spleen and parasite rates among the resident children. It was then considered to be an example of 'malaria *sine* anopheline'; more properly it is an instance of 'imported malaria', the strain of ascent through 7 miles, of fasting and other religious ceremonies facilitating relapses of infections acquired elsewhere. A subsequent survey in 1935 revealed some local transmission with definite seasonal and periodical exacerbations in common with the behaviour of malaria epidemics in the ceded districts of the province. Obviously in normal years malaria was of the 'introduced' type, the local anophelines capable of some degree of transmission only because of the loading of the reservoirs of infection among the pilgrims from all over the country. The survey in 1935 coincided with post-epidemic conditions in many parts of the district.

* The original manuscript (12 pages, 2 charts and 8 tables) has been placed in the Library of the Malaria Institute of India and is available on loan to workers who wish to consult it.—EDITOR.

Since 1935, the temple authorities took up more detailed malaria survey and control. At that time the spleen rate was about 21 per cent. In 1944, another epidemic broke out and the spleen rate was 48·7 per cent and it dropped to 4·3 per cent early in 1947. During 1944 and 1946, the parasite rate was 12·2 per cent, *P. falciparum*, *vivax* and *malariæ* accounting for 32·7, 54·7 and 12·6 per cent respectively of the total infections.

As many as 20 species of anophelines are recorded (*aitkeni*, *annularis*, *barbirostris*, *culicifacies*, *culiciformis*, *fluviatilis*, *hyrcanus*, *jamesi*, *jeyporiensis*, *karwari*, *maculatus*, *moghulensis*, *minimus*, *pallidus*, *splendidus*, *subpictus*, *tessellatus*, *theobaldi*, *vagus* and *varuna*). The only species found naturally infected was *fluviatilis*, one out of 354 showing sporozoites.

Antilarval control through paris greening, minor engineering works and stocking water collections with fish coupled with quinine treatment of malaria cases was carried out since 1935. In 1946, bi-weekly spray-killing with pyrethrum was carried out from dusk till 10 p.m. to deal, according to the authors, with the vector species of *fluviatilis* at the time of their entry into houses for purposes of feeding. In 1947, two rounds of indoor residual spray with D.D.T. using a 3·7 per cent solution in kerosene was carried out at a six-weekly interval.

The authors, comparing the results of antilarval control, spraying with pyrethrum and indoor residual spray with D.D.T., conclude that the last method has proved to be the most promising.

COMMUNICATIONS.

THE EDITOR,

INDIAN JOURNAL OF MALARIOLOGY,

MALARIA INSTITUTE OF INDIA,

22, ALIPORE ROAD, DELHI.

DEAR SIR,

Owing to the delay in printing, for reasons which are well understood by me, my letter to you of March 3, 1949, published in your December 1948 issue is actually being read by subscribers during the last week of September or the first week of October 1949. My letter, therefore, might appear to conflict with the recommendations of the Medical Research Council on 'Paludrine', published in the *Lancet* and the *British Medical Journal* of April 2, 1949. This appearance of conflict was of course due to the delay in the publication of your journal's December 1948 issue, and I would like to stress the fact that the recommendations of the Medical Research Council are accepted by my Company in entirety, and that wherever sub-paragraphs 1 to 5 of my letter differ from the Medical Research Council's recommendations for India, the latter's recommendations should be followed.

The recommendations of the Medical Research Council particularly emph e the value of a dose of 0·3 gm. of 'Paludrine' for prophylaxis and suppression, or for clinical cure of malaria in village or rural populations, and we have, therefore, made a special point of ensuring adequate supplies of the 0·3 gm. 'Paludrine' tablet throughout India.

Yours faithfully,

J. M. MUNGAVIN,

M.B., B.Ch., D.T.M. & H. (Eng.),

Imperial Chemical Industries (India) Ltd.,

Calcutta.

12*th October*, 1949.

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DIAGNOSTIC CHARACTERS FOR THE DIFFERENTIATION OF THE LARVÆ OF *A. SUBPICTUS* AND *A. SUNDAICUS*.

BY

P. SEN, M.Sc. (Cal.), Ph.D. (Lond.), D.I.C.

(*Malaria Officer and Entomologist, Directorate of Health Services, West Bengal.*)

[July 26, 1949.]

SWELLENGREBEL and SWELLENGREBEL (1919: 1920) studying in Malaya and Dutch East Indies did not succeed in differentiating the larvæ of *rossi* (*subpictus*) from those of *ludlowi* or *sundaicus*. Walch and Soesilo (1929), however, thought that the arrangement and shape of the pecten teeth differ in the two species, but according to Puri (1931) the character of the pecten is highly variable.

Ghose (1932) for the first time showed that it was possible to differentiate the larvæ of *A. sundaicus* (*ludlowi*) from those of *A. subpictus* on the character of the mesothoracic hair No. 5 and on the banding on the head. According to him the mesothoracic hair in *A. sundaicus* has three or more branches in about 90 per cent of specimens. In a small percentage (10 per cent) of cases, this hair may have three branches on one side and two branches on the other. In *A. subpictus*, on the other hand, the branches may be two or less on both sides in most specimens (93 per cent). In only 1 per cent cases in *A. subpictus*, there may be three branches on either side as in *A. sundaicus*, but these branches never originate from the base of the hair as in the latter species. A certain amount of difficulty has all along been felt in differentiating the larvæ of *A. sundaicus* with 2-branched hair No. 5 and of *A. subpictus* with 3-branched hair No. 5.

Venhuis (1938) described some more characters such as the pigment of the anal setæ on the 10th segment, the branching of the post-spiracular hair, the form of the pecten and of the pleural hairs which according to him would make the identification a little more definite. But Sundaresan and Rao (1943) have shown that these characters are not constant in either of the two species. Although the characters described by Ghose were found fairly reliable, for some reasons they were not made use of by Senior White and Adhikari (1939) while working in the Chilka Lake area, as they remarked that they had to discard three months' larval survey at Chilka owing to the impossibility in separating the larvæ of the two species.

A large number of larvæ of both *sundaicus* and salt water form of *subpictus* were critically examined by the author during 1940 to determine how far the characters described by Ghose could be depended upon for the differentiation of the two species.

An analysis of the observations made has shown that in 94 per cent of *sundaicus* larvæ, the tri-radiate character of the mesothoracic hair No. 5 together with the head band offers infallible differentiation. This hair, in at least 95 per cent of *subpictus* larvæ, does not exceed bi-radiate condition (Table I). No other

TABLE I.

Differentiating characters of A. sundaicus *and* A. subpictus *larvæ.*

		Sub-basal hair No. 13 (branches).				Orbital hair No. 14 (branches).		
		3 branches.	4 branches.	5 branches.	6 branches.	2 branches.	3 branches.	4 branches.
A. subpictus (brackish water)	Number	12	106	8	...	1	113	12
	Percentage	9·5	84·1	6·3	...	0·8	89·7	9·5
A. sundaicus ...	Number	13	100	15	2	3	108	19
	Percentage	10·0	76·9	11·5	1·5	2·3	83·0	14·6

		Infra-orbital hair No. 15 (branches).			Head band.		Mesothoracic dorsal hair No. 5 (branches).			
		2 branches.	3 branches.	4 branches.	Fully developed.	Partially developed or without band.	2 branches; same number on both sides or in combination.	2 in one side and 3 in another.	3 branches on either side.	4 or more branches on one or both sides.
A. subpictus (brackish water)	Number	92	34	...	95	31	120	6	...	—
	Percentage	73·0	26·9	...	75·4	24·6	95·2	4·7	...	—
A. sundaicus ...	Number	77	51	2	130	...	...	8	104	18
	Percentage	59·2	39·2	1·5	100·0	...	...	6·1	80·0	13·8

TABLE I—*concld.*

		Mesothoracic pleural hair (branches).			Post-spiracular hair (branches).							
		2 branches.	3 branches.	4 branches.	5 branches.	6 branches.	7 branches.	8 branches.	9 branches.	10 branches.	11 branches.	12 branches.
A. subpictus (brackish water)	Number	109	17	...	8	20	58	28	10	2	...	...
	Percentage	86·5	13·5	...	6·3	15·9	46·0	22·2	7·9	1·6	...	...
A. sundaicus ...	Number	110	20	...	...	9	32	52	28	6	2	1
	Percentage	84·6	15·4	...	...	6·9	24·6	40·0	21·5	4·6	1·5	0·7

		Setæ on 10th abdominal segment.				Pecten.			
		Fine, not pigmented.	Coarse, not pigmented.	Coarse and pigmented.	Fine and pigmented.	1st small tooth ½ or more of tooth I.	1st small tooth less than ½ of tooth I.	Succeeding teeth decrease gradually.	Succeeding teeth of same length.
A. subpictus (brackish water)	Number	117	9	...	...	118	...	...	...
	Percentage	92·8	7·1	...	...	...	...	...	...
A. sundaicus ...	Number	...	16	96	14	118	...	...	...
	Percentage	...	12·7	76·2	11·1	...	...	...	...

characters gave such a high percentage of correct identification. Moreover, in *sundaicus*, the branches of the hair arise near the base while in *subpictus* the branching is more distal.

In the remaining small percentage of disputed larvæ, the characteristic blotched head band and the pigmented setæ on the 10th abdominal segment can safely be utilized to differentiate *sundaicus* larvæ from those of the other species which usually have the setæ unpigmented and the head without a band.

The mesothoracic pleural hairs, post-spiracular hair and pecten, the characters which according to Venhuis are very helpful in differentiating *A. sundaicus* larvæ

in Dutch East Indies have even great variations among the larvæ from Lower Bengal, and are of no importance as diagnostic characters for differentiating these two species.

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CONTROL OF RURAL MALARIA WITH D.D.T. INDOOR RESIDUAL SPRAYING IN KANARA AND DHARWAR DISTRICTS, BOMBAY STATE*: THIRD YEAR'S RESULTS, 1948-49.

BY

D. K. VISWANATHAN,

AND

T. RAMACHANDRA RAO.

(*Malaria Organization, Bombay State.*)

[August 13, 1949.]

THE results of an extensive rural malaria control scheme for the first two years since its inauguration in 1946 in Dharwar and Kanara districts were described in two previous papers (Viswanathan and Ramachandra Rao, 1947, 1948). The results of the third year's work are briefly presented in this paper. The epidemiology of malaria, the nature of the control organization, the technique and formulation and dosage of D.D.T. have already been fully described and the following represent new features during the third year—

(1) *Inclusion of more villages.*—As in 1947-48 many new villages were added to the list in 1948-49, if on representation from the villagers it was found that the villages satisfied the established criteria regarding spleen rate (more than 10 per cent) and population (more than 200 in the Dharwar District and more than 100 in the Kanara District).

(2) *Extension of the scheme to all malarious villages of Shirhatti Taluka, a part of Sangli State, which has been merged with Dharwar District.*—Shirhatti Taluka has practically the same epidemiology and climatic conditions as the eastern parts of Dharwar District. The total number of villages included in the spraying programme in Shirhatti Taluka is 58 with a total population of 38,000. An extra staff of one Malaria Supervisor, 2 Havildars, one Insect Collector and 10 Sepoys,

* With the advent of the new constitution of the Republic of the Indian Union, all units including those previously called 'Provinces' are now called 'States'.

together with a truck, was sanctioned for the purpose. With the inclusion of more villages and Shirhatti Taluka in the Dharwar District, the grand totals of the number of villages included in the scheme and their population at the close of the third year were :—

	Number of villages.	Population (1941).
Dharwar District (including Shirhatti) ...	486	561,881
Kanara District	579	308,724
Belgaum and Khanapur Taluka ...	66	42,693
	1,131	913,298

(3) *A slight modification in the formula of the D.D.T.-aromex-soap emulsion, by preparing a more concentrated solution of D.D.T. than before.*—The new formula is :—

D.D.T.	... 100 lbs.
Aromex	... 25 gallons.
Soap $7\frac{1}{2}$ lbs., water $3\frac{3}{4}$ gallons	... Dissolved first.
Mother emulsion.	

This mother emulsion is diluted with 5 times its own volume of water to give a final concentration of D.D.T. of approximately 5 per cent.

The total quantities of D.D.T. (technical) used by the several units during the third year (July 1948 to June 1949) were as follows :—

Unit No. 1	... 15,795 lbs.
Unit No. 2	... 24,625 ,,
Unit No. 3	... 19,440 ,,
Unit No. 4	... 16,247 ,,
Unit No. 5	... 13,666 ,,
Dharwar Municipality ...	... 4,358 ,,
Total	... 94,131 lbs.

RESULTS.

DENSITIES OF ANOPHELES ADULTS.

Data regarding the Anopheles collections are presented in Tables I and II. The incidence of *fluviatilis* adults in unsprayed villages of Kanara District was much higher in the monsoon and the post-monsoon months of 1948 than in the previous years. Still only three specimens of this species were collected in sprayed dwellings during the entire year. A noteworthy feature was that though adult *fluviatilis* were still collected in larger numbers in the unsprayed houses than in the unsprayed cattlesheds, their proportions occurring in cattlesheds during 1948-49 were much higher than previously.

Culicifacies adults were found to prevail at practically negligible levels in the sprayed houses and cattlesheds of Dharwar District, while in the unsprayed villages, their densities were well above the critical limit throughout the transmission season.

Judged by the reduction of the numbers of the vector species in sprayed dwellings, the D.D.T. indoor residual spraying continued to be as effective in the third year of work as in the previous two years.

SPLEEN AND PARASITE RATES.

Kanara and Dharwar districts.—The spleen and parasite rates in the sprayed and unsprayed villages of Kanara District are presented in Tables III and IV and of Dharwar District in Tables V and VI. The cumulative rates in the two districts, every year from the commencement of the spraying operations, are given below :—

	Sprayed villages.		Unsprayed villages.	
	Cumulative spleen rate, per cent.	Cumulative parasite rate, per cent.	Cumulative spleen rate, per cent.	Cumulative parasite rate, per cent.
Kanara District.				
1946-47	14·4	3·8	72·2	14·6
1947-48	11·6	2·7	47·1	19·5
1948-49	7·1	2·1	52·9	21·6
Dharwar District.				
1945-46	...	...	39·0	...
1946-47	19·7	4·3	28·3	7·5
1947-48	10·6	0·9	25·1	5·2
1948-49	7·7	1·1	18·0	3·2

The above figures very clearly reveal that further reduction has taken place during the third year. The rates in a few selected towns of Kanara District from 1942 to date are given below :—

Town.	1942.	1943.	1944.	1945.	1946.	1947.	1948.
Spleen rates.							
Sirsi	50	20	20	12	8	4	1
Yellapur ...	70	45	58	24	19	9	6
Mundgod ...	75	58	45	35	25	11	11
Haliyal ...	...	67	56	34	7	9	4
Parasite rates.							
Sirsi	18	6	8	5	5	1	0
Yellapur ...	22	20	18	15	5	0	0
Mundgod ...	18	22	22	23	0	0	0
Haliyal ...	...	20	29	7	3	0	0

Belgaum District.—The spleen and parasite rates in selected villages of Khanapur Taluka are given in Table VII. It may be noted here that the spraying operations have been carried out in this taluka for two seasons only. The spleen and parasite rates have shown a further reduction as a result of the second season's work. The cumulative spleen rates in sprayed and unsprayed villages at the end of the 1948-49 season were 15·4 and 37·6 per cent respectively and the cumulative parasite rates 2·8 and 18·6.

Shirhatti Taluka.—The spleen, parasite and infant rates in selected villages of Shirhatti Taluka after one season of spraying are given in Table VIII. It has to be noted that the spraying operations commenced in September instead of in July and only two rounds were completed instead of the scheduled three rounds.

INFANT PARASITE RATES.

In Tables IX and X are presented the data in respect of the infant parasite surveys carried out in the two districts. Once again the rates in the sprayed villages have been practically at the zero level indicating the effective stoppage of malaria transmission, while in the unsprayed villages the rates were still 2·6 and 27·3 per cent in Dharwar and Kanara districts, respectively.

DISPENSARY STATISTICS.

The total number of malaria cases treated in 12 public dispensaries in Kanara District and 8 in Dharwar District during 1948 are given in Table XI. The grand total of cases in 1948 was 31,213 as against a total of 44,983 in 1947. The overall reduction since the commencement of the scheme has been by nearly two-thirds. Estimating that these dispensaries serve only about one-sixth of the entire population, the total reduction in the number of cases in the two districts may be estimated as 360,000 in 1948. It has once again to be emphasized that these data are based on clinical diagnosis carried out in out-patient departments and are subject to considerable limitation. One would expect an even more marked reduction in dispensary cases in view of the rapid decline in all other measures of malariometry. But the medical man in the tropics will take long to forget malaria.

VITAL STATISTICS.

A detailed statistical study of the vital statistics of the two districts before and after the inauguration of the scheme, together with the data for the whole state, has been made and its results separately described (Viswanathan, 1949). The salient points arising out of that study are :—

(1) Morbidity has been greatly reduced.

(2) Mean death rates have declined in Kanara from 26·2 per mille to 16·3 per mille and in Dharwar from 27·4 per mille to 19·6 per mille. The malaria death rates have declined from 2·1 per mille to 0·8 per mille in Kanara District and from 3·6 per mille to 0·8 per mille in Dharwar District.

(3) Birth rates have increased from 31·1 to 34·7 in Kanara and from 36·2 to 38·4 in Dharwar.

(4) As a result of these, it is confidently expected that the next census in 1951 would show a considerable increase in population in Kanara District instead of a stationary population during the sixty years from 1881–1941. This increase will principally occur in the hyperendemic ghat talukas of Kanara where during the past there has been a steady decimation of population.

DISCUSSION.

The scheme has completed three years of activity at the end of June 1949, with increasingly good results accruing year after year. The initial successes, spectacular as they were, required confirmation. That has now been obtained and it can now be taken as definitely proved that indoor residual spray with D.D.T. in a dosage of 56 mg. per sq. foot, three times during the malaria season (with a few modifications to suit local conditions), will effectively prevent malaria transmission in Dharwar and Kanara districts. Extremely low spleen and parasite rates have been reached. Morbidity has reached further low levels. House to house and village to village inquiries have almost always yielded one answer that there has been no case of malaria in the family during the previous year. Birth and death rates already show significant shifts. Population has, at last, shown an upward trend in Kanara District. Now that the weapon has proved adequate and the enemy has been laid low, the question is what next ? How can the results already achieved be consolidated and extended ?

It has to be emphasized that the enemy has only been forced to lie low and is not completely eliminated. Small unsprayed villages within the area still continue to show all evidences of high endemicity. This is as expected, for malaria is a locally transmitted disease and however effective may be the measures adopted in one village, they will not have any effect on another situated beyond the flight range of the mosquito. The first reform that is to be contemplated is to extend the scheme to all the malarious villages, particularly in Kanara District, irrespective of size. In the whole of Kanara District, it is estimated that the extra number of houses to be sprayed for that purpose would not exceed 10 per cent of the number now being sprayed. But they are extremely scattered and located in most inaccessible and difficult places. The cost *per capita* will undoubtedly be higher but not unreasonably so and the time would now seem ripe to extend the benefits of the scheme to all malarious villages.

Before determining upon such an extension to smaller sized villages in Kanara District, one relevant point for consideration on the part of Government will be whether the benefit of malaria control should not be extended to other malarious districts in the state. While other malarious districts should naturally take precedence, inasmuch as a considerable degree of governmental effort has already been made in Kanara, one has to consider that in the latter district malaria constitutes by far the most important public health problem, that vast tracts of the district, naturally fertile, remain uncultivated on account of the sparsity of population rendered worse by being stricken with malaria and neither is the movement of villagers from untreated smaller sized village sites to treated areas possible nor is colonization of the treated areas of the district with displaced persons from elsewhere in the country being attended with any great promise. Hence while

further extension is vitally necessary to bring more lands under plough in the district, such a measure will also benefit the health of the people of the smaller villages. Furthermore, psychologically speaking, islands of lack of attention on the part of Government are likely to provoke a more vigorous criticism than complete lack of attention. Yet again, although, malaria, as stated earlier, is an extremely local disease and untreated islands would continue to have malaria notwithstanding its total absence in the vast surrounding treated villages, *fluviatilis* with high anthropophilic indices and a natural high infection rate is almost entirely confined to the Malnad Tract in Dharwar and Kanara districts. Hence although geographically speaking Kanara, not being an island, is not particularly suitable for any scheme of species eradication, the nearest approach to an attempt in this direction can best be made in Kanara District inasmuch as zoologically speaking it is surrounded by tracts in which there is extreme scarcity, if not total absence, of the same vector species with the same biological behaviour except for narrow tracts in Mysore in the south and Goa in the north. If therefore a complete and comprehensive malaria control programme is established throughout the district including all small sized villages, it is conceivable that after a few years of such work the number of *fluviatilis* may be brought to a vanishing point. The factor of importation of this species will be limited to the narrow tracts from the north and the south respectively. The findings so far recorded do not give any warrant for such a probability but one can only determine its possibility with more certainty if the entire district is included within the scope of D.D.T. operations.

Finally, if such an extension of D.D.T. scheme to all the villages in Kanara District can be brought about without material additional expenditure by way of labour costs and supervisory staff, and the cost of additional quantities of insecticidal material and necessary solvents and accessories is alone to be incurred, such an extension becomes an extremely worth while proposition. Proposals with the above aim and organization in view are now under the active consideration of Government.

The next point for consideration relates to the need for making the scheme permanent. The sanction which has been accorded to this scheme would expire at the end of March 1951. Well, before the expiry of that sanction, the proposed re-organization making use of other public health staff in the district and unifying malaria control and other public health measures under the same staff would have yielded sufficient experience to determine whether the revised organization should not straightway be made permanent from the first of April 1951. As far as the upper supervisory staff is concerned, they would be needed in any case on a permanent basis and there should be no bar to make them permanent straightway. Permanency of tenure would be the surest means of ensuring continuity of service of trained personnel and as D.D.T. trained staff are highly specialized it is even more imperative that such staff should be made permanent as early as possible.

The results of the scheme set forth earlier amply illustrate how extremely useful it has been in improving the health and economics of the people. Computed purely in terms of sordid cash the results of operation have paid dividends to the State equal to several times the annual expenditure. At a very

conservative estimate of rupees 2 per day as the wage for the working adult and six days as the average number of days lost by an individual due to an attack of malaria and assuming that one-third of the cases prevented related to the adult population, there is actually a saving of Rs. 15 lakhs as against the actual expenditure of only Rs. 5 lakhs. But the indirect benefits on account of freedom from malaria are far larger than even the direct ones. With the recent lowering of the prices of D.D.T. it is expected that the *per capita* cost would be only six annas per annum. Even so it will be for consideration whether the State can bear the entire burden from its own general revenues or whether now that the benefits of the scheme have been amply demonstrated the State may embark upon a levy of taxation or a service fee from every householder, adequate to meet the entire cost of the scheme. Perhaps such a proposal for the levy of service fee would receive a lesser degree of opposition if it is promised that the amount thus recovered would be utilized for further public health amenities in the district. When in village after village the question was put what the villagers would do if the Government is unable to find further funds for the scheme in this district in view of their similar obligations to other districts, the answer had invariably been that, if obliged, they would have to pay for the service. But on no account would they countenance any termination of the scheme. This is a matter of some considerable importance. Enlightened opinion at the Fourth International Congresses of Tropical Medicine and Malaria, held in Washington in May 1948, seemed to canvass the D.D.T. spraying programmes on a self-help plan in the tropics in view of the magnitude of the area and cost involved. The authors are of the opinion that it would seem more desirable that the operations should be entirely carried out as a State enterprise and that the financing of the scheme may be made dependent upon the levy of a small service fee two or three years after the benefits of the scheme have been demonstrated by the State from its own general revenues in the first instance. The authors do not believe that for the conditions prevailing in this state or in any other state in India, such a measure of vital importance to the public health of large sections of population should be made dependent on any self-help plan especially when the vector is a winged insect of considerable range of flight with scant respect for proprietary or geographical frontiers and the laudable efforts of the few may be entirely vitiated on account of the apathy of their neighbours.

Public co-operation of the scheme continued unabated and there were comparatively a few critics during the third year under report. The bulk of the criticism was however related to the need for extension of the scheme to smaller villages. Occasionally one still hears that the formulations used during this year were less efficacious than in the past mainly based on the degree of culex mosquito nuisance. While every such criticism is invariably looked into, it is to be placed on record that the formulations used have been at least as efficacious as in the past years, judging from the degree of prevalence of the vector species and malarial incidence. As considerable reduction in malarial prevalence had already been effected in the first two years, there is naturally not the same degree of capacity for further reduction during the third year. So the results could not obviously be so spectacular by comparison from second to the third year as from the pre-D.D.T. period to the first and second years.

A more relevant criticism that has been raised by technical experts from other countries of the world is whether a single application of a larger dose of D.D.T. would not bring about as good a reduction in the incidence of malaria as against our programme of three applications of divided doses. Our programmes were based upon our preliminary experiments and upon the degree of reappearance of adult anophelines as judged by day-time catches made in the morning. It has been asked whether early morning catches of Anopheles really indicate any hazard of transmission in view of the possibility that such mosquitoes may only have fed late at night and would have received enough contact with the D.D.T.-sprayed surfaces and would die eventually. In combating this view it is to be pointed out that, while there is almost a total disappearance of Anopheles for about 15 days after spraying operations, there is a very rapid recovery and by the sixth week in Dharwar District and by the end of eight weeks in Kanara District reappearance of adults of the vector species was observed as judged by morning collections well above the assumed critical densities. It is argued that critical densities after the D.D.T. spray would need to be far higher than in the pre-D.D.T. period to bring about effective transmission in the community in view of the bulk of them being much shorter lived than before. The authors feel that in the last week or two prior to the next round of spray that had to be resorted to, the rate of increase of adult Anopheles as judged by morning collections is so great that it will not be justified to presume that they are all primiparous. If they were so, one would expect not the steep rise in the densities that were found but rather a series of waves with increasing amplitude, the interval between any two waves being equal to the period required for the aquatic life cycle of the mosquito. The authors are therefore of the opinion that the rate of increase of adult Anopheles even as judged by morning collections is a sufficient evidence to prove the hazard of transmission and they would therefore continue to adopt the previously determined frequency of spraying and the dosage. In order, however, to throw some light on the age composition of the mosquitoes after a round of D.D.T. spray, experiments have been formulated during the current year which may throw further light. In order also to determine if previous conclusions regarding the non-utility of resorting to a single massive dose of D.D.T. are still valid, experiments are now in progress in Ahmedabad District during the current year to test the comparative merits over large areas with different doses of D.D.T. In three talukas, it is proposed to adopt a single dose of 180 mg. In three other talukas, it is proposed to experiment with first dose of 120 mg. and second dose of 60 mg. and in the remaining talukas of Ahmedabad District it is proposed to carry out three applications of about 60 mg. of D.D.T. per sq. foot. In each group it is proposed to have some villages for comparative study.

Yet another question is asked whether with large-scale reduction in the incidence of malaria brought about by repeated applications of D.D.T. in two or three years and with the greatly reduced gametocyte carriers in the community the critical density of the vector species should not be of much higher order than before to bring about effective transmission in the community. This is largely a speculative matter, but inasmuch as in the experience of the great regional epidemics in the Punjab and in Sind, even a very low-grade factor of reservoir of infection in the pre-epidemic week is consistent with the emergence of large epidemics so long as the primary factors necessary for their genesis, viz. great

flooding due to increased rainfall locally or in the catchment area and persistence of high levels of humidity, are found to prevail, it is not safe to assume that a larger critical threshold of vector species would be necessary even when there is a marked reduction in the transmission of malaria. Without any bias however in favour of the authors' present programme of three rounds of application of D.D.T., they have planned a series of experiments on the dynamics of mosquito populations in relation to D.D.T. operations during the current year which, they hope, will throw further light on various aspects.

The collateral benefits of D.D.T. spraying operations have been maintained. Yet another year is past without a single human case of plague proved to have been acquired locally occurring in any of the sprayed villages. Even though there may have been one or two imported cases from the adjoining Mysore State, there has been no further spread of the disease although Dharwar District was previously endemic for plague. It is a fairly common experience along the frontier between Dharwar District and Mysore State to find time and again that the village reporting cases of human plague was an unsprayed village either in the State of Bombay or in Mysore. There was a considerable degree of prevalence of plague in the adjoining territory of Mysore in 1948 and yet there was not a single case of human plague in the adjoining sprayed villages of Dharwar District.

In this district there is a continued reduction in the number of deaths due to diarrhœa and dysentery but no such significant changes were observed in Kanara District. One wonders whether this is because of the inclusion of cattlesheds for spraying purposes in Dharwar District and their exclusion in Kanara. If this is confirmed by further data, it would seem desirable to include cattlesheds also in Kanara District within the scope of D.D.T. operations, as deaths due to diarrhœa and dysentery form a substantial proportion of the total deaths in that district.

SUMMARY.

(1) The results of the third season's working of D.D.T. scheme in Dharwar and Kanara districts are presented.

(2) The previous year's results have been maintained and in some respects improved.

(3) Cumulative spleen rates have further declined to 7·0 per cent in Kanara and to 7·8 per cent in Dharwar from 11·6 per cent and 10·6 per cent respectively in the previous year. In the unsprayed areas they are 52·8 and 18·0 per cent respectively as compared with 47·1 and 25·1 per cent in the previous year.

(4) Infant parasite rates are almost nil in the present year in the sprayed areas as compared with 2·6 per cent in Dharwar and 27·7 per cent in the unsprayed villages. While there has been some natural decline in malaria in Dharwar in unsprayed areas as well, there is no such decline noticed in the unsprayed villages of Kanara.

(5) The dispensary statistics show a further decline in malaria cases to the extent of about 13,700 cases. The total reduction in the third year in the areas served by dispensaries is about 60,000 or about 360,000 in the entire scheme areas.

(6) Increase in birth rates and decrease in death rates and malaria death rates have been demonstrated after a statistical analysis of the relevant data. The

downward trend in population is definitely arrested and the next census will show an increase in population in the hyperendemic malarious tracts.

(7) There has been a considerable increase in man-power for agricultural pursuits and there are indications of several acres of land having been brought under plough for the first time.

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TABLE I.

Density of Anopheles adults—Kanara District.

Month.	SPRAYED VILLAGES.						UNSPRAYED VILLAGES.					
	HUMAN AND MIXED DWELLINGS.			CATTLESHEDS.			HUMAN AND MIXED DWELLINGS.			CATTLESHEDS.		
	Time spent, hours.	Per 10 man-hours.		Time spent, hours.	Per 10 man-hours.		Time spent, hours.	Per 10 man-hours.		Time spent, hours.	Per 10 man-hours.	
		Fluviatilis.	All Anopheles.		*Fluviatilis.*	All Anopheles.		*Fluviatilis.*	All Anopheles.		*Fluviatilis.*	All Anopheles.
						1948.						
Jul. ...	124	0·0	6·94	49	0·0	182·7	99	2·92	57·69	35	0·57	216·57
Aug. ...	97	0·0	3·94	56	0·54	189·2	61	3·44	116·8	31	5·57	262·62
Sep. ...	137	0·22	7·71	84	8·02	218·44	87	17·58	172·64	45	14·22	329·33
Oct. ...	89	0·0	0·0	50	3·03	374·5	57	13·1	127·4	26	9·02	376·9
Nov. ...	161	0·0	0·9	109	0·2	13·97	131	4·9	68·04	87	3·2	16·6
Dec. ...	126	0·0	2·22	13	0·48	169·76	127	4·56	9·89	82	2·19	24·92
						1949.						
Jan. ...	225	0·0	2·76	151	0·66	122·52	197	3·09	54·27	128	1·40	156·48
Feb. ...	265	0·0	0·37	144	0·13	98·17	180	0·89	36·15	108	3·24	155·6
Mar. ...	316	0·0	0·85	155	0·96	89·25	210	1·05	25·09	121	2·31	116·19
Apr. ...	357	0·0	0·36	177	0·28	65·19	239	1·33	23·23	135	0·74	84·88
May ...	326	0·0	0·0	171	0·05	21·91	188	0·64	22·88	106	0·94	96·20
Jun. ...	192	0·0	0·0	117	0·08	93·16	159	1·32	29·15	90	1·56	135·64

Table II.

Density of Anopheles adults—Dharwar District.

Month.	SPRAYED VILLAGES.						UNSPRAYED VILLAGES.					
	Human dwellings.			Cattlesheds.			Human dwellings.			Cattlesheds.		
	Time spent, hours.	Per 10 man-hours.		Time spent, hours.	Per 10 man-hours.		Time spent, hours.	Per 10 man-hours.		Time spent, hours.	Per 10 man-hours.	
		A. culicifacies.	All Anopheles.		*A. culicifacies.*	All Anopheles.		*A. culicifacies.*	All Anopheles.		*A. culicifacies.*	All Anopheles.
						1948.						
Jul. ...	257	0·62	6·69	84	2·85	39·17	258	37·26	66·81	79	49·38	145·4
Aug. ...	216	0·56	3·47	68	4·10	38·68	186	48·34	139·9	55	106·82	324·0
Sep. ...	134	0·21	2·95	81	1·49	16·26	210	31·92	112·25	64	60·16	297·48
Oct. ...	220	0·22	2·4	74	0·95	16·8	207	34·4	109·3	60	57·7	236·4
Nov. ...	208	0·14	1·34	67	4·16	13·23	216	86·42	163·09	62	185·8	364·19
Dec. ...	134	0·0	0·22	43	0·0	1·87	175	6·04	115·42	50	108·08	231·11
						1949.						
Jan. ...	65	0·0	1·41	21	0·0	1·42	60	32·26	57·49	17	38·21	70·45
Feb. ...	18	0·0	6·12	6	0·0	3·83	18	70·85	104·57	5	46·0	16·0
Mar. ...	57	0·0	8·74	18	0·0	1·66	58	45·51	87·24	17	60·0	125·29
Apr. ...	100	0·9	15·05	24	0·0	32·76	83	25·94	100·3	23	34·7	158·6
May ...	151	0·72	1·39	39	0·21	3·84	119	15·57	53·75	31	33·92	113·92
Jun. ...	159	0·0	0·0	53	7·42	52·57	53	6·8	18·6	62	52·5	17·06

TABLE III.

Kanara District spleen and parasite rates in sprayed villages, 1948-49.

Taluka and village.	SPLEEN RATE, PER CENT.		SPLEEN RATE IN 1948-49.			PARASITE RATE IN 1948-49.					
									Parasite species.		
	1946-47.	1947-48.	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number showing malaria parasites.	Parasite rate, per cent.	V.	F.	M.
1	2	3	4	5	6	7	8	9	10	11	12
Bhatkal Petha.											
Muttoli	...	13·5	30	3	10·0	21	0	0	0	0	0
Kuntawani	61·1	33·3	14	4	28·5	12	1	8·3	1	0	0
Kothand	52·9	7·1	20	1	5·0	17	0	0	0	0	0
Marukeri	59·5	29·7	29	2	6·8	19	2	10·5	0	1	1
Bhatkal	4·4	6·1	200	7	3·5	...	...	...	...	...	...
Kitre	52·9	12·5	24	3	12·5	19	0	0	0	0	0
Shirali	...	4·0	36	1	2·7	31	0	0	0	0	0
Mavalli	...	26·1	22	4	18·18	20	1	5·0	0	1	0
Kaikeni	...	87·0	52	7	13·5	39	3	7·7	1	2	0
TOTAL ...	...	...	227	32	14·0	178	7	3·9	2	4	1

V=*vivax*. F=*falciparum*. M=*malariæ*.

TABLE III—*contd.*

Taluka and village.	SPLEEN RATE, PER CENT.		SPLEEN RATE IN 1948-49.			PARASITE RATE IN 1948-49.					
	1946-47.	1947-48.	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number showing malaria parasites.	Parasite rate, per cent.	Parasite species.		
									V.	F.	M.
1	2	3	4	5	6	7	8	9	10	11	12
Sirsi Taluka.											
Sirsi Town	4·6	4·0	422	4	0·9	40	0	0·0	0	0	0
Islur.	...	...	35	3	8·6	25	1	4·0	0	1	0
Amminhalli	...	...	40	2	5·0	20	0	0·0	0	0	0
Targod	...	...	30	2	6·6	20	0	0·0	0	0	0
Hanumanti	...	...	25	1	4·0	20	0	0·0	0	0	0
Banavasi	...	...	100	5	5·0	25	0	0·0	0	0	0
TOTAL ...	...	...	652	17	2·6	150	1	0·6	0	1	0
Siddapur Taluka.											
Bilgi	15·5	6·6	50	4	8·0	44	1	2·3	1	0	0
Harshikatta ...	45·0	8·0	32	2	5·2	30	0	0	0	0	0
Tyagali	24·2	6·6	36	2	5·6	34	0	0	0	0	0
Kansur	13·3	11·5	29	2	6·8	29	1	3·4	0	1	0

Kangod	...	8·7	72	4	5·5	67	2	2·9	1	1	0
Nidgod	...	10·0	20	1	5·0	19	0	0	0	0	0
Manmane	...	7·3	45	2	4·4	42	0	0	0	0	0
Halgeri Husur ...	...	...	45	3	6·7	24	1	4·2	1	0	0
Jog Ferry and Mavingundi	16·0	19·0	20	3	15·0	20	1	5·0	1	0	0
Siddapur	38·0	2·0	183	9	4·9	48	2	4·2	1	1	0
TOTAL ...	...	...	532	32	6·01	357	8	2·2	5	3	0
Yellapur Taluka.											
Yellapur Town ...	18·8	9·3	211	12	5·7	40	0	0	0	0	0
Kirwatti	15·4	15·6	52	8	15·4	25	1	4·0	0	1	0
Nandolli	...	53·3	25	6	24·0	20	1	5·0	1	0	0
TOTAL ...	...	...	288	26	9·02	85	2	2·3	1	1	0
Supa Taluka.											
Supa	...	...	54	7	12·8	...	...	...	...	...	...
Castle Rock	...	...	93	11	11·8	...	...	...	...	...	...
Asu	...	...	41	7	1·7	...	...	...	...	...	...
TOTAL ...	...	...	188	25	13·2	170	4	2·4	...	...	...

V=*vivax*. F=*falciparum*. M=*malariæ*.

TABLE III—*contd.*

Taluka and village.	SPLEEN RATE, PER CENT.		SPLEEN RATE IN 1948-49.			PARASITE RATE IN 1948-49.					
	1946-47.	1947-48.	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number showing malaria parasites.	Parasite rate, per cent.	Parasite species.		
									V.	F.	M.
1	2	3	4	5	6	7	8	9	10	11	12
Mundgod Taluka.											
Mundgod Town ...	17·9	11·0	127	14	11·0	50	0	0	0	0	0
Pala	10·9	6·2	128	5	8·9	30	0	0	0	0	0
Chigalli	21·8	12·0	60	6	10·0	50	0	0	0	0	0
Katur	...	...	35	3	8·6	25	0	0	0	0	0
TOTAL ...	...	...	350	28	8·0	155	0	0	0	0	0
Haliyal Taluka.											
Haliyal	6·8	9·3	300	12	4·0	30	0	0	0	0	0
Sambrani	...	...	32	2	6·3	...	...	...	...	...	...
Havgi	...	3·5	60	2	3·3	35	0	0	0	0	0
Kerwad	...	3·7	30	1	3·3	22	0	0	0	0	0
Guttigeri	...	...	15	1	6·7	8	0	0	0	0	0
Tergaon	5·0	2·5	85	1	1·2	73	0	0	0	0	0

Dashgi	...	15·2	30	2	6·7	23	0	0	0	0	0
Madnalli	...	...	40	2	5·0	27	0	0	0	0	0
Nagshetti	...	17·4	18	3	16·7	18	1	5·5	1	0	0
Mundwad	...	30·0	50	9	18·0	31	1	3·2	1	0	0
Kanchanhalli Budruk ...	...	13·1	72	3	4·2	45	0	0	0	0	0
Kanchanhalli Khurd ...	...	...	24	3	12·5	18	0	0	0	0	0
Joginkop	...	...	28	6	21·6	...	...	...	...	...	...
TOTAL ...	...	...	784	47	5·9	330	2	0·6	2	0	0
Kumta Taluka.											
Kalve	...	52·0	27	11	40·7	25	3	12·0	1	2	0
Santiguli	...	43·0	25	8	32·0	25	2	8·0	0	2	0
Karkimakki ...	...	4·0	50	2	4·0	25	0	0	0	0	0
Harodi	...	12·0	30	1	3·3	25	0	0	0	0	0
Kujalli	...	8·0	35	3	8·6	25	1	4·0	0	1	0
Mirjan	...	10·0	50	2	4·0	25	0	0	0	0	0
Bargi	...	8·0	50	3	6·0	25	0	0	0	0	0
Madangeri	...	2·0	40	2	5·0	25	0	0	0	0	0
Divgi	...	...	50	1	2·0	25	0	0	0	0	0
TOTAL ...	...	...	357	33	9·2	225	6	2·6	1	5	0

V=*vivax*. F=*falciparum*. M=*malariæ*.

TABLE III—*concld.*

Taluka and village.	SPLEEN RATE, PER CENT.		SPLEEN RATE IN 1948-49.			PARASITE RATE IN 1948-49.					
									Parasite species.		
	1946-47.	1947-48.	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number showing malaria parasites.	Parasite rate, per cent.	V.	F.	M.
1	2	3	4	5	6	7	8	9	10	11	12
Karwar Taluka.											
Honkan	44·4	25·0	52	5	9·6	35	3	8·6	...	...	...
Kadra	36·3	25·0	22	2	9·0	11	0	0	...	...	...
Sawantwada ...	78·9	30·7	13	4	5·9	13	0	0	...	...	...
Madhewada ...	67·9	19·3	50	4	8·0	33	2	6·0	...	...	...
Kolge	62·9	30·7	15	2	13·0	11	1	9·0	...	...	...
Gotegalli	...	...	43	4	9·5	25	2	8·0	...	...	...
Bhaire	...	...	38	3	7·8	20	1	5·0	...	...	...
Mallapur	...	...	40	3	7·5	21	3	14·0	...	...	...
TOTAL ...	...	...	273	27	9·8	169	12	7·1	...	...	...
Honnavar Taluka.											
Gundbal	...	12·8	42	3	7·14	41	1	2·4	0	1	0
Honnavar	...	3·2	100	2	2·0	...	...	...	...	...	...

Arollimundgod ...	31·4	7·1	50	3	6·0	47	0	0	0	0	0
Balkur	35·5	5·4	50	2	4·0	48	0	0	0	0	0
Hosad	10·0	8·0	62	4	6·4	62	0	...	...	...	...
Gersappa	75·0	9·5	60	5	8·3	56	0	0	0	0	0
Chandavar	62·8	6·1	50	2	4·0	48	0	0	0	0	0
Kadle	11·1	10·0	30	2	6·6	30	1	3·3	0	1	0
Kekkar'	...	13·5	50	4	8·0	50	1	2·0	0	1	0
Kadtoka	...	17·1	88	8	9·09	70	2	2·9	1	1	0
Navilgone	...	12·7	70	3	4·2	34	2	5·9	1	1	0
Madgeri	...	13·2	38	2	5·2	...	...	...	...	...	...
TOTAL ...	...	...	690	40	5·7	486	7	1:4	2	5	0
GRAND TOTAL ...	...	...	4,341	307	7·1	2,305	49	2·1	13	19	1*

* Identification of species was made in only 33 out of 49 positive smears.

V=*vivax*. F=*falciparum*. M=*malariæ*.

TABLE IV.

Kanara District spleen and parasite rates in unsprayed villages, 1948-49.

Taluka and village.	SPLEEN RATE, PER CENT.		SPLEEN RATE IN 1948-49.			PARASITE RATE IN 1948-49.					
	1946-47.	1947-48.	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number showing malaria parasites.	Parasite rate, per cent.	Parasite species.		
									V.	F.	M.
1	2	3	4	5	6.	7	8	9	10	11	12
Bhatkal.											
Herur	...	...	8	4	...	6	2	...	0	2	0
Bilalkhand	...	41·6	14	5	35·7	6	2	...	0	2	0
Kulvadi	...	...	4	4	...	4	2	...	0	2	0
Hedvath	...	75·0	9	7	...	8	4	...	2	2	0
Vonibogil	...	...	6	4	...	4	1	...	0	1	0
Beshi	...	...	13	10	76·9	12	5	41·7	1	4	0
Kekkod	...	...	8	8	...	8	3	...	0	3	0
Hallari	...	...	4	4	...	4	2	...	0	2	0
Hegli	...	41·9	4	4	...	4	1	25·0	0	1	0
Belki	...	...	29	10	34·4	28	6	21·4	0	6	0
Venktapur	...	6·2	35	3	8·5	28	4	14·2	1	3	0
TOTAL ...	...	...	134	63	47·01	112	32	28·5	4	28	0

Siddapur.											
Chandraghatgi ...	...	...	6	4	...	3	1	...	0	1	0
Kahanhalli ...	...	...	2	1	...	1	1	...	0	1	0
Hangerkhand ...	...	33·3	11	4	36·4	8	3	...	1	2	0
Haltot ...	...	...	2	1	...	2	0	...	0	0	0
Hoskop ...	...	...	7	2	...	7	1	...	0	1	0
Padvanbail ...	...	...	2	1	...	2	1	...	0	1	0
Matigar ...	...	...	6	3	...	6	2	...	1	1	0
Nagarbhavi and Dogdimane	...	...	5	1	...	4	1	...	1	0	0
Hiremaghi ...	...	...	6	1	...	4	1	...	0	1	0
Mankod ...	...	10·0	10	2	20·0	9	2	...	0	2	0
TOTAL ...	...	...	57	20	35·08	46	13	28·3	3	10	0
Karwar.											
Gopshitta ...	...	37·9	22	10	45·4	20	7	3·5	...	...	...
TOTAL ...	...	...	22	10	45·4	20	7	3·5	...	...	...

V=*vivax*. F=*falciparum*. M=*malariæ*.

TABLE IV—*contd.*

Taluka and village.	Spleen rate, per cent.		Spleen rate in 1948-49.			Parasite rate in 1948-49.					
	1946-47.	1947-48.	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number showing malaria parasites.	Parasite rate, per cent.	Parasite species.		
									V.	F.	M.
1	2	3	4	5	6	7	8	9	10	11	12
Honnavar.											
Sampoli	...	...	6	4	...	5	2	...	1	1	0
Kot	...	...	8	6	...	8	3	...	0	3	0
Tumbibail	...	...	4	2	...	4	2	...	1	1	0
Manki	...	54·5	20	6	30·0	20	5	25·0	2	3	0
Kervalli	...	...	30	7	23·3	30	5	16·7	2	3	0
Malkod	...	20·0	20	5	25·0	20	4	20·0	1	3	0
Heggargaddi	...	...	2	2	...	2	1	...	0	1	0
Baleniat	...	...	8	4	...	...	...	...	...	...	...
TOTAL ...	...	...	98	36	36·7	89	22	24·7	7	15	0
Haliyal.											
Kurigadde	...	68·8	16	11	68·8	...	...	...	...	...	...
TOTAL ...	...	...	16	11	68·7	...	...	...	...	...	...

Sirsi.												
Landkanhalli	...	...	9	6	...	9	0	...	0	0	0
Umblekop	...	...	12	9	75·0	12	2	16·6	0	2	0
Uplekop	...	...	10	8	80·0	10	2	20·0	1	1	0
Gondalli	...	...	8	7	...	8	2	...	1	1	0
Narebail	...	...	13	8	61·5	13	2	15·4	0	2	0
Heggar	...	...	10	7	70·0	10	1	10·0	0	1	0
Yashe	...	...	8	6	...	8	1	...	0	1	0
TOTAL ...	...	...	70	51	72·8	70	10	14·2	2	8	0
Yellapur.											
Huttanmane	...	...	8	6	...	8	1	12·5	0	1	0
Tatgar	...	...	6	4	...	6	0	0	0	0	0
Shirmala	...	...	12	10	83·3	12	2	16·6	1	1	0
Kundur	...	...	11	9	81·8	11	2	18·2	0	2	0
TOTAL ...	...	...	37	29	78·3	37	5	13·5	1	4	0

V=*vivax*. F=*falciparum*. M=*malariæ*.

TABLE IV—*concld.*

Taluka and village.	Spleen rate, per cent.		Spleen rate in 1948-49.			Parasite rate in 1948-49.					
									Parasite species.		
	1946-47.	1947-48.	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number showing malaria parasites.	Parasite rate, per cent.	V.	F.	M.
1	2	3	4	5	6	7	8	9	10	11	12
Mundgod.											
Nyasargi	...	...	12	7	58·3	12	1	8·3	0	1	0
Kagankrip	...	...	10	8	80·0	10	1	10·0	1	0	0
Kyasankeri	...	...	8	6	...	8	2	25·0	0	2	0
Malwalli	...	...	15	10	66·6	15	1	6·7	0	1	0
Aggihalli	...	...	6	5	...	6	0	0	0	0	0
TOTAL ...	...	...	51	36	70·5	51	5	9·8	1	4	0
Kumta.											
Santur	...	...	8	4	...	8	0	0	0	0	0
Soppinhosalli	...	...	6	4	...	6	1	16·6	0	1	0
TOTAL ...	...	...	14	8	57·1	14	1	7·1	0	1	0
GRAND TOTAL ...	...	...	499	264	52·9	439	95	21·6	18	70	0*

* Identification of species was made in only 87 out of 95 positive smears.

V=*vivax*. F=*falciparum*. M=*malariæ*.

TABLE V.

Dharwar District spleen and parasite rates in sprayed villages, 1948-49.

Taluka and village.	Spleen rate, per cent.		Spleen rate in 1948-49.			Parasite rate in 1948-49.					
									Parasite species.		
	1946-47.	1947-48.	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number showing malaria parasites.	Parasite rate, per cent.	V.	F.	M.
1	2	3	4	5	6	7	8	9	10	11	12
Dharwar.											
Garag	...	2·4	94	2	2·1	...	0	0	0	0	0
Navalur	27·4	9·8	108	7	6·5	32	0	0	0	0	0
Rayapur	...	7·4	72	4	5·6	12	0	0	0	0	0
Sattur	...	7·1	46	2	4·3	10	0	0	0	0	0
Kalgeri	37·6	20·6	87	12	13·8	36	0	0	0	0	0
Mummigatti ...	14·3	4·9	56	3	5·4	12	0	0	0	0	0
Narendra	14·5	7·6	102	12	11·8	...	...	0	1	2	0
Tegur	44·8	21·3	170	40	23·5	60	3	5·0	...	...	...
Talshinkop	...	9·4	35	2	5·7	12	0	0	...	...	...
Alnavar	30·8	8·3	107	6	5·6	38	0	0	...	...	...
Tadakop	...	...	104	10	9·6	24	0	0	...	...	...
Somapur	...	...	42	4	9·5	...	...	...	...	...	...
Mugad	32·9	9·4	135	12	8·9	...	...	...	...	...	...
Mandihal	...	10·4	53	5	9·4	...	...	...	0	0	0
Nigadi and Bunkankatti	...	...	137	9	6·6	37	0	0	...	...	...
Devarhulli	...	...	92	6	6·5	...	...	0	...	...	...
Arvatgi-Amboli * ...	...	81·8	10	6	6·0	10	1	10·0	1	0	0
Dharwar Town ...	...	...	155	2	1·7	32	0	0	0	0	0
(Line Bazaar only).											
Kadabagatti ...	...	...	46	4	8·7	...	...	0	...	...	...
Kogilgeri	...	...	48	9	18·7	15	0	0	...	...	...
Kumbarkop ...	...	...	52	10	19·2	20	1	5·0	1	0	0
TOTAL ...	...	...	1,751	167	9·5	350	5	1·4	3	2	0

* Sprayed for the first time in 1948-49.

V=*vivax*. F=*falciparum*. M=*malariæ*.

TABLE V—*contd.*

Taluka and village.	SPLEEN RATE, PER CENT.		SPLEEN RATE IN 1948-49.			PARASITE RATE IN 1948-49.					
	1946-47.	1947-48.	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number showing malaria parasites.	Parasite rate, per cent.	Parasite species.		
									V.	F.	M.
1	2	3	4	5		7	8	9	10	11	12
Hubli.											
Bhaidwarkop ...	...	17·5	47	6	12·7	21	0	0	0	0	0
Unkal	26·9	5·7	118	6	5·1	26	0	0	0	0	0
Keshwapur	...	2·9	36	0	0	...	...	...	...	...	...
Adarganchi	4·2	4·2	38	0	0	...	...	...	...	...	...
Nulvi	...	2·4	28	0	0	...	...	...	...	...	...
Sherwad	...	...	32	1	3·1	12	0	0	0	0	0
Rayandoll	...	3·5	31	1	3·2	...	...	...	...	...	...
Revdihal	...	4·7	28	1	3·6	...	...	...	...	...	...
Gokul	12·8	3·2	29	1	3·4	...	...	...	...	...	...
Kodgunchi	...	2·7	35	2	6·7	18	0	0	0	0	0
Amargol	20·2	10·0	102	9	8·8	...	...	...	...	...	...
TOTAL ...	...	...	524	27	5·1	77	0	0	0	0	0

Kalghatgi.												
Gangigatti	...	...	84	11	13·1	...	...	...	...	...	...
Kalghatgi	8·7	6·9	127	3	2·4	...	...	...	...	...	...
Devikop	...	4·6	78	8	10·2	...	...	...	...	...	...
Bammingatti ...	...	7·5	32	2	6·3	...	...	...		...	...
Dasanur	...	8·5	53	3	5·7	15	0	0	0	0	0
Dummad	...	5·2	44	1	2·3	10	0	0	0	0	0
Tamehal	...	...	36	2	5·5	15	0	...	...	...	...
Hirehongal	...	7·9	48	3	6·2	12	0	...	...	...	...
Dastikop	13·5	4·5	58	5	8·6	...	0	0	0	0	0
Hulginkatti ...	...	...	48	3	6·2	12	0	0	0	0	0
Begur	16·3	7·6	42	2	4·0	...	...	...	...	...	...
Rangapur	...	...	38	2	5·3	...	0	0	0	0	0
TOTAL ...	...	...	688	45	6·5	64	0	0	0	0	0
Mundargi.												
Dambal	14·0	19·8	84	8	9·5	20	0	0	0	0	0
Kadampur	...	58·5	80	30	37·5	42	1	2·3	0	1	0
Meundi	...	12·5	47	4	9·1	...	...	...	...	...	...
Randur	...	13·0	51	4	7·9	...	...	...	...	...	...
Mundargi	17·9	9·7	114	7	6·2	32	0	0	0	0	0
Kalkeri	...	...	64	17	26·5	30	2	6·7	1	1	0
TOTAL ...	...	...	440	70	15·9	124	3	2·4	1	2	0

V=*vivax*. F=*falciparum*. M=*malariæ*.

TABLE V—*contd.*

Taluka and village.	SPLEEN RATE, PER CENT.		SPLEEN RATE IN 1948-49.			PARASITE RATE IN 1948-49.					
	1946-47.	1947-48.	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number showing malaria parasites.	Parasite rate, per cent.	Parasite species.		
									V.	F.	M.
1	2	3	4	5	6	7	8	9	10	11	12
Hangal.	...	7·1	105	2	1·9	...	...	...	...	...	...
TOTAL ...	...	...	105	2	1·9	...	...	...	...	...	...
Bankapur.											
Shiggaon	20·8	12·1	96	4	4·2	24	0	0	0	0	0
Bankapur	...	4·0	102	4	3·9	...	...	...	...	...	...
Tadas	...	13·3	56	3	5·4	...	...	...	...	...	...
Adursomapur ...	...	21·2	58	4	6·9	12	0	0	0	0	0
Kunnur	...	...	71	2	2·8	10	0	0	0	0	0
Dhundshi	...	10·2	48	3	6·3	10	0	0	0	0	0
TOTAL ...	...	...	431	20	4·6	56	0	0	0	0	0
Gadag Taluka.											
Asundi	...	11·0	68	4	5·9	36	0	0	0	0	0
Malsamudra ...	...	15·7	110	9	8·2	...	...	...	...	...	...

Narsapur	...	...	42	6	14·3	...	...	...	...	...	...	
Chickop	...	25·0	35	4	11·4	...	...	...	...	...	...	
Hirekop	...	21·8	44	5	11·3	...	...	...	...	...	...	
Nagsamudra ...	...	26·7	57	6	10·5	12	0	0	0	0	0	
Papanashi	...	13·7	42	4	9·5	...	...	...	...	...	...	
Advisomapur ...	...	13·8	86	5	5·9	11	0	0	0	0	0	
TOTAL ...	...	...	484	43	8·8	59	0	0	0	0	0	
Kod Taluka.												
Rattihalli	6·0	3·3	54	1	1·8	...	...	...	...	...	...	
Masur	30·0	19·5	67	7	10·9	14	0	0	0	0	0	
Hirekerur	20·8	8·6	41	3	7·3	16	0	0	0	0	0	
Chikerur	16·0	7·5	38	2	5·31	...	...	...	...	...	...	
	...	...	42	2	4·4	...	...	...	...	...	...	
	...	...	46	2	4·2	...	...	...	...	...	...	
TOTAL ...	...	...	288	17	5·9	30	0	0	0	0	0	
Haveri Taluka.												
Devinkoper	...	...	117	4	3·4	...	...	...	...	...	...	
Sangur	...	...	66	8	12·3	...	...	...	...	...	...	
Haveri	8·5	3·5	52	1	1·9	6	0	0	0	0	0	
Kankapur	...	...	39	3	7·7	22	1	4·5	0	1	0	
TOTAL ...	...	...	274	16	5·8	28	1	3·5	0	1	0	

V=*vivax*. F=*falciparum*. M=*malariæ*.

TABLE V—*concld.*

Taluka and village.	SPLEEN RATE, PER CENT.		SPLEEN RATE IN 1948-49.			PARASITE RATE IN 1948-49.					
	1946-47.	1947-48.	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number showing malaria parasites.	Parasite rate, per cent.	Parasite species.		
									V.	F.	M.
1	2	3	4	5	6	7	8	9	10	11	12
Ranebennur Taluka.											
Chatra	...	...	42	1	2·4	...	...	...	...	...	...
Kakol	...	...	38	1	2·63	...	...	...	...	...	...
Ranebennur ...	...	...	112	2	1·8	20	0	0	0	0	0
Chatgeri	...	...	36	1	2·8	...	...	...	...	...	...
Karur	...	...	38	0	0	...	...	...	...	...	...
Itgi	...	...	31	1	3·2	...	...	...	...	...	...
Halgeri	...	...	42	1	2·4	...	...	...	...	...	..
Motebennur ...	...	...	86	3	3·6	25	0	0	0	0	0
TOTAL ...	...	...	425	10	2·3	45	0	0	0	0	0
GRAND TOTAL ...	...	...	5,410	418	7·7	833	9	1·08	4	5	0

V=*vivax.* F=*falciparum.* M=*malariæ.*

TABLE VI.

Dharwar District spleen and parasite rates in unsprayed villages, 1948-49.

Taluka and village.	Spleen rate, per cent.		Spleen rate in 1948-49.			Parasite rate in 1948-49.					
									Parasite species.		
	1946-47.	1947-48.	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number showing malaria parasites.	Parasite rate, per cent.	V.	F.	M.
1	2	3	4	5	6	7	8	9	10	11	12
Dharwar.											
Guladkop	...	52·4	58	30	51·7	20	0	0	0	0	0
Shinganhalli ...	...	52·2	32	17	53·1	...	...	...	...	...	...
Jogyellapur	...	21·9	35	7	20·0	20	0	0	0	0	0
Khanapur	...	16·6	48	9	18·7	...	...	...	...	...	...
Maradgi	...	15·7	46	7	15·2	...	...	...	...	...	...
Inamali Hebli ...	...	4·0	96	4	4·2	18	0	0	0	0	0
Kamalapur	...	...	50	12	24·0	15	2	13·3	1	1	0
Nuggikeri	...	...	32	8	25·0	...	...	...		...	...
Managalgali ...	...	...	69	14	20·3	...	...	...	...	...	...
Erikop	...	...	48	10	20·8	...	...	...	...	...	...
Mavinkop	...	...	25	11	44·0	...	...	...	...	...	...
Mangachale ...	...	...	69	14	20·5	...	...	...	...	...	...
TOTAL ...	...	...	608	143	23·5	73	2	2·7	1	1	0

V=*vivax*. F=*falciparum*. M=*malariæ*.

TABLE VI—*contd.*

Taluka and village	Spleen rate, per cent.		Spleen rate in 1948-49.			Parasite rate in 1948-49.					
									Parasite species.		
	1946-47.	1947-48.	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number showing malaria parasites.	Parasite rate, per cent.	V.	F.	M.
1	2	3	4	5	6	7	8	9	10	11	12
Hubli Taluka.											
Bungeri	...	0·0	21	1	3·5	...	...	...	...	...	...
Anchalgeri	...	75·6	44	32	72·7	15	2	6·6	2	0	0
Birmal	...	2·7	29	1	3·4	...	...	...	...	...	...
Bundursinghi ...	...	23·5	28	7	25·0	14	0	0	0	0	0
Nannur	...	17·5	18	3	16·7	10	0	0	0	0	0
Gorgyol	...	78·5	25	18	72·0	7	0	0	0	0	0
Nagashittikop ...	...	2·5	32	1	3·1	14	0	0	0	0	0
TOTAL ...	...	...	197	63	31·9	60	2	3·3	2	0	0
Kalghatgi.											
Tambur	77·7	77·7	28	15	53·6	12	1	8·3	0	1	0
Bisanhalli	47·6	47·6	23	9	39·1	12	1	8·3	1	0	0
Ganjgatu	...	...	84	11	13·1	...	...	...	...	...	...
TOTAL ...	...	...	135	35	25·9	24	2	8·3	1	1	0
Gadag.											
Betgeri	...	...	320	19	5·9	68	1	1·4	0	1	0
Hulkoti	...	24·6	68	16	23·4	14	0	0	0	0	0
Hirechandinhal ...	...	25·0	46	11	23·9	12	0	0	0	0	0
Binkankatti ...	...	20·4	48	9	18·7	11	1	9·9	1	0	0
TOTAL ...	...	...	482	55	11·4	105	2	1·9	1	1	0

Kod Taluka.											
Dodgubbi	2·1	38	3	7·9	...	...	...	...	...	...	
Sangubbi	21·5	42	8	19·0	...	...	...	...	...	...	
Hiremorab	10·0	22	2	9·1	...	...	...	...	...	...	
Khandibagal	16·1	28	4	14·3	...	...	...	...	...	...	
Yettinhalli	80·2	82	35	42·6	25	1	4	1	0	0	
Yellapur	12·9	26	2	7·7	...	...	...	...	...	...	
Hirbadihal	...	50	11	22·0	...	...	...	...	...	...	
Battikop	...	55	7	12·7	...	...	...	...	...	...	
Balambid	...	42	6	14·2	...	...	...	...	...	...	
Divigihalli	...	40	9	22·5	...	...	...	...	...	...	
Gimdagatti	...	100	13	13·0	...	...	...	...	...	...	
Satgihalli	...	32	9	28·1	...	...	...	...	...	...	
Madlun	...	118	18	15·2	...	...	...	...	...	...	
Ghalpuj	...	55	5	9·1	...	...	...	...	...	...	
Guddadmallapur	...	20	3	15·0	...	...	...	...	...	...	
Bhirankop	...	30	4	13·3	...	...	...	...	...	...	
TOTAL		780	139	17·5	25	1	4	1	0	0	
Haveri Taluka.											
Mammankatti	9·3	41	3	7·3	12	0	0	0	0	0	
Basapur	2·7	32	0	0	8	0	0	0	0	0	
Basankatti	...	28	1	3·6	10	0	0	0	0	0	
Mallapur	7·8	28	2	...	12	0	0	0	0	0	
Yellapur	4·3	48	2	4·2	21	0	0	0	0	0	
Lingapur	8·3	28	1	3·6	...	...	...	...	...	...	
Tattinhalli	7·8	42	1	2·4	...	...	...	...	...	...	
Budgatti	...	45	10	22·2	...	...	...	...	...	...	
Devginiyellapur	...	35	3	8·5	...	...	...	...	...	...	
Sanripur	...	30	4	13·3	...	...	...	...	...	...	
TOTAL		357	27	7·5	63	0	0	0	0	0	

V=*vivax*. F=*falciparum*. M=*malariæ*.

TABLE VI—*concld.*

Taluka and village.	SPLEEN RATE, PER CENT.		SPLEEN RATE IN 1948-49.			PARASITE RATE IN 1948-49.					
									Parasite species.		
	1946-47.	1947-48.	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number showing malaria parasites.	Parasite rate, per cent.	V.	F.	M.
1	2	3	4	5	6	7	8	9	10	11	12
Mundargi.											
Bennihalli	...	21·0	32	8	25·0	14	1	7·2	0	1	0
Shirol	...	...	33	8	24·2	13	0	0	0	0	0
TOTAL ...	...	...	65	16	24·6	27	1	3·7	0	1	0
Bankapur.											
Silvansomapur ...	...	9·5	38	3	7·9	24	1	4·2	0	1	0
Ghodgadi	...	...	42	1	2·4	10	0	0	0	0	0
Kinimilhalli ...	...	14·0	51	8	15·7	21	0	0	0	0	0
Bishantalli	...	...	52	30	57·7	32	4	12·5	0	4	0
Belvalkop	...	...	60	9	15·0	...	...	...	...	...	...
Bardur	...	...	40	4	10·0	...	...	...	...	...	...
Attigeri	...	...	100	16	16·0	...	...	...	...	...	...

Halsur	...	...	40	5	12·5	...	...	...	...	...	...
Tirth	...	...	25	7	28·0	...	...	...	...	...	...
Chakapur	...	...	34	8	23·5	...	...	...	...	...	...
TOTAL ...	...	...	482	91	20·9	87	5	5·7	0	5	0
Ranebennur Taluka.											
Yemihosahalli ...	...	2·5	45	3	6·6	10	0	0	0	0	0
Harogappa	...	22·2	46	5	10·9	22	1	4·5	1	0	0
Damdgihalli ...	...	15·0	42	4	9·5	12	0	0	0	0	0
Guddadhosalli ...	...	...	60	10	16·6	...	...	...	...	...	...
Guddadbeninhalli ...	...	...	45	8	17·7	...	...	...	...	...	...
Devgerdankatti ...	...	...	50	11	22·2	...	...	...	...	...	...
TOTAL ...	...	...	288	41	14·2	44	1	2·2	1	0	0
GRAND TOTAL ...	...	..	3,394	610	18·0	508	16	3·2	7	9	0

V=*vivax*. F=*falciparum*. M=*malariæ*.

TABLE VII.

Belgaum District spleen and parasite rates, 1948-49.

Taluka and village.	SPLEEN RATE, PER CENT.		SPLEEN RATE IN 1948-49.			PARASITE RATE IN 1948-49.					
	1946-47.	1947-48.	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number showing malaria parasites.	Parasite rate, per cent.	Parasite species.		
									V.	F.	M.
1	2	3	4	5	6	7	8	9	10	11	12
Khanapur.					**Sprayed villages.**						
Khanapur	37·5	19·4	178	31	17·4	80	2	2·5	1	1	0
Nagargali	100·0	66·7	33	12	36·4	15	2	13·3	1	1	0
Bidi	62·5	22·2	72	10	13·9	56	1	1·8	1	0	0
Nandgad	40·0	11·2	149	13	8·7	60	1	1·7	0	1	0
Tavargatti	100·0	51·8	54	10	18·5	21	1	4·8	1	0	0
Narambal	21·4	16·1	30	4	13·3	16	0	0	0	0	0
Rumawali	5·5	9·1	26	3	11·5	10	0	0	0	0	0
Honkal	...	27·8	32	6	18·8	14	0	0	0	0	0
Tunjiwad (N) ...	...	...	39	5	12·8	...	...	...	...	...	...
Nandgad (J) ...	39·2	25·9	30	5	16·6	18	1	5·5	0	1	0
TOTAL ...	...	...	643	99	15·4	290	8	2·8	4	4	0

Unsprayed villages.

Kupatgari ...	...	...	14·3	45	9	20·0	11	2	18·2	0	2	0
Kauntal ...	...	30·8	26·8	36	9	25·0	20	2	10·0	1	1	0
Lalwadi ...	...	21·4	26·1	25	7	28·0	...	...	...	...	...	...
Nangi ...	...	...	24·1	30	7	23·3	...	...	...	...	...	...
Kinkikop ...	...	30·0	33·3	28	10	35·7	14	3	21·4	1	2	0
Baikwad ...	...	...	29·4	20	8	40·0	17	2	11·8	2	0	0
Garbenhatti	...	35·6	33·3	18	6	33·3	11	2	18·2	0	2	0
Khumbharwada	...	94·1	90·5	23	16	69·6	23	5	21·7	2	3	0
Sawantwada	...	91·0	88·9	20	17	85·0	18	4	22·2	1	3	0
Bamanatkop	...	...	...	8	6	...	4	2	...	0	2	0
TOTAL	...	...	...	253	95	37·6	118	22	18·6	7	15	0

V=*vivax*. F=*falciparum*. M=*malariæ*.

TABLE VIII.

Shirhatti Taluka spleen and parasite rates and infant parasite rate in sprayed villages, 1948-49.

Taluka and village.	Spleen rate, 1947-48, per cent.	Spleen rate in 1948-49.			Parasite rate in 1948-49.					
		Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number showing malaria parasites.	Parasite rate, per cent.	Parasite species.		
								V.	F.	M.
1	2	3	4	5	6	7	8	9	10	11
Shirhatti.		**Spleen and parasite rates.**								
Halalpur *	85*	21	10	48·0	14	1	7·2	0	1	0
Mugdi	62	108	50	46·3	52	2	3·8	0	2	0
Parsapur	88	15	6	40·0	...	...	...	...	...	...
Khanapur	70	30	12	40·0	10	0	0	0	0	0
Haripur	...	57	9	15·8	51	0	0	0	0	0
Shirhatti	19	166	10	6·1	...	...	...	...	...	...
Varvi	79	48	14	50·0	...	...	...	...	...	...
Chebbi	46	111	53	47·8	48	1	2·1	0	1	0
Sojapur	...	42	12	28·6	12	0	0	0	0	0
Akigund	63	34	12	35·5	12	0	0	0	0	0
TOTAL ...	...	632	188	29·9	199	4	2·01	0	4	0

							Infant parasite rate.					
Magadi	...	...	...	...	...	...	15	1	6·7	0	1	0
Halalpur *	...	...	...	...	...	...	6	0	0	0	0	0
Chebbi	...	...	...	...	...	...	18	1	5·6	1	0	0
TOTAL		...	...	...	...	...	39	2	5·1	1	1	0

* Spraying in Shirhatti Taluka was commenced in September 1948 and only two rounds were completed by end of December 1948.

V=*vivax*. F=*falciparum*. M=*malariæ*.

TABLE IX.

Summary of infant malaria indices in Kanara District, 1948-49.

Taluka.	Village.	Number examined.	Number showing malaria parasites.	Infant parasite rate, per cent.	Species.		
					V.	F.	M.
1	2	3	4	5	6	7	8
	Sprayed villages.						
Sirsi ...	Sirsi Town	60	0	0	0	0	0
	Islur	6	0	0	0	0	0
	Targod	5	0	0	0	0	0
	Banavasi	12	0	0	0	0	0
Yellapur ...	Yellapur Town ...	30	0	0	0	0	0
	Kirwatti	6	0	0	0	0	0
Kumta ...	Kalve	5	1	—	0	1	0
	Kujalli	8	0	0	0	0	0
	Heroda	7	0	0	0	0	0
	Mirjan	15	0	0	0	0	0
	Bargi	10	0	0	0	0	0
	Madangeri	6	0	0	0	0	0
	Divgi	9	0	0	0	0	0
Mundgod ...	Mundgod Town ...	24	0	0	0	0	0
	Chigalli	10	0	0	0	0	0
Bhatkal ...	Mottoli	3	0	0	0	0	0
	Kuntwani	2	0	0	0	0	0
	Mavankeri ...	3	0	0	0	0	0
	Kitre	1	0	0	0	0	0
	Shivali	1	0	0	0	0	0
	Mavalli	2	0	0	0	0	0
	Kaikini	3	1	—	0	1	0
Siddapur ...	Kangod	5	0	0	0	0	0
	Nidgod	1	0	0	0	0	0
	Tyagali	2	0	0	0	0	0
	Manmane	3	0	0	0	0	0
Honnavar ...	Arollimundgod ...	3	0	0	0	0	0
	Gersappa	4	0	0	0	0	0
	Chandavar ...	2	0	0	0	0	0
	TOTAL ...	248	2	0·8	0	2	0

V=*vivax*. F=*falciparum*. M=*malariæ*.

TABLE IX—*concld.*

Taluka.	Village.	Number examined.	Number showing malaria parasites.	Infant parasite rate, per cent.	Species.		
					V.	F.	M.
1	2	3	4	5	6	7	8
	Unsprayed villages.						
Bhatkal ...	Herur	1	0	...	0	0	0
	Hedvalli	1	1	...	0	0	0
	Beshi	1	1	...	0	1	0
	Venkatapur ...	1	0	...	0	0	0
Siddapur ...	Nagarbhavi and Begdimane.	1	1	...	1	0	0
	Kananhalli ...	1	1	...	0	0	0
	Mankod	1	0	...	0	0	0
	Hiremaghi ...	1	0	...	0	0	0
	Hangerkhand ...	3	1	...	0	1	0
	Chandraghatgi ...	3	2	...	1	1	0
Sirsi ...	7 villages	12	2	16·6	0	2	0
Yellapur ...	4 villages	9	2	...	1	1	0
Mundgod ...	5 villages	7	1	...	0	1	0
Kumta ...	2 villages	3	0	...	0	0	0
	TOTAL ...	44	12	27·3	3	7	0

V=*vivax.* F=*falciparum.* M=*malariæ.*

TABLE X.

Summary of infant malaria indices in Dharwar District, 1948-49.

Taluka.	Village.	Number examined.	Number showing malaria parasites.	Infant parasite rate, per cent.	Species.		
					V.	F.	M.
1	2	3	4	5	6	7	8
	Sprayed villages.						
Dharwar ...	Navalur	18	0	0	0	0	0
	Tadakod	21	0	0	0	0	0
	Garag	23	0	0	0	0	0
	Kelgeri	14	0	0	0	0	0
Hubli ...	Bhairedevikop ...	11	0	0	0	0	0
	Unkal	18	0	0	0	0	0
Kalghatgi ...	Devikop	14	0	0	0	0	0
	Kalghatgi	14	0	0	0	0	0
Gadag ...	Malsamudra ...	14	0	0	0	0	0
Mundargi ...	Mundargi	18	0	0	0	0	0
	Kadampur ...	12	0	0	0	0	0
Bankapur ...	Shiggaon	12	0	0	0	0	0
Haveri ...	Haveri	12	0	0	0	0	0
Ranebennur ...	Agadi	6	0	0	0	0	0
	Motibennur ...	11	0	0	0	0	0
	Herekerur	8	0	0	0	0	0
	Yattinhalli ...	6	0	0	0	0	0
	TOTAL ...	242	0	0	0	0	0

V=*vivax*. F=*falciparum*. M=*malariæ*.

TABLE X—*concld.*

Taluka.	Village.	Number examined.	Number showing malaria parasites.	Infant parasite rate, per cent.	Species.		
					V.	F.	M.
1	2	3	4	5	6	7	8
	Unsprayed villages.						
Dharwar ...	Inamati Hebli ...	16	0	0	0	0	0
	Guledkop	18	0	0	0	0	0
Hubli ...	Anchadgeri ...	12	1	8·3	0	1	0
Kalghatgi ...	Bishanhalli ...	12	0	0	0	0	0
Gadag ...	Hirehandihal ...	8	0	0	0	0	0
	Bhinkadkatti ...	7	0	0	0	0	0
Mundargi ...	Bennihalli	10	1	10·0	0	1	0
Bankapur ...	Bishanhalli ...	10	1	10·0	1	0	0
Haveri ...	Yattinhalli ...	4	0	0	0	0	0
Ranebennur ...	Harogopp	8	0	0	0	0	0
	Sangubbi	8	0	0	0	0	0
	TOTAL ...	114	3	2·6	1	2	0

V=*vivax.* F=*falciparum.* M=*malariæ.*

TABLE XI.

Number of malaria patients treated in public dispensaries in Kanara and Dharwar districts.

Dispensary.	1943.	1944.	1945.	1946.	1947.	1948.
Kanara District.						
Sirsi ...	5,567	8,763	6,509	4,327	4,254	3,075
Mundgod ...	4,145	2,934	2,045	1,796	1,962	1,248
Yellapur ...	3,750	3,083	2,742	2,717	2,064	666
Haliyal ...	7,603	5,632	4,159	3,782	2,347	2,205
Supa ...	2,369	1,907	1,772	1,397	1,074	1,177
Dandeli ...	2,871	2,337	1,610	910	1,725	690
Manchikeri ...	2,304	1,792	1,819	1,926	1,325	981
Kumta ...	9,956	10,279	9,401	4,269	3,364	1,382
Ankola ...	3,626	5,175	2,868	1,761	Not available	1,392
Gokarn ...	2,151	4,924	2,176	2,261	2,762	1,863
Siddapur ...	1,150	1,630	2,305	1,786	1,133	1,045
Bhatkal ...	4,746	5,710	6,407	5,263	4,071	1,497
TOTAL ...	50,278	54,166	43,813	32,095	26,081 or 28,252*	17,455
Dharwar District.						
Dharwar ...	4,747	4,719	4,878	2,863	2,930	1,847
Haveri ...	9,437	7,919	5,267	2,622	2,644	2,195
Hangal ...	4,963	7,321	3,141	1,767	1,423	1,317
Ranebennur ...	7,311	9,611	7,416	3,578	3,512	2,129
Hirekerur ...	2,993	3,392	1,321	1,479	3,457	2,010
Shiggaon ...	4,602	3,603	2,613	1,016	1,120	1,079
Kalghatgi ...	6,009	7,294	5,161	3,405	2,810	2,783
Mundargi ...	783	1,296	1,956	692	602	598
TOTAL ...	40,845	45,155	31,753	17,422	18,498	13,958
GRAND TOTAL ...	91,123	99,321	75,566	49,517	44,579 or 46,750*	31,413

* Assuming Ankola has the same number of cases as in the previous year.

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A CRITICAL REVIEW OF MALARIA CONTROL MEASURES IN INDIA.

BY

V. VENKAT RAO, F.R.S.T.M. & H., M.R.San.I.

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THE phenomenal speed with which D.D.T. has practically superseded all other methods of malaria control in many parts of the world is certainly striking. This is due partly to certain defects and difficulties inherent in other methods and partly to some unique advantages which D.D.T. possesses. Till about twenty years ago, oiling of mosquito breeding places was the main method employed in India in malaria control. Oil is a costly substance. Its transport, specially to submontane areas where malaria is the biggest problem, is a costly and difficult matter. Its inflammability renders its storage a matter of some concern and it is liable to be pilfered and misused. When subsequently paris green was introduced, there was, therefore, some relief and it was thought that malaria control had become somewhat less difficult and less expensive. But, procurement and storage of a suitable diluent is a great difficulty with paris green as also the necessity to apply it at more frequent intervals than oil. In the case of both, weather conditions act as a limiting factor and their application on growing food-crops like rice, specially during periods of heavy breeding, is objected to on the ground, sometimes justified, that the crop is thereby damaged to a considerable extent. Above all is the supreme difficulty of locating and treating the numerous breeding places at weekly, or even shorter, intervals.

The skill and patience required in antilarval measures of the above kind are such that the schemes could be successful only when the services of enthusiasts could be secured. The cost according to pre-war standards was high enough to limit operations to industrial and military requirements. Control of malaria in those vast rural areas where malaria is the greatest scourge and which in consequence cannot be properly developed, could not even be thought of.

These difficulties were sought to be overcome by what are known as 'permanent measures', which include drainage,—open and subsoil—fillings, etc., with a view to obviate the need for repeated chemical treatment. In the Malay Peninsula, subsoil drains were constructed on an extensive scale, indicating that such works had a definitely important place in antimalaria schemes. But, their high initial cost stood in the way of their being adopted in this country on a wide

scale. They are not equally effective in respect of certain Anopheles like *A. culicifacies*, *A. philippinensis* and *A. annularis*.

Theoretically, bonification measures are the best and most effective in the long run as they do not involve the use of chemical larvicides but, on the other hand, include works which gradually raise the economic level of the people and increase their resistance to disease. Such works are, however, so costly in the beginning and their social and political implications are so far-reaching that they have not so far been tried in any country except in Italy under a Dictator.

In order to render antilarval measures cheaper and more rational, ' naturalistic measures ' were evolved and recommended for large-scale adoption. Williamson (1935) practised his ' herbage-cover ' method in slow-running streams and succeeded in showing the way to eliminate the breeding of *A. maculatus* in the submontane regions of the Malay Peninsula. He was quickly followed by Senior White (1936) in India, who achieved similar success with the same method against the *fluviatilis* group of vectors in the Jeypore Hills. Russell and Jacob (1939) eliminated the breeding of *A. culicifacies* in the Ennore area near Madras through similar methods. Following the clue given by Hackett *et al.* (1938), Venkat Rao (1942) flooded ricefields with crude sullage at periodical intervals during the dry season when they were lying fallow and found that such fields not only failed to breed the local vector (*A. annularis*) in the next rice-growing season but also yielded better crop.

Other naturalistic measures which were adopted to suit varying local conditions included shading, sluicing, flushing, deweeding, silting, etc., and it appeared that such measures would hold the field for a long time, if only, as Hackett *et al.* (*loc. cit.*) emphasized, they could ultimately be carried out by the local people themselves.

Meanwhile, species sanitation was practised in certain countries. This method, first envisaged by Watson (1921) and adopted by him in Malay Peninsula, is cheaper and more effective than general anti-anopheline measures when applied against certain species with selective breeding habits. The work of Senior White (1938) in India has shown that a very considerable saving in cost can be effected by the application of this method against the *fluviatilis* group even in hyperendemic areas. Similar success can also be achieved against *A. sundaicus* (Senior White *et al.*, 1947), *A. philippinensis* (Iyengar, 1944) and possibly *A. stephensi*. But, species sanitation against *A. culicifacies*, a vector of great importance in this country, is unlikely to be a money-saving device, owing to this insect being capable of breeding everywhere, except in dirty or saline waters.

The various methods mentioned above are antilarval measures which, as already stated, are difficult to carry out, requiring a good deal of work in the field under trying conditions. They involve an amount of physical labour and personal risk which many workers would like to avoid. Therefore, as also for other reasons, when malaria control by anti-adult measures was envisaged through the use of pyrethrum extracts, it was hailed with evident enthusiasm and claimed to be easier, cheaper and more effective than other methods. When Russell and Knipe (1939, 1940 and 1941) in South India, and Covell (1941) in Delhi, succeeded in achieving the objective, the hypothesis was advanced that spray-killing of adult mosquitoes rendered rural malaria control quite possible and economical.

That the spraying could ultimately be carried out by the village people themselves, as in the case of naturalistic measures, was emphasized by the former authors.

The limitations of this method became apparent only some time later. As the above-mentioned authors succeeded in their areas by once-a-week spraying, it was generally assumed that the same interval would apply in most, if not all, cases. It was accordingly tried against *A. sundaicus* in the Chilka Lake area and against the *fluviatilis* group in the Jeypore Hills but the results were not very encouraging. Viswanathan (1942) obtained some success against *A. minimus* in Assam but not against *A. fluviatilis* in Bombay Province (Viswanathan *et al.*, 1944). It was then realized that Russell and Knipe (*loc. cit.*) as well as Covell (*loc. cit.*) were working against the same species, viz. *A. culicifacies*, in their respective areas and that the different results obtained by workers in other areas might be due to differences in the bionomics of species other than *A. culicifacies*. It was also realized that whereas a good deal of attention was paid to the ecology of anopheline larvæ, sufficient study was not made of the bionomics of the adults. Elaborate studies were then made on adult mosquito bionomics by Viswanathan *et al.* (1944) and Senior White *et al.* (1945). The results indicated that in anti-adult spraying with pyrethrum, the spacing of the intervals should be based on rational methods, depending on the tropisms of the particular Anopheles concerned. It was observed by Senior White *et al.* (1945) that *A. culicifacies* spends all but a small part of each gonotrophic cycle inside the house and is thus always available to be sprayed indoors whereas *A. fluviatilis* and the other two members of the group spend but one day-light period after feeding in the house and then leave the house for some unknown outdoor shelter to spend the remaining part of the gonotrophic cycle there, irrespective of whether the cycle is completed in two, three or four days. Some of these mosquitoes are found in outdoor shelters even at a period when they are generally accepted as properly being at the feeding site (Senior White, 1946), an observation which was also made by Viswanathan *et al.* (1944).

Pyrethrum spraying against *A. sundaicus* has been found to be unsatisfactory under certain conditions. In one instance, a railway station and the nearby village were sprayed daily but infected mosquitoes were still found in sprayed houses. This was observed to be due to the nightly influx of infected mosquitoes from villages two or more miles farther away (Senior White, quoted by Covell and Pritam Singh, 1942). Long-range infiltration up to six miles from the breeding focus was reported by these authors (*loc. cit.*).

No work appears to have been done against *A. stephensi* but it is believed that this mosquito is so rarely seen resting in houses, probably because it 'leaves the table directly after meals', that pyrethrum spraying is unlikely to be effective.

Thus, in respect of certain dangerous anophelines, very frequent and even daily spraying is indicated, which renders the method costly and generally impracticable.

D.D.T. came into the field at this stage during World War II, when effective malaria control made all the difference between victory and defeat. Antilarval measures were useless for protection of troops except in permanent camps. Pyrethrum spraying was not always dependable for absolute safety. What was

required was an insecticide which was immediately effective and could be applied at intervals long enough to cause minimum interference to other defence arrangements. Having fulfilled both the conditions in the emergency, D.D.T. has come to be popularly accepted as an ideal insecticide.

There is no doubt that D.D.T. is a great advance over other insecticides and constitutes a landmark in the history of disease control. Its utility in long-range antimalaria policies is, however, still under study and cannot yet be taken as proved. Reports have been received from various countries that mosquitoes have disappeared for months after D.D.T. treatment. But, some workers have found as a result of further work that such disappearance of mosquitoes from houses did not always mean death. Kennedy (1947) observed that *A. atroparvus* can absorb doses that are only sublethal causing all symptoms up to and including knock-down but not resulting in death. Hocking (1947) concluded that, in Kenya, *A. gambiæ* and *A. funestus* tend to leave treated premises when these are lightly treated, though higher dosage such as 200 mg. per square foot would achieve a high mortality approaching 100 per cent among the mosquitoes. Muirhead Thomson (1947) however used dosages up to 250 mg. per square foot and found that still *A. gambiæ* entered the huts to feed and did not die during the 48 hours that followed. In India, Puri and associates (1947) carried out experimental sprayings and reported enormous reductions in anopheline mosquitoes, specially *A. minimus* and *A. culicifacies*. Viswanathan and Ramachandra Rao (1947 : 1948), in the course of malaria control operations with D.D.T. on a large scale, also observed similar reductions in the numbers of *A. fluviatilis* and *A. culicifacies*. But, they do not appear to have ascertained by means of window trap collections, etc., that all missing mosquitoes have been actually killed by D.D.T. and that they have not left the houses after having no contact or insufficient contact with D.D.T.

Reports have also been received from several parts of the world of reduction in malaria incidence as a result of D.D.T. spraying. From Kenya comes the report that during the epidemic of 1946, malaria swept the district, leaving the oasis of health where D.D.T. had been sprayed (Granham, 1948). Similar reports have also been received from Italy (Missiroli, 1947), Greece (Vine, 1947), Ethiopia (Tonking and Gebert, 1947) and from India (Viswanathan and Ramachandra Rao, 1947 : 1948). On the other hand, Ribbands (1947) found in Assam that treatment of whole tea estates with doses adequate to eliminate *A. minimus* often yielded no apparent malaria reduction. Kennedy (*loc. cit.*) admitted that mosquitoes failed to appear in house catches after D.D.T. treatment but stated that unfortunately there was as yet no published evidence that the treatment had *an equal effect* on malaria. There is at present evidence that D.D.T. can control malaria where malaria is transmitted by species like *A. fluviatilis* and *A. culicifacies* but it is too early to forecast what will happen in the future even in such regions (Pampana, 1948).

Different species of mosquitoes react differently to D.D.T. treatment. Puri (1947) found that the effect of D.D.T. on culicines was of shorter duration than on any anopheline species. As between different species of Anopheles, the *fluviatilis* group and *A. jeyporiensis* expose themselves considerably more to D.D.T. than do *A. culicifacies* and the *subpictus* group (Senior White and Ghosh, 1946). Viswanathan and Ramachandra Rao (1947) found a 90 per cent reduction

of *A. fluviatilis* after D.D.T. spraying but only a 70 to 80 per cent reduction in *A. culicifacies* and, therefore, recommended a two-months' spraying interval against the former and six weeks' interval against the latter.

Hadaway and Barlow (1947) emphasize that, on account of mosquitoes leaving the houses after 'some contact' or after absorbing sublethal doses, it is necessary that the surface deposit applied should be sufficiently large and lethal to destroy mosquitoes even after a brief contact. They have shown that mud surfaces absorb as much as 85 to 94 per cent of D.D.T. in oil solutions and 65 to 73 per cent of D.D.T. in emulsions, leaving only small fractions for mosquitoes to contact on the surface. Other surfaces absorb lesser quantities which would still be considerable. No such considerations appear to have weighed with malariologists in general, and those of India in particular, as they apply D.D.T. (mostly emulsions) uniformly at a certain rate (in India at the rate of 50 to 60 mg. per square foot), irrespective of the materials of which the walls and roofs are made. The reductions in mosquito density obtained by such application have to be subjected to a careful and critical scrutiny.

The above observations may be sufficiently indicative of the fact that the future of malaria control by D.D.T. will depend on the solution of a large number of problems which need further study. Indeed, it has been stressed by Pampana (*loc. cit.*) that the spectacular success so far obtained should not lead to generalizations or to a disregard of the need for a critical appraisal of any apparent decrease of malaria. It has to be remembered that Buxton (1947) has said that users of D.D.T. have failed to look far enough, or deep enough, and have been satisfied with the more superficial observation, that after putting D.D.T. on the wall, you did not find mosquitoes there for three months.

The development of D.D.T.-resisting races of insects has not perhaps received the attention that it deserves. The emergence of a D.D.T-resisting race of *Musca domestica* has been reported from Italy (Missiroli, *loc. cit.*) and other countries including India. Missiroli (*loc. cit.*) has also reported the emergence of a D.D.T.-resisting race of *C. pipiens*. It is perhaps because *A. darlingi* is exhibiting a similar tendency that attempts are being made to kill off the species by exceptionally heavy doses before a resisting race develops (private communication to the author). Whether gammexane (benzene hexachloride gamma isomer) will go the same way will probably depend on whether it is also a nerve poison or an antibiotic but according to *Nature* quoted in a private communication to the author from Senior White, two Danish workers have shown that D.D.T.-resistant Musca is also resistant to many other compounds with the same type of chemical structure but not to cyclohexanes like gammexane.

Development of resistance is often due to unexpected and slight mutations which appear at random in every species. If the new mutatory character presents some advantage, it is likely to be retained and passed on to the succeeding generations (Missiroli, *loc. cit.*). Even if the new character is selectively neutral, it will establish itself in half the individuals in 10^5 generations but if it confers an advantage of only one per cent, it would establish itself in half the individuals in 10^2 generations. But, a reduction of 0·1 per cent in viability would result in adverse selection which would override mutation at the highest rate ever observed in nature (Huxley, 1942). As a mosquito under optimum conditions has about

twenty-four generations in a year, this means that a useful mutation, which does not affect viability adversely, would spread through half the population in a little more than four years (Senior White, 1948).

This possibility may perhaps be overcome by completely killing off any particular species before a mutant race has had time to emerge. The results of attempting to eradicate *A. darlingi* with very heavy doses of D.D.T. have not yet been published*. That eradication of a particular species from a particular area is possible has already been shown by Soper and Wilson (1943), who worked against *A. gambiæ* in Brazil in the pre-D.D.T. days. But, as these authors have emphasized, they succeeded with a mosquito which was not native to the country but was imported from Africa and did not yet acquire a firm foothold in the adopted country. They doubted if such measures would ever succeed with local species of mosquitoes. In this instance, antilarval treatment with paris green was supplemented by house-spraying with pyrethrum. Shousha (1948) obtained similar success against the same species, imported into Egypt, using paris green almost exclusively as pyrethrum was then in short supply. After the advent of D.D.T., eradication measures were tried successfully against local vector species in Cyprus (Aziz, 1948). But, in such measures, antilarval operations are the primary means of attack and house-spraying against adult mosquitoes is only a supplementary measure (Pampana, *loc. cit.*). Though Brazil is the largest country in South America covering an area of over $3\frac{1}{4}$ million square miles, the *gambiæ*-infested area covered only about 10,000 square miles. Egypt is just over one-tenth of Brazil in extent and the infested area is much less. Cyprus is a small island in the Mediterranean Sea with an extent of less than 3,600 square miles.

In India, *A. fluviatilis*, *A. minimus* and *A. varuna* (commonly called the *fluviatilis* group) exist jointly and severally and are responsible for hyperendemic conditions in the montane and submontane areas which, on a rough estimate, are about 200,000 square miles in extent. The terrain is difficult and inaccessible and the breeding places are numerous. Even for indoor spraying, this area may present great difficulties, and thorough and regular antilarval treatment of all vector breeding places which is necessary in eradication schemes is, in the author's experience, an exceedingly difficult proposition.

In the plains of India, specially of the Punjab and Peninsular India, *A. culicifacies* is the vector *par excellence*. In the Punjab, this mosquito is responsible for widespread epidemics occurring at intervals of 5 to 10 years. In other areas like Pattukkottai (S. India) and the Irwin Canal area of Mysore, *A. culicifacies* maintains endemic conditions through annual outbreaks of short duration, occurring in the rainy months of July–September. In either case, it breeds almost everywhere, including vast areas under rice cultivation. Here also antilarval measures are very difficult to apply owing to the large extent of breeding surface and the heavy monsoon showers rendering such measures ineffective.

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* These results have since been published. Giglioli (1948) claims to have eradicated *A. darlingi* (and also *Aëdes ægypti*) from the coast-lands of British Guiana. He, however, points out that D.D.T. control must continue indefinitely to avoid reinfestation from the uncontrollable, uninhabited hinterland.

Malaria in the coastal districts of Bengal, Orissa and the North Madras Coast is transmitted by *A. sundaicus*, a very elusive insect. As Covell and Pritam Singh (1942) have said, *A. sundaicus* may be prevalent in any locality either in the spring or in the autumn or at both these times, and may disappear, possibly for years, whilst on the other hand, it may suddenly make its appearance in a place where its presence has never been recorded previously. Senior White (1948) reported that after the evident disappearance from the entire area on the west bank of River Hooghly in 1940–44, *A. sundaicus* suddenly re-appeared in the year 1945 and colonized a wider area than it ever did before. During the year 1943, this insect invaded and colonized fresh water areas along the coast of the Bay of Bengal in South Orissa and North Madras Coast but subsequently disappeared from the entire area and has not yet re-appeared (Senior White *et al.*, 1947). *A. sundaicus* may be responsible for endemic malaria or for violent epidemics which occur at irregular intervals. Eradication schemes in such cases are likely to be far too complicated and prolonged to be easily adopted.

Pampana (*loc. cit.*), however, hopes that repeated indoor spraying with D.D.T. over many years (without antilarval work) will ultimately replace vector species by non-vector anophelines. There can as yet be no published or accumulated evidence to establish this as correct. D.D.T. spraying has not been carried out in any country for a sufficiently long period for such evidence to accumulate, as D.D.T. has been made available for civilian use only about three years ago. In those areas where indoor spraying has been continuously done for at least two years, vector species continue to exist, though in greatly reduced numbers. After spraying was carried out for over eighteen months, *A. fluviatilis* continued to appear in sprayed houses (in Jeypore Hills) in almost the same density in the second year as during the first year of spraying (Senior White and Ghosh, 1946). Viswanathan and Ramachandra Rao (1948), however, recorded a greater reduction in the second year than in the first in the case of both *A. fluviatilis* and *A. culicifacies* but stated that as long as D.D.T. is sprayed at the dosage and with the intervals that are at present generally adopted in this country, eradication of vector species could not be possible even after continued spraying over a number of years. They have also pointed out that eradication schemes have undoubted utility only under restricted and specialized conditions and that this indicates their unsuitability for a vast country like India.

It would thus appear that D.D.T. is as much a short-term measure of malaria control as other insecticides are, and though eminently suitable for emergencies and during periods in which permanent or biological measures are under way, it cannot constitute by itself an economical long-term policy of malaria control, even if meanwhile the vector insects do not develop chemico-resistance against its action. If, however, such an eventuality materializes, it would cease to be useful even as a temporary measure. In such a case, the hygienist is perforce driven to biological measures, even when funds for chemical methods are available (Senior White, 1948).

One such measure as envisaged by Senior White (1948) is exploration of the possibility of malaria control by what he describes as 'race substitution' as an extension of the known concept of 'species sanitation'. This presupposes the existence of crypto-species or biological races, of which Huxley (*loc. cit.*) is so certain

that his references to them are given the force of axioms. Malariologists generally adopt the easier and more convenient method of classifying the various species by external morphological characters. Where biological differences exist, the fact is recognized but no serious attempt has so far been made to pursue the subject. Sometimes, biological differences were sought to be explained by differences in morphological characters with a view to classify the concerned species into well-defined races but such attempts have not usually been successful. Therefore, for instance, why a particular species is a potent vector of malaria in one area but is harmless in another in the same region has not been understood and it cannot perhaps be correctly understood as long as the species are distinguished by visible external characters alone. According to Huxley (*loc. cit.*), much of speciation is concerned with invisible physiological characters and groups may remain perfectly distinct though morphologically indistinguishable. Differences in ecological preferences may isolate groups as effectively as geographical barriers or spatial distance. Certainly in most phyla, and probably in all, there exist groups of individuals which are undoubtedly distinct in every sense except the accepted morphological one.

If these axioms are accepted to be as applicable to mosquitoes as to other animals, one can but ponder on how many crypto-species lie hidden in the portmanteaux-names of current systematic nomenclature (Senior White, 1948). In this view, Senior White (*loc. cit.*) listed certain anophelines of the Oriental Region in each of which differences in vectorial status exist without any apparent explanatory factors and, therefore, concluded that the various species concerned must be composed of more than one biological race incapable of occular differentiation. The concept of biological races would assume added importance when the following facts are considered along with those described by Senior White (*loc. cit.*) :—

(*a*) Besides being a vector in certain areas and harmless in others, *A. fluviatilis* is observed to be much more dangerous in one part and not so dangerous in another within the area of its importance as a vector. Thus, in the Wynaad Hills of South India, Covell and Harbhagwan (1939) found the infection rate of this species to be at times as high as 30 per cent whereas in the Jeypore Hills, the same species, which along with *A. minimus* and *A. varuna* is responsible for hyperendemic conditions, is found to exhibit an infection rate of not more than 5 per cent. In the sub-Himalayan foothills, *A. fluviatilis* is not a vector at all (Covell, 1939 : 1940).

(*b*) In the Chilka Lake area and in Lower Bengal, *A. sundaicus* has been shown to be responsible for both epidemic and endemic conditions. The infection rate of this species ranges between 2 and 4 per cent in Lower Bengal (Iyengar, 1931), whereas, in the Chilka Lake area, it is only 0·3 per cent (Senior White and Adhikari, 1939, and Covell and Pritam Singh, 1942). But, on the North Vizagapatam Coast, which had been the scene of a virulent epidemic during 1943-44, the infection rate of the same species was found to be as high as 10 per cent. It has been suggested that in areas south of Lower Bengal, *A. sundaicus* breeds freely in both fresh and saline waters, though in much larger numbers in the latter than in the former, and that, as the fresh water form is probably the real vector, the low infection rates found in the Chilka area are due to the mixing up of a smaller number of the vector (fresh water) race with a much larger number of the harmless (saline water) race (Senior

White *et al.*, 1947). Subsequently, Venkat Rao and Ramakrishna (unpublished) have found differences in the structure of the phallosome of the two forms which, in their opinion, are valid enough to differentiate them into two distinct varieties.

(*c*) Having observed that *A. culicifacies* is of no practical importance as a malaria vector in a large section of East Central India, Senior White suggested that this species is a composite of at least two races, morphologically indistinguishable, one of which alone was the vector. Russell and Ramachandra Rao (1942) calculated the average life of *A. culicifacies* in the Tanjore Delta of South India to be two days but found that a small proportion of them lived much longer and carried on transmission.

(*d*) Periodical epidemics are stated generally to be the result of loss of balance between infection and immunity but, in the Punjab particularly, they are also based on the amount of rainfall in March, April and May, indicating that there is a definite and significant relationship between such rainfall and the malaria incidence in the subsequent autumn (Yacob and Swaroop, 1947).

In the case of *A. fluviatilis*, it would appear that in the Wynaad area, this species consists entirely, or almost entirely of the vector race, whereas in the Jeypore Hills, it consists of at least two races, one of which is the vector and the other non-vector, the latter being the more populous. Otherwise, why, in spite of the very large number of human carriers, only a small percentage of the mosquitoes show infections in this area, cannot be explained ? Of course, the *fluviatilis* population of the sub-Himalayan foothills consists entirely of the non-vector race or races.

The co-existence of the fresh water form and the saline water form of *A. sundaicus* in the same area has not so far been reported except on the Chilka Lake and North Vizagapatam Coast. In the other countries of its prevalence, *A. sundaicus* breeds only in saline waters and is everywhere a recognized vector. *A. ludlowi*, a closely related species, breeds in fresh waters in the Philippine Islands and is not a vector. In India too, where *A. sundaicus* was first recorded in Bengal, it has been observed to prefer saline waters for its breeding. Thus, the brackish water form, which is a vector wherever it exists, is shown to be at least relatively harmless in the Chilka Lake and North Vizagapatam areas, whereas a fresh water form, not recorded elsewhere, is a potent vector there. Taylor (1944) has stated that, in view of the lack of correlation between *sundaicus* prevalence and malaria incidence in certain areas in Singapore, *A. sundaicus* there may consist of more than one species or variety. Therefore, the existence of different races within the species is strongly indicated.

That the *A. culicifacies* of East Central India consists only of non-vector races has already been indicated. Even in areas where it is the undoubted vector, its infection rate is as low as 0·1 per cent on an average, which may indicate an admixture of vector and non-vector races, the latter being much more populous than the former. In the Tanjore Delta, it appears *prima facie* that there is a short-lived race, which is obviously harmless and a long-lived race, which alone can be responsible for transmission.

The findings of Yacob and Swaroop (*loc. cit.*) may indicate that special meteorological conditions, which occur cyclically at regular or somewhat irregular intervals, provide optimum requirements for the prevalence of a particular vector race in

sufficient numbers to cause an epidemic outbreak. In mosquitoes, conditions which favour increase or decrease in numbers are in the main climatic and may be cyclical in appearance (Senior White, 1948).

The existence of biological races among vector species *per se* does not lighten the malariologist's burden unless such races have varying bionomics and can be isolated demonstrably into distinct groups. If the various races of a particular species, for instance, have similar breeding and feeding habits, their existence may provide academic interest to the pure zoologist but means nothing to one engaged in applied biology. If there are differences between the vector and non-vector groups, these may be due to variations in ecological preference. In that case, the wide difference in the infection rates of *A. fluviatilis* mentioned above becomes understandable. Covell and Harbhagwan (*loc. cit.*) found the breeding of this mosquito only in running waters like *nullahs* in the Wynaad area but not in ricefields, seeping or otherwise, and this appears to hold good also in the Malnad area of the Western Ghats. In the Jeypore Hills on the other hand, ricefields of the seeping variety, the extent of which is considerable, are responsible for a major part of the breeding (Senior White, 1946). Similar conditions are also reported from Bombay Province by Ramachandra Rao (1945). Do the lower infection rates in *A. fluviatilis* (and in the other two members of the group) observed in the Jeypore Hills indicate, as in the case of *A. sundaicus*, that these mosquitoes emerging out of particular breeding places (like *nullahs* and other running waters) are the real vectors and that, in dissections, they are mixed up with a large number of mosquitoes of the same species which emerge out of other types of breeding places (like seeping ricefields) and which may be relatively or entirely harmless? If there are groups based on variations in ecological preference, such variations would produce differences in infection rates quite as definite as would differences in anthropophilic index. In the case of *A. culicifacies*, the question of longevity observed by Russell and Ramachandra Rao (*loc. cit.*) may be studied further with a view to ascertain the ecological differences if any in the two groups. That such differences exist leading to difference also in vectorial capacity has been shown by Senior White (1936) in the case of *C. fatigans*. He observed that whereas *C. fatigans* breeding in natural sullage waters is a vicious biter of man and carries the infection of filariasis, that which breeds in waters rendered impure by herbage cover does not attack man and is thus incapable of transmitting the disease.

One method of distinguishing groups or crypto-species in their relation to malaria transmission has been suggested to the author by Senior White (private communication). If one could radio-active 'tag' vector larvæ from various types of breeding places and distinguish the tagged adults as they mingle with other adults, one could see whether larvæ from some particular breeding place produce more infective adults than others. Such work is reported to be in progress under the auspices of the Rockefeller Foundation in West Africa. Such a method would also indicate if certain groups tend to enter into a condition described by Venkat Rao (1947) as gonotrophic discordance, whereby they are fixed in houses and so establishing greater contact with man, are enabled to carry on transmission to a much greater extent than when they behave normally.

The possibility of a fresh approach to the problem of malaria control may also be considered. It may be emphasized that malaria is essentially 'a disease of waste land, waste water and waste men'. Where the land is allowed to deteriorate,

where water is not properly conserved and utilized and where man is too indolent and too apathetic for his own welfare, there malaria is firmly established. Malaria control may then be considered in terms of conservation of land, water and man-power and their diversion into useful and productive channels.

In the hills generally, malaria is transmitted, as already stated, by anophelines of the *fluviatilis* group, which breed in slow-running clear waters like *nullahs*, seepages and seeping ricefields. In the last analysis, most of these breeding places, as they at present exist, are the result of continuous and indiscriminate deforestation and the consequent soil erosion. As more and more soil is washed away with each flood, the subsoil is exposed to a greater extent, giving rise to a number of seepages, both along the hill-foot and in the ricefields. The *nullahs* are also widened and deepened on an ever-increasing scale, rendering their effective flushing impossible and giving rise to numerous side-seepages, which are more dangerous than the main current itself. The land also becomes more sterile with the lapse of time. If the erosion is stopped by proper methods of soil conservation and limited re-forestation, the reverse process begins to operate, exposed seepages are buried under the fast accumulating silt and dangerous mosquito breeding is checked. These methods, so essential in agriculture, are also antimalaria measures of first-rate importance.

The fear expressed in certain quarters that seepages in ricefields are their only source of irrigation and the crop suffers if the seepages are buried or drained away, does not appear to be correct as it is shown by an experienced paddy specialist (unpublished) that in areas where the average annual rainfall is not less than 50 inches, ricefields do not stand in need of further irrigation. The rainfall is not less than this figure in most hill tracts.

As supplementary measures, extensive cultivation and shading of hill streams are likely to prove very useful. It has been shown by Senior White (1946) that in hill tracts, fallow fields of the seeping variety breed a much larger number of vector anophelines than cultivated fields and Muirhead Thomson (1940) has indicated the possibility of controlling the breeding of *A. minimus* by heavily shading running waters.

Thus, in the hill tracts, malaria can be reduced, and even ultimately eradicated, by (1) soil conservation and re-forestation; (2) extensive cultivation; and (3) shading hill streams. The first two methods are also calculated at the same time, to develop the agricultural wealth of the area.

Malaria in the plains is mainly due to *A. culicifacies* except in Bengal and Orissa. This Anopheles, as stated above, is usually responsible for epidemics, but in certain areas it maintains endemic conditions through annual outbreaks of short duration. In endemic areas, malaria has followed the execution of large irrigation works such as the Irwin Canal Scheme in Mysore and the Mettur Project in South India. Russell (1938) and Rao (1945) have proved that most of this malaria is the result of defective and untidy irrigation, indicating that the remedy lies in irrigation works being carried out with an eye on antimalaria efficiency in consultation with malariologists.

In the Bengal plains, the vector is mainly *A. philippinensis* breeding in overgrown tanks and ponds but not ricefields, whereas in Orissa, the vector is *A. annularis*, which breeds in tanks and ponds with heavy aquatic vegetation as

well as in ricefields. Breeding in tanks and ponds may be dealt with by deweeding on the lines described by Venkat Rao and Ramakrishna (1947) or by growing plants which are inimical to the particular vector like *Eichhornia speciosa* against *A. philippinensis* (Iyengar, 1944). Vector breeding in ricefields is shown to be due to defective agriculture which can be improved by heavy manuring as shown by Venkat Rao (1942), a procedure which besides inhibiting vector breeding, also ensures better crop output.

The factors responsible for epidemics in areas other than the Punjab do not appear to have been well studied. They may be related to altered climatic conditions which may occur cyclically or to the development or invasion of a new vector race. Or, as suggested below, they may be related to sudden disturbances in the economic balance of the population. These, it need hardly be stated, require intensive study.

The human factor involved in the epidemiology of malaria has not perhaps received the emphasis that it deserves. Malaria is mainly a disease of poor and economically backward countries. Though Russell *et al.* (1946) say that nutrition is a factor in the epidemiology of malaria only in so far as a poorly nourished individual is less liable to withstand the effects of disease, Gill (1928) observed that at the close of an epidemic, other things being equal, the spleen rate among the less well-to-do classes is relatively high and that economic conditions probably play some part in the variation of spleen rates. A study of most epidemics indicates the presence of this economic factor. The great epidemic of 1917 in the U.S.S.R. followed widespread famine caused by a total breakdown of agriculture after the revolution. In Ceylon, failure of the south-west monsoon, which must affect the delicately balanced economy of the people, was the prime factor of the 1934 epidemic. The famines of Bengal and the North Madras Coast in 1942-43 were quickly followed by large-scale epidemics in the affected areas. The chronic low economic condition of the inhabitants of most hyperendemic areas is too well known to require emphasis. One cannot, therefore, entirely agree with Russell *et al.* (*loc. cit.*) that susceptibility to infection is not dependent on the state of nutrition. However, what exactly is the factor, which is involved in the interplay of economic disorder and malaria, is not known but such a study may well repay the effort.

The studies indicated above as necessary for a fresh approach to the problem of malaria control, require the co-operation of biologists, engineers and agriculturists no less than that of medical men. As Senior White (1948) states, our ignorance of this subject is greater than our knowledge and we should have, not only workers who can apply existing methods of investigation and control, but also investigators who will apply and extend the methods of pure zoological (and other) research to the problems of malaria control. If this paper dispels, even to some extent, the popular notion that, after the advent of D.D.T., malaria research has become a luxury and all that needs to be done is only periodical spraying of D.D.T. on the walls, its purpose is served and, if the matter is sought to be pursued with a view to render antimalaria work more rational and less costly, the author is amply rewarded.

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A PRELIMINARY NOTE ON THE PRESERVATION OF UNSTAINED BLOOD SMEARS.

BY

LIEUT.-COLONEL JASWANT SINGH, M.B., ch.B., D.P.H., D.T.M. & H.,

MAJOR A. P. RAY, M.B., B.S.,

AND

MAJOR C. P. NAIR.

(*Malaria Institute of India.*)

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INTRODUCTION.

ORDINARY blood smears for examination of malaria parasites or other protozoa should be stained as soon as they are taken, if not within at least 24 hours, as otherwise the staining gives poor results with any of the Romanowsky stains and the desired classical picture is not obtained. Delay in staining favours the growth of moulds, and the length of time that elapses before staining, influences the staining technique employed, as good chromatin staining of aged smears is more difficult than in the case of fresh smears (Boyd, 1930). Preliminary fixation of thin smears before staining is quite often unnecessary in the case of slides stored for a few days in the tropics. This is due to auto-fixation by heat. In the same way, summer heat and age of the smear fixes the thick film also with the result that complete dehæmoglobinization is impossible. If such films are stained with J.S.B. stain (Jaswant Singh and Bhattacharji, 1944) or Giemsa, the red blood corpuscles and the cytoplasm of the malaria parasites stain deep dark blue, and chromatin sometimes remain unstained and at other times stained either lightly pinkish or even blue.

Field (1949) states that after a week, stored unstained thick smears may well be useless. According to Craig (1948), blood films will stain poorly even a few days after preparation and therefore suggests the method of Daniels (1907) to stain old blood films which involves a preliminary acid alcohol bath followed by washing and staining in the usual manner. But in practice, for slides stored for long periods (some weeks to months), this method of staining has not given satisfactory

results. Ratcliffe (1946) found that smears of blood containing numerous parasites of *P. vivax* which stained readily when stained in Texas, could not, after transportation to Indiana, be stained to show organisms of diagnostic acceptability. To get satisfactory staining results of such old slides he recommended therefore the bathing in strong alkali and subsequently staining for 24 hours in Giemsa or Wright's stain. This technique however is more time-consuming and requires at the same time further development and improvement.

The unsuitability of using old slides stored in the laboratory and the difficulty of obtaining fresh smears that are required at all times of the year for teaching purposes, prompted the authors in the past 2 years to attempt to devise some method of preserving unstained blood films and the progress so far made in this direction is recorded in this paper.

APPROACH.

During the height of the winter season in Delhi when atmospheric temperatures are low and it is comparatively dry (Table), it has been observed that thick and thin unstained smears do not as a rule undergo any appreciable auto-fixation during the course of a few days as the colour contrast of the different elements of the blood and parasites is more or less retained after routine staining.

TABLE.

Mean temperature and humidity during winter months in Delhi for 5 *years* (1944 *to* 1948).

Particulars.	January.	February.	March.	October.	November.	December.
Temperature maximum °F.	70·1	75·7	85·9	89·54	81·40	74·6
Temperature minimum °F.	43·8	51·14	57·7	65·63	53·1	44·5
Humidity at 8-00 a.m. (per cent).	71·9	67·4	54·8	66·3	60·2	71·3

It has been claimed by Wilcox (1943) that during dry summer months thick smears can be successfully stored in a cold room on a week-end but care should always be taken to prevent condensation of moisture on the slides since it may loosen the smears and cause them to be lost during the staining process. Shute (1946) has found that old films kept at 50°F. stain better than similar films kept at 80° to 90°F. These observations indicate that the most essential conditions to be satisfied in any attempt to preserve blood smears are low temperatures and dryness of the atmosphere and to meet these requirements the following procedure was found in these laboratories to give satisfactory result.

METHOD AND THE RESULTS.

Fresh thick and thin blood smears taken on the same slide from microscopically diagnosed malaria cases were wrapped after preliminary fixation of the thin films in methyl alcohol in a sheet of tissue paper in such a way that each slide is kept separate from the other by a layer of paper. In the case of thick smear if it were too thick, the film was lysed when fresh, and dried before being wrapped in the paper. A number of similar packets were kept on the same day of taking the smear in a desiccator (with calcium chloride at the bottom chamber) placed in a refrigerator. After varying periods from 6 to 24 months, the smears were stained either with J.S.B. or Giemsa and examined. In the case of smears preserved in this manner for six months or less, the results compared very favourably with those obtained from freshly made films. Slides stored for more than a year often gave very good staining reactions but occasionally the red cells stained light slate blue, thus exhibiting only a slight differentiation. This defect however was easily overcome by J.S.B. stain by prolonging the time of contact of the film (varying with different batches of smears) in the buffered wash water. Thick smears that were too thick gave rise to partial auto-fixation within 5 to 6 months but even this was prevented by preliminary dehæmoglobinization of the thick smears before keeping them in the refrigerator.

Further observations on the preservation of blood slides under varying degrees of humidity using desiccators with different concentrations of sulphuric acid are in progress and results will be published later.

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INCIDENCE OF *ANOPHELES FLUVIATILIS* JAMES LARVÆ IN A D.D.T. SPRAYED AREA IN WYNAAD, SOUTH INDIA.

BY

J. C. VEDAMANIKKAM.
(*Bureau of Malariology, Madras Presidency.*)

[September 27, 1949.]

INTRODUCTION.

THE Wynaad is a hilly taluk of the Malabar District, Madras Presidency, South India, with an average elevation of 3,000 feet above mean sea-level. The average rainfall is about 150 inches and is brought about by both the south-west and north-east monsoon.

A typical area in Wynaad consists of a valley bounded by low hills with uncultivated marsh at the head of a central channel in the middle and contour drains at the sides running almost parallel to the central channel and finally both the contour and central channels uniting to form a bigger stream lower down. On the slopes of the hills forming the boundary of the valley, coffee, pepper and oranges are usually grown, while paddy is extensively cultivated in the valley. All the houses and cattlesheds are generally situated on the slopes just above the margins of the valley.

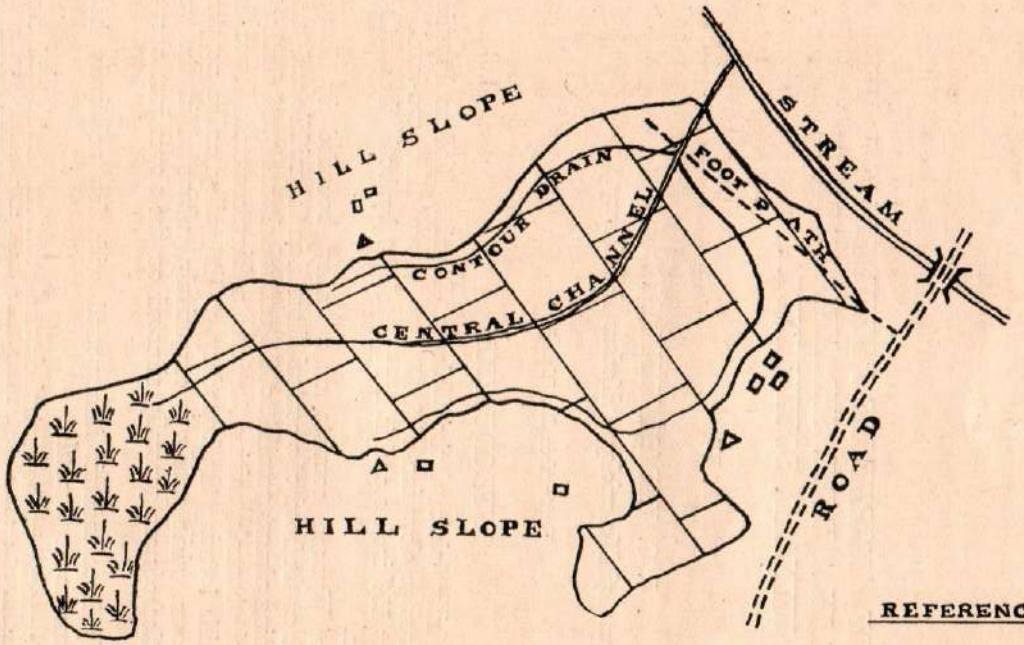
Malaria is generally hyperendemic interspersed with few relatively healthy areas. The carrier species is *A. fluviatilis*.

A. fluviatilis breeds mostly in the central channel, contour drains and streams and feeds on human beings residing in the slopes and rests in houses. Each valley in fact forms a breeding and feeding unit in respect of *A. fluviatilis*. Significant breeding of this species occurs in December and continuous breeding is observed till the onset of heavy rains in June, when due to the flushing action of the streams and channels, such breeding is considerably reduced.

The maximum density of adult mosquitoes is during the months April to June, which is also the peak of the transmission season for malaria though there is chance transmission at all times of the year.

In December 1943, a Land Colonization Scheme known as the Wynaad Colonization Scheme, extending over 60 square miles of hyperendemic area around

Map 1.



TYPICAL AREA IN WYNAAD.

Sultan's Battery (which is 60 miles from Calicut on the Calicut-Mysore Road), was started for reclamation of the land for settlement of ex-service men. The observations recorded in this paper were made while malaria control measures were being carried out in the area selected for land colonization.

The entire area was divided for purposes of entomological investigations into six circles. Each circle was in charge of a Health Inspector assisted by a Field Assistant responsible for making both larval and adult mosquito collections. Each circle was further divided into twelve divisions and a Field Assistant visited each of these divisions once every fortnight. Collections were made from fixed collecting stations in every division. These included six houses and a cattleshed for adult mosquitoes; larval collections were made from streams, ricefield channels, contour drains and other types of breeding places such as wells, drinking water pits, paddy fields, marshes, etc. The collecting stations were peg-marked and serially numbered. In the case of breeding places with flowing water, 100 feet of stream length was taken to be the extent of a larval collecting station. Fifteen minutes were spent in each adult mosquito collecting station and 10 minutes in each larval collecting station.

ANTIMALARIA MEASURES.

Only antilarval measures, such as flushing of streams by hand-operated sluices, trimming and canalization were being carried out till the end of the *A. fluviatilis* season in 1945-46. During the season 1946-47, spray-killing with pyrethrum extract was done in the entire area till January 1947. This measure was gradually replaced by D.D.T. spraying so that by the end of May 1947 all the houses in the entire colonization area were sprayed with D.D.T. with the exception of Nulpuzha circle which continued to be under pyrethrum spray.

During the season December 1947 to June 1948, the houses in the entire area were sprayed with D.D.T. The first round of spraying was completed by the middle of January 1948 and subsequent sprays were continued at six-weekly intervals till the first week of July that year.

During 1946-47, D.D.T. was applied as a 5 per cent solution in kerosene at the rate of 100 mg. per square foot and the interval between spraying was one month for the first few rounds. Later, this was replaced by 5 per cent D.D.T.-M.K.E.-soap-emulsion, the dosage being 50 mg. per square foot and the interval between sprays six weeks. During 1947-48, the same technique was continued.

Only the inner surface area of walls and furniture were sprayed. Verandahs of houses were sprayed only if they were partially enclosed and used for sleeping purposes. Stirrup pumps with suitable nozzles were used for spraying during the two seasons.

COLLECTION OF ADULT *A. FLUVIATILIS*.

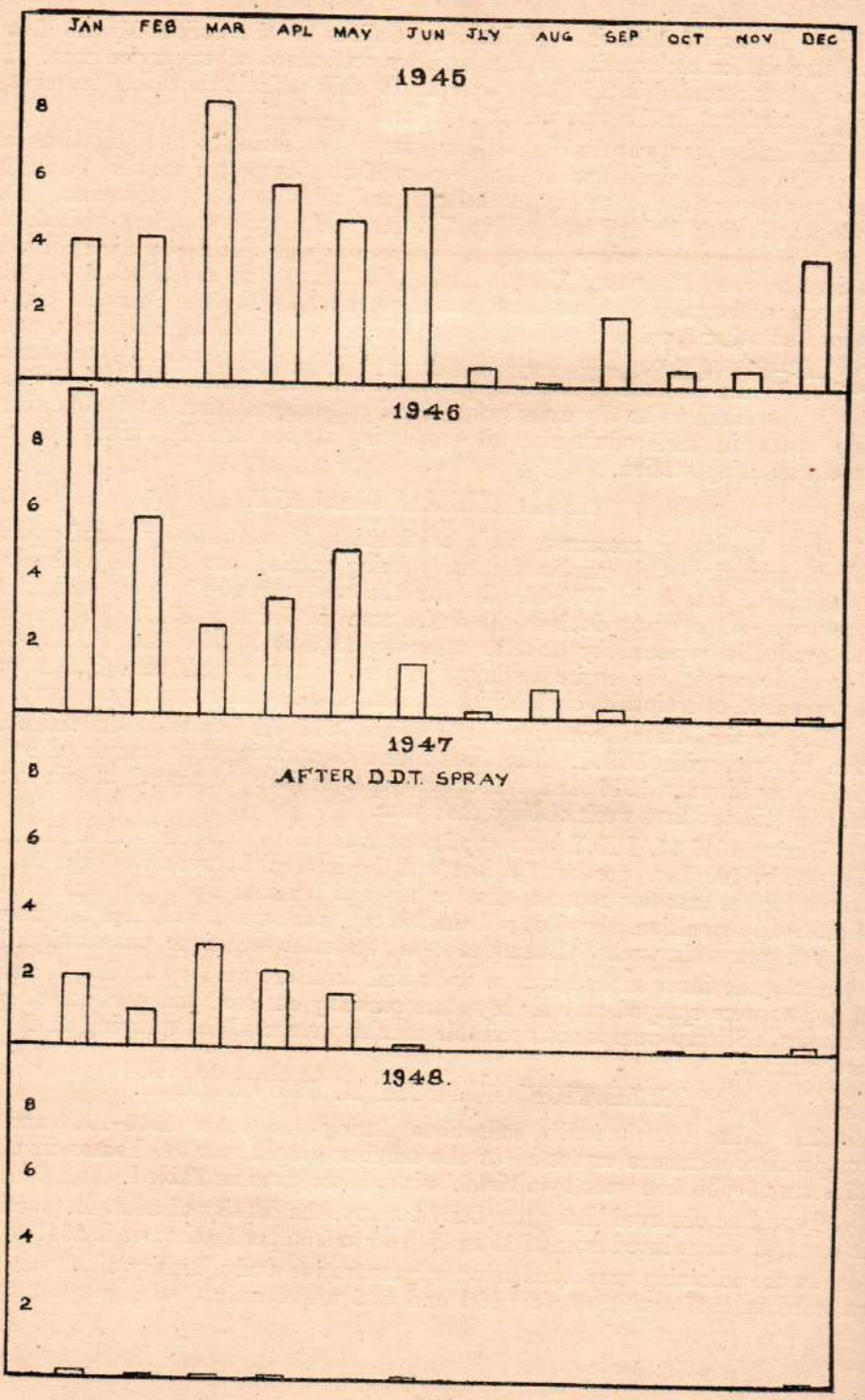
The adult *A. fluviatilis* collections during the years 1945–1948 and the comparative incidence expressed as the number caught per 100 houses involving 25 hours of collection work by a Field Assistant, are given in Table I.

It will be observed that after D.D.T. spray, the density of adult *A. fluviatilis* in the area was reduced from 37·15 in 1945-46 to 0·05 per 100 houses in 1947-48.

In the adjoining unsprayed area for comparative study, density of *A. fluviatilis* recorded in 1947 and 1948 was 88·1 and 19·9 respectively.

CHART 1.

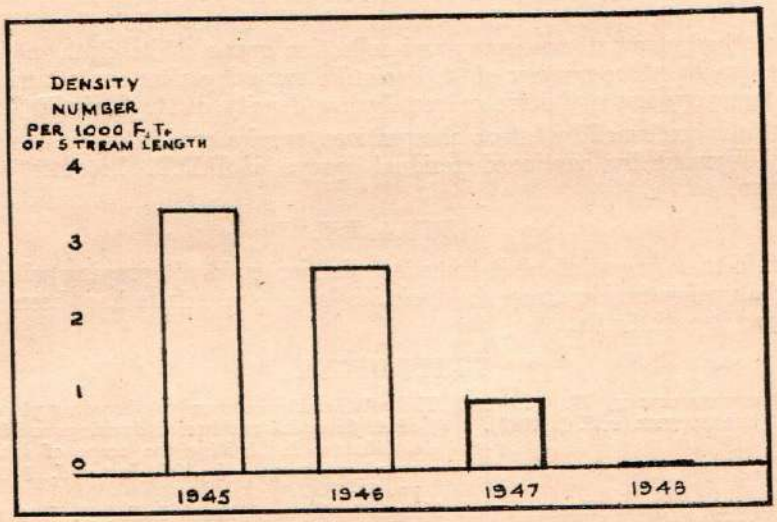
Monthly density of larvæ.



COLLECTIONS OF LARVÆ OF *A. FLUVIATILIS.*

Table II gives the number of larvæ collected in the area every month during the years 1945–1948. The comparative annual incidence for the period and the number of larvæ collected from breeding places with flowing water, are shown in Charts 1 and 2.

CHART 2.



A perusal of the Tables and the number of larvæ collected from breeding places with flowing water is alone taken up for comparative purposes and the charts will indicate that the incidence of larvæ in all types of breeding places has been reduced in a remarkable manner after the commencement of indoor D.D.T. residual spray.

DISCUSSION.

Within two seasons of spraying with D.D.T. in Wynaad where *A. fluviatilis* is the only vector, the incidence of adults became almost nil and there is also a corresponding marked decrease of *A. fluviatilis* larvæ.

Covell and Harbhagwan (1939) recorded that *A. fluviatilis* in Wynaad is strongly anthropophilic with 97 per cent containing human blood. Its strongly anthropophilic nature makes it prefer human dwellings for feeding and also for resting. It prefers to lay its eggs in closeby breeding places, for larvæ are found usually in close proximity to human dwellings (Covell and Harbhagwan, 1939; Adisubramaniam and Vedamanikkam, 1943; Jaswant Singh and Jacob, 1944).

Thus, in every Wynaad valley, *A. fluviatilis* forms a feeding and breeding unit with human dwellings on the slopes and streams and channels in the valley. It is because of the formation of such a unit, that control of breeding in 1,000 feet length of channel within 200 to 1,000 feet from the human dwellings reduced the

incidence of adult *A. fluviatilis* in the human dwellings (Adisubramaniam and Vedamanikkam, 1943).

Similarly, D.D.T. spraying in houses or the attack on the adult *A. fluviatilis* in its feeding and resting units, viz. the human dwellings, has very markedly reduced the incidence of larvæ in the breeding unit. D.D.T. applied as a residual spray against adult *A. fluviatilis* seems to be a far more potent method than the old-time measures of larval control. But intensive searches of houses and cattlesheds in the neighbourhood of areas where *A. fluviatilis* larvæ have been caught, have failed to show the presence of any resting adult *fluviatilis*. Even by the method of pyrethrum spray and collection over a white cloth, no mosquito could be caught. The number of *A. fluviatilis* surviving after contact with D.D.T. is therefore very low and have extremely low density of *A. fluviatilis* larvæ.

If this is confirmed by future observations, species eradication in limited areas such as Wynaad by continued residual sprays of D.D.T. becomes a feasible proposition.

SUMMARY.

Within two seasons of adult control by indoor residual spraying with D.D.T., a remarkably low incidence of *A. fluviatilis* larvæ and adults has been recorded in Wynaad, South India.

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COVELL, G., and HARBHAGWAN (1939). Malaria in Wynaad, South India. *Ibid.*, **2**, 4, pp. 341–376.

JASWANT SINGH and JACOB, V. P. (1944). Malaria investigations in North Kanara. *Ibid.*, **5**, 3, pp. 267–303.

TABLE I.

Total number of A. fluviatilis *caught in daytime resting places before and after D.D.T. spraying.*

Year.	Total number caught.	Number per 100 houses.
1945	3,195	37·15
1946	3,090	35·93
After D.D.T. spraying.		
1947	108	1·53
1948	4	0·05
Comparison resting places.		
1947	484	88·1
1948	305	19·9

TABLE II.

The number of A. fluviatilis *larvæ collected in the area every month before and after D.D.T. indoor residual spraying.*

Month.	1945.				1946.			
	Number of collecting stations searched.	Number of *A. fluviatilis* caught.	Number caught per 1,000 feet of stream length.	Number of larvæ caught from other breeding places.	Number of collecting stations searched.	Number of *A. fluviatilis* caught.	Number caught per 1,000 feet of stream length.	Number of larvæ caught from other breeding places.
Jan. ...	640	268	4·19	8	658	642	9·75	42
Feb. ...	656	285	4·34	18	699	409	5·85	13
Mar. ...	668	564	8·44	15	686	182	2·65	5
Apr. ...	538	322	5·98	35	602	210	3·48	30
May ...	496	242	4·88	30	632	317	5·01	49
Jun. ...	550	329	5·98	20	516	84	1·62	21
Jul. ...	424	25	0·59	23	478	8	0·16	33
Aug. ...	472	4	0·09	20	492	45	0·91	28
Sep. ...	586	122	2·08	52	504	15	0·29	24
Oct. ...	604	29	0·48	43	678	3	0·04	9
Nov. ...	766	42	0·54	7	594	1	0·02	16
Dec. ...	754	301	3·99	7	674	5	0·07	4
TOTAL ...	7,154	2,533	3·54	288	7,213	1,921	2·66	274

TABLE II—*concld.*

Month.	1947.			1948.		
	Number of collecting stations searched.	Number of *A. fluviatilis* caught.	Number caught per 1,000 feet of stream length.	Number of collecting stations searched.	Number of *A. fluviatilis* caught.	Number caught per 1,000 feet of stream length.
Jan. ...	723	191	2·06	703	13	0·18
Feb. ...	746	79	1·06	795	9	0·11
Mar. ...	696	216	3·10	672	4	0·05
Apr. ...	692	165	2·38	812	9	0·11
May ...	815	128	1·57	750	...	0·00
Jun. ...	742	8	0·11	770	1	0·01
Jul. ...	783	1	0·01	793	...	0·00
Aug. ...	755	...	0·00	812	...	0·00
Sep. ...	836	...	0·00	749	...	0·00
Oct. ...	704	1	0·01	808	...	0·00
Nov. ...	776	2	0·02	812	...	0·00
Dec. ...	777	14	0·18	777	2	0·02
TOTAL ...	9,045	805	0·89	9,253	38	0·04

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KAKNAR NYAY PANCHAYAT MALARIA CONTROL CO-OPERATIVE SCHEME.

BY

R. SUBRAMANIAN,

B. K. VAID,

AND

D. T. DIXIT.

(*Malaria Organization, C.P. and Berar.*)

[October 13, 1949.]

EARLY in 1948 the Government of Central Provinces and Berar suggested that the provincial malaria organization should work on co-operative basis with the villagers in controlling malaria, and further suggested that the Government should supply D.D.T. and equipment free of cost and provide for technical supervision. The villagers on their part should arrange for labour to be engaged in spraying operations. It was decided to carry out control measures on these lines in a selected area for one year and, if successful, to extend it to other areas as well. The reasons for selecting Kaknar Nyay Panchayat Circle for this experiment were: (1) the residents of the circle had made a representation to the Government to include their circle in the malaria control scheme and promised every help; (2) this circle is located in the Kaknar tract which is intensely hyperendemic with an average spleen rate of 88 per cent; and (3) in the same district an antimalaria unit was operating which could easily supervise the scheme.

In April 1948, a meeting was held at Kaknar which was attended by the senior author, the Civil Surgeon of the District, Chairman of the Nyay Panchayat, Kaknar, the Sarpanch of the Grampanchayats, Kaknar, Manjrod and Doiphoria and other local influential persons. It was explained to all present that the Government would undertake antimalaria work in the Nyay Panchayat Circle provided adequate help was forthcoming. The Government would provide D.D.T., spraying equipments and technical supervision but the labour should be provided by the grampanchayats. Seven labourers were to be employed on a monthly basis throughout the season and given a fixed pay of Rs. 40 per month. The grampanchayats agreed to contribute adequate funds for engaging the labour and assured of their co-operation in making the scheme a success.

AREA AND TOPOGRAPHY.

The Kaknar Nyay Panchayat Circle which forms part of the southern portion of Nimar District was an intensely hyperendemic foothill area. The tract lies between the two portions of the Western Satpura Range separated by the narrow Tapti Valley. The range is covered by forests and drained by a large number of perennial streams. This tract affords a most interesting study in malarial endemicity. It was formerly a populous pargana but was devastated in 1803 by war and famine; in consequence it became almost deserted and overgrown with forest. It is now being reclaimed by ryotwari settlement but malaria offers a serious obstacle to successful colonization. The soil is good for cotton, maize and rice crops.

POPULATION AND HOUSING CONDITION.

The Kaknar Nyay Panchayat Circle comprises the three grampanchayats, Kaknar, Doiphoria and Manjrod. There are in all 34 villages in the circle with an aggregate population of 15,000 (1941 census). These villages are situated in an area approximately 16 miles long and 4 miles wide along the Burhanpur-Dharni Road with Kaknar in the centre (*see* Map). The houses in this circle may be broadly classified into two types:—

(*a*) Huts with bamboo walls and thatched roofs, mainly occupied by Korku (aboriginal) settlers.

(*b*) Small houses with mud plastered walls, roofs made of thatch or tiled. These types of houses are generally occupied by settlers from the plains.

Every village possesses a fair-sized herd of cattle which are kept in close proximity to the villagers either in the verandah of the house or in adjoining shed.

EPIDEMIOLOGY.

Both *A. culicifacies* and *A. fluviatilis* have been incriminated as vectors in this part, infection rates being 0·21 and 1·28 per cent respectively (Subramanian and Dixit, 1948). Nearly 60 per cent of the Anopheles density is due to *A. culicifacies*. *A. fluviatilis* occurs in comparatively small numbers. The transmission season is from September to February*. Transmission could possibly occur throughout the year but the limiting factor is the non-availability of suitable and adequate breeding grounds which in turn depend on rainfall.

The anopheline fauna includes:—

A. subpictus, *A. culicifacies*, *A. stephensi*, *A. fluviatilis*, *A. vagus* and *A. theobaldi*.

DETAILS OF THE SCHEME.

Staff.—The services of one trained antimalaria supervisor were lent to the scheme for six months—July to December 1948. Seven labourers were employed locally on a monthly basis at Rs. 40 per month for six months. The scheme was supervised in the beginning by No. 3 Antimalaria Unit and later by No. 5 Antimalaria Unit.

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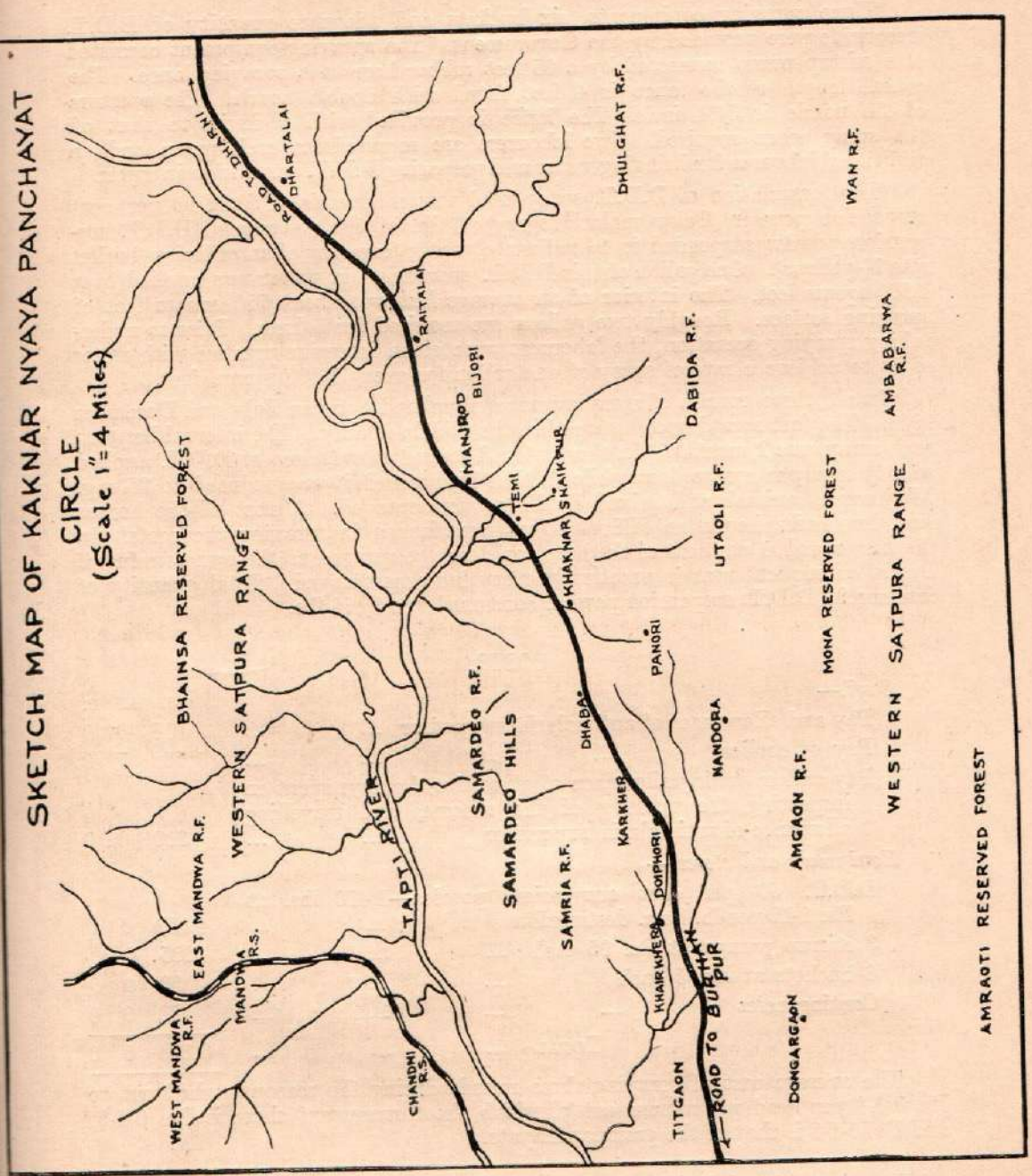
* Further dissections carried out in 1949 revealed natural gland infections in *A. culicifacies* in July and August.


SKETCH MAP OF KAKNAR NYAYA PANCHAYAT CIRCLE
(Scale 1"=4 Miles)
ROAD TO DHARNI
DHARTALAI
BHAINSA RESERVED FOREST
WESTERN SATPURA RANGE
RAITALAI
MANJROD
BIJORI
TEMI
KHAKNAR
SHAIKPUR
DHULGHAT R.F.
DABIDA R.F.
UTAOLI R.F.
WAN R.F.
AMBABARWA R.F.
MONA RESERVED FOREST
WEST MANDWA R.F.
EAST MANDWA R.F.
MANDWA R.S.
CHANDNI R.S.
TAPTI RIVER
SAMARDEO R.F.
SAMARDEO HILLS
SAMRIA R.F.
DHABA
KARKHER
PANORI
KHAIRKHED
DOIPHORI
NANDORA
TITGAON
ROAD TO BURHANPUR
DONGARGAON
AMGAON R.F.
WESTERN SATPURA RANGE
AMRAOTI RESERVED FOREST

Three spraying equipments, six buckets and adequate quantity of D.D.T. insecticide were provided by the Government. The spraying equipment consisted of a stirrup pump connected by a 25 feet rubber hose to a spraying lance. The overall length of the lance is 4 feet fitted with double nozzle. The aperture of the nozzle is 3/64 inch. The lance is provided with a device to shut off the spray when required. Two labourers are required for each pump and an additional labourer for bringing water and preparing D.D.T. suspension in water.

The formulation of D.D.T. spray used was commercial D.D.T. 50 per cent suspension powder (Geigy malaria spray); 1 lb. of the commercial D.D.T. suspension powder suspended in 2½ gallons of water (capacity of an ordinary bucket was 2½ gallons). One gallon of this D.D.T. suspension in water was applied over 1,800 square feet. The interior of an average hut was about 720 square feet of spraying surface. Roughly two gallons are required for 5 huts. Prior to undertaking spraying operation, the labourers were given intensive practice with water so as to achieve a rate of spray of one gallon for every 1,800 square feet.

In all, three rounds of spraying were completed between July and December 1948. The first spraying commenced in the middle of July. The interval between each spray was 2 months. In the first and second round, dose of D.D.T. applied was 50 mg. per square foot while in the third round it was reduced to 25 mg. All human, animal and mixed dwellings were sprayed. Almost all the houses were low built, hence no difficulty was experienced in spraying the roofs. In the first round, 1 per cent of the huts could not be sprayed as the owners refused. But in subsequent rounds practically every hut was sprayed. Total quantity of commercial D.D.T. suspension powder consumed was 1,476 lbs.

COSTS.

	Rs.	A.	P.
Staff.—			
Pay and allowances of antimalaria supervisor	618	0	0
Pay of coolies	1,522	7	0
Pay and travelling allowance of officer for the days spent on inspecting the working of the scheme, and travelling allowance of antimalaria supervisor	300	0	0
Equipment and stores.—			
D.D.T. 50 per cent suspension powder—1,476 lbs. at Rs. 2-12 per lb. f.o.r. destination	4,059	0	0
3 stirrup pumps at Rs. 95 each	285	0	0
6 buckets at Rs. 5 each	30	0	0
Contingencies	73	9	0
TOTAL	6,888	0	0

The average cost of spraying three rounds during the season worked out to Re. 0-7-4 per head per annum out of which the Government share is Re. 0-5-8, and Re. 0-1-8 is that of the grampanchayats.

Note.—A provision of a little less than 5 per cent of the cost of non-recurring items on its maintenance is included.

In subsequent years, the Government share in the expenditure is likely to work out to Re. 0-5-2.

This reduction in cost is possible by :—

(*i*) Reduction in the price of commercial D.D.T. suspension powder.

(*ii*) Elimination of cost of non-recurring items.

RESULTS.

DENSITY OF ANOPHELES ADULTS.

In the Graph, the fortnightly per man-hour Anopheles density recorded in an unsprayed village Raitalai and in a sprayed village Kaknar, is shown.

The mosquito collections were made from these villages by a trained insect collector at fortnightly intervals. In each village, 8 catching stations were fixed : 4 human dwellings and 4 cattlesheds. Twenty minutes' catch was made in each station.

Study of the Graph shows that in the middle of July, Anopheles density per man-hour in both sprayed and unsprayed villages was 30. On the 19th and 20th, Kaknar was sprayed. On the 26th when the next routine catch was made, the per man-hour density in Kaknar was 22, whereas in the unsprayed village Raitalai it rose to 64, nearly 3 times more. Five weeks after the first round of spray, the Anopheles density in Kaknar registered the lowest figure of 16 per man-hour. This reduction in the total anopheline density in the sprayed village as compared with the mosquito density obtained in the unsprayed village for the same week, is highly significant. Then the mosquito density started rising. Kaknar received its second spray on the 19th September after which the Anopheles density dropped practically to zero.

The remarkable drop after the second spray is probably a cumulative effect of the second over the first spray. After this drop in the mosquito density to zero, in Kaknar, it never rose again throughout the season. A third round of spray of 25 mg. D.D.T. per square foot was given early in November, immediately after the Diwali festival when the houses are white-washed in this part of the country. It is difficult to say whether in the absence of the third round the Anopheles density in Kaknar would have continued at zero level till the end of February.

EFFECT ON SPLEEN RATE.

In Table I are presented the spleen rates recorded in three villages (both before the introduction of the malaria control scheme, and after the completion of one season's operation).

There is a definite reduction in the spleen rate recorded in the sprayed village whereas the unsprayed village shows an increase. The reduction is not much, because—

1. The antimalaria measures were not long in operation.
2. The average size of the enlarged spleen in these villages is 3 (Hackett's method). Such big spleens naturally take a long time to contract.

In areas with a very high spleen rate and big spleens, recording of spleen rates in children of age groups 0–2, 3–5, 6–10, 11 and above, might be of value in assessing results of control measures.

GRAPH.

Per man-hour anopheline mosquito density.

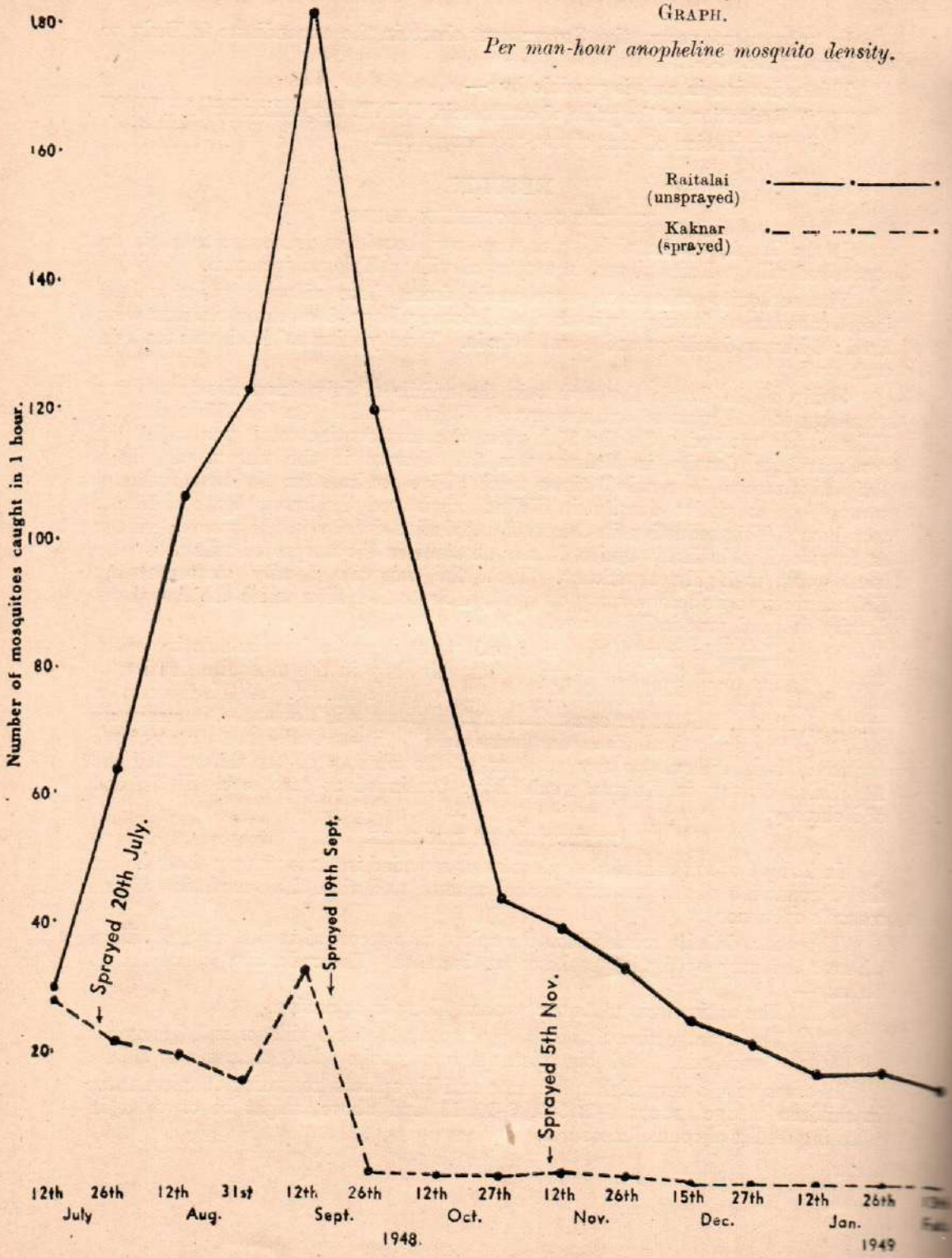


TABLE I.

Spleen rates in sprayed and unsprayed villages.

Month and year.	Village Kaknar (sprayed).			Village Manjrod (sprayed).			Village Raitalai (unsprayed).		
	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.
1948.									
Sep. ...	40	35	87·5	35	34	97	75	61	81·3
Dec. ...	45	35	89	35	32	91·4	58	51	87·7

INFANT PARASITE RATE.

The details of the infant parasite rate carried out every month, June 1948 to January 1949, in sprayed and in an unsprayed village, are recorded in Table II.

TABLE II.

Infant parasite rate in a sprayed and an unsprayed village, June 1948 to January 1949

Month and year.	Village Kaknar (sprayed).			Village Raitalai (unsprayed).		
	Number examined.	Number showing malaria parasite.	Parasite rate, per cent.	Number examined.	Number showing malaria parasite.	Parasite rate, per cent.
1948.						
Jun. ...	3	...	0	8	...	0
Jul. ...	10	1	10	8	2	25
Aug. ...	13	2	15	7	4	57
Sep. ...	14	1	7·1	10	3	30
Oct. ...	9	0	0	11	7	63·6
Nov. ...	10	0	0	6	1	16·7
Dec. ...	7	1	14·3	6	4	66·6
1949.						
Jan. ...	10	1	10·0	4	3	75·0
TOTAL ...	76	6	7·8	60	24	40·0

Out of 76 infant blood slides examined in Kaknar, only 6 slides showed malaria parasites. All of them were of *vivax* infection. In Raitalai, the unsprayed village, out of a total of 60 infant blood slides, as many as 24 were found with malaria parasites, of which only one was *falciparum* infection and the rest were all *vivax* infection

DISPENSARY DATA.

In the Nyay Panchayat Circle, there is only one dispensary at Kaknar.

In Table III are given the total number of patients and the number of malaria patients treated in the dispensary in 1945, 1946, 1947 and 1948. Prior to July 1948, no antimalaria activity was undertaken in this circle. Therefore, the drop in the total number of patients recorded at the Kaknar dispensary in 1948 is highly significant.

TABLE III.

Kaknar dispensary record.

	1945.	1946.	1947.	1948.
Total number of patients treated in the dispensary ...	11,000	11,402	11,485	6,952
Number of malaria patients treated in the dispensary	3,279	3,420	3,311	2,206

But the figures of malaria patients do not reveal a proportionate reduction in 1948.

To illustrate the full effect of the control measures in the sprayed villages in lowering the sickness due to malaria, the dispensary figures of malaria patients from the sprayed villages are presented in Table IV for the four years 1945 to 1949.

TABLE IV.

Number of malaria patients who attended the dispensary from the villages under control.

Month.	1945-46.	1946-47.	1947-48.	1948-49.
Jul. ...	137	110	234	95*
Aug. ...	151	155	206	99
Sep. ...	217	314	288	94
Oct. ...	614	428	348	174
Nov. ...	399	554	359	135
Dec. ...	316	446	268	90
Jan. ...	169	207	225	62
Feb. ...	186	229	191	83
Mar. ...	198	305	219	85
Apr. ...	131	211	223	67
May ...	140	211	139	53
Jun. ...	97	202	79	38
TOTAL ...	2,755	3,372	2,779	1,075

* Malaria control measures instituted.

The consistently low figures registered during the period July 1948 to June 1949, as compared with the other years, are highly significant.

DISCUSSION.

The scheme is a novel one in the sense the villagers voluntarily came forward to contribute a share in the expenditure on the control of malaria. This naturally reduced the cost to the Provincial Government in launching the scheme. Having paid some money for their own benefit, the villagers took a very keen interest in the operation of the scheme. The staff were well received wherever they went and their comforts were attended to.

The immediate benefit of the spraying appreciated by the villagers was that they could get peaceful and undisturbed sleep at night, free from the annoying presence and bites of mosquitoes. They got equally good relief from the destruction of bugs. The villagers also reported that their cattle improved in their health and the milk yield of cows increased. The reduction in the sickness due to malaria is expressed by the villagers in terms of rupee value. According to them the reduction was 14 annas in the rupee. The result has been so spectacular that within 6 months of the commencement of the scheme, the Chairmen of the neighbouring Nyay Panchayats Dertalai and Haidarpura submitted applications to the Government to extend the activities to their areas as well and promised to pay the cost of labour engaged in the spraying operation.

The scheme which started as an experiment is rapidly getting popular. It is no exaggeration to say that this is the only Governmental activity which is earning tremendous popularity to the Government. The unique feature of this D.D.T. spraying is that it equally benefits one and all, irrespective of the fact whether one belongs to a particular caste or creed, and whether rich or poor, high or low.

SUMMARY.

At the suggestion of the Government of Central Provinces and Berar, malaria control scheme on a co-operative basis was started in Kaknar Nyay Panchayat Circle. The Government supplied free of cost D.D.T. insecticide, spraying equipment, buckets and technical supervision. The villagers contributed funds to pay labour engaged in the spraying operations.

The scheme covered three grampanchayats, Kaknar, Manjrod and Doiphoria, consisting of 34 villages with an aggregate population of 15,000.

Cost per head per annum on malaria control measures worked out to Re. 0-7-4, of which the Government share was Re. 0-5-8 and the grampanchayats Re. 0-1-8.

The results of antimalarial activities extended over six months—July to December 1948—have been so satisfactory that the neighbouring Nyay Panchayats Dertalai and Haidarpura have requested the Government to extend similar activities to their areas as well and have promised full co-operation and contribution.

ACKNOWLEDGMENT.

The authors express their appreciation of the excellent work carried out by Shri Surjit Singh Darwesh, Antimalaria Supervisor.

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Indian Journal of Malariology, **3**, *4, December 1949.*

STAINING AND RE-STAINING OF OÖCYSTS AND SPOROZOITES FROM INFECTED MOSQUITOES.

BY

LIEUT.-COLONEL JASWANT SINGH, M.B., ch.B. (Edin.), D.P.H. (Eng.), D.T.M. & H. (London),

AND

A. DAVID.

(*Malaria Institute of India.*)

[October 30, 1949.]

MODIFICATIONS of Romanowsky stains like Leishman and Giemsa have for a number of years been used for staining blood smears for diagnosis of malaria parasites and sporozoites from salivary glands of infected mosquitoes. For staining of oöcysts in midgut of mosquitoes, Green's technique (1932), modified as detailed below, has been followed in these laboratories for years.

Several experimentally infected midguts showing oöcysts at various stages have been successfully stained with Leishman and Giemsa stains and recently with J.S.B. (Jaswant Singh and Bhattacharji, 1944) stain. After considerable experience, the following procedure of staining has been adopted in each case :—

1. GREEN'S TECHNIQUE.

Green has devised a method giving good results and in which the pigment of young oöcysts is not obscured in the process. The time taken in Green's method of staining oöcysts from the completion of fixation to the mounted specimen is only about 10 minutes.

Fixation.—Run in formol-saline solution* to the preparation under the coverglass. During this process pressure should be kept on the coverglass so that the specimen is well flattened and spread out†.

* This consists of one part of formalin (40 per cent formaldehyde) to 9 parts of normal saline solution.

† This pressure is easily and effectively applied by partially overlapping one end of the coverslip with a small piece (about ½ inch wide) cut from microscopical slide. Run the fixing fluid into the cavity made between this tilted piece and the surface of the saline. This forms a reservoir from which the fluid is drawn under the coverglass by small pieces of filter or blotting paper applied to the opposite side of the coverglass. As the reservoir is depleted, more fluid is added.

The completion of fixation is recognized when the dissected midgut turns white and opaque and when this occurs, the coverglass is floated off with excess of the fixative. The preparation will remain flattened and attached either to the glass slide or to the coverglass. The specimen may then be carefully flooded with the fixative and set aside for about 5 minutes to ensure complete fixation. The fixative should now be washed off carefully with distilled water.

Staining.—Stain with aqueous toluidine blue solution (0·1 per cent) instead of Pugh's stain* recommended by Green (*loc. cit.*) for a couple of minutes. Wash off the stain with distilled water and examine the depth of staining under low power of the microscope. (The colour should be a medium shade of blue, and if too light the stain should be re-applied.)

Dehydration and mounting.—Remove any excess of water and dip the slide or the coverglass carefully into the following mixtures† in rotation for the times indicated :

(*i*) Acetone 95 parts plus xylol 5 parts (30 seconds).
(*ii*) Acetone 70 parts plus xylol 30 parts (1 minute).
(*iii*) Acetone 30 parts plus xylol 70 parts (1½ minutes).
(*iv*) Pure xylol (2 minutes).

Examine the preparation against a dark background, and if it is not perfectly translucent, and shows any opaque cloudy or milky appearance, pass it back again to (*iii*) for 1½ minutes and bring up to pure xylol again. When the specimen is clear, remove the excess of xylol, and apply a drop of canada balsam or euparal for making a permanent preparation.

2. LEISHMAN'S STAIN.

(*i*) Fix in 10 per cent formol-saline solution as in Green's technique.
(*ii*) Wash excess of fixative with distilled water.
(*iii*) Stain with concentrated stock solution (Leishman) for 2 to 3 minutes.
(*iv*) Wash with distilled water.
(*v*) Dehydrate as in Green's technique ending in xylol.
(*vi*) Mount in canada balsam.

3. GIEMSA STAIN.

Giemsa stain can also be used in 1 to 5 dilution with water, the duration of staining being 2 to 3 minutes.

4. J.S.B. STAIN, SOLUTION I.

The technique of staining the gut is the same as in above three methods, except for the stain used. The preparation is stained in this case with J.S.B. Solution I only for 2 to 3 minutes.

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* Dissolve 1 gm. toluidine blue in 20 c.c. absolute alcohol; to this solution add 20 c.c. glacial acetic acid and make up to 1,000 c.c. with distilled water. A stock solution (1 per cent), which is diluted as required, has been found more convenient.

† These should be kept in closed jars to prevent the absorption of moisture from the air.

Some preparations which were originally stained with Green's method in 1940–45 and had faded away during these years were re-stained in 1949, some with J.S.B. stain, some with toluidine blue, some with Leishman and others with Giemsa. The results in all cases were satisfactory.

The details of the procedure are as follows :—

The faded preparations are submerged in xylol for 18 to 24 hours in order to remove all traces of canada balsam. The coverglass is then carefully removed. The midgut usually remains attached either to the coverslip or on the slide. It is kept in xylol for a further period of 5 minutes after which the preparation is passed successively through the following grades of alcohol :—

(1) Absolute alcohol, (2) 90 per cent alcohol, (3) 75 per cent alcohol, (4) 50 per cent alcohol, (5) 30 per cent alcohol, and finally through distilled water. The time allowed for each change is 5 minutes.

The preparation is then stained with 0·1 per cent toluidine blue for 10 to 11 seconds.

The subsequent dehydration process is the same as in Green's technique, that is, passing through the acetone-xylol mixture. The preparation is then mounted in canada balsam before examination.

Re-staining of oöcysts has also been done with Leishman, Giemsa and J.S.B. stains. Time for staining is the same as for original staining with Leishman and Giemsa, but with J.S.B. Solution I, it takes only 30 seconds.

Uniformly good results were achieved in staining sporozoites of *P. præcox* by the J.S.B. technique (vector—*Culex fatigans*). The procedure adopted is as follows :—

(*i*) Use a very tiny drop of 0·6 per cent normal saline for dissecting out the salivary glands.
(*ii*) Place a clean coverglass on the dissected glands.
(*iii*) Exert very slight pressure on the coverslip with the point of the dissecting needle to crush the gland and force out the sporozoites.
(*iv*) Carefully remove the coverslip by gently lifting one edge with the point of the dissecting needle.
(*v*) Dry both the coverslip as well as the slide by waving about in the air. Never expose them to heat as it tends to contract and distort the sporozoites out of shape.

When completely dry, the slide and coverslip should be fixed with methyl alcohol and stained as below :—

(*i*) Dip slide and coverslip in Solution I for 4 to 5 minutes.
(*ii*) Wash in a jar containing buffer water (3 dips are needed) containing 0·021 per cent disodium hydrogen phosphate and 0·038 per cent potassium acid phosphate.
(*iii*) Stain with Solution II for one second (one dip).
(*iv*) Wash with buffer water (3 dips).
(*v*) Stain with Solution I for 4 to 5 minutes.
(*vi*) Wash in the same buffer water (4 dips).

Dry as usual and examine.

The side of the coverslip which has the sporozoites should always be mounted facing upwards.

The different staining techniques being followed in these laboratories have shown very encouraging results and it has been found that with J.S.B. stain not only the differentiation is somewhat more marked but the cost is negligible. For future observations and study of fading effects, series of stained and unstained preparations of oöcysts and sporozoites have been kept. Use of J.S.B. stain for demonstrating malaria parasites in blood smears, oöcysts, sporozoites, trypanosomes, and Leishman-Donovan bodies has been fully established.

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CLINICAL TRIALS WITH NEOCHIN IN THE TREATMENT OF SIMIAN MALARIA.

BY

LIEUT.-COLONEL JASWANT SINGH, M.B., ch.B. (Edin.), D.P.H. (Eng.), D.T.M. & H. (London).

(*Director, Malaria Institute of India, Delhi.*)

[October 22, 1949.]

NEOCHIN is manufactured by Shri Subodh Chandra Ghosh at the Indian Drugs and Chemical Industries, Calcutta. According to the particulars furnished, it is a synthetic product from cinchonine with the absence of the methoxy (CH_3O) group of the quinoline base. Chemically it is di-cinchonine carbonate and is an almost tasteless white powder melting at 246–247°C. It is insoluble in water and ether, sparingly soluble in alcohol and highly soluble in chloroform and dilute acids and is dextro-rotatory.

The drug is easily hydrolysed by dilute hydrochloric acid and is quickly absorbed. It has been claimed by the manufacturers that the drug brings down the temperature with 2 doses of 5 grains each, and can cure acute cases of malaria with 4 or 5 days' treatment (5 grains twice a day for 2 days and thereafter 5 grains daily for 2 days; total 30 grains) and that it is the only drug which after 3 such courses can cure chronic cases of malaria with enlarged spleen. Further, it has been stated that it is both a curative and a prophylactic drug and that one or two doses per week is sufficient as preventive. Best time of administration of this drug is when there is a fall of body temperature by 1°F., whatever be the maximum temperature. The period of remission is also suitable but it is not to be used above 100°F. in the rising stage as, though it controls the highest temperature, the patient if weak, may be exhausted to a degree.

The tests carried out at the Biochemical Standardization Laboratory* showed that parasites disappeared from peripheral circulation two hours after administration of 2 grains neochin to two monkeys (approximate weight 7·5 kg.) infected with *P. knowlesi*.

*Abstracts from Report No. 719 dated July 13, 1946, of the Director, Biochemical Standardization Laboratory, Government of India, as quoted by S. C. Ghosh, under Biological Assay in his memorandum on neochin submitted to the Government of India.

A small quantity of this drug was originally received at the Malaria Institute of India from the Director-General of Health Services in March 1948 for testing its antimalarial properties. In September that year, the Therapeutic Trials Committee of the Indian Research Fund Association recommended that since this drug contains a cinchonine salt, investigations should be carried out both on monkeys and human cases. On behalf of the Indian Research Fund Association, a detailed investigation was accordingly carried out under the direction of the Malaria Institute of India in 1948-49. The present report is based on the effect of this drug as observed in these trials in 15 cases of simian malaria at the Malaria Institute. Sixteen cases of human malaria were treated at the Calcutta School of Tropical Medicine and results obtained have been given on page 357 of this issue of the journal (Chaudhuri and Rai Chaudhuri, 1949).

DOSAGE AND METHOD OF TREATMENT.

Simian malaria.—Healthy monkeys weighing 6 to 8 lbs. each were experimentally inoculated intraperitoneally with blood from donor monkeys suffering from *P. knowlesi* infection at the rate of approximately 3 million parasites per kg. body-weight. Thick and thin blood smears from these monkeys were taken daily in the morning at 10 a.m. and stained with J.S.B. method (Jaswant Singh and Bhattacharji, 1944). After a prepatent period of 5 to 6 days, parasites appeared in the peripheral blood when neochin was administered orally in all cases.

Recommended dosage for human malaria cases (adults) consists of a total of 30 grains distributed as one tablet of 5 grains twice a day for 2 days and thereafter one tablet once daily for the following two days. Calculated according to body-weight, the proportionate dose of this drug to a monkey weighing 7 lbs. is $1\frac{1}{2}$ grains. Four monkeys were placed on this dosage (two monkeys received this as a single dose whereas the remaining two received it in divided doses of half grain a day for three days). To two other monkeys (5th and 6th), a single dose of three and six grains respectively was administered. To another one (7th monkey) a total of 12 grains was given distributed over 2 days. A batch of another two monkeys (8th and 9th) was placed on a still higher dosage of 24 grains. This total dosage was administered in one monkey as six grains once a day for four days, and in the other as four grains twice a day for the first two days and four grains daily thereafter for two days. Finally, to six more monkeys (10th to 15th), the drug was administered in the same dosage as advocated for human adult (i.e. a total of 30 grains in 4 days).

RESULTS.

Infection in monkeys 1 to 7, that received neochin in total doses varying from $1\frac{1}{2}$ to 12 grains, failed to react to the drug and the infection ran a normal course in all of them. Of the two animals that received 24 grains, one (No. 8) reacted well initially and the blood smears proved negative after the third day of drug administration, but after a week it developed a heavy infection to which it succumbed. In the other (No. 9), there was no change in the degree of infection up to three days after the completion of the drug administration and on the fourth day, it died of intercurrent disease. In the case of monkeys Nos. 10 to 15 that received the recommended human adult doses (30 grains), parasites disappeared from the peripheral circulation 24 to 120 hours from the commencement of treatment. Within an

observation period of 63 days, four animals had 5 to 10 relapses while the remaining two remained negative throughout. The drug was not toxic to any of the monkeys even in these high dosages.

DISCUSSION.

The statement in the memorandum on neochin submitted by the manufacturer to the Government of India, that 2 grains of the drug cleared parasites from peripheral circulation of two monkeys infected with *P. knowlesi*, is at variance with the findings in the present trials as well as in the human cases treated at the Calcutta School of Tropical Medicine.

It is a well-established fact that plasmodicidal action of cinchonine is less potent than quinine and that neither of the drugs has any effect whatsoever on exo-erythrocytic (primary or secondary tissue phases) forms of the parasite. Quinine in daily doses of 5 grains has been found ineffective as a prophylactic in man. It is therefore not understood how this drug neochin which contains cinchonine can act both as a prophylactic and a curative as claimed by the manufacturer. Further it is not clear how this drug can cure cases of chronic malaria with splenomegaly and enlarged liver any more than other drugs already known.

SUMMARY AND CONCLUSIONS.

Neochin, an alleged antimalaria drug containing cinchonine salt, was subjected to a detailed investigation on monkeys against *P. knowlesi*.

It was tried on 15 monkeys in varying doses ranging from a total dose of $1\frac{1}{2}$ grains (corresponding dose for a monkey of 7 lbs. as per body-weight) to 30 grains (recommended human dose), and failed to show effective plasmodicidal action in doses proportionate to the body-weight of the monkeys. In higher doses up to 24 grains, it yielded poor results but in monkeys which received a total dosage of 30 grains each, the parasites from peripheral blood disappeared within an average of 82 hours from the commencement of treatment but 66 per cent relapsed frequently thereafter.

No toxic symptoms were observed with any of the dosage regimes.

In the treatment of *P. knowlesi* infections in *S. rhesus* monkeys of northern India, neochin has not shown any properties comparable to quinine or other synthetic antimalarial drugs in common use.

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A NOTE ON CLINICAL TRIALS WITH NEOCHIN.*

BY

R. N. CHAUDHURI, M.B., M.R.C.P., T.D.D.,

AND

M. N. RAI CHAUDHURI, M.B.

(*Department of Tropical Medicine, School of Tropical Medicine, Calcutta.*)

[November 1, 1949.]

SIXTEEN indoor patients whose blood showed a fair number of asexual malarial parasites were selected for the purpose in the Carmichael Hospital for Tropical Diseases. They included 7 cases of *P. falciparum*, 6 cases of *P. vivax*, 1 case of *P. malariæ* and 2 cases of mixed infection (*P. falciparum* and *P. vivax*). Four-hourly temperature charts were maintained and thick blood films, stained by Field or J.S.B. technique, were examined twice daily until the blood became negative for asexual parasites, and then only once daily throughout the period of stay in the hospital unless there was a recurrence of fever or reappearance of parasites when blood was examined twice daily again.

The distribution of patients according to race, sex and age is shown in Table I:—

TABLE I.

Race, sex and age of patients.

Infection.	Race.			Sex.		Age in years.		
	Bengalee.	Other Indians.	Non-Indians.	Male.	Female.	Up to 12.	13 to 20.	Above 20.
P. falciparum ...	4	2	1	7	0	1	2	4
P. vivax ...	4	2	0	5	1	2	3	1
Mixed (*falciparum* and *vivax*).	1	1	0	2	0	1	0	1
P. malariæ ...	1	0	0	1	0	0	0	1
TOTAL ...	10	5	1	15	1	4	5	7

* Neochin is 'di-cinchonine carbonate', melting point 246–247°C.; almost tasteless white powder; insoluble in water and ether; sparingly soluble in alcohol; highly soluble in chloroform and dilute acids; dextro-rotatory.

DOSAGE.

The drug was administered by mouth in the form of tablets, 5 grains each, two regimes being used, viz. (A) One tablet twice daily for two days and then one daily for the next two days; (B) One tablet three times a day for 4 or 5 days. Regime A was the dosage recommended by the manufacturer but was found to be unsatisfactory and hence changed to B.

RESULTS.

The immediate effect on the temperature and parasites is shown in Table II:—

TABLE II.

Regime.	Serial number.	Infection.	The day of treatment.		Gametocytes first seen.			Observation period (days).	Relapse after completion of treatment (days).
			Temperature became normal.	Asex. parasites became negative.	Before treatment.	During treatment.	After treatment.		
A	1	F	No effect in 4 days; patient became worse; chloroquine was administered to control infection.		—	—	—	22	—
A	2	VF	3	5	+	—	—	9	—
A	3	V	5	5	—	+	—	10	—
B	4	F	3	5	—	+	—	14	—
B	5	F	5	4	—	—	—	38	17
B	6	V	Sulphadiazine and penicillin.	3	+	—	—	48	31
B	7	V	4	3	—	+	—	23	—
B	8	F	No more fever	3	+	—	—	6	—
B	9	V	3	4	+	—	—	10	—
B	10	F	2	4	+	—	—	38	16
B	11	V	5	3	+	—	—	30	—
B	12	F	5	4	+	—	—	37	4
B	13	FV	5	7	+	—	—	27	6
B	14	M	No more fever	4	+	—	—	28	—
B	15	F	5	5	—	+	—	30	9
B	16	V	5	4	+	—	—	30	15

F = *falciparum*. V = *vivax*. M = *malariæ*.

The day-to-day effect of neochin on temperature and asexual parasites is shown in Table III :—

TABLE III.

Regime.	Infection.	Number of cases.	Last day of fever.					Last day of asexual parasite.					
			0	1	2	3	4	1	2	3	4	5	6
A ...	F	1	No effect in 4 days					Patient worse ; chloroquine given.					
	V	1	0	0	1	0	0	0	0	0	1	0	0
	FV	1	0	0	0	0	1	0	0	0	1	0	0
TOTAL ...		3	0	0	1	0	1	0	0	0	2	0	0
Percentage ...		...	0	0	50·0	0	50·0	0	0	0	100·0	0	0
B ...	F	6	1	1	1	0	3	0	0	1	3	2	0
	V*	5	0	0	1	1	2	0	3	2	0	0	0
	FV	1	0	0	0	0	1	0	0	0	0	0	1
	M	1	1	0	0	0	0	0	0	1	0	0	0
TOTAL ...		13	2	1	2	1	6	0	3	4	3	2	1
Percentage ...		...	16·0	8·3	16·0	8·3	50·0	0	23·0	30·8	23·0	15·5	7·7

F = *falciparum*. V = *vivax*. M = *malariæ*.

* One case (No. 6) had lung complications and the temperature became normal after sulphadiazine and penicillin treatment.

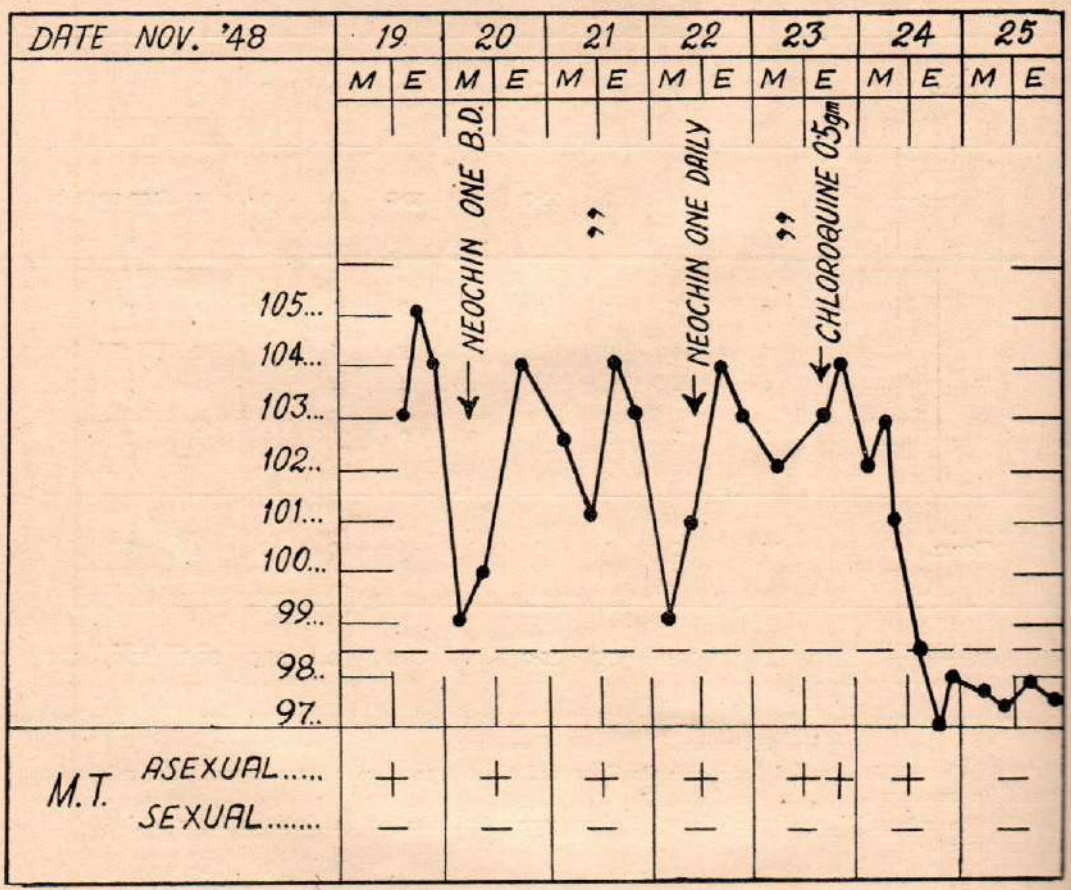
In the column 'last day of fever' 0 day means that the patient had no fever with the institution of treatment.

Only three patients were treated with regime A, of whom one with *falciparum* infection not only failed to respond to it but became very seriously ill with heavy

parasitæmia after the last (fourth) day of treatment when chloroquine was given with prompt recovery. Thirteen patients were treated with regime B. The temperature became normal by the fourth day of treatment in 4 cases and on the fifth day in 6 cases. Two patients (Nos. 8 and 14) had no fever with the institution of treatment while in another (No. 6) the fever persisted owing to lung complications. The asexual parasites were cleared of the peripheral blood in 3 to 7 days (on the third day of treatment in 4 cases and on the 4th day in 6 cases). They persisted after completion of treatment in 3 cases (fifth day in 2 cases and seventh day in one).

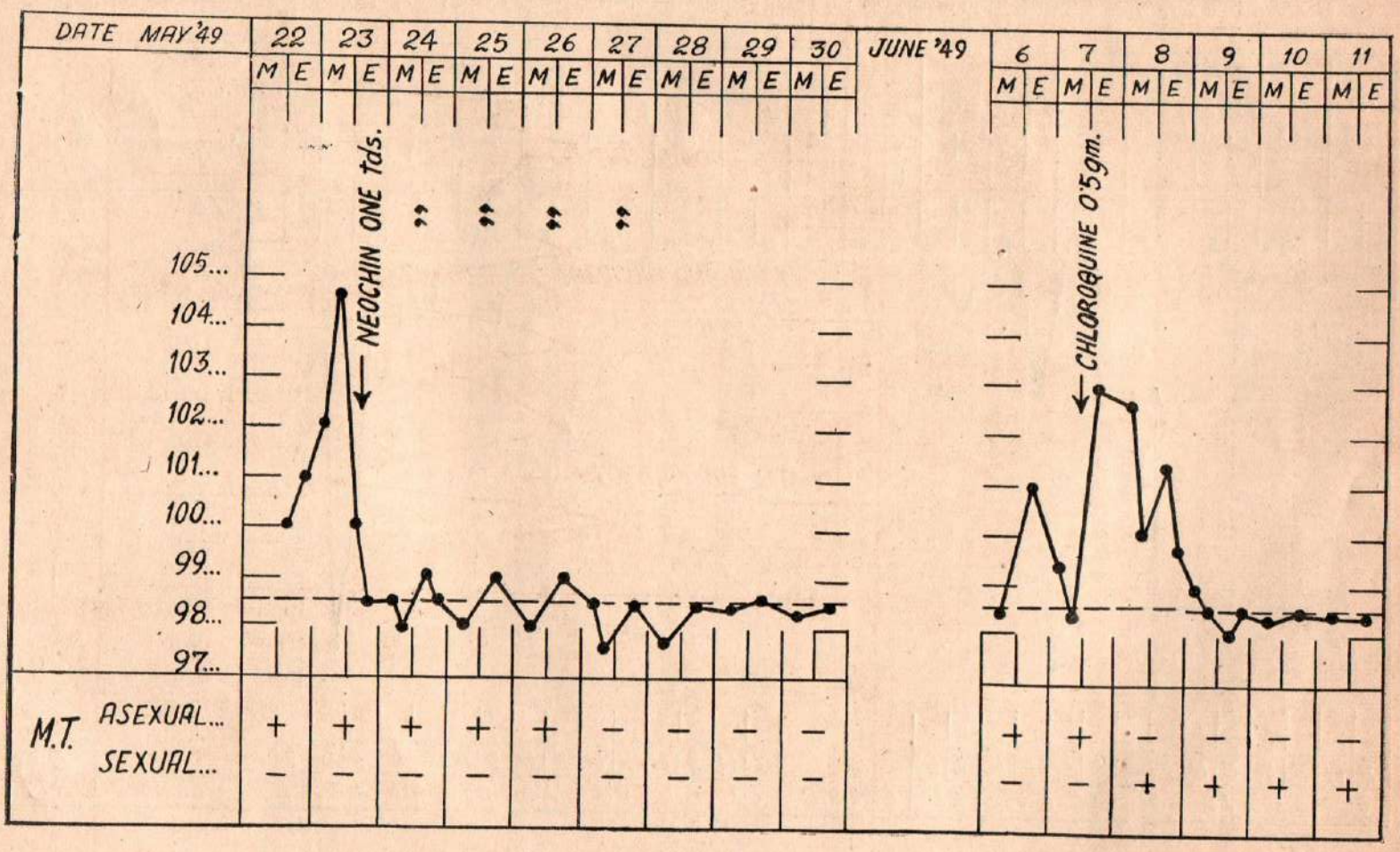
The average period by which the temperature became normal and the parasites disappeared from the peripheral blood with regime B was 3·5 and 4·1 days. The clinical response is illustrated in Charts 1 to 3.

CHART 1.



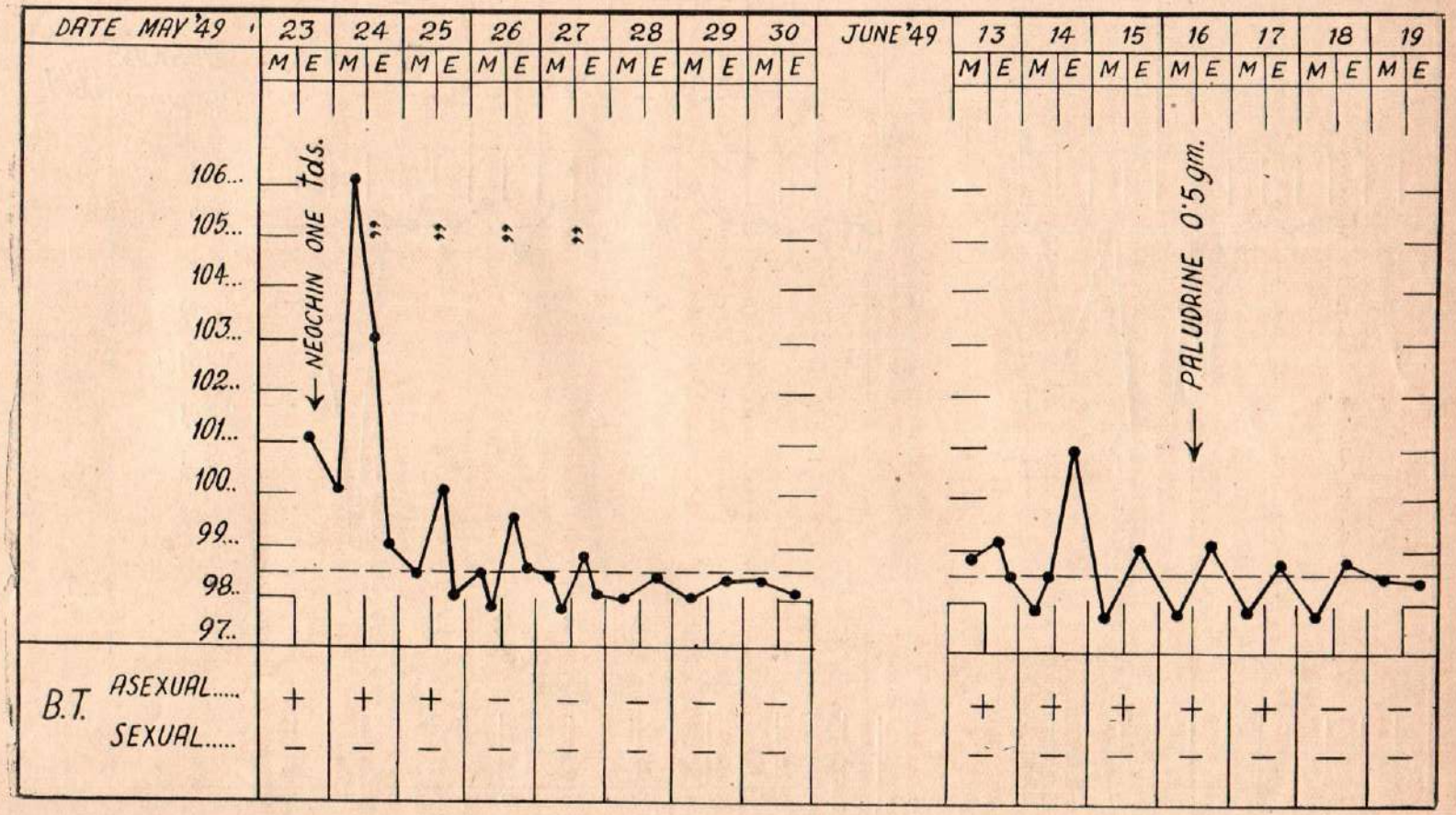
Case 1.—A case of *falciparum* malaria failed to respond to regime A. Prompt recovery with a single dose of chloroquine 0·5 gm.

CHART 2.



Case 15.—A case of *falciparum* malaria. Recrudescence 9 days after completion of treatment.

CHART 3.



Case 16.—A case of *vivax* malaria relapsing 15 days after completion of treatment.

The effects on *P. falciparum* and *P. vivax* cases are shown in Table IV :—

TABLE IV.

Infection.		Last day of fever.					Last day of asexual parasites.				
		0	1	2	3	4	1	2	3	4	5
6 *falciparum* cases.	Number ...	1	1	1	0	3	0	0	1	3	2
	Per cent ...	16·7	16·7	16·7	0	50·0	0	0	16·7	50·0	33·3
6 *vivax* cases	Number ...	0	0	2	1	2	0	3	2	1	0
	Per cent ...	0	0	40·0	20·0	40·0	0	50·0	33·3	16·7	0

RELAPSE OR RECRUDESCENCE.

Eleven out of 13 patients treated with regime B could be kept under observation in the hospital for 14 to 48 days. Seven (63·7 per cent) of them relapsed (fever as well as homologous parasites in the blood) 4 to 31 days after completion of treatment (5 *falciparum* and 2 *vivax* cases) when they were given different antimalarial drugs.

CONCLUSION.

The small number of cases would not justify in drawing a conclusion beyond stating that neochin in recommended dosage is unsatisfactory for treatment of malaria. Even regime B with double the dosage appears to be inadequate. Over 60 per cent cases observed for 2 to 7 weeks relapsed after an average interval of 14 days after completion of treatment. Tablets were given even in presence of high fever; no obvious toxic symptoms were encountered.

ACKNOWLEDGMENT.

The authors wish to record their thanks to the Indian Research Fund Association on whose behalf the above investigation was carried out, and the Director, Malaria Institute of India, Delhi, for the supply of a part of neochin tablets received by him from the Indian Drugs and Chemical Industries, Calcutta.

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FALCIPARUM INFECTION REFRACTORY TO PALUDRINE.

BY

R. N. CHAUDHURI, M.B., M.R.C.P., T.D.D.,

AND

M. N. RAI CHAUDHURI, M.B.

(*Department of Tropical Medicine, School of Tropical Medicine, Calcutta.*)

[November 8, 1949.]

RECENTLY attention has been drawn to cases of malaria which failed to respond to paludrine. Chaudhuri (1948) reported a case of *falciparum* infection treated with this drug, 0·3 gm. daily for ten days; the patient had a second attack nine days after the completion of treatment, and a third, eleven days after the second course was completed. He also encountered a patient (unpublished report, 1948) with heavy *falciparum* infection, who died despite intensive intravenous therapy with paludrine; the autopsy showed intense proliferation of parasites in internal organs, which was uninfluenced by the drug though they had been considerably reduced in the peripheral blood. It should however be mentioned that this was a case of overwhelming infection in an old weak man of 75 years. Covell *et al.* (1949) reported failure of paludrine to effect radical cure of infection with the West African (Lagos) strain, a fact which is in marked contrast with the New Guinea strain as observed by Fairley (1946). In the circumstances, the following case, in which a strain of *P. falciparum* did not respond to paludrine, is worth reporting.

CASE REPORT.

A boy, aged 12, was admitted to hospital on April 29, 1949, with history of high remittent fever and intense headache for three days. The onset was sudden; there was no rigor or vomiting. The temperature was said to be very high on the second day when, it was stated, he was almost unconscious for about a couple of hours.

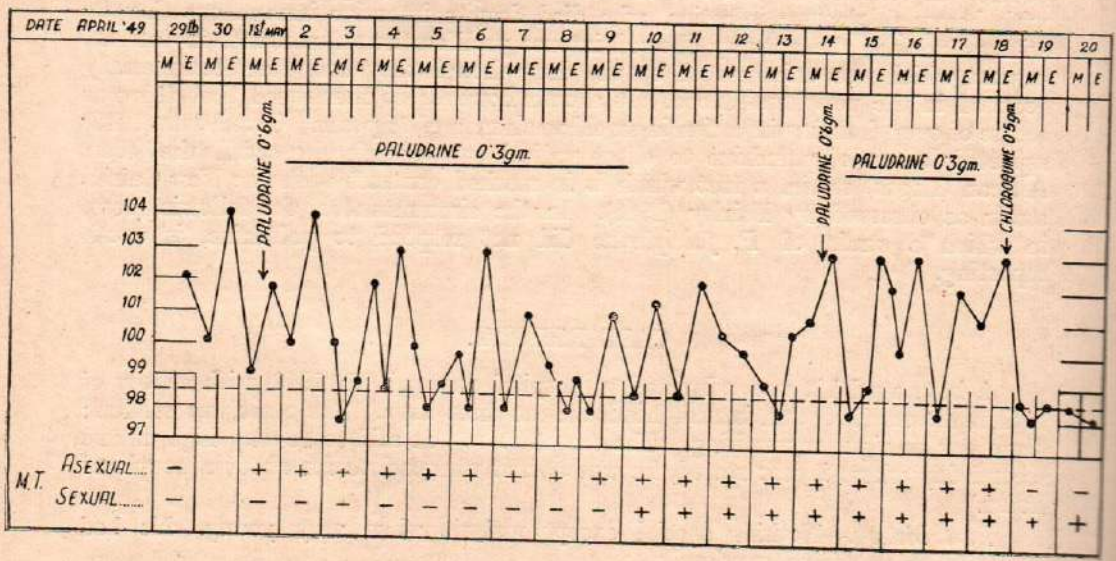
Past history.—Nothing special except malaria when very young. He had never taken paludrine previously.

On admission.—The temperature was 102°F., pulse rate 120 and respiration 24 per minute. The patient was drowsy but conscious; headache was very severe, photophobia present. Liver enlarged, one inch below the costal margin, spleen was not palpable. Chest—no abnormality detected. Blood examination showed no malarial parasites. The white cell count was 9,150 per c.mm. with neutrophils 80 per cent and lymphocytes 20 per cent. Urine showed no abnormality. Lumbar puncture—clear fluid under normal pressure.

The day after admission, the temperature rose high but the general condition of the patient was fair. Blood was sent for culture while only palliative treatment was given.

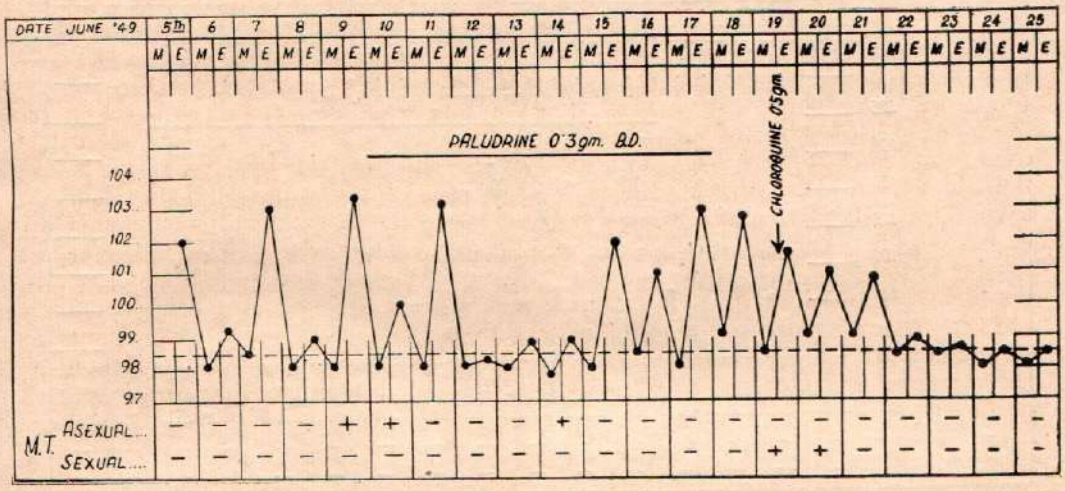
On the following day, i.e. May 1, another blood smear was examined, and M.T. rings were found. He was then given paludrine 0·6 gm. by mouth followed by 0·3 gm. daily for the next eight days, i.e. 3 gm. in nine days. There was no effect on the fever or parasites, although the general condition was in no way bad. M.T. rings were found in the blood daily and also crescents from the 10th day onwards. Both blood and urine gave a strongly positive reaction for paludrine. After four days, i.e. on May 14, paludrine was repeated, 0·6 gm. on the first day followed by 0·3 gm. daily for the next 3 days but still without any effect. This made the authors consider that the particular strain of parasite might be resistant to paludrine. For further study of the effect of paludrine on this strain, a volunteer was inoculated with the patient's blood and then the patient was given a single dose of chloroquine 0·5 gm. (Chart 1). The response was immediate. Three days after the inoculation the volunteer was purposely given paludrine 0·3 gm. daily for 4 days. Nevertheless, he developed fever on June 5, i.e. 17 days after the inoculation and later blood

CHART 1.



showed M.T. rings. He was again put on paludrine 0·3 gm. twice daily but without effect on fever even after 8 days when it was discontinued. Both blood and urine gave a strongly positive reaction to paludrine. One day after stopping paludrine, i.e. on June 19, he was given chloroquine 0·5 gm. after inoculating another individual with his blood. There was a positive response to chloroquine (Chart 2) though not

CHART 2.



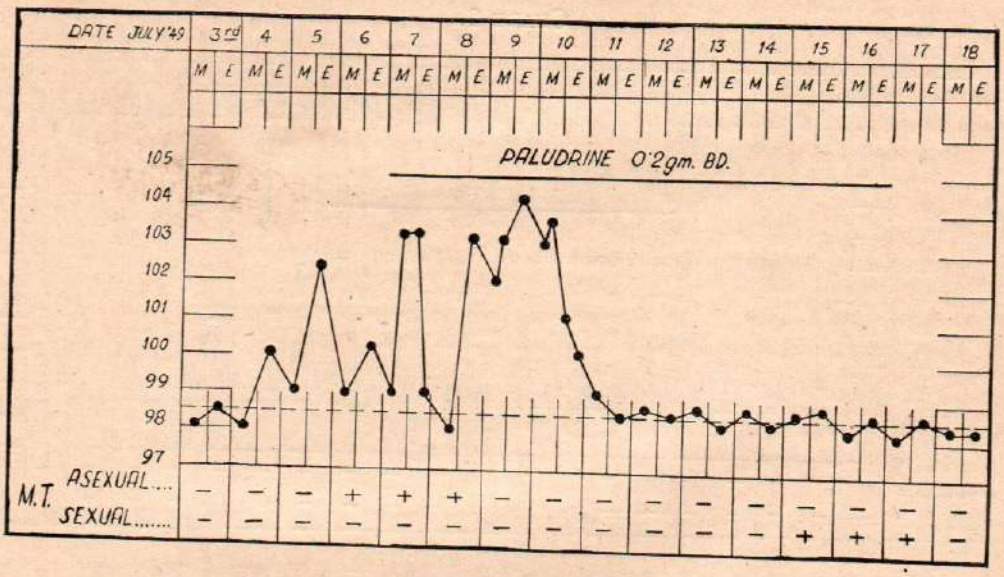
so striking as in the previous case. The second volunteer was a boy of 10 years suffering from tropical eosinophilia but responding poorly to acetylarsan injections. He had had 6 injections of acetylarsan 3 c.c. each with very little effect on symptoms and eosinophilia. Incidentally it may be mentioned that the authors had previously treated three such cases with malaria therapy with satisfactory response. This patient developed malaria 15 days after the inoculation when he was put on paludrine 0·2 gm. twice a day for ten days. Parasites disappeared from the peripheral blood two days after and fever four days after starting the treatment (Chart 3).

DISCUSSION.

The case illustrates a rare instance of natural resistance of a strain of *falciparum* parasite to paludrine. The drug also failed to cure the infection induced by inoculation of a volunteer with trophozoites. The possibility of drug inertness was excluded by treating successfully several other cases with tablets from the same container. There was no question of failure of absorption as both blood and urine gave strongly positive reaction. It may be due to the particular strain of parasite, or there may be other possible factors such as lack of immunity or non-formation of the proper metabolite in the body on which the antimalarial activity of paludrine

depends. The positive response in the second inoculated case might be due to more effective host and drug relationship, or to attenuation of the parasite through passage, or probably to arsenical injections, which the patient had both before and after inoculation with the infected blood.

CHART 3.



Several reports have appeared from Africa of failure of paludrine but in our experience they have been rare. Experimentally, irreversible paludrine resistance has been developed in chicks infected with *P. gallinaceum* (Williamson and Lourie, 1947; Bishop and Birkett, 1947, 1948; Bishop and McConnachie, 1948) and in monkeys (*P. cynomolgi*) by Hawking and Perry (1948). Thompson *et al.* (1948) observed that resistance to paludrine could be developed also in *P. lophure* but so far not to chloroquine or camoquin. Cooper *et al.* (Editorial, 1949) have observed human infection to develop paludrine resistance. Blackie (1947) observed that *P. falciparum* (Rhodesian strain) is not suppressed by systematic paludrine prophylaxis. In studies on paludrine prophylaxis among 500 individuals in a hyperendemic rural area of West Bengal with 0·1 gm. bi-weekly or 0·3 gm. weekly, the authors encountered only a small number of persons showing parasites in the blood, mostly without fever. Except in a few non-conclusive cases, no such resistance among the village population kept under prophylactic regimen for six months from July to December 1948, was encountered.

In any case, it is apparent that paludrine has certain limitations which the earlier trials failed to reveal. There are rare strains of parasites with a natural resistance to the drug, and certain others which are less sensitive than in the majority of cases. Its antimalarial action is relatively slow. So until there are further experiences, it is better not to use paludrine alone for serious cases of malaria.

Covell *et al.* (1949) suggests mepacrine or quinine on the first day of treatment followed by the usual course of paludrine in *falciparum* infection. All this, however, shakes the confidence in paludrine especially in the light of the fact that it is not so quite rapid in its action as chloroquine and camoquin, although, in general, it is an effective, cheap and non-toxic drug for treatment and in particular suppression of malaria.

ACKNOWLEDGMENT.

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THE CONTROL OF MALARIA IN A RURAL AREA OF WEST BENGAL.

BY

RADHAKRISHNA BANERJEA, M.B., B.S., D.T.M., D.P.H.

(All-India Institute of Hygiene and Public Health, Calcutta.)

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INTRODUCTION.

FAIRLEY (1946) demonstrated the schizonticidal effect of paludrine and its ability to resolve overt attacks of *falciparum* or *vivax* malaria in non-immune volunteers when administered as a single dose of 100 mg. or more. He suggested a single dose regimen instituted at weekly intervals for controlling overt malaria and malaria in populations with premunity.

The present experiment was conducted in a rural area of West Bengal which is an endemic home for malaria, with the object of finding out :—

(*i*) Whether a dose of 100 mg. paludrine administered once a week to persons living in an endemic area during a malaria season would reduce the incidence of malaria fever cases.

(*ii*) If a single weekly dose is not sufficient to control fever incidence, whether bi-weekly administration would yield better results.

(*iii*) What dosage is necessary to effect cure of malaria and prevent relapses.

(*iv*) Whether paludrine is effective as a causal prophylactic and, if so, what dosage is to be used for people in endemic areas.

The experiment was begun in November 1946 and was continued till February 1947, the period being the later part of the malaria season of 1946-47, and then it was taken up during the following spring malaria season from March to May 1947. Some tentative conclusions were drawn and these were further tested for corroboration in the following malaria season comprising the period August 1947 to January 1948.

For the experiment proper, a village was chosen near Singur in the Hooghly District, having a population of 406 persons. Another village, for comparison, was chosen in an adjoining area, having features somewhat similar to the experimental village with regard to malaria and having a population of 308 persons. A survey of the experimental village was conducted in November 1946 and family lists giving particulars of name and age of individual members were prepared.

393 persons in the village at the time were available for examination in the first survey. Their blood was examined for malaria parasites and spleen for enlargement, the extent of enlargement being measured in finger breadths below costal margin. A similar survey of the control village was conducted at the same time, where only 295 persons were available for examination in the survey.

The results of these surveys are given in Table I.

TABLE I.

		Experimental area.	Comparison area.
Group A	(1) Parasite positives with enlarged spleen ...	16	6
	(2) Parasite positives without enlarged spleen	6	2
Group B	Parasite negatives with enlarged spleen ...	171	142
Group C	Parasite negatives without enlarged spleen ...	200	145
TOTAL PARASITE POSITIVES		22 (5·6 per cent)	8 (2·7 per cent)
TOTAL ENLARGED SPLEEN		187 (47·6 per cent)	148 (50·2 per cent)

All the above groups have been used to note reduction of parasite rate, spleen rate and size of the spleen of the entire population. But as group A are positive cases and group B comprise individuals who were previously infected as shown by the enlargement of the spleen, these two groups have been excluded for study in causal prophylaxis.

STUDIES ON THE EFFECT OF A SINGLE DOSE OF PALUDRINE AT WEEKLY INTERVALS.

For this, each family in the experimental village was visited on a specific day in the week and all the adults were administered one tablet of 100 mg. of paludrine, children between 2 and 12 years of age half a tablet each, and infants below 2 a quarter of a tablet each. On other days of the week, house-to-house enquiries were made for fever cases, temperature was recorded of such cases and blood was examined for malaria parasites. The parasite positive cases were treated with paludrine according to methods described under the heading 'treatment'. In the comparison area mepacrine was administered to actual cases only, the dosage being two tablets of 100 mg. per day for adults, 50 mg. twice a day for children between 2 and 12 years of age, 25 mg. twice a day for infants below 2 years, till the fever stopped. For studying the effects of a weekly dose of paludrine, the period utilized was middle of November 1946 to end of malaria season in the second week of February 1947. The prolonging of the malaria season up to the middle of February is ascribable to the delayed rainfall in 1946. This helped us in observing the effect of the drug for full three months.

After the observation period of weekly administration of paludrine was over, a final survey was made. Out of a total of 393 individuals in the experimental

TABLE II.

Spleen and parasite rates in experimental and comparison villages.

	Spleen rates.										Parasite rates.		
				Description of enlargement of spleen.									
	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Palpable.	1F.	2F.	3F.	4F.	5F.	U.	BU.	Number examined.	Number with malaria parasites in blood.	Parasite rate, per cent.
A. Experimental village.														
Initial survey ...	349	170	48·7	29	30	36	35	28	1	8	3	349	22	6·3
Final survey ...	349	82	23·5	36	6	14	12	9	1	2	2	349	5	1·4
B. Comparison village.														
Initial survey ...	260	133	51·2	85	7	22	9	5	2	1	2	260	7	2·7
Final survey ...	260	61	23·5	45	4	6	3	1	1	1	0	260	2	0·8

village who offered themselves for the first survey, there were some who left the village just after the survey and others who did not take the drug regularly or did not take it at all. After the necessary exclusions, there were 349 individuals left in the experimental village, during the three months, who presented themselves for both the surveys. Similarly in the comparison village 260 individuals presented themselves for both the surveys. The results of the two surveys after the above deductions are given in Table II.

So far as the results of the two surveys show, there is not much improvement in the experimental village as a result of weekly single dose administration of 100 mg. of paludrine to all persons when compared with the comparison village where only malaria cases were treated with mepacrine.

The lowering of the spleen and parasite percentages in the final survey in the comparison area is due to the fact that malaria season came to a close during the final survey. If weekly paludrine had any effect on malaria prophylaxis, the corresponding figures in the experimental area would have shown more decrease than in the comparison area.

A survey at the beginning and another at the end of a malaria season does not give a complete picture of the malaria incidence, so the number of cases that occurred in the intervening period amongst the three groups into which the population was classified originally, namely group A the parasite positives, group B the parasite negatives with enlarged spleen, and group C the parasite negatives without enlarged spleen, are given in Table III. Total numbers dealt with were those present in both surveys.

TABLE III.

Number of malaria cases during the malaria season—middle of November 1946 to middle of February 1947.

	Total number examined.	Number found parasite positive. Group A.	Number found parasite negative with enlarged spleen. Group B.	Number found parasite negative without enlarged spleen. Group C.
A. Experimental area.				
Number of malaria cases during the malaria season.	349 14	22 1	155 6	172 7
Percentage of malaria cases ...	4·01	4·55	3·87	4·07
B. Comparison area.				
Number of malaria cases during the malaria season.	260 9	7 1	128 7	125 1
Percentage of malaria cases ...	3·46	14·29	5·47	0·80

These results corroborate the conclusions already arrived at, that a single dose of paludrine given once a week, does not materially help in the control of malaria.

STUDIES ON THE EFFECT OF BI-WEEKLY DOSE OF PALUDRINE.

In view of the ineffectiveness of a single weekly dose of paludrine to control malaria, the next problem was to find out an effective dose. It was found by Fairley *et al.* (*loc. cit.*) that 100 mg. dose given 3 hours before exposure or more than 144 hours, i.e. 6 days after exposure, failed to protect a volunteer, and this dose administered 2 to 5 days after exposure, gave protection. Therefore, it is clear that a single dose given weekly leaves a gap period during which a person remains unprotected, when he might contract infection. This explains why, in the present experiment also, a single 100 mg. dose once a week failed to protect all persons. In order to protect fully a man, therefore, a second dose should be given at the latest on the 5th day. Hence it was thought that bi-weekly 100 mg. dose was likely to be more effective than a single weekly dose. So, this method was adopted in the following spring malaria season, when two consecutive doses were given with an interval either of three or four days.

Spring malaria cases cropped up at the end of March 1947 and a survey preliminary to the administration of paludrine was made in the last week of that month. Bi-weekly administration of paludrine went on from the last week of March to the end of the first week of May and again a survey was made. The administration of paludrine during spring malaria, however, could not be made continuous. Owing to a temporary suspension of the supply of the drug, it had to be stopped for a brief period of about 6 to 14 days. The results of spleen and parasite surveys before and after the spring malaria season, are given in Table IV.

It is seen that improvement in the spleen figures of the experimental area from initial to final survey is not more than the corresponding improvement in the comparison area. But in the parasite survey, the improvement is statistically significant in the experimental area ; the probability to obtain a rate of 0 in a sample of 326 from a population in which 1·5 per cent are positives, being as small as 0·0065. For the comparison village, no such significant change took place.

With regard to spleen figures, it should be stated that, during the spring, altogether only about 9 tablets were administered which may not have been sufficient to reduce the size of the spleen to a greater extent than due to natural causes on account of spring epidemic coming to a close. This is specially likely to be so when, after taking 5 doses, the persons did not get any paludrine for a period varying from 6 to 14 days. Though there were no parasite positive cases in the final survey in spring in the experimental village, there were several cases there, in the interval between the initial and the final surveys, due to partial cessation of drug administration mentioned above. The relationship of these cases with the number of bi-weekly doses after which these cropped up or the number of subsequent days

TABLE IV.

Spleen and parasite rates before and after the administration of paludrine.

	Spleen rates.											Parasite rates.		
	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Description of enlargement of spleen.								Number examined.	Number with malaria parasites in blood.	Parasite rate, per cent.
				Palpable.	1F.	2F.	3F.	4F.	5F.	U.	BU.			
A. Experimental village.														
Initial survey made in the last week of March; before the administration of paludrine.	326	75	23·0	33	6	14	11	4	3	2	2	326	5	1·5
Final survey made in May; after the administration of paludrine.	326	60	18·4	26	6	9	10	3	1	2	3	326	0	0·0
B. Comparison village.														
Initial survey made in the last week of March.	162	32	19·8	27	1	3	1	0	0	0	0	162	5	3·1
Final survey made in May.	162	20	12·3	14	1	4	0	0	0	1	0	162	4	2·5

of non-administration of paludrine after which the cases appeared, are given in Table V.

TABLE V.

Relation of parasite positive cases with the number of bi-weekly doses.

Species of parasite.	Relationship of the case with the number of bi-weekly dose and period of non-administration of drug.
1. Benign tertian ...	After 2 bi-weekly doses followed by 10 days' gap.
2. Malignant tertian ...	,, 5 ,, ,, ,, 10 ,, ,,
3. Malignant tertian ...	,, 5 ,, ,, ,, 10 ,, ,,
4. Malignant tertian ...	,, 5 ,, ,, ,, 6 ,, ,,
5. Malignant tertian ...	,, 2 ,, ,, ,, no gap.
6. Malignant tertian ...	,, 4 ,, ,, ,, 14 days' gap.

It is thus seen that cases may occur after 2 bi-weekly doses. Cases that occurred after more than 2 bi-weekly doses, had gap periods of non-administration of drug for 6 or more days following the last bi-weekly dose. The results indicate that for prevention of cases, the interval between successive administration of the drug should be less than 6 days, and that at least two bi-weekly doses are necessary, in addition, to start with.

FURTHER STUDIES ON THE EFFECT OF BI-WEEKLY DOSE OF PALUDRINE.

The tentative conclusion arrived at from the above work done in the spring season of 1947 that there should be less than 6 days' interval between the administration of two successive doses of drug would naturally lead one to find out what effect a bi-weekly dose of paludrine will produce, when given regularly throughout a malarial season. This was done during the next malaria season comprising the period August 1947 to January 1948.

As the village which was used in the preceding work as comparison did not show malaria incidence and the people at the same time did not co-operate much in the work, a different comparison area was taken in hand in August 1947 which was also near to the experimental village. In this comparison area, the malarial fever cases with parasitæmia, were given quinine sulphate 5 grains thrice a day till the patient became afebrile which generally was not more than three days.

In the experimental area, the dose of paludrine given to the people was the same as before, namely 100 mg. for an adult, 50 mg. for persons of 2 to 12 years of age, and 25 mg. for children below 2 years. But all of them were given the drug twice a week, the interval between the two being either 3 or 4 days.

As usual, a survey at the beginning of the season and another at the end of the season was done both for the experimental and the comparison area and the results are given in Table VI. The figures include only those who consented to be examined for both the initial and the final surveys. Thus the actual figures kept under observation were higher because some persons declined to get examined or were absent in the village in the final survey.

TABLE VI.

Spleen and parasite rates before and after the malaria season comprising the period August 1947 to January 1948.

	Spleen rates.											Parasite rates.		
				Description of enlargement of spleen.										
	Number examined.	Number with enlarged spleen.	Spleen rate, per cent.	Palpable.	1F.	2F.	3F.	4F.	5F.	U.	BU.	Number examined.	Number with malaria parasites in blood.	Parasite rate, per cent.
A. Experimental village.														
Initial survey before the administration of paludrine.	337	126	37·4	65	6	17	19	6	2	9	2	337	18	5·3
Final survey after the administration of paludrine.	337	52	15·4	7	3	13	16	7	2	4	0	337	1	0·3
B. Comparison village.														
Initial survey ...	293	98	33·4	19	14	19	21	6	2	9	8	293	30	10·2
Final survey ...	293	137	46·8	24	11	33	31	14	0	14	10	293	30	10·2

It will be seen that there is definite improvement in the enlarged spleen figure in the experimental area, from 37·4 per cent it came down to 15·4 per cent, a figure much lower than any of the previous final survey figures. In the comparison area, the figure instead of improvement shows deterioration, as is to be expected in an endemic area during this period. The parasite incidence also showed pronounced improvement in the experimental area, and this improvement was found to be statistically significant, though in the comparison village the parasite incidence was the same in final survey as in the initial survey.

In the experimental area, the people co-operated in the preventive work readily and rather eagerly in this particular season, because just after the initial survey was over, and before any drug administration could be taken up, there was a sudden flare up of malaria. A delay of about 20 days in drug administration after the initial survey was caused due to disturbed conditions in Delhi City wherefrom the drug was being mainly supplied. During these 20 days, there were as many as 32 cases of malaria as against only 14 cases within a long period of about 6 months of drug administration (Table VII).

TABLE VII.

Number of malaria cases during 20 days of non-administration of drug.		Number of malaria cases during 6 months of drug administration.	
Malignant tertian	29	Malignant tertian	5
Benign tertian	3	Benign tertian	8
		Quartan	1

The cases were immediately treated and made parasite free and then taken for prophylactic experiment whereas the non-infected persons were at once taken up for prophylaxis. The value of preventive work was more realized by the people of the locality, because whereas in their village very few cases cropped up during the administration of the drug, the surrounding villages were full of malaria cases. This was coincident with a high incidence of malaria in the whole of Hooghly District this year when compared with the previous year. This fact is evident from the number of new malaria cases registered at the outdoor dispensary of R. N. Mullick Hospital, Singur, for two consecutive seasons (Table VIII).

TABLE VIII.

Number of new malaria cases registered in the R. N. Mullick Hospital Outdoor Dispensary, Singur, 1947.

	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.
Malaria season of 1946-47	474	389	490	504	507	452
Malaria season of 1947-48	712	1,384	1,605	1,066	660	529

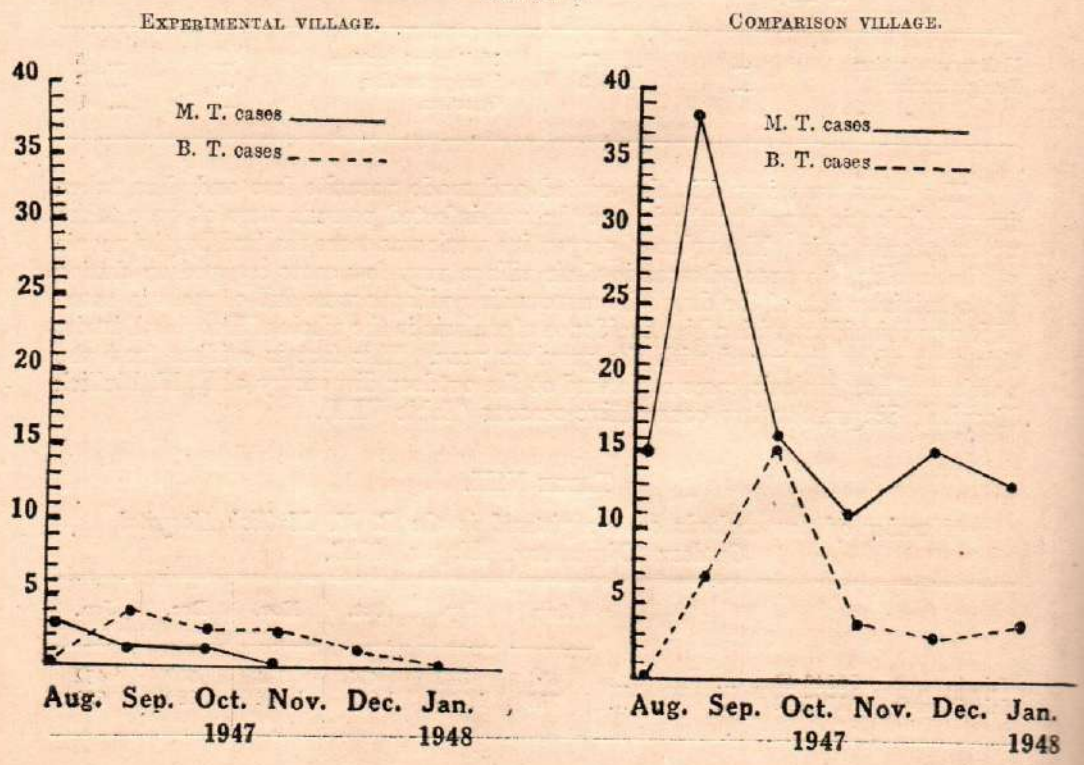
The incidence of malaria, both in the experimental and in the comparison area, during the observation period in 1947-48, is given in Table IX and Chart 1, month by month, and according to the species of malaria infection.

TABLE IX.

Malaria cases during the period August 1947 to January 1948.

	Experimental village.							Comparison village.						
	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	TOTAL.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	TOTAL.
M.T. ...	3	1	1	...	...	...	5	15	38	16	11	15	13	108
B.T. ...	...	3	2	2	1	...	8	...	7	15	4	3	4	33
Quartan	...	...	...	...	1	...	1	...	...	2	1	...	...	3
TOTAL ...	3	4	3	2	2	0	14	15	45	33	16	18	17	144

CHART 1.



Total number of persons under observation were 364, both in the experimental and in the comparison area. In the experimental area, the number represents the total population available at the beginning of the experiment under consideration. In the comparison area, the houses were distributed, more or less in a line, and the number of houses taken along the line were such as would give approximately the same number of persons as in the experimental area. After excluding those persons who were absolutely unwilling to present for examination, the number left behind became by chance exactly 364.

In the experimental village, out of 3 M.T. cases in August, one developed fever after one dose and two after two doses of paludrine. In spite of paludrine administration, there was one M.T. case in September as also one in October. The latter had two relapses during December. There were two more M.T. cases which are not shown above, because they did not take the drug regularly, one having refused to take any paludrine for 10 days and another for 17 days. It may be concluded that excepting the two cases in September and October, amongst those who took regularly paludrine bi-weekly in the prescribed dosage, none else developed M.T. infection after more than two bi-weekly doses. The B.T. cases which began to crop up in September did so in spite of prolonged bi-weekly administration of the drug and were more in number than the M.T. cases. There was a case of quartan malaria developing, in spite of paludrine administration, shown in the table and another not shown there, because the person in question, a child, vomited out the drug very often just after ingestion.

In the comparison village, the incidence of malaria was quite high. With regard to the comparative incidence of the three species of malaria parasites, the number of M.T. cases, instead of being lower than B.T. cases as in the experimental area, is on the other hand more than three times the number of B.T. cases. This corroborates the fact that paludrine is more efficacious in suppressing M.T. cases than B.T. cases.

TREATMENT OF CASES.

Though the main purpose of this enquiry was to find out how to prevent malaria with paludrine, many overt cases of malaria had to be dealt with from time to time ; for example, when dosage was insufficient or when there were cases occurring just before malaria control work was taken up in the early part of a malaria season. Such cases were treated with paludrine in preference to quinine or mepacrine to get some experience of the curative value of paludrine in a malaria endemic area like the present one.

In the earlier part of the experiment when only weekly doses of paludrine were being given to everybody, and later in the spring malaria season when due to want of supply, bi-weekly doses of paludrine were stopped for 6 to 14 days, several malaria cases cropped up. No extra dose of paludrine was given to such cases at the beginning to see how they behaved with the usual dose of paludrine given to everybody. In 15 out of 38 parasite positive malaria cases, the fever and parasitæmia disappeared in spite of not increasing the single weekly dose that used to be given at the time. But there were many cases which did not respond to the usual dose and these had to be given one dose every day till the fever subsided. There were 11 such cases and required the daily dosage from 3 to 5 days. But,

4 out of these 11 cases relapsed, in spite of the usual weekly doses that followed; the number of such weekly dose after which the relapse occurred being 2, 4, 5 and 6 respectively. With a view to prevent such relapses in all fever cases, in 10 such cases, which developed during either the autumnal malaria or in the following spring of 1946-47, the dose was further increased to 3 tablets per day. The number of days of treatment which these cases required to become free from fever, were one day in 5 cases, two days in 1 case, three days in 1 case, four days in 2 cases and six days in 1 case. In the last case, though the fever subsided on the second day, relapse occurred for a day each on the third and sixth day before fever was completely stopped. In this series, therefore, excepting the last case, fever was controlled after 4 days of administration of 300 mg. paludrine per day. All the above cases were M.T. infections.

There was an opportunity to give a further trial of paludrine, three doses a day, to 46 frank malaria cases, 32 of which developed within 20 days before the bi-weekly administration in the malaria season of 1947-48 and the remaining 14 cases occurring earlier but after the final spring survey. Out of these 46 cases, 38 were M.T. infections and 8 B.T. infections. These were made afebrile after administration of paludrine three times a day for a period ranging from 1 to 7 days in different cases (Table X).

TABLE X.

Malaria cases made afebrile after one to seven days of paludrine administration.

Species of malaria parasites.	After (days)						
	1	2	3	4	5	6	7
Benign tertian	0	1	0	1	1	1	4
Malignant tertian	4	3	4	6	8	6	5
TOTAL ...	4	4	4	7	9	7	9

Thus the number of days required to make M.T. cases afebrile varied from 1 to 7 days, while for B.T. cases the period ranged from 2 to 7 days. The average number of days for the two types, however, did not differ to the extent to declare it statistically significant.

CAUSAL PROPHYLAXIS.

A causal prophylactic drug is one which after the occurrence of infection prevents parasites appearing in red blood corpuscles by virtue of its action either on sporozoites or on a stage existing between sporozoites and the blood parasites. Paludrine however has been shown not to have any action on sporozoites. But it may be causal prophylactic drug acting on the stage intermediate between the

sporozoites and the blood parasites which is known as pre-erythrocytic form. Thus in a healthy person, taking the drug regularly in sufficient dosage, malaria may not develop in spite of the exposure to bites by infected mosquitoes. The experimental area in this work being situated in the midst of a malaria endemic place, the people living here are constantly exposed to the bite of infected mosquitoes. Again, in this experiment, measures against control of malaria were strictly limited to antimalarial drug administration, no measures against adult mosquitoes or mosquito larvæ being taken. So when it is found that in spite of prophylactic dose of the drug cases of parasitæmia are coming up, it may be said that the particular dose used is not sufficient for causal prophylaxis.

One difficulty about persons living in a malaria endemic area like the present one, being used for causal prophylactic experiment, is that many of them will be already infected before the experiment is started. Besides the persons showing evident parasitæmia, those possessing enlarged spleen should be excluded from consideration for the present purpose, though even after this it may not be said for certain that all the rest would be free from malarial infection. The most suitable persons for the purpose will however be newborn babies and those of them who, before they could get infection, were given regular doses of paludrine. So, after dealing with the prophylactic action of the drug on adults who did not give evidence of infection like parasitæmia, fever, or enlarged spleen just before the malaria season, the prophylactic action will be studied in babies.

CAUSAL PROPHYLAXIS IN ADULTS.

(1) *Weekly one dose.*—During the three months' period, middle of November to middle of February 1947, out of the persons having no parasitæmia and spleen enlargement at the initial survey, 170 took paludrine regularly but once a week. Out of these, 8 persons developed parasitæmia and fever during the period of their taking the drug (Table XI).

TABLE XI.

Person number.	Number of weekly doses after which parasitæmia developed.	Species of parasite.
16/2	4	M.T. ring.
39/4	5	M.T. ring.
59/2	14	M.T. ring.
61/2	6	M.T. ring and crescent.
62/2	9	M.T. ring.
62/4	5	M.T. ring.
62/3	6	M.T. ring.
65/4	7	M.T. ring and crescent.

(2) *Bi-weekly one dose.*—During the period from the last week of March 1947 to the end of second week of May 1947 when bi-weekly doses of paludrine were being given, cases occurred mostly after the period of non-administration of drug for 6 to 14 days as mentioned already. Hence, these are not considered here. Better observation however was possible in this regard during the next autumnal malaria of 1947-48. During this period, amongst 218 persons who did not show any parasites in the blood or enlargement of spleen in the initial survey and regularly took bi-weekly doses of paludrine thereafter, seven cases occurred (Table XII).

TABLE XII.

Person number.	Number of bi-weekly doses after which parasitæmia developed.	Species of parasites.
7/6	1	M.T. ring.
18/1	22	B.T. ring.
19/6	33	B.T. ring and gametocytes.
27/2	9	M.T. ring.
28/4	2	M.T. ring.
63/4	14	B.T. ring.
69/1	6	B.T. ring.

Regarding the M.T. cases amongst these persons, excepting person No. 27/2, none developed malignant malarial infection after more than two bi-weekly doses. The B.T. infections however occurred after as long as 6 to 33 bi-weekly doses.

CAUSAL PROPHYLAXIS IN NEWBORN BABIES.

The infants born in the village either after the first survey was taken up in November 1946, or within two months before its commencement, were 12 in number. All these babies got the drug at a dose of 25 mg. bi-weekly. Out of these, one did not get the drug before he got the infection due to the parents not allowing. Out of the remaining 11, four were born one to two months before the first survey. Of these 4, three took the drug regularly once a week in the autumn of 1946-47 and bi-weekly in the spring of 1947 as well as in the autumn of 1947-48 and they did not have any malaria. One was present in the village and took the drug only during the spring malaria of 1947 and did not show malaria during this period. Out of the remaining 7, one who was born two months before spring malaria, took the drug bi-weekly in the spring as well as in the following autumn of 1947-48 and did not have the disease. The remaining 6 took bi-weekly doses in the autumnal malaria of 1947-48 and did not suffer from malaria. Thus there was not a single case amongst the 11 babies that took the prophylactic dose of the drug regularly at a dose of 25 mg. bi-weekly.

As a contrast it may be mentioned here that amongst 131 babies of a few days to 6 months of age in the Singur Health Centre area, there were 5 M.T. cases and 10 B.T. cases during the malaria seasons of 1947 and 1948.

DISCUSSION.

The results of the experiments show that under the conditions existing in rural areas of West Bengal, paludrine taken bi-weekly will prevent the occurrence of most of the M.T. infections and a considerable portion of B.T. infections. Thus, a person visiting such a malarious locality for a short period of a few days, will get reasonable protection from malignant malaria, and the chance of getting benign tertian or quartan infection will be very much lessened provided he begins to take paludrine bi-weekly at least one week before the visit, finishing two bi-weekly doses during that week.

But is it worth while to take up malaria prophylaxis in the villages by paludrine administration in a mass scale ? This question will be considered from two points of view : firstly, the amount of benefit to be derived out of it, and secondly, the cost that it will entail on the health department.

With regard to the first point, it cannot be said at the present moment from the evidence obtained in this work that paludrine will stop the occurrence of all malaria cases. In a place where benign tertian cases predominate, a number of such cases are likely to develop in spite of paludrine administration. The same can probably be said of quartan infections, but considering the very small number of such cases dealt with in this paper, it is better to reserve judgment on this point. As regards malignant tertian infection, it can definitely be said that most of these cases, though not all, may be prevented. So, it appears that if complete freedom from malaria is to be achieved, then over and above drug prophylaxis, anti-mosquito and antilarval measures must be taken as supplement.

Though the benefit is limited, still the scheme is worth while, if the cost is inconsiderable. Therefore, the financial implications of the measure may be considered. For prophylactic use of paludrine, it will have to be administered to the population for the whole of the main malaria season of the year from July to January which approximates 30 weeks and for the whole of the spring season from middle of March to middle of May which will be about 10 weeks. For these 40 weeks, 80 rounds of paludrine administration will have to be undertaken. For a population of 500, having 30 per cent persons under the age of twelve, 425 tablets per round will be required and this means 34,000 tablets will have to be used per year at an approximate cost of Rs. 680 a year for the drug, calculating at the rate of Rs. 20 per 1,000 tablets. This means the cost of drug at the rate of Re. 1-5-9 *per capita* per annum. To it will be added the expenditure on personnel. This is too much when we consider the Indian Health Survey and Development Committee's (1946) proposal of Re. 1-3-11 *per capita* per annum in the first five years of the plan recommended by them to meet the expenditure of all the health activities.

SUMMARY AND CONCLUSIONS.

1. A weekly dose of 100 mg. of paludrine, administered to persons living in a malaria endemic area, is not sufficient for reducing malaria fever incidence amongst

the population. For more efficient prevention, the time interval between the two consecutive doses should be less than 6 days. Bi-weekly administration of paludrine at a dose of 100 mg. for an adult has proved to be a suitable procedure for getting definite preventive value of the drug.

2. The prophylactic value of paludrine is not as effective against benign tertian and probably also quartan malaria as against malignant tertian infection, though the incidence of the benign tertian cases is kept very much lowered under paludrine regime.

3. For treatment of malaria in a rural area, a dose of 100 mg. paludrine given thrice a day was found to be effective. But it took 1 to 7 days to control malignant tertian malaria and 2 to 7 days in the case of benign tertian infection.

4. For causal prophylaxis in newborn babies, 25 mg. given bi-weekly proved to be quite effective in 11 babies under observation. In adults, amongst 218 spleen negative and parasite negative persons, 100 mg. paludrine given bi-weekly proved an efficient causal prophylactic against malignant tertian malaria after at least two such doses, though not so effective against benign tertian infection.

ACKNOWLEDGMENTS.

The writer is grateful to Lieut.-Colonel C. K. Laksmanan, Director, All-India Institute of Hygiene and Public Health, at whose initiative this work was undertaken, and who all along during the enquiry took keen interest into minute details and helped with advice, supply of personnel and medicine. The writer's grateful thanks are also due to Dr. K. V. Krishnan, Professor of Microbiology, for his valuable advice and guidance and to Dr. C. Chandrasekharan, Professor of Statistics, for his help in the preparation of the plan of the work in collaboration with Professor K. V. Krishnan, and to Dr. N. C. Mullick, who helped in the field work in the control area in the early part of the work. The author's special thanks are due to Mr. K. K. Mathen for statistical treatment of the paper.

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Indian Journal of Malariology, **3**, 4, December 1949.

PRELIMINARY INVESTIGATIONS ON THE CHEMOTHERAPEUTIC ACTIVITY OF ATEBRIN, PALUDRINE, RESOCHIN, CAMOQUIN, METACHLORIDINE AND APHACRINE ON SIMIAN MALARIA.

BY

LIEUT.-COLONEL JASWANT SINGH, M.B., Ch.B. (Edin.), D.P.H. (Eng.), D.T.M. & H. (London),

MAJOR A. P. RAY, M.B., B.S.,

AND

MAJOR C. P. NAIR.

(*Malaria Institute of India.*)

[June 30, 1949.]

THE present-day knowledge of the chemotherapeutic values of antimalarials is far from complete. In all preliminary trials of new remedies, tests in animals are usually necessary, and experience has shown that experimentally induced infections in monkeys, birds and other laboratory animals offer a reasonable parallel to the results usually achieved in tests against human malaria. Experiments in animals can be standardized with advantage which cannot easily be realized in clinical trials in human beings. A number of authors (Buttle, Henry and Trevan, 1934; Marshall, 1942; Marshall and Dearborn, 1946; Davey, Curd and Rose, 1946; Hughes and Brackett, 1946; Davey, 1946; Gingrich and Schoch, 1947; Tonkin and Hawking, 1947; Butler *et al.*, 1947; Walker and Richardson, 1948) have worked on the chemotherapy in birds but more extensive work is still required in *simian* malarias.

P. knowlesi was first described as a distinct species of plasmodium by Sinton and Mulligan (1932). This parasite gives rise to a quite benign infection in *Silenus irus* but produces high degree of parasitæmia in *Silenus rhesus* monkeys resembling very acute *P. falciparum* infection in man. After an intraperitoneal inoculation of infective blood, monkeys begin to show the parasites in peripheral blood after an average incubation period ranging from 4 to 7 days and invariably die within 5 to 6 days of an established infection. As the disease is acute with scanty chances of spontaneous cure and the course being more constant, the results of chemotherapy of this infection have thus been far easy to assess.

Chopra and Das Gupta (1934), Chopra, Ganguli and Roy (1935), Nauck (1934) and others have demonstrated the superiority of atebrin over quinine in respect of intensity of action in their laboratory experiments on monkeys infected with *P. knowlesi*. Chopra (1935) studied the effect of tebetren on *P. knowlesi* infection in monkeys and found it to stand between quinine and atebrin in its action. Coggeshall (1930 : 1938) have shown that large doses of sulphonamides of various types exert a profound plasmodicidal effect on *P. knowlesi* but there is no clear-cut overall picture of the effects of these drugs on this infection on account of the different factors, such as varying dosage regimes, routes of administration of infective blood and the time of starting treatment during the course of the disease. By following a standard technique of inoculation, Richardson *et al.* (1946) used the host parasite relationship for quantitative chemotherapeutic studies and showed that sulphadiazine is approximately 175 times as active as quinine.

In the last few years, the authors have been trying a number of indigenous and synthetic remedies to test their suitability for the treatment of malarial infections in monkeys. During these investigations, opportunities were availed of to make a comparative study of the relative effectiveness of a few selected compounds (atebrin, paludrine, resochin, camoquin, metachloridine and aphacrine) on *P. knowlesi* to determine their respective critical dosages for complete cure of blood induced infections and to determine their toxicity, if any. It may be specifically mentioned here that chemotherapeutic studies on these drugs were carried out before the new standardized techniques were applied, and the results achieved have been presented in this preliminary paper for whatever worth they may be. The results obtained in the subsequent work, using standardized techniques, will be published in the near future.

METHOD USED FOR CHEMOTHERAPEUTIC STUDIES.

Selected healthy monkeys of an average weight of 3 to 5 kg. were infected by intraperitoneal inoculations of 2 c.c. blood in 2 per cent citrate saline taken from the saphenous vein of a donor monkey showing a very low infection (500 to 1,000 parasites per c.mm.) of *P. knowlesi*. All experimental monkeys in various batches were inoculated simultaneously with the same dose and strain of parasites.

After inoculation the monkeys were given a diet consisting of vegetables like carrots, spinach, etc., 0·25 lb. of soaked Bengal gram and green fruit like bananas, guavas, pears, etc., and one loaf of bread (*chapatti*) daily throughout the course of the tests.

Examination of blood smears, both thick and thin, taken at 10.00 a.m. and stained with J.S.B. (Jaswant Singh and Bhattacharji, 1944) was made every day. The infection rate of the red blood corpuscles was determined by counting the number of parasitized red cells and the stages encountered per each field of 400 to 500 red blood cells in thin film in the following arbitrary manner. At least 10,000 R.B.Cs. were counted.

'Very very scanty infection' (v.v.s.) means 1 parasitized R.B.C. in every ten or more fields (less than 1,000 parasites per c.mm.). 'Very scanty infection' (v.s.) means 1 parasitized R.B.C. per 5 to 9 fields (1,000 to 2,000 parasites per c.mm.). 'Scanty infection' (s) means 1 parasitized R.B.C. per 2 to 4 fields (2,500 to 5,000 parasites per c.mm.). 'One plus' (+) means 1 to 5 parasitized R.B.C. per field

(10,000 to 50,000 parasites per c.mm.). 'Two plus' (++) means 6 to 10 parasitized R.B.C. per field (60,000 to 100,000 parasites per c.mm.). 'Three plus' (+++) means 11 to 25 parasitized R.B.C. per field (110,000 to 250,000 parasites per c.mm.). 'Four plus' (++++) means 26 to 50 parasitized R.B.C. per field (260,000 to 500,000 parasites per c.mm.). 'Five plus' (+++++) means above 50 parasitized R.B.C. per field (above 500,000 parasites per c.mm.).

The treatment was started as soon as parasites were detected in the peripheral blood (very very scanty infection) and in some cases at a time when the infection was heavy. In each test, at least two monkeys were placed under each dosage regime of the drug under trial.

A chart and a register were maintained wherein the number of each monkey under experiment and the following particulars against each, viz. (1) strain of parasite, (2) date of inoculation, (3) dose and route of inoculation, (4) weight, (5) results of daily blood examination, (6) treatment given, (7) anæmia and other symptoms, and (8) causes of death were recorded.

For purposes of oral administration, the drug under trial, if solid, was either dissolved or suspended in 1 to 3 c.c. water and administered, care being taken to see that the whole of it was swallowed. Treatment as a rule was started with one-third of the dose recommended for a human being of an average weight of 120 lb. in order to find out (1) if the drug in question showed any demonstrable influence on the parasitic multiplication and (2) ability to tolerate such a dose. The treatment was generally continued for 2 to 7 days. In the evaluation of the properties of an antimalarial remedy, the following points were taken into consideration :—

(1) Its immediate effect on the parasite and the host.

(2) Its ability in preventing relapses by effecting a complete cure or sterilization.

In most of the cases where the drug showed no specific antimalarial properties, progressive rise of infection was noticed and the animals usually died along with the untreated controls within a week of showing parasites. In some cases, drugs were exhibited much above the recommended dose without any change in the course of the infection and the animals died subsequently. Further trials in respect of such (so-called) antimalarials were discontinued.

In cases where the drug exhibited any specific action on the parasites, the monkeys either survived death, but still showed a small number of parasites or the rate of parasitic multiplication was fully checked with complete disappearance of the parasites from the peripheral blood. The average number of days and the dose required were noted in each case. In addition to this, the degenerative changes in the parasites, the stages disappearing from the peripheral blood and the general blood picture were studied and recorded and further tests, if necessary, were carried out with smaller doses.

In cases where a monkey died during a course of treatment after becoming parasite-free, post-mortem examinations were made and the likely cause of death ascertained.

In order to study the effect of the drug with regard to relapses, all animals which became parasite-free after a course of treatment, were kept under observation for recrudescence of parasites in the peripheral blood and thick and thin smears

were examined once daily for 3 to 12 months (minimum 3 months). Where no parasitic relapses were encountered within this period, the following methods were adopted to ensure sterilization of infection :—

(1) The animals were given injections of adrenaline hydrochloride (1 in 1,000 solution 0·5 c.c.) intravenously and thick blood smears were taken every 5 minutes up to 30 minutes, stained and examined for parasites.

(2) The blood of such monkey as did not show any parasites after injection of adrenaline hydrochloride was sub-inoculated to another normal monkey and the latter again kept under observation to see whether parasites showed up or not. Absence of infection developing at all, indicated complete cure.

(3) The experimental monkeys were subjected to super-infection intra-peritoneally with the homologous strain of parasites in the same dosage as originally employed. If infection developed almost as severely as in primary infections, the chain of evidence regarding cure was then somewhat complete.

(4) In few cases splenectomy was also undertaken instead of super-inoculation.

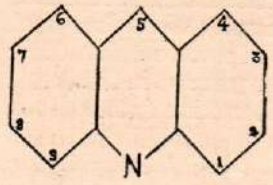
The drugs tested were mepacrine, paludrine, resochin, camoquin, meta-chloridine and aphacrine.

CHEMICAL FORMULÆ—PARTICULARS OF DOSAGE REGIMES EMPLOYED AND RESULTS.

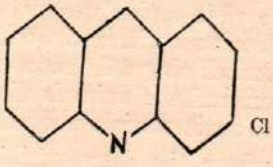
ATEBRIN.

Synonyms.—Atabrine : mepacrine : quinacrine : acriquine : erion : haffkenine : italchine : metoquina : malaricida.

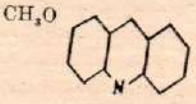
Chemical name.—2-chloro-5 (4-diethylamino-1 methyl butylamino)-7 methoxy-acridine 2 hydrochloride.



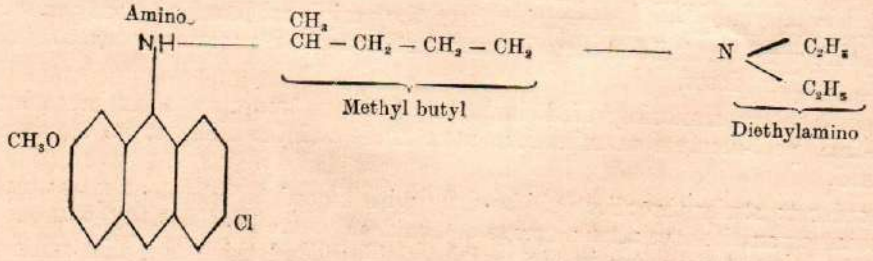
Acridine (the basis of atebrin)



2 chloro acridine



2-chloro-7-methoxy-acridine



Particulars of the trials.—Eighteen monkeys were treated with this drug. The dosage regimes employed, the severity of infection at which drug was administered and the results achieved are given in Table I.

TABLE I.

Particulars of the therapeutic trials with atebrin.

Serial number.	Dosage.	Nature of infection at the time of drug administration.	Number of monkeys treated.	Results.		
				Mortality (number).	Average time taken for disappearance of parasites (hours).	Number relapsed during an observation period of 3 to 6 months.
1	100 mg. daily for 3 days ...	+ to ++	4	0	72–96	4
2	100 mg. daily for 3 days ...	v.v.s.	5	0	48–96	5
3	100 mg. daily for 5 days ...	v.v.s.	1	0	48	1
4	100 mg. daily for 7 days ...	++++	1	1	...	...
5	100 mg. daily for 7 days ...	+ to ++	3	0	72–96	0
6	100 mg. daily for 7 days ...	v.v.s.	2	0	60	0
7	100 mg. daily for 14 days ...	v.v.s.	2	0	72	0

For explanation to abbreviations in column 3, please *see* para 6 on page 388.

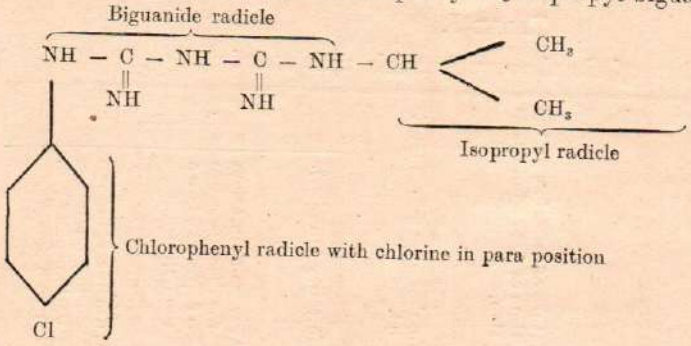
Nine monkeys were given a daily dose of 100 mg. atebrin for 3 days when the infection was v.v.s. in five and + to ++ in the remaining four. The initial infection was promptly controlled within 48 to 96 hours after the commencement of treatment but 6 to 10 relapses occurred in all during a minimum observation period of 3 months. One monkey at the stage of v.v.s. infection was given 100 mg. of the drug daily for 5 days. Parasites completely disappeared from the peripheral blood within 48 hours of the commencement of treatment but it relapsed 4 times during the observation period of 3 months. Drug was administered to 6 monkeys at a dosage of 100 mg. daily for 7 days (total 700 mg.). The infection at the time of drug administration was v.v.s. in two, + to ++ in three and ++++ in one.

The monkey, which received the drug at the height of the infection, succumbed but in the rest parasites disappeared completely from the peripheral blood 60 to 96 hours after the commencement of treatment and no relapses occurred during the whole of the observation period. Two monkeys were placed on 100 mg. daily dose for 14 days when the infection was v.v.s. Within 72 hours of treatment, parasites disappeared from peripheral blood and the monkeys remained healthy without showing any relapses during the whole of the observation period.

PALUDRINE.

Synonyms.—Proguanil : chlorguanide : M 4888.

Chemical name.—N_1-P-chlorophenyl-N_5-isopropyl-biguanide hydrochloride



Particulars of the trials.—Seventy-four monkeys were treated with paludrine in various dosage schedules and at different stages of infection and their particulars are given in Table II.

Fifteen monkeys were treated with daily doses of 50 mg. paludrine hydrochloride for 14 days. Thirteen monkeys became parasite-free in an average of 78 hours and the other two died of heavy infection after the second dose. Of the 13 which became parasite-free, two died—one after receiving a total of 300 mg. and the other after the full course of 700 mg. On post-mortem examination, no parasites were observed in smears taken from heart, liver, spleen and brain. Death in these two animals is presumed to have been due to the toxic effects of the drug. During an observation period of 150 to 210 days, none of the other treated monkeys showed parasites in the peripheral blood.

Four monkeys receiving 50 mg. paludrine hydrochloride daily for 7 days became parasite-free after an average of 90 hours, and out of those, one collapsed 5 days after the completion of treatment. In this case no parasite was seen in the heart, spleen, liver or brain and death was presumed to be due to the toxic effects of the drug. The three surviving monkeys showed parasitic relapses 6 to 8 days after the completion of the treatment.

Similarly eleven monkeys were given a 5-day course of 50 mg. paludrine hydrochloride daily. Of these, 10 became parasite-free after an average of 86 hours whilst the remaining one died from severe infection within two days. Six monkeys relapsed after an average period of 5 days, the shortest interval being 2 days and the longest 12 days.

In the 50 mg. daily dosage, there were no relapses after 14 days treatment. After 7 days treatment, all the monkeys that survived suffered from relapses but in the case of 5 days treatment, only six out of eleven had relapses.

Fourteen monkeys were given paludrine hydrochloride in 100 mg. daily doses for 4 days. In seven monkeys, the drug was administered when the infection was v.v.s. and to the rest when it was +. Out of these, one monkey with + infection series died on the third day of treatment and the remaining 13 monkeys

TABLE II.

Particulars of the therapeutic trials with paludrine hydrochloride.

Serial number.	Dosage.	Nature of infection at the time of drug administration.	Number of monkeys employed.	RESULTS.			
				Mortality (number).	Average time taken for disappearance of parasites (hours).	Number relapsed.	Toxic effect (number).
1	50 mg. daily for 14 days ...	v.v.s.	15	2	78	0	2
2	50 mg. daily for 7 days ...	v.v.s.	4	0	89	3	1
3	50 mg. daily for 5 days ...	v.v.s.	11	1	86	6	...
4	100 mg. daily for 4 days ...	v.v.s.	7	0	108	6	...
5	100 mg. daily for 4 days ...	+	7	1	110	5	...
6	100 mg. daily for 6 days ...	v.v.s.	7	1	108	5	...
7	100 mg. daily for 6 days ...	+	7	1	108	6	...
8	100 mg. daily for 7 days ...	v.v.s.	4	1	108	1	...
9	100 mg. twice daily for 7 days	v.v.s.	4	...	53	2	...
10	100 mg. twice daily for 7 days	+	4	...	53	2	...
11	100 mg. thrice daily for 7 days	+	4	...	48	0	1

For explanation to abbreviations in column 3, please *see* para 6 on page 388.

were cured of the initial infection in an average of 110 hours (range 96 to 192 hours). However, 11 out of these relapsed within a period of 4 to 12 days of stopping drug administration, proving fatal in two and passing on to chronic infection in the others.

Fourteen monkeys were given paludrine in 100 mg. dosage for 6 days. To seven of these the drug was administered when the infection was v.v.s. and to the rest when it was +. One monkey of v.v.s. infection series and another of + infection series died on fifth and fourteenth day of treatment respectively due to progressively increasing infection. The remaining twelve monkeys got over the initial infection, parasites disappearing from peripheral blood on an average of 108 hours (96 to 144 hours). But all, with the exception of one, relapsed frequently 5 to 6 days after the cessation of treatment and among these, four succumbed to infection in spite of giving a daily dose of 200 mg. during relapse. Of the 14 treated, one remained healthy during the whole of the observation period of 180 days.

Four monkeys were given 100 mg. for 7 days when the infection was v.v.s. The drug failed to control infection in one monkey which was later placed on some other drug (resochin). In others the parasites disappeared from peripheral blood in an average of 108 hours (range 96 to 144 hours) but one out of them relapsed on fifth day after the end of the treatment.

Eight monkeys were given 100 mg. twice a day for 7 days when the infection came up to v.v.s. in four and + in the rest. Infection was controlled in an average of 53 hours but four monkeys relapsed (2 in v.v.s. infection series and 2 in + infection series) 12 to 18 days later with the exception of one which relapsed after 34 days.

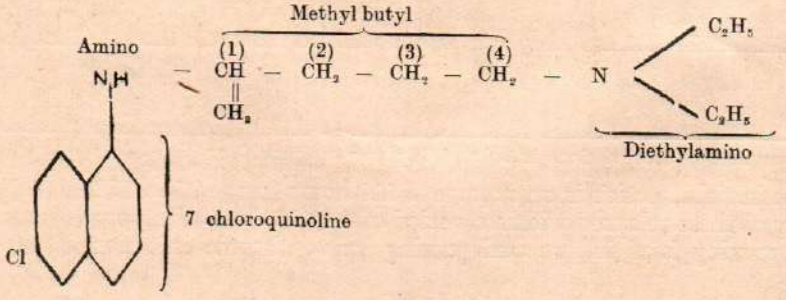
Four monkeys received 100 mg. thrice a day for 7 days at the stage of + infection. There was prompt response in all and parasites disappeared from circulation in an average of 48 hours. The day after the course of treatment, one monkey died due possibly to the toxic effect of the drug whereas the remaining three remained healthy during the period of observation of 180 days.

The drug was tested as shown above in various dosage regimes ranging from 50 to 300 mg. a day, and it is rather strange to find that mortality, due probably to the toxic effects of the drug, was noticed only in the minimum and maximum daily dosages and there was no death due to this effect in the 100 to 200 mg. daily doses administered over a period of 4 to 7 days. These findings are rather difficult to reconcile and further detailed and conclusive investigations are in progress on this line.

RESOCHIN.

Synonyms.—Chloroquine : SN 7618 : aralen : nivaquine B.

Chemical name.—7-chloro-4 (diethylamino-methyl-butyl-amino) quinoline.



Particulars of the trials.—Twenty-three monkeys, ten showing v.v.s. and the others with heavy infection, were treated with resochin with various dosage regimes. The details are shown in Table III.

TABLE III.

Particulars of the therapeutic trials with resochin.

Serial number.	Dosage.	Nature of infection at the time of drug administration.	Number of monkeys employed.	Results.		
				Mortality (number).	Average time taken for disappearance of parasites (hours).	Number relapsed.
1	125 mg. single dose ...	v.v.s.	6	1	48	2
2	125 mg. single dose ...	++	1	0	48	1
3	125 mg. a day for 2 days ...	++	1	0	48	0
4	125 mg. a day for 3 days ...	v.v.s.	4	0	48	1
5	125 mg. a day for 3 days ...	+	7	0	48	2
6	250 mg. a day for 3 days ...	+	4	0	48	0

For explanation to abbreviations in column 3, please *see* para 6 on page 388.

The tests were performed on the daily doses of 125 mg. for a period of one to three days and 250 mg. for 3 days. Seven monkeys were given a single dose of 125 mg. of the drug. Out of these, the drug was given at v.v.s. stage to six

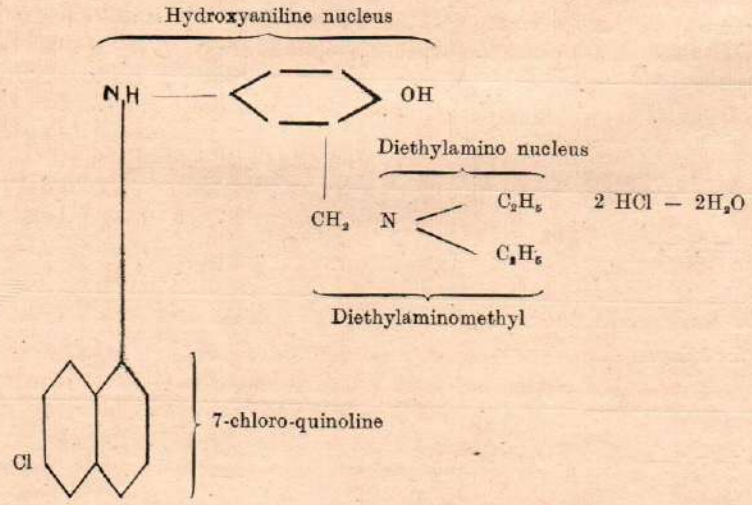
and to one when the infection was heavy. With the exception of one monkey in the v.v.s. infection series, which succumbed to the disease four days after the drug administration, in all the rest, parasites disappeared from peripheral blood quickly but 50 per cent of the monkeys relapsed within 10 to 39 days.

Twelve monkeys received 125 mg. of the drug for 2 to 3 days when the degree of infection was v.v.s. in four, + in 7 and ++ in one. In all, prompt response in initial infection was effected but 25 per cent of the monkeys relapsed within 10 to 39 days. It is observed that a dose of 250 mg. daily for 3 days given to four monkeys when the degree of infection was +, eradicated the infection altogether, the parasites failing to appear in peripheral blood during a long period of observation (10 months). Clearance of parasites from peripheral blood in all the successful cases under all dosage regimes occurred within an average of 48 hours. No toxic reaction was observed at the dosages employed for tests.

CAMOQUIN.

Synonyms.—Cam-aqi : camaquin : miaquin : SN 10751.

Chemical name.—4-(3-diethylamino-4-hydroxyanilino)-7 chloroquinoline dihydrochloride dihydrate.



Similar to resochin except for a hydroxyaniline nucleus being incorporated in place of alkyl side chain at 4 position of the quinoline group.

Particulars of the trials.—The full recommended dose of camoquin to man (adult) is 100 mg. initial plus 50 mg. every 12 hours up to 7 doses (total 450 mg.).

Eight monkeys were treated with the drug; two monkeys each received $\frac{1}{3}$, $\frac{2}{3}$, full, and double the recommended dosage for human adults. The details are shown in Table IV.

TABLE IV.

Particulars of therapeutic trials with camoquin.

Serial number.	Dosage.	Nature of infection at the time of drug administration.	Number of monkeys employed.	Results.		
				Mortality number.	Average time taken for disappearance of parasites (hours).	Number relapsed.
1	100 mg. twice daily for 4 days (total 800 mg.).	v.v.s.	2	0	24	0
2	100 mg. initial + 50 mg. every 12 hours up to 7 doses (total 450 mg.).	v.v.s.	2	0	60 (24 to 96)	0
3	100 mg. daily for 3 days (total 300 mg.).	v.v.s.	2	0	48 (24 to 72)	0
4	50 mg. daily for 3 days (total 150 mg.).	v.v.s.	2	0	24	2

For explanation to abbreviations in column 3, please *see* para 6 on page 388.

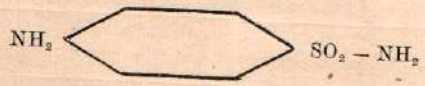
In all cases the rate of multiplication of parasites was successfully checked resulting in their complete disappearance within an average of 48 hours (range 12 to 96 hours).

The administration of the drug had significant effect on the ultimate rate of relapse; a total of 300 mg. or more resulting in the ultimate cure. Relapse, however, occurred in the two monkeys which had received one-third the recommended dosage for human adult (total 150 mg.) in an average of 36 days. There was no evidence of the toxic effect of the drug at any of the dosages tried.

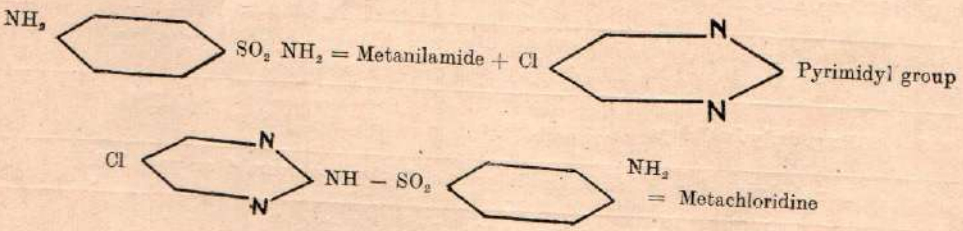
METACHLORIDINE.

Synonym.—SN 11437.

Chemical name.—N^1-5-chloro-2-pyrimidyl metanilamide.



Sulphanilamide



Details of the trials.—Metachloridine was given to ten monkeys and the degree of infection at the time of trial was v.v.s. in four and heavy (+) in the rest. Eight monkeys received 300 mg. daily for 3 days, one 300 mg. daily for 4 days and another 600 mg. daily (in divided doses) for 3 days. In all the monkeys, the initial infection was cured in 120 to 168 hours from the commencement of treatment. The follow-up showed frequent relapses in seven monkeys, the first relapse occurring 6 to 25 days after the end of treatment and they were not governed by the severity of infection at which the treatment was started or the total quantity of drug administered.

APHACRINE.*

Two monkeys were put on 100 mg. of aphacrine a day for 5 days and 5 monkeys on 100 mg. a day for 7 days. Five other monkeys were given 200 mg. of the drug for 7 days. In all, the initial infection was cured within 48 to 168 hours but 57 per cent of the monkeys in the daily 100 mg. dosage and 40 per cent in the daily 200 mg. dosage regimes relapsed, the relapses occurring regardless of the degree of infection at which the drug was administered. The details are shown in Table V.

TABLE V.

Particulars of therapeutic trials with aphacrine.

Serial number.	Dosage.	Nature of infection at the time of drug administration.	Number of monkeys employed.	Results.		
				Mortality number.	Average time taken for disappearance of parasites (hours).	Number relapsed.
1	100 mg. daily for 5 days ...	+	2	0	120 to 168	2
2	100 mg. daily for 7 days ...	v.v.s.	3	0	48 to 168	1
3	100 mg. daily for 7 days ...	+	2	0	72 to 168	1
4	200 mg. daily for 7 days ...	v.v.s.	3	0	72 to 120	2
5	200 mg. daily for 7 days ...	+	2	0	72 to 120	0

For explanation to abbreviations in column 3, please *see* para 6 on page 388.

* Manufactured by Lister Antiseptic Co., Ltd., Calcutta.

DISCUSSION.

Clearance of all parasites from peripheral blood was obtained with chloroquine and camoquin in an average after 48 hours; with atebrin after 73 hours, with paludrine after 80 hours, with aphacrine after 120 hours and with metachloridine after 144 hours. The disappearance of parasites was very slow with aphacrine and metachloridine and somewhat slower with atebrin and paludrine than with resochin and camoquin.

The dosage schedules used to determine the minimum dose required for the control of initial infection and the survival of all animals treated were small, but from the few series tried it may be said that the dosage required with atebrin was 100 mg. daily for 3 days, with paludrine 100 mg. twice daily for 7 days, with resochin 125 mg. daily for 2 days, with metachloridine 300 mg. daily for 3 days and with aphacrine 100 mg. daily for 5 days. Further trials are necessary to find out whether lower dosages than the above are compatible with the required result.

Pre-erythrocytic schizogony of *P. cynomolgi* has been described by Shortt, Garnham and Malamos (1948) and the exo-erythrocytic schizogony by Shortt and Garnham (1948) in sporozoite induced infections. The knowledge of the tissue phase in mammalian malaria has to a great extent clarified the complexities of relapses. It is now deemed to be due to the persistence of the tissue phases. With certain species such as *P. gallinaceum*, exo-erythrocytic forms develop directly even from blood schizonts after trophozoite induced infections (Corradetti, 1941) which give rise to the usual late relapse and persistence of infection throughout the life span of the bird. In blood induced infections in man, relapses tend to be less in number, late relapses are absent, and usually with small amounts of drug, complete termination of infection is possible. Schmidt *et al.* (1949) found that blood induced infection with *P. cynomolgi* in *rhesus* monkeys generally dies out spontaneously in a period of four months, during which time the primary attack lasting approximately 45 days and a series of about 3 relapses each of less intensity and length than the first attack, occur. These indirect evidences indicate that although in certain species of malarial parasites (avian) exo-erythrocytic stages are found in infections produced by blood inoculations, such may not be the case in the malarial parasites of mammals. But it has been observed in these laboratories in the follow-up of *S. rhesus* monkeys inoculated with blood forms of *P. inui* for maintenance of the strains that after the primary attack and initial recrudescences, renewed clinical activity occurs after six to twelve months. Such relapses after long periods of latency are more characteristic of the type that follow as a result of parasitic activity in the exo-erythrocytic than in the conventional erythrocytic cycle. However, there is no direct evidence to show that in *P. inui* exo-erythrocytic forms develop from blood schizonts. The fact that radical cure in trophozoite induced *P. knowlesi* infection can be gained by the use of drugs capable of only preventing erythrocytic relapses indicate that secondary exo-erythrocytic stages may not be existing in such monkeys infected with blood forms of this species also.

The testing of curative action of drug requires the use of mosquito induced infections and the need for a long-term observation period. Infection established by the simple transfer of infected blood permits only the examination of the suppressive type of activity (Shannon, 1946). In the treatment of the latter

type of infection with *P. knowlesi*, it is believed that only erythrocytic activity occurs which, if treated with inadequate doses of suppressive drugs only, gives rise to parasitic attacks either fatal or mild following shortly after the primary infection. Such renewed activity occurring after a few days of latency is known as recrudescence (Sapero, 1947) contrary to the relapse which occurs due to the re-activation of exo-erythrocytic parasites after a latent period of several months. Chopra (1935) found that quinine has a much less powerful immediate effect on *P. knowlesi* parasites than atebrin but with the latter, fatal relapses sometimes occurred as against the advantages of quinine, in that relapses did not as a rule occur after the treatment of primary blood induced infections and even when they did occur, were seldom fatal. Similarly, Row, Dalal and Gollerkeri (1933) stated that even after a sufficiently long course in adequate doses of quinine, atebrin and plasmochin, the relapses came on periodically with the return of the asexual forms of the parasite and in their usual virulence. In this connection it may be mentioned that with ' suppressive group of drugs ' it is spectacularly successful in aborting an individual attack of malaria induced by sporozoites though it may fail in effecting a complete cure. But in treated blood induced infections the relapses (recrudescences) occur mainly due to deficiency in therapy as radical cure is obtained by repeated schizonticidal action of the drug when it is maintained at effective blood level throughout the period. So one method to compare the effects of different drugs is to determine the critical dose of the new antimalarial drugs (Curd *et al.*, 1945), i.e. the lowest dose of a drug which under a particular dosage regime excites what is materially the maximum effect of the drug and compare them with that of a standard drug such as mepacrine or quinine. For purposes of as accurate an assessment as possible, the critical dose in these experiments is arbitrarily chosen as the minimum dosage required for completely eradicating the trophozoite induced infection without giving rise to any relapse or recrudescence. This critical dosage was as follows :—

Mepacrine 100 mg. daily for 7 days ; paludrine 100 mg. thrice daily for 7 days ; resochin 250 mg. daily for 3 days ; and camoquin 100 mg. daily for 3 days. The maximum dosage regimes of 300 mg. metachloridine twice a day for 3 days, and aphacrine 200 mg. daily for 7 days, tried during the experiments, did not produce a complete cure.

For treatment, atebrin was used in the form of bihydrochloride (atebrin bihydrochloride), paludrine as hydrochloride (paludrine hydrochloride), resochin as diphosphate (chloroquine diphosphate) and camoquin as dihydrochloride dihydrate, and these were administered in the way described in the preceding pages.

On the basis of dosage of the free base administered orally, atebrin is 3·26 times as effective as paludrine, whereas resochin and camoquin are 1·2 and 1·8 times as effective as atebrin respectively. Similarly, the curative dose (free base) of atebrin in these animals is 0·263 of the usual recommended human (adult) dose, whereas the proportion in the case of paludrine, resochin and camoquin comes to 0·7, 0·3 and 0·66 respectively.

Even with the heavy dosages required for the complete sterilization of infection, no toxic manifestations were observed in any of the animals except in those treated with paludrine which however showed 25 per cent mortality.

In addition to the trials carried out with camoquin against initial infection, it was also tested in 19 monkeys during relapses that occurred after inadequate treatments with one or the other drugs such as atebrin, paludrine, resochin, etc., with equal dosages as recommended for man (100 mg. initial dose followed by 50 mg. every twelve hours up to 7 doses, i.e. total 450 mg.). Of these, five were treated when the infection during the relapses was v.v.s. and 14 at the stage of heavy infection. Though there was immediate effect in all the monkeys tried, the relapse rate mounted to 42 per cent which is in marked contrast to the complete absence of the same noted in fresh infections treated with the same as well as a lower dosage of a total of 300 mg. of camoquin. This may suggest the resistance of the relapsing blood forms of parasites to camoquin, and this point is being further investigated.

When compared with mepacrine, resochin and camoquin, the action of paludrine on simian plasmodia (*P. knowlesi*) appears to be comparatively less pronounced in blood induced infections and is slightly more toxic to the animals. This seems to be contrary to the findings in bird and human malaria. Butler *et al.* (1947) found that under various treatments chicks tolerated much higher concentration of the drug than mice and rats and concluded that probably man behaved like the chicks rather than like the rat or the mouse. Similarly, it appears that monkeys behave like mice and rats in their tolerance to the drug rather than like man or chicks. More detailed investigations are needed before this can be finally confirmed. Sapero (1947) and Fairley (1946) believed that in human malaria pre-erythrocytic forms are more susceptible to paludrine than the erythrocytic forms, and that in blood induced malaria where it is almost certain that only erythrocytic activity occurs, the response is poor if treatment is attempted with drugs more active against pre-erythrocytic forms. Perhaps the relatively poor results obtained with paludrine in blood induced malaria infection in *rhesus* monkeys as compared with those obtained with resochin, atebrin and camoquin stands to confirm the views of these two workers.

SUMMARY AND CONCLUSIONS.

Synthetic drugs, mepacrine, paludrine, resochin, camoquin, metachloridine and aphacrine were tested for their therapeutic properties in 164 *S. rhesus* monkeys weighing 3 to 5 kg. infected with blood forms of *P. knowlesi*. These tests were carried out on different dosage schedules under certain identical standardized conditions and these have been described in detail.

All the drugs were efficient in clearing the parasites from the peripheral blood. The minimum dose required for this varied with different drugs and the same was a total of 150, 250, 300, 500, 900 and 1,400 mg. respectively in divided doses for camoquin, resochin, atebrin, aphacrine, metachloridine and paludrine respectively. The complete disappearance of parasites occurred earlier (i.e. in an average 48 hours) after the administration of resochin and camoquin than after treatment with atebrin (in 73 hours) and paludrine (in 80 hours). Aphacrine and metachloridine on the other hand were very slow in clearing the parasites and took 120 and 144 hours respectively.

A blood induced *P. knowlesi* infection can be completely sterilized by drugs possessing powerful erythrocytic activity, the failure to eradicate such infection after treatment is presumed to be due to deficiency in therapy.

It is considered that one of the best methods of comparing the efficacy of the action of drugs is to do it in terms of the critical dose of the respective drugs. This dose in blood induced infections (*P. knowlesi*) can to the best advantage be taken arbitrarily as the minimum dose required for completely eradicating the infection without giving rise to relapses (recrudescences). Hence calculated in terms of the free base of the different drugs, atebrin is 3·26 times as effective as paludrine in *P. knowlesi* infection in monkeys whereas resochin and camoquin are 1·2 and 1·8 times respectively as effective as atebrin.

When compared in terms of the human adult recommended dose, that in monkeys, the dosage for the complete eradication of infection is in the ratio of 1 : 0·263, 1 : 0·3, 1 : 0·66 and 1 : 0·7 respectively for atebrin, resochin, camoquin and paludrine.

The comparatively poor results obtained with paludrine in trophozoite induced *P. knowlesi* infection as compared with resochin, camoquin and atebrin are attributed to the fact that the former is a drug of an exo-erythrocytic activity unlike the rest which possess erythrocytic activity only and as such the latter activity is considered to be less pronounced with paludrine. Toxic manifestations were observed in some of the monkeys treated with larger doses of paludrine.

The plasmodicidal effect of camoquin in the relapsing form of parasites is not as promising as in the primary forms of the parasites during initial infection.

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SCREENING OF SOME BIGUANIDE DERIVATIVES* FOR ANTIMALARIAL ACTIVITY.

BY

LIEUT.-COLONEL JASWANT SINGH, M.B., ch.B. (Edin.), D.P.H. (Eng.), D.T.M. & H. (London),

MAJOR A. P. RAY, M.B., B.S.,

MAJOR C. P. NAIR,

AND

CAPTAIN P. C. BASU, M.B. (Cal.).

(*Malaria Institute of India, Delhi.*)

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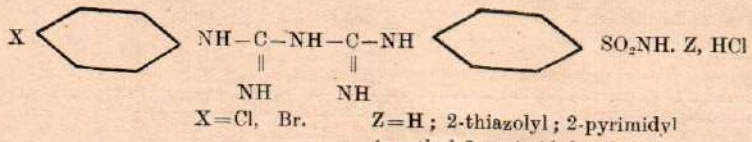
FOLLOWING the discovery of paludrine (proguanil, chlorguanide) and its establishment as an efficacious antimalarial remedy, great interest was shown in the biguanide structure as a potential basic molecule for antimalarial research. In 1946, Bami *et al.* (1947*a*, 1947*b*, 1948*a*, 1948*b*) and Bami and Guha (1949*a*, 1949*b*) undertook the synthesis of a large number of new biguanide derivatives in the Organic Chemistry Laboratories of the Indian Institute of Science, Bangalore. The screening of various synthetic and indigenous drugs for antimalarial properties was in progress in the laboratories of the Malaria Institute of India, and included during 1947 and 1948, different types of biguanide derivatives synthesized by the above workers and received through the Indian Research Fund Association.

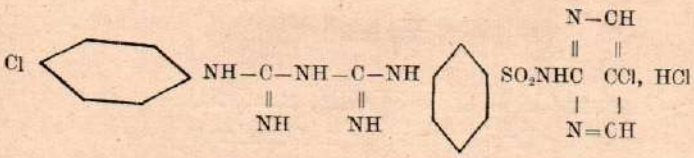
* The authors wish to record their thanks to Dr. H. L. Bami for forwarding the drugs and helping in the study of the chemical compounds.

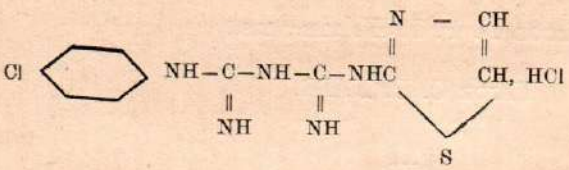
Eleven compounds, whose chemical group formulæ are given below, were tested :—

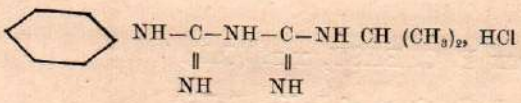
GROUP FORMULÆ.

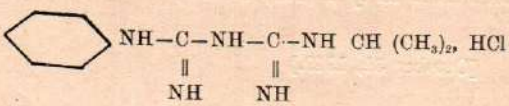
I 

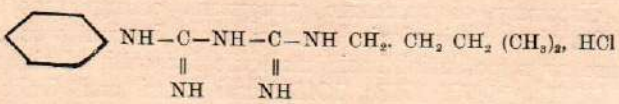
X=Cl, Br. Z=H ; 2-thiazolyl ; 2-pyrimidyl 4-methyl-2-pyrimidyl and 4 : 6-dimethyl-pyrimidyl.

II 

III 

IV 

V 

VI 

Compounds 1 to 6 are sulpha-biguanide derivatives (represented by Formula I) where chlorophenyl group is present at one end of the biguanide chain while the other end is combined with various potent sulphonamide groups derived from sulphanilamide (Compounds 1 and 2), sulphathiazole (3), sulphadiazine (4), sulphamerazine (5) and sulphamethazine (6) respectively.

Compound 7 contains aryl and metachloridine groups at either end of a biguanide chain as represented in Formula II, while Compound 8 is having a potent heterocyclic ring, viz. thiazole, attached to a substituted biguanide chain (Formula III).

TABLE I.

Chemical name and formulæ of compounds.

Serial number of drug.	Chemical name.	Chemical formula.	References.
1	N′-*p*-chlorophenyl-N⁵-*p*-phenylsulphonamido-biguanide hydrochloride.	(I) where x = Cl, y = H	Bami, Iyer and Guha (1947*a*).
2	N′-*p*-bromophenyl-N⁵-*p*-phenylsulphonamido-biguanide hydrochloride.	(I) where x = Br, y = H	Do.
3	N′-*p*-chlorophenyl-N⁵-*p*-(2-thiazolyl) phenylsulphonamido-biguanide hydrochloride.	(I) where x = Cl, y = 2-thiazolyl	Do.
4	N′-*p*-chlorophenyl-N⁵-*p*-(2-pyrimidyl) phenylsulphonamido-biguanide hydrochloride.	(I) where x = Cl, y = pyrimidyl	Do.
5	N′-*p*-chlorophenyl-N⁵-*p*-(4-methyl-2-pyrimidyl) phenylsulphonamido-biguanide hydrochloride.	(I) where x = Cl, y = 4-methyl-2-pyrimidyl.	Bami, Iyer and Guha (1947*b*, 1948*a*).
6	N′-*p*-chlorophenyl-N⁵-*p*-(4 : 6-dimethyl-2-pyrimidyl) phenylsulphonamido-biguanide hydrochloride.	(I) where x = Cl, y = 4 : 6-dimethyl 2-pyrimidyl.	Do.
7	N′-*p*-chlorophenyl-N⁵-*m*-(5-chloro-2-pyrimidyl) phenylsulphonamido-biguanide hydrochloride.	(II)	Bami, Iyer and Guha (1948*b*).
8	N′-*p*-chlorophenyl-N⁵-2-thiazolyl-biguanide hydrochloride ...	(III)	Bami and Guha (1949*a*).
9	N′-*m*-chlorophenyl-N⁵-isopropyl-biguanide hydrochloride ...	(IV)	Bami and Guha (1949*b*).
10	N′-*p*-bromophenyl-N⁵-isopropyl-biguanide hydrochloride (*Bromoguanide*).	(V)	Do.
11	N′-2 : 4-dichlorophenyl-N⁵-*iso*-amyl-biguanide hydrochloride ...	(VI)	Do.

In the case of Compound 9 (Formula IV), the parachlorine atom in the phenyl ring of the paludrine molecule has been shifted to the *meta* position, while in the case of Compound 10 (bromo-guanide) (Formula V), the chlorine atom present in paludrine molecule has been replaced by bromine. Lastly, Compound 11 (Formula VI) is similar to paludrine but has an additional chlorine atom in the phenyl ring and a longer alkyl chain.

SCREENING AGAINST *P. GALLINACEUM* IN CHICKS.

As Compounds 1 and 3 to 9 were screened against *P. gallinaceum* in chicks previously by Bami *et al.* (1949), the results of which are extracted in Table II, only Compounds 2, 10 and 11 were tested against *P. gallinaceum* in these laboratories. These tests were carried out in fowls, weighing one to one and a half kg., inoculated with 30 million parasites per kg. body-weight. The infection was allowed to develop to the extent of 0·1 to 1 per cent cell infection and at this stage the compounds in solution/suspension were administered orally by catheter according to the different dosage regimes. The state of infection was recorded by 12-hourly examination of blood smears stained with J.S.B. stain (Jaswant Singh and Bhattacharji, 1944). Control fowls with the same inoculum were kept for comparison. The time required for complete disappearance of parasites from the peripheral blood was considered as the criterion of activity. The results are recorded in Table III.

TABLE II.

Extracts from results of testing of compounds against P. gallinaceum.

Compound.	Dosage regime mg. per kg. body-weight.	Activity.
1	60	*Nil.*
2	120	*Nil.*
3	200	*Nil.*
4	400	Slightly active.
5	400	Active.
6	400	Slightly active but toxic.
7	400	Active but highly toxic.
8	400	Doubtful and toxic.
9	200	Active.

TABLE III.

Results of testing of compounds against P. gallinaceum.

Compound.	Dosage regime per kg. body-weight.	Number of fowls used.	Results.	REMARKS.
2	30 mg. twice a day (morning and evening) for 4 days : Total 240 mg.	1	Infection increased in spite of the drug ...	No activity.
2	15 mg. twice a day (morning and evening) for 4 days : Total 120 mg.	1	Do.	Do.
10 (Bromo-guanide)	25 mg. twice a day (morning and evening) for 4 days : Total 200 mg.	2	Complete absence of parasites in 64 hours from commencement of treatment.	Active (relapsed after 10 days).
,,	20 mg. twice a day (morning and evening) for 4 days : Total 160 mg.	2	Complete absence of parasites in 108 hours from commencement of treatment.	Active (relapsed within 7 to 12 days).
,,	15 mg. twice a day (morning and evening) for 4 days : Total 120 mg.	2	Complete absence of parasites in 72 hours from commencement of treatment.	Active (relapsed within 10 days).
,,	10 mg. twice a day (morning and evening) for 4 days : Total 80 mg.	2	Complete absence of parasites in 72 hours from commencement of treatment.	Active (relapsed after 9 days).
,,	5 mg. twice a day (morning and evening) for 4 days : Total 40 mg.	2	Complete absence of parasites in 72 hours from commencement of treatment.	Active (relapsed after 9 days).
,,	$2\frac{1}{2}$ mg. twice a day (morning and evening) for 4 days : Total 20 mg.	2	Complete absence of parasites in 96 hours from commencement of treatment.	Active (relapsed after 7 days).
11	40 mg. twice a day (morning and evening) for 3 days : Total 240 mg.	1	Died on the second day of drug administration with increased parasitæmia.	Toxic and inactive.
11	20 mg. twice a day (morning and evening) for 4 days : Total 160 mg.	1	Died 2 days after drug administration with very heavy infection.	Do.

Comparison fowls died on the sixth day following inoculation with 90 per cent of cell infection in most cases.

SCREENING AGAINST *P. KNOWLESI.*

Compounds 1 to 11 were tested against *P. knowlesi* using healthy *Silenus rhesus* monkeys weighing 2 to 3 kg. each, inoculated with 2 c.c. of blood in 2 per cent citrated saline intravenously from a donor monkey harbouring the strain of *P. knowlesi* with very scanty infection. As soon as the infection became evident in the experimental monkeys to the extent of one parasite per five thin fields (100 R.B.C. in each field), the compounds were given orally in solution or suspension according to the different dosage regimes. Daily examination of blood smears was carried out and the extent of parasitæmia noted on similar lines as described by Jaswant Singh *et al.* (1949).

Those monkeys in which apparent sterilization of infection was effected without relapses during a period of 3 months were subjected to curative tests.

The results are given in Table IV.

RESULTS.

P. gallinaceum.—It is observed from Table II that Compounds 5, 7 and 9 are active at relatively large dosage while Compound 7 is also toxic at that dosage. Remaining compounds are either inactive or slightly active but associated with toxicity.

Compounds 2 and 11 (Table III) have been found to be inactive (11 being toxic also) while bromo-guanide (Compound 10) has given encouraging results. A dosage as low as 5 mg./kg. body-weight a day for 4 days has caused complete disappearance of the parasites within 96 hours. It has been observed however that increase of this dosage up to 50 mg./kg. body-weight has not resulted in an increase in antimalarial activity.

P. knowlesi.—From Table IV it will be evident that Compounds 1, 2, 3, 4, 6, 7, 8 and 11 showed no antimalarial activity at the dosage at which they were tested. In all the cases infection invariably increased in the drug treated groups. Compound 5 showed some antimalarial activity. It was only in the case of Compounds 9 and 10 that activity coupled with non-toxicity was encountered. Bromo-guanide (Compound 10) was however found to be more active than Compound 9 and is being further investigated.

DISCUSSION.

It is evident that results of screening in chicks and monkeys can be favourably compared in the above tests. Compounds 1 to 6 have basic structural features of paludrine and sulpha drugs. Activity in the case of Compound 5 indicates that, out of the different sulphabiguanide derivatives, the one associated with sulphamerazine possesses antimalarial activity although of a lower order. Compounds derived from metachloridine and thiazole group have been found to be inactive. Compound 9 is active against both the *strains* and it may be said that presence of chlorine at the para position of the phenyl ring is not absolutely necessary for activity. Activity in the case of bromo-guanide (Formula V) indicates that perhaps chlorine atom in the case of paludrine may be favourably replaced with bromine although evidence in this respect is not yet complete.

TABLE IV.

Results of testing of compounds against P. knowlesi.

Compound.	Dosage regime per kg. body-weight.	Number of monkeys used.	Results.	REMARKS.
1 to 4	100 mg. daily (single dose) for 3 days: Total 300 mg.	1 each	Infection increased in spite of drug administration in all and in the case of Compound 1 the animal died.	No activity.
1 to 4	100 mg. daily (single dose) for 7 days: Total 700 mg.	1 each	Infection increased in spite of the drug administration in all and in the case of Compounds 2 and 3 the animal died.	Do.
5	Do.	2	Peripheral blood became free either during or 3 days after cessation of treatment. Relapses occurred after an interval of 10 to 13 days.	Active but frequent relapses occurred.
6	Do.	2	Infection increased in spite of the drug	No activity.
7	Do.	2	Do.	Do.
8	Do.	2	Do.	Do.
9	Do.	2	Peripheral blood became free either during or 3 days after the cessation of treatment. Complete sterilization of infection in one case. Frequent relapses in the other.	Active.
10	Do.	3	Peripheral blood became free within an average of 8 days from the commencement of treatment (7 to 9 days). No relapse. 2 to 3 transient relapses lasting for one day in two. No relapse in the third.	Do.
10	100 mg. daily (single dose) for 3 days: Total 300 mg.	1	Peripheral blood became free within 8 days from the commencement of treatment. No relapse.	Do.
10	50 mg. daily (single dose) for 7 days: Total 350 mg.	1	Peripheral blood became free within 7 days from the commencement of treatment. No relapse.	Do.
10	50 mg. daily (single dose) for 3 days: Total 150 mg.	1	Peripheral blood became free within 8 days from the commencement of treatment. No relapse.	Do.
11	100 mg. daily (single dose) for 7 days: Total 700 mg.	1	Infection increased in spite of drug administration	No activity.

SUMMARY.

The screening of eleven biguanide derivatives for their antimalarial activity against *P. gallinaceum* in chicks and *P. knowlesi* in *rhesus* monkeys at various dosages was made. Out of these, para-bromo-analogue of paludrine (termed as bromo-guanide) has been found to be most active and is being further studied.

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RECENT RESEARCHES ON ANTIMALARIALS: REVIEW OF PROGRESS.*

BY

LIEUT.-COLONEL JASWANT SINGH, M.B., Ch.B. (Edin.), D.P.H. (Eng.), D.T.M. & H. (London).

(*Director, Malaria Institute of India, Delhi.*)

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MALARIA continues to be a major health problem in tropical and subtropical countries and its magnitude in India is confronting the nation all the time. Interruption of transmission of malaria at the mosquito level by the use of modern insecticides has proved highly effective, but even then for obvious reasons, search for a more suitable drug than those that are already available, must continue. Such a drug should not only serve as a true causal prophylactic but should also be capable of completely sterilizing malarial infections in man. Present position of some of the well-known antimalarial drugs and a review of some of the important pharmacological and biological developments in this field are discussed.

SUPPRESSIVE ANTIMALARIALS.

Quinine.—Even though some of the recent and more potent synthetic compounds have proved in many respects superior to quinine (Chaudhuri, 1948; Gordon *et al.*, 1947; Pullman *et al.*, 1948), the popular concept of treatment of malaria with quinine in this country still prevails. The general belief that quinine is relatively non-toxic and can therefore be safely administered by non-medical personnel has not been borne out by the recent series of controlled experiments by Coatney *et al.* (1948) where it has been observed that of all the antimalarials tested at maximum dosages thought to be safe, quinine is the only one which has been demonstrated to produce irreversible damaging effects. With regard to suppression with quinine, the World Health Organization Expert Committee on Malaria in its meetings held at Geneva in August 1949 confirmed its previous opinion that 'the newer drugs are so much more active that few health authorities would be prepared to consider the use of quinine for this purpose'. They have

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also recommended that it would be preferable to concentrate on the production of synthetic drugs rather than on growing more cinchona as it would use up the acreage needed for agricultural and other purposes. Such steps are likely to bring about a drop in the price of antimalarials which is an important factor for mass chemotherapy, particularly in under-developed areas.

Mepacrine.—Out of the synthetic suppressive antimalarials, mepacrine has been thoroughly investigated in the field trials during the war years. While it proved highly effective as a suppressant with generally low toxicity, its many limitations have now been fully determined. For example, it does not possess any prophylactic action, gives a yellow discoloration of the skin and may produce certain toxic effects (Fairley, 1945; Cooper *et al.*, 1949; Shannon *et al.*, 1944; Wiselogle, 1946; Chaudhuri, 1948).

Chloroquine and its analogues.—As a result of extensive researches in America, a potent suppressive in the field of 4-aminoquinolines, viz. chloroquine, which rapidly alleviates acute attacks of malaria and generally results in a radical cure of *falciparum* infections, has been put into large-scale use in that country (Berliner *et al.*, 1948; Coatney *et al.*, 1949). It has proved more effective than mepacrine and is usually well tolerated, but it is somewhat more toxic than paludrine (Alving *et al.*, 1948; Schmidt, 1946; Cooper, 1949). A single dose cure has also been claimed in respect of this drug mostly against human malaria parasites of American and South Pacific regions, and in a few instances in this country (Chaudhuri *et al.*, 1948; Ray, 1948; Goldsmith, 1946). In 1947, chloroquine was tried out as a prophylactic in a tea garden in Assam where in weekly doses of one tablet regularly for six months, it gave satisfactory results (Ray, 1948). There are other 4-aminoquinoline drugs, namely santochin, oxychloroquine and camoquin, which closely resemble chloroquine in their chemical structure and antimalarial properties (Cooper, 1949; Wiselogle, 1946; Field, 1949). They are, however, still under trial and are not yet commercially available in this country.

Paludrine and its analogues.—Another notable contribution in this field has been the discovery of paludrine (proguanil) by the workers of the Imperial Chemicals (Pharmaceuticals) Ltd., Manchester (Curd and Rose, 1946). Early trials with this drug led one to the belief that it was probably an ideal antimalarial (Afridi, 1947; Fairley *et al.*, 1946; Field, 1947), but further investigations, while confirming the unusual properties of this compound, have at the same time served to uncover certain of its limitations (Ray, 1948; Fairley, 1949; Editorial in *B.M.J.*, 1949). There exists a wide range between the minimum effective dose and the maximum tolerated dose and in this respect paludrine is superior to all the other known antimalarials (Fairley *et al.*, 1946; Maegraith *et al.*, 1946). It not only exerts a causal prophylactic action against *P. falciparum*, a property not possessed by quinine, mepacrine or the 4-aminoquinolines (Covell *et al.*, 1949; Maegraith *et al.*, 1946; Field, 1947), but also exerts a suppressive action against all species of human malarias. Its suppressive action is however less rapid than that of quinine, mepacrine or chloroquine. Covell *et al.* (1949) have recommended that for quicker action, in the beginning of the treatment, paludrine may be reinforced by other antimalarials, such as quinine or mepacrine. In cost, it is about the cheapest of all antimalarials and this is a factor of some considerable importance. The efficacy and toxicity of paludrine lactate when given parenterally

is being investigated at the Malaria Institute of India and elsewhere, and until these studies are completed it cannot be recommended for general use.

Due to the novel chemical structure of paludrine, researches have been conducted on biguanide derivatives in a search for a more potent compound. During the course of screening tests of a number of new synthetic antimalarials prepared at the Indian Institute of Science, Bangalore, a compound closely resembling paludrine, viz. para-bromo analogue of paludrine (Bami and Guha, 1948), which compares favourably with the parent drug when tested against avian and simian malarias, has been encountered (Jaswant Singh *et al.*, 1949). Further trials with this compound have given encouraging results.

CURATIVES AND PROPHYLACTICS.

Plasmoquine and its analogues.—The advantage of the 8-aminoquinolines, viz. plasmoquine, pentaquine (SN 13276) and isopentaquine (SN 13274), is that when given with quinine and possibly other antimalarials, a radical cure of *vivax* malaria is obtained (Fairley, 1949; World Health Organization Expert Committee on Malaria, 1949). This is a matter of great importance in the case of individuals who are not likely to be re-exposed to malaria infections. Other special attributes of this group of drugs are that they are capable of producing causal prophylaxis against all species of malaria parasites and are also effective gametocidals (Jones *et al.*, 1948; Alving, 1948). In these respects, however, they are not of much practical value, as they are effective only if given in dosages which are otherwise highly toxic. Their gametocidal property does not materially avert transmission of malaria in all communities except in those where the population can be placed under rigid discipline and medical supervision. Within the group, the various compounds differ from one another mainly in the degree of toxicity, plasmoquine being the most toxic, followed by pentaquine and isopentaquine in that order (Alving, 1948; Coatney, 1949). Further researches on this group of drugs are desirable.

The discovery of a true causal prophylactic against tissue forms of all types of human malarias should receive active attention in the light of the recent discovery of exo-erythrocytic forms of *P. vivax* and *P. falciparum* (Shortt *et al.*, 1948; Shortt *et al.*, 1949). So far, a number of synthetic antimalarials, viz. sulphonamides (sulphadiazine type), naphthoquinones, acridones, 8-aminoquinolines and biguanides (paludrine type), have been reported to possess some action against the tissue forms of human malarias (Wiselogle, 1946).

Miscellaneous compounds.—A number of new chemical compounds derived from pyrimidines (M. 3349), metanilamides (metachloridine—SN 11437), quinolyl methanols (resembling quinine), phenanthrenes, naphthoquinones, amino-cresols and pantothenic acid derivatives have shown some antimalarial properties but none of them is potent enough to compete with the well-established antimalarials like mepacrine, paludrine or chloroquine (Wiselogle, 1946; Bami *et al.*, 1947; Ganapathi, 1947; Field, 1949). Although researches on the above chemical groups have not so far yielded the desired results, the knowledge and experience gained may prove most useful. It may be stated here that although the chemical synthesis programme has slackened since the termination of the war, yet the efforts

are being continued to improve upon the biological techniques, and other tests which are used in the assessment of antimalarial activity of chemical compounds.

PHARMACOLOGICAL AND BIOLOGICAL INVESTIGATIONS.

Testing of drugs in vivo.—With regard to the action of a particular drug *in vivo* it is possible that quantitatively it may affect both the tissue as well as the blood form of parasites. In that case, the question of dosage and toxicity will have to be constantly borne in mind. 8-aminoquinoline derivatives for example, although highly effective against gametocytes and the tissue forms, are not schizonticidal in the prescribed doses. If action is qualitative, then the effect will be specific against one or the other forms of parasites in which case it will fall short of the ideal. It is essential therefore that the new drugs should be tested against both sporozoite and blood induced infections.

Testing of drugs in vitro.—The study of *in vitro* cultures of erythrocytic forms may give a better insight into the physiology and nutritional requirements of the parasites than has been possible so far (Geimen *et al.*, 1946 ; Hawking, 1947 ; Hawking and Perry, 1948 ; Tonkin, 1948 ; Woolley, 1944). Experience has shown that drugs structurally similar to the metabolites necessary for the growth of malaria parasites possess some antimalarial properties ; for instance, calcium pantothenate (a part of vitamin B complex) was found necessary for the survival of *P. lophuræ in vitro* and study of chemical compounds allied to pantothenic acid have resulted in the discovery of pantothenophenone (SN 12610) which has given promising results as an antimalarial (Woolley, 1944). Similarly M. 3349 (a forerunner of paludrine and an active pyrimidine derivative) is structurally related to riboflavin, which is one of the metabolites required by the avian malaria parasites (Adams and Sanderson, 1945). Yet another example in this connection is the exceptional activity of sulpha drugs against simian malaria which is due to the fact that para-amino-benzoic acid (structurally akin to sulpha drugs) is one of the necessary requirements for the growth of *P. knowlesi* (Ball *et al.*, 1945 ; Geimen *et al.*, 1946).

It will be interesting to note that, when naphthoquinone type of antimalarials were tested against *in vitro* cultures of *P. lophuræ*, a fair correlation between chemical structures and antimalarial activity *in vivo* could be obtained by simply determining the extent of respiratory inhibition caused to the parasites *in vitro* (Ball *et al.*, 1947 ; Heymann and Fieser, 1948).

Drug metabolism.—Another important aspect of antimalarial research is the study of degradation products of drugs *in vivo* which may reveal the mode of action, detoxication mechanism and forms in which they are excreted finally. Preliminary studies in this respect have been made with some of the more well-known antimalarials (Armored Med. Res. Lab., 1946 ; Taggart *et al.*, 1948 ; Zubrod *et al.*, 1948 ; Shannon *et al.*, 1944) but a detailed examination of the more recent drugs is still incomplete (Maegraith, Tottey *et al.*, 1946 ; Loeb *et al.*, 1946 ; Hawking and Perry, 1948 ; Gordon *et al.*, 1947 ; Berliner *et al.*, 1948). This aspect of chemotherapy of malaria is gaining ground and although such studies are difficult to conduct and progress may be slow, they are likely to simplify the task of synthesizing chemical compounds displaying the required type of antimalarial activities.

Drug resistance.—Drug resistant strains of avian, simian and human malarias have been described in the case of several antimalarials, viz. quinine, atebrin, sulphadiazine and paludrine. Active attention is now being focused on the latter (Editorial in *B.M.J.*, 1949; Covell *et al.*, 1949; Seaton and Lourie, 1949; Fairley, 1949; Field and Edeason, 1949).

CONCLUSIONS.

Summing up the progress of antimalarial research in the recent past, it may be said that although notable advances have been made in the field of chemotherapy of malaria, efforts to develop an ideal antimalarial have not met with much success.

The available antimalarials have various limitations and, even for a particular drug, there are considerable differences in the reaction of different geographical strains of parasites to drug prophylaxis and therapy. In practice, the choice and the dosage of an antimalarial drug will depend upon such factors as rapidity of action, toxicity, length of treatment period, nature of the relapse pattern, natural or acquired strain resistance, the presence or absence of immunity in a community, and the cost and availability of the drug (Chaudhuri, 1948; Cooper, 1949; Field, 1949; Fairley, 1949).

In view of the great importance to India of research work in malaria chemotherapy, it is hoped that an active programme of co-ordinated research in antimalarials will soon be put into operation under the auspices of the Council of Scientific and Industrial Research, Indian Research Fund Association, army medical authorities and other scientific institutions receiving the co-operation of experts in the various fields.

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