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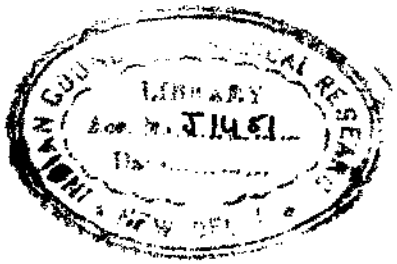

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Director, Malaria Institute of India, Delhi.

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C. R. Soc. Biol.
Geneesk. Tijd. Ned.-Ind.
Ind. J. Mal.
Ind. J. Med. Res.
J. Mal. Inst. Ind.
Jl. R. A. M. C.
J. Trop. Med. Hyg.
Meded. Volk. Ned.-Ind.
Rec. Mal. Surv. Ind.
Rev. App. Ent.
Riv. Malariol.
Trans. Roy. Soc. Trop. Med. Hyg.
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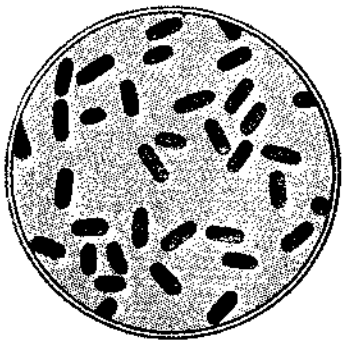

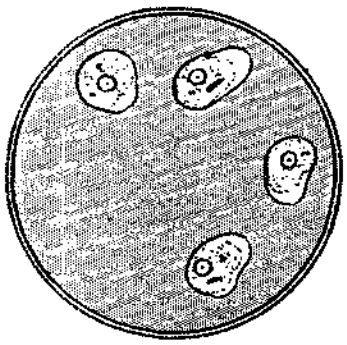

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OBSERVATIONS ON MOSQUITOES OF POONA DISTRICT, INDIA, WITH SPECIAL REFERENCE TO THEIR DISTRIBUTION, SEASONAL PREVALENCE AND THE BIOLOGY OF ADULTS.

BY

T. RAMACHANDRA RAO

AND

P. K. RAJAGOPALAN.

(*Virus Research Centre, Poona, India.*)*

[October 22, 1956.]

INTRODUCTION.

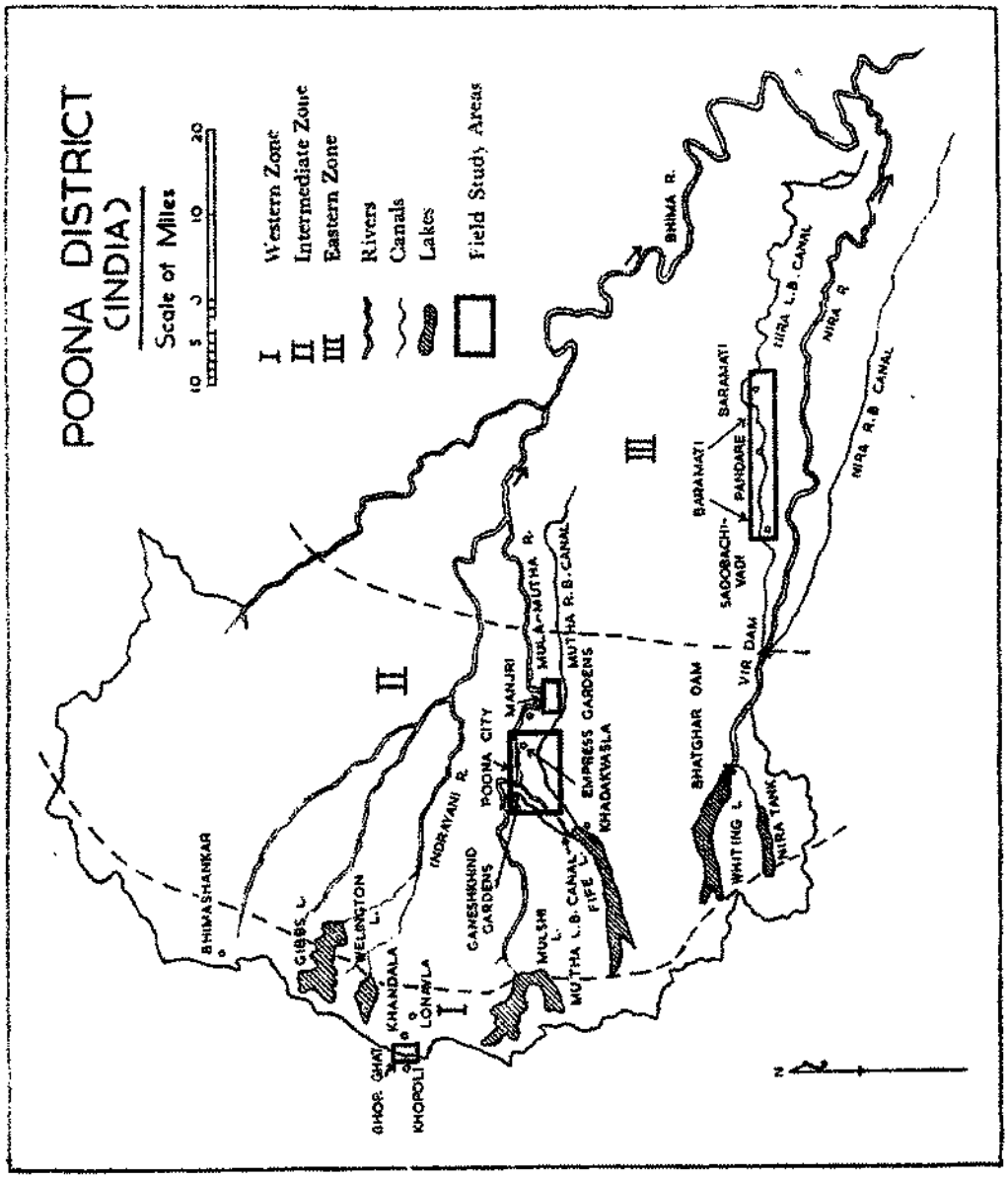
THE observations recorded in this paper were made in Poona District, Bombay State, India, between April 1953 and March 1955, in connection with virological investigations. The studies were particularly directed towards: (*a*) determination of the species of mosquitoes occurring in the district; (*b*) determination of their day-time shelters; (*c*) finding out their seasonal prevalences; and (*d*) elucidating their biting habits, particularly as regards their man-biting propensities. The studies now reported deal exclusively with adult mosquitoes. It is realized that a study of the larval stages in their natural habitats will yield valuable additional information regarding the seasonal prevalences but no attempts were made in that direction.

The studies were carried out in four field study areas, selected to include representatives of the major topographical and climatological divisions of the district. In such areas collections of mosquitoes were made mainly at fixed routine catching stations, supplemented by numerous general collections at other places.

The previous detailed mosquito surveys in Poona District have all been mainly connected with Anopheles. The reports of Barber and Rice (1938) and Viswanathan (1950) may be specially mentioned in this connection. There are practically no published records of surveys on the other genera and the little information that was available was utilized by Barraud (1934) in his monograph on Indian Culicini and Megarhinini.

*The Virus Research Centre, Poona, is maintained jointly by the Indian Council of Medical Research and The Rockefeller Foundation with the assistance of the Government of Bombay.

MAP 1.



Map of Poona District showing the location of the field study areas.

TOPOGRAPHY, CLIMATE AND HYDROLOGICAL FEATURES OF POONA DISTRICT.

Poona District, Bombay State, India, has approximately an area of 6,028 sq. miles with a total population of 19,50,976 (1951 Census). It is roughly triangular in outline and extends about 130 miles west to east and 80 miles north to south (Map 1). The western boundary of the district coincides roughly with the foot of the escarpment of the Western Ghats and is roughly 40 to 50 miles from the Arabian Sea. The elevation is between 2,000 and 3,000 feet above sea level at the western boundary and gradually falls off to 1,600-1,800 feet in the easternmost sections of the district.

From the point of view of physiography and climate the district may be divided roughly into three regions: (1) The western hilly region with high rainfall (100-150 inches per year), (2) the intermediate region of moderately undulant country broken by isolated hills and having a moderate rainfall (30-50 inches per year), and (3) the eastern plateau with low rainfall (less than 30 inches per year). The mean maximum and the mean minimum temperatures and the mean relative humidity in Poona City are given in Table I and the average and the actual rainfall during the different months recorded at (1) Lonavla, (2) Poona and (3) Baramati, typifying the western, intermediate and the eastern regions respectively, are given in Table II. The figures have been obtained from, and with the courtesy of, the Poona Observatory.

The rainfall is distinctly seasonal and most of it falls between July and October. The rest of the year is comparatively dry.

The district has several perennial rivers, the general direction of flow being from west to east. Chief among them are the Bhima, Mula-Mutha and the Nira; the last actually forms the southern boundary of the district. All rivers are flooded during monsoon but from November to end of May they have a much restricted flow. There are innumerable *nullahs* (small streams) which dry up soon after the rains.

Several of these rivers have been used for irrigation. Two major irrigation systems in the district are: (1) The Mutha Canals system taking off from a large reservoir across the Mutha at Khadakvasla, 10 miles south-west of Poona City and (2) the Nira Canals system arising from a weir across the Nira River at Vir. In the latter case, the main reservoir (Lloyd's dam) is actually some twenty miles higher up the river. Both the irrigation systems are perennial, in that the canals carry water throughout the year, but the flow is not always continuous. They will be referred to in more detail in a later section.

Apart from the reservoirs used for irrigation, there are a few which are exclusively used for hydro-electric power generation, such as the Mulshi Lake, and Gibbs and Sydenham lakes near Lonavla. These are located very close to the Western Ghats and the water is drawn away towards the west in penstock pipes. There are also a few large tanks (reservoirs) which fill up with rain water during monsoon, such as the Pashan Tank, close to Poona City. They are used for minor irrigation. Irrigation from wells by lifts, power or bullock operated, is widespread.

TABLE I.

Mean maximum and minimum temperatures and mean relative humidity at Poona City by months and the actuals for the period January 1954 to March 1955.

	Actuals for the period of study				Mean for several years			
	Mean daily maximum temperature°F.	Mean daily minimum temperature°F.	Mean relative humidity 08·00 hrs. (per cent)	Mean relative humidity 17·00 hrs. (per cent)	Mean daily maximum temperature°F.	Mean daily minimum temperature°F.	Mean relative humidity 08·00 hrs. (per cent)	Mean relative humidity 17·00 hrs. (per cent)
1954								
January	87·3	52·1	71	30	86·5	53·0	69	25
February	93·2	58·5	62	23	90·5	55·1	64	23
March ...	96·6	63·6	56	23	96·9	61·7	57	19
April ...	100·8	70·2	45	23	100·9	68·3	53	22
May ...	99·8	73·6	55	35	98·9	72·4	61	32
June ...	87·8	72·9	81	69	89·4	73·5	76	64
July ...	81·9	71·5	88	82	82·5	71·7	82	78
August ..	82·3	70·3	88	79	81·7	70·5	84	74
September	80·7	69·5	85	79	84·6	68·9	84	71
October	88·8	64·6	75	46	89·4	66·3	80	47
November	88·2	55·4	65	30	86·5	58·5	76	35
December	87·1	54·9	77	38	84·9	53·0	74	29
1955								
January	88·4	56·4	72	34	86·5	53·0	69	25
February	92·6	58·3	58	23	90·5	55·1	64	23
March ...	97·5	64·9	59	26	96·9	61·7	57	19

DESCRIPTION OF THE FIELD STUDY AREAS.

The study areas chosen were:

(1) Poona City; (*a*) Urban.
(*b*) Gardens.

(2) Manjri; a part of the area irrigated by the Mutha Right Bank Canal.

(3) Baramati; a part of the irrigated area under the Nira Left Bank Canal.

(4) Bhorghat; located on the face of the scarp of the Western Ghats.

Poona City and Manjri are in the intermediate zone described above, Baramati is in the eastern zone and Bhorghat in the western zone.

TABLE II.

Actual rainfall (in inches) by months, January 1954 *to March* 1955, *and mean rainfall by months in Poona, Lonavla and Baramati (Poona District).*

	Poona		Lonavla		Baramati	
	Actual	Mean	Actual	Mean	Actual	Mean
1954						
January ...	0	0·06	0	0·05	0	0·14
February ...	0	0·06	0	0·02	0	0·12
March ...	1·50	0·06	0	0·02	0·57	0·12
April ...	0·30	0·59	0	0·24	0·06	0·47
May ...	0·19	1·06	0	0·93	0·35	1·23
June ...	5·96	4·49	22·08	26·53	7·43	3·55
July ...	9·97	6·56	78·62	67·92	5·39	2·20
August ...	4·00	3·55	46·63	48·44	2·48	1·98
September ...	4·28	5·29	59·58	20·27	2·07	5·57
October ...	1·59	3·54	1·80	4·65	1·70	3·06
November ...	0	1·08	0	0·84	0	1·31
December ...	0·16	0·15	0·36	0·15	1·14	0·33
1955						
January ...	0	0·06	0	0·05	0	0·14
February ...	0	0·06	0	0·02	0	0·12
March ...	1·04	0·06	0	0·02	0·28	0·12

POONA CITY.

Poona City (composed of the areas of Poona Municipal Corporation proper and Poona and Kirkee Cantonments) has a population of approximately 6,00,000. It has a central very thickly populated section called the ' City ', approximately a mile in diameter, and extensive less populated outlying sections, the whole area being about eight miles long and eight miles wide. Two rivers, Mutha and Mula, flow into the area from the south-west and north-west respectively, meet within the municipal area and flow away together to the east. The Mutha Right Bank Canal, the major canal taking off from the Khadakvasla Dam, skirts the southern boundary of the city. It is nearly 40 miles long and is used mainly for irrigation. No water outlet from the canal is permitted within the ' City ' limits except for the town water supply. But there are several leakages which fill up

the *nullahs* in the locality. The Mutha Left Bank Canal, which is much smaller than the Right Bank one, flows through the less populated suburbs of the city and ends in the Ganeshkhind Botanical Garden, within the limits of the town.

Except for the heart of the ' City ' the area is moderately well provided with trees, and there are a few gardens which are well wooded. The few low hills in the proximity of the city are, however, very bare and rocky.

The localities chosen for routine collection of mosquitoes were (1) The urban area: 48 selected houses in two sections of the city, Kasbapeth and Ravivarpeth, for collection of mosquitoes resting inside houses. (2) Empress Garden and Ganeshkhind Garden, for collection (*a*) of mosquitoes resting in the outdoor shelters, such as bushes, tree stumps, mudholes, etc. and (*b*) of biting mosquitoes.

Many of the houses in the city proper have been sprayed with D.D.T. since 1950 twice a year as an antimalaria measure. It is stated that even in the two gardens referred to above, certain antimosquito measures, such as spraying oil in channels, etc., are being carried out by the military authorities but such measures have neither been regular nor thorough.

The city has a piped water supply and an underground drainage system. But there are large sections still not connected to the central drainage system and the practice of building septic tanks is prevalent. Several minor drains flow directly into the river.

MANJRI.

Manjri is an area approximately five miles long and a mile wide, commencing some eight miles to the east of Poona City on the Poona-Sholapur Road. It is an entirely rural area and it includes sections of the Manjri and Loni Kalbhor villages. It is irrigated from the Mutha Right Bank Canal which flows through it. Apart from direct irrigation, there are innumerable wells with lifts, worked by oil engines or by bullocks. Most of the land is under intense cultivation and there are many trees both on the sides of the roads and along the margins of the cultivated fields. One section of this area receives the sewage effluent from Poona City. Dotted throughout the area are groups of houses of farmers. Cattle are moderately abundant as are other domestic animals and chickens.

The chief crops grown are sugarcane, jowar (a millet-*Andropogon sorghum*), wheat and several varieties of leguminous plants. There are many citrus, guava and banana groves.

As the water supply from the canals is intermittent, no field can have standing water for more than a day or two, except during the monsoon months. Even during the monsoon, water is not allowed to stand in the fields and is generally drained away.

The routine mosquito collection stations established here were in three groups, in each of which were stations for collection of mosquitoes (1) resting indoors; and (2) resting outdoors in sugarcane fields and bushes. Routine collections of mosquitoes biting at dusk were also made in two stations.

The houses in this area are also included in the D.D.T. spraying programme of the Government and are scheduled to be sprayed twice between June and October each year. Actually some of the houses were found to have been sprayed and others not.

BARAMATI.

This study area is in the irrigated area below the Nira Left Bank Canal. The Nira irrigation system consists of a large reservoir at Bhatghar, situated practically in the Western Ghats; a pick-up weir across the Nira River at Vir, some twenty miles lower down; and two large canals on either side of the Nira River. The left Bank Canal is nearly 100 miles long, and all along its length, irrigates an area of an average width of five miles between it and the river. The water in the canal and its distributaries flows in all months of the year but a practice of intermittency has been in vogue and the canals flow for eight to ten days and the water is turned off for three or four days during the non-monsoon months. Fields are watered by turns for two to three days only during the period when the canal actually flows. Because of this practice and because of the nature of the crops grown, standing water rarely occurs in the fields for more than a day.

There are, comparatively speaking, few seepage outcrops below the main canals, quite unlike many other irrigation systems in India. One effect of the irrigation system, however, is the rise in the natural sub-soil water level in the area and many of the *nullahs* (natural drainage water courses) which normally would have carried water only during the rainy season, have become perennial. Many of them have heavy growth of weeds and are good mosquito breeding places.

The Nira Canal Zone was moderately endemic for malaria till 1952. Since then, D.D.T. spraying of houses and cattlesheds has been in operation and the incidence of the disease is now practically nil.

The main crops grown are sugarcane, jowar, wheat and legumes. There are also a few scattered banana and citrus groves. Such of the trees and shrubbery which are found in the area, are there entirely as a result of the introduction of the irrigation system some seventy years ago. There is a striking contrast between the lands immediately above and below the main canals, those above the canals presenting the appearance of an arid semi-desert with dry rocky soil.

The farming community is prosperous and livestock is abundant.

Three groups of routine mosquito collection stations were selected in this area. (1) In the outskirts of Baramati Town, (2) near Pandhara Village (seven miles to the west of Baramati) and (3) near Sadobachivadi in Hol Village (16 miles west of Baramati). All these localities were typically rural. The collecting stations in each group were: (*a*) houses, for mosquitoes resting indoors and (*b*) sugarcane fields, for mosquitoes resting outdoors. Collection of mosquitoes attracted to men, were also made at dusk in all the three locations.

BHORGHAT.

Bhorghat is the pass (four miles long) in the Western Ghats through which the main Poona-Bombay highway descends to the coastal plain. The head of the ghat is 45 miles to the north-west of Poona. Situated on the steep western escarpment of the Western Ghats, this area receives the full impact of the monsoon. Khandala Village (a part of Lonavla Municipality), above the ghat, has an annual rainfall of nearly 200 inches but practically all the rain falls between June and October and only a few scattered showers in the other months.

The ghat is well wooded, comprised chiefly of low deciduous trees interspersed with patches of broad leaf evergreen. Because of the predominance of deciduous flora, the area presents two strikingly different aspects during the year. During and immediately after the monsoon, from June to November, the aspect is extremely green, all trees with full foliage and the grass, tall and green. From December onwards, the area rapidly assumes a very dry brown appearance, only a few patches of evergreen forest standing out in marked contrast.

There are several small hill streams rapidly descending through the ghats. During the monsoon, they are all flooded and are so repeatedly flushed that no mosquito larvæ can breed in them. Immediately after the monsoon, most of the streams dry up rapidly but a few of the larger ones continue to have clear flowing water till March and all streams dry up completely in April and May.

During the monsoon and the post-monsoon months, the innumerable ground pools and tree holes provide ample opportunities for mosquito breeding.

There is practically no cultivation on the slopes of the ghat itself. But immediately above and below the ghat, rice is extensively cultivated during the monsoon months.

There are only a few scattered huts of the local people in the slopes of the ghat. But both above and below the ghats, there is a fair-sized human population, living in the townships of Lonavla (population 16,771) and Khaopoli (population 1,693) respectively. The area has a moderate wild life including birds and small mammals.

The stations selected in this study area were in two localities: (1) in a patch of evergreen forest, a mile from Khandala, at an elevation of 1,800 feet and (2) in another patch of evergreen, some 300 feet lower down. The two localities were separated by a natural ridge on which is situated the old railway reversing station, a well known landmark in this region. Passing through both the stations, were small streams with rocky beds which contained water till the middle of February. Collections made at each of the stations were:

(*a*) Mosquitoes resting inside houses,

(*b*) Mosquitoes in outdoor resting places, such as in bushes and in holes in rock along the stream margin, etc., and

(*c*) Mosquitoes attempting to bite men at dusk.

METHODS.

Data were gathered in two ways: by routine collections in fixed collecting stations and by general collections in other places. The routine collections were made at each station for a fixed duration. In general collections also, though there was no time limit, the actual time spent was always recorded. Printed cards were used and all relevant data, except the identification of the species, were directly recorded on them in the field. The identifications were made in the laboratory and recorded on the same cards.

The methods adopted for catching mosquitoes were:

MOSQUITOES RESTING INDOORS.

Hand collecting with standard glass and rubber suction tube, with the aid of a two-cell electric flashlight were necessary. Mosquitoes collected in suction tubes were immediately transferred individually into rimless culture tubes, 16 × 100 m.m., and plugged with cotton.

MOSQUITOES RESTING OUTDOORS.

In bushes or sugarcane fields.—The usual practice was for the mosquito collector to walk into the bushes and disturb the mosquitoes resting on them. When the mosquitoes started to fly, they were caught by means of the standard suction tube either when they were actually on the wing or when they came to rest. Although seemingly difficult, in practice it did not prove so. During day time most of the species of mosquitoes were not very active and could be easily caught while on the wing or after they came to rest, which they usually did quite quickly. Use of collecting nets was not routine because there was hardly any space for sweeping them.

In holes in mud and rocks or tree-trunks.—The light of the flashlight was beamed into the holes and the resting mosquitoes caught with the suction tube. Sometimes a thin stick was introduced into the hole to disturb the mosquitoes and they were quickly captured when they started flying about.

Biting mosquitoes.—Individual mosquitoes which alighted on the exposed portion of the human body, were caught directly in the test tubes. In routine collections, both forearms and legs below the knee were exposed.

Collections in traps.—Small portable traps, baited with birds or small mammals, were used. These have been described in detail in another paper (Ramachandra Rao, *in press*). Such traps were usually set up between 18.00 and 06.00 hours.

A light trap, modelled after the New Jersey light trap, powered by a 6-volt battery, was tried but was not found satisfactory.

OBSERVATIONS.

The observations recorded here were made between April 1953 and March 1955. During the first twelve months (April 1953 to March 1954), many collections were made, but as the collecting staff was new and the methods not

yet standardized, the data for the period are regarded as unsuitable for quantitative studies, and have been used for qualitative purposes only.

The routine stations were systematically visited once a fortnight from April 1, 1954 to March 31, 1955 and the data, subject to certain qualifications given below, are adequate for quantitative analysis.

The observations will be dealt with under five separate headings.

(1) Genera and species of mosquitoes found in Poona District.

(2) Seasonal prevalences of mosquitoes as determined from routine collections from April 1, 1954 to March 31, 1955 in each of the four study areas.

(3) Mosquitoes attracted to man and observations on their activity.

(4) Mosquitoes attracted to baits other than man.

(5) Detailed notes on each species of mosquito.

(1) GENERA AND SPECIES OF MOSQUITOES FOUND IN POONA DISTRICT.

In Table III is given a list of all genera and species of mosquitoes collected, together with numbers of each species captured, during the entire period of study from April 1953 to March 1955. Their occurrence in the four field study areas is also indicated.

TABLE III.

List of mosquitoes taken in Poona District, April 1, 1953 to March 31, 1955, distributed by type of collection and area.

Note: Occurrence in any area is indicated by *X*.

Serial number	Name of the species	Number collected					Taken in			
		Indoor.	In outdoor shelters	Biting outdoors.	In other ways.	Total	Poona City.	Manjri.	Bhorghat.	Baramati.
1	2	3	4	5	6	7	8	9	10	11
1	*Aedes* (*Aedimorphus*) *alboscutellatus* Theobald 1905	...	2	...	...	2	*X*	...	...	...
2	,, ,, *jamesi* Edwards, 1914	...	6	...	...	6	*X*	*X*	...	...
3	,, ,, *pipersalatus* Giles, 1901	...	21	4	...	25	...	...	...	*X*
4	,, ,, *sexans* Meigen, 1830	4	373	76	...	453	*X*	*X*	...	*X*
5	,, ,, species indet. ...	...	64	105	...	169	...	...	...	*X*
6	,, (*Banksinella*) *lineatopennis* Ludlow, 1905	1	39	12	...	52	*X*	*X*	...	*X*

TABLE III. (*Contd.*)

Serial number	Name of the species	Number collected					Taken in			
		Indoor.	In outdoor shelters.	Biting outdoors.	In other ways.	Total.	Poona City.	Manji.	Bhorghat.	Baramati.
1	2	3	4	5	6	7	8	9	10	11
7	*Aedes* (*Christophersiomyia*) *annulirostris* Theobald, 1905 ...	...	19	86	...	105	X	X	X	...
8	,, ,, *thomsoni* Theobald, 1905	...	1	...	...	1	X	...	...	...
9	,, (*Diceromyia*) species indet. ...	...	1	...	...	1	...	...	X	...
10	,, (*Finlaya*) *cogilli* Edwards, 1922 ...	...	5	13	...	18	X	X	X	...
11	,, ,, *dissimilis* Leicester, 1908 ...	...	1	6	...	7	...	...	X	...
12	,, ,, *greeni* var. *kanaranus* Barraud, 1924 ...	...	8	13	...	21	X	...	X	...
13	,, ,, *gubernatoris* Giles, 1901 ...	...	2	9	...	11	X	...	X	...
14	,, ,, sp. belonging to *niveus* group	...	...	1	...	1	...	...	X	...
15	,, ,, *pseudotæniatus* Giles, 1901	1	2	1	...	4	X	...	X	...
16	,, ,, species indet. ...	...	...	1	...	1	...	...	...	...
17	,, (*Mucidus*) *scatophagoides* Theobald, 1901	...	30	4	...	34	...	X	...	X
18	,, (*Stegomyia*) *ægypti* Linnæus, 1762 ...	1	...	...	...	1	X	...	...	...
19	,, ,, *albopictus* Skuse, 1894 ...	2	632	3270	...	3,904	X	X	X	X
20	,, ,, *vittatus* Bigot, 1861 ...	1	94	156	2	253	X	X	X	X
21	,, ,, *w-albus* Theobald, 1905	...	343	1,277	...	1,620	X	X	X	...
22	,, subgenus and species indet. ...	...	1	...	...	1	...	...	...	...
23	*Armigeres* (*Armigeres*) *obturbans* Walk, 1860	8	45	13	...	66	X	X	X	X
24	,, ,, *theobaldi* Barraud, 1934	..	1	...	...	1	...	X	...	...
25	,, (*Leicesteria*) *magnus* Theobald, 1908	...	...	1	...	1	X	...	...	...
26	*Culex* (*Culex*) *bitæniorhynchus* Giles, 1901 ...	23	1,288	51	42	1,404	X	X	X	X
27	,, ,, *cornutus* Edwards, 1922 ...	...	20	...	...	20	X	X	X	X
28	,, ,, *edwardsi* Barraud, 1923 ...	...	1	...	...	1	X	...	...	...
29	,, ,, *fatigans* Wiedemann, 1828 ...	19,666	4,305	159	684	24,814	X	X	X	X
30	,, ,, *fuscocephalus* Theobald, 1907	1	5	...	...	6	X	X	...	...

TABLE III. (*Contd.*)

Serial number	Name of the species	Number collected					Taken in			
		Indoor.	In outdoor shelters.	Biting outdoors.	In other ways.	Total.	Poona City.	Manjri	Bhorghat.	Baramati.
1	2	3	4	5	6	7	8	9	10	11
31	*Culex* ,, *gelidus* Theobald, 1901 ...	...	122	...	...	122	X	X	...	X
32	,, ,, *mimulus* Edwards, 1915 ...	...	71	...	4	75	X	X	X	X
33	,, ,, *univittatus* Theobald, 1901 ...	7	147	1	...	155	X	X	...	X
34	,, ,, *vishnui* Theobald, 1901 ...	59	1,939	32	5	2,035	X	X	X	X
35	,, ,, *whitmorei* Giles, 1904 ...	...	69	...	...	69	X	X	...	X
36	,, (*Culicomyia*) *fragilis* Ludlow, 1903 ...	...	1	...	...	1	...	...	X	...
37	,, ,, *nigropunctatus* Edwards, 1926	...	20	...	...	20	X	X	X	X
38	,, ,, *pallidothorax* Theobald, 1905	1	184	...	...	185	X	X	X	X
39	,, ,, species indet. ...	...	9	...	...	9	...	...	...	...
40	,, (*Lophoceratomyia*) *flavicornis* Barraud, 1924	...	124	...	...	124	X	...	X	...
41	,, ,, *minor* Leicester, 1908	...	22	...	...	22	...	X	X	...
42	,, ,, *minutissimus* Theobald, 1907	...	198	...	...	198	X	X	X	X
43	,, ,, *plantaginis* Barraud, 1924	...	3	...	...	3	...	X	...	...
44	,, ,, species indet. ...	...	624	...	...	624	...	...	...	...
45	,, (*Lutzia*) *fuscanus* Wiedemann, 1820	6	161	...	...	167	X	X	X	X
46	,, ,, *raptor* Edwards, 1922 ...	...	1	...	...	1	...	X	...	...
47	,, ,, species indet. ...	...	1	...	...	1	...	...	...	...
48	,, (*Mochthogenes*) *khazani* Edwards, 1922	4	273	1	...	278	...	X	X	...
49	,, ,, *malayi* Leicester, 1908	...	40	...	...	40	X	X	...	...
50	,, ,, *pluvialis* Barraud, 1924	...	250	...	...	250	X	X	X	X
51	,, (*Neoculex*) *brevipalpis* Giles, 1902 ...	...	19	...	...	19	...	...	X	...
52	,, subgenus and species indet. ...	1	246	...	...	247	...	...	...	...

TABLE III. (*Contd.*)

Serial number	Name of the species	Number collected					Taken in			
		Indoor.	In outdoor shelters.	Biting outdoors.	In other ways.	Total.	Poona City.	Manjri.	Bhorghat.	Baramati.
1	2	3	4	5	6	7	8	9	10	11
53	*Ficalbia* (*Etoriphiomyia*) *luzonensis* Ludlow, 1905	...	11	...	...	11	X	X	X	...
54	,, ,, species indet. ...	...	2	...	...	2	...	...	X	...
55	*Heizmannia viridis* Barraud, 1929 ...	...	1	...	...	1	...	...	X	...
56	,, species indet.	...	1	...	...	1	...	...	X	...
57	*Mansonia* (*Coquillettidia*) *crassipes* Van der Wulp, 1892	...	118	11	66	195	X	X	...	...
58	,, (*Mansonioides*) *annulifera* Theobald, 1901	...	3	...	...	3	X	...	...	...
59	*Mansonia* (*Mansonioides*) *uniformis* Theobald, 1901	2	5	1	...	8	X	...	...	...
60	*Megarhinus splendens* Wiedemann, 1819 ...	...	...	...	4	4	...	...	X	...
61	*Orthopodomyia anopheloides* Giles, 1903 ...	...	10	...	...	10	...	...	X	...
62	*Tripteroides affinis* Edwards, 1913 ...	...	11	...	...	11	...	...	X	...
63	,, *aranoides var. serrata* Theobald, 1901	2	189	1	...	192	X	X	X	...
64	*Uranotænia campestris* Leicester, 1908 ...	...	289	...	...	289	X	...	X	...
65	,, *maculipleura* Leicester, 1908 ...	...	3,703	...	...	3,703	X	X	X	X
66	,, *recondita* Edwards, 1922 ...	11	4,120	...	...	4,131	X	X	X	X
67	,, *stricklandi* Barraud, 1926 ...	...	322	...	...	322	...	...	X	...
68	,, species indet. (n. sp. ?) ...	...	364	...	...	364	...	...	X	...
69	*Anopheles aitkeni* James, 1903 ...	...	3	...	...	3	...	...	X	...
70	,, *annularis* Van der Wulp, 1884 ...	7	...	...	...	7	...		X	...
71	,, *barbirostris* Van der Wulp, 1884	1	1	1	...	3	X	X	...	X
72	,, *culicifacies* Giles, 1901 ...	791	120	3	1	915	X	X	X	X
73	,, ,, Cogill, 1903 ...	...	2	2	...	4	X	...	X	...
74	,, *fluviatilis* James, 1902 ...	199	107	4	...	310	X	X	X	X
75	,, *hyracanus* Giles, 1900 ...	4	26	8	...	38	X	X	...	X

TABLE III. (*Contd.*)

Serial number	Name of the species	Number collected					Taken in			
		Indoor.	In outdoor shelters	Biting outdoors.	In other ways.	Total	Poona City.	Manjri.	Bhorghat.	Baramati.
1	2	3	4	5	6	7	8	9	10	11
76	*Anopheles jamesi* Theobald, 1901 ...	48	13	4	...	65	X	X	X	X
77	,, *jeyporiensis* James, 1902 ...	14	2	...	...	16	...	X	X	X
78	,, *karwari* James, 1903 ...	...	...	1	...	1	...	...	X	...
79	,, *maculatus* Theobald, 1901 ...	3	4	9	...	16	...	X	X	...
80	,, *moghulensis* Christophers, 1924 ...	118	1	10	...	129	...	X	X	X
81	,, *pallidus* Theobald, 1901 ...	13	1	...	...	14	...	X	X	...
82	,, *splendidus* Koidzumi, 1920 ...	4	2	...	...	6	...	...	X	...
83	,, *stephensi* Liston, 1901 ...	11	...	...	...	11	...	...	...	X
84	,, *subpictus* Grassi, 1899 ...	1,106	68	3	...	1,117	X	X	X	X
85	,, *tessellatus* Theobald, 1901 ...	80	39	2	...	121	X	X	X	X
86	,, *theobaldi* Giles, 1901 ...	11	5	1	...	17	X	X	X	...
87	,, *turkhudi* Liston, 1901 ...	52	14	...	...	66	...	X	X	X
88	,, *vagus* Donitz, 1902 ...	22	12	1	...	35	...	X	X	X
89	Genus and species indet. ...	...	1	1	...	2	...	...	...	2
	Grand Total ...	22,285	21,403	5,355	808	49,851				

Eightynine species of mosquitoes, including eleven undetermined ones, belonging to eleven genera have been found in Poona District. The following twentyfour species are now being recorded for the first time in the district:

Name of the species.*	Nearest place from which recorded hitherto.
1. *Aedes alboscutellatus*	Tavargatti, Belgaum District.
2. ,, *annulirostris*	Belgaum and Nagargali, Belgaum District.
3. ,, *cogilli*	Nagargali, Belgaum District.
4. ,, *dissimilis*	Karwar, North Kanara District.
5. ,, *niveus group*	Nagargali, Belgaum District.

*The order in which the species are listed here and in other sections of this paper, follow the one adopted in Table III.

6.	*Armigeres theobaldi*	Karwar, North Kanara District.
7.	*Culex cornutus*	Hog Island, Bombay harbour.
8.	,, *edwardsi*	Coorg.
9.	,, *univittatus*	Punjab and Uttar Pradesh.
10.	,, *nigropunctatus*	Mudgere, Mysore State.
11.	,, *fragilis*	Kadra, North Kanara District.
12.	,, *flavicornis*	Nilgiris, Madras State.
13.	,, *minor*	Tavargatti, Belgaum District.
14.	,, *khazani*	Kadra, North Kanara District.
15.	,, *pluvialis*	Kadra, North Kanara District.
16.	*Ficalbia luzonensis*	Saharanpur, Uttar Pradesh.
17.	*Heizmannia viridis*	Nagargali, Belgaum District.
18.	*Megarhinus splendens*	Sawantwadi, Ratnagiri District.
19.	*Tripteroides aranoides* var. *serrata*	Nagargali, Belgaum District.
20.	,, *affinis*	Belgaum, Belgaum District.
21.	*Uranotaenia campestris* var. *zelena*	Delhi; Saharanpur, Uttar Pradesh.
22	,, *stricklandi*	Coorg.
23	,, *maculipleura*	Darjeeling, Bengal.
24	,, *recondita*	Kadra, North Kanara District.

Thirtyeight other species of mosquitoes, belonging to seven genera which are known to occur in other parts of Bombay State (mostly in the Western Ghats of Kanara and Belgaum districts), were not collected by the authors in Poona District but some of them may be expected to occur.

(2) SEASONAL PREVALENCES OF MOSQUITOES IN THE DIFFERENT FIELD STUDY AREAS.

The seasonal prevalences of mosquitoes can be accurately determined only by careful periodical collections made at fixed collection stations during all twelve months of the year. Stations once chosen should not be changed and adequate precautions taken to eliminate as many variable factors as possible, not the least important of which is the human factor.

Adequate precautions were taken and the insect collectors were not allowed to carry out the routine work without either or both of the two authors being present at the place of collection or in the vicinity. The collectors have been interchanged frequently and surprise checks often made. The duration of collections in routine stations have been kept to the optimum of 10 to 15 minutes in each house, and one hour to three hours when making collections in extensive outdoor areas. The total duration of continuous routine work by any insect collector was not allowed to exceed three hours on any one occasion. Collections about which the slightest doubt existed on any point, have been discarded.

Determination of seasonal prevalences of any species requires the study of the species from many aspects. For instance, the numbers collected in day-time

shelters may not truly reflect the abundance of species which are normally active during day time, or the numbers collected biting man or any other selected bait may not have significance if the preferred host for the species of mosquitoes is different. The study of the larval stages, in their natural habitats, is also often necessary. Valid conclusions would be possible only after the data gathered from several types of collection are considered together. These limitations have been kept in view in the discussion which follows on the seasonal prevalences of mosquitoes in the four field study areas.

The routine collecting stations were visited once a fortnight on a fixed day of the week between April 1, 1954 and March 31, 1955. Data for all collections in any one month are averaged and are presented in Tables IV, V, VI and VII. For each type of collection, viz., in indoor shelters, in outdoor shelters and biting outdoors, the total duration of collection and the densities of each species of mosquitoes expressed as the number collected per man-hour, have been given. To economize space, the actual numbers collected are not given but they may be easily calculated.

The total number presented include both males and females. Comments about the sex ratios, where necessary, are given in the section dealing with the individual species.

Poona City (Table IV).

(*a*) *Urban area: Indoors* (Table IV-A).—The routine station consisted of 48 houses in two sections in the heart of the city (Ravivarpeth and Kasbapeth). The houses were mostly two, three or four storeyed buildings made of brick and mud on a wooden framework and tenanted by lower middle class people. The rooms were generally dark and ill-ventilated, and on the average measure 8 to 10 feet in width and length and about 8 to 10 feet in height. Cattle were uncommon, having been noticed only in two of the 48 houses. Four insect collectors searched 12 houses each, spending 15 minutes per house and, therefore, a total of 12 man-hours on each visit.

Culex fatigans was the only species collected in these houses and was prevalent throughout the year. December, January and February were the months of the highest prevalence and April, May and June, the months of least prevalence.

The total absence of anopheline species calls for comment. Till 1944, Poona City was moderately malarious, particularly the sections adjacent to the rivers and the canals and several species such as *Anopheles culicifacies*, *A. stephensi* and *A. subpictus* were quite commonly found resting inside houses. The densities of these species were greatly reduced by extensive antilarval measures undertaken by the Government beginning in 1944. Since 1950, many of the houses in the city proper have been sprayed twice a year with D.D.T. as an indoor residual spray. This may explain the total absence of adults of Anopheles spp. But the persistence of *Culex fatigans* in sprayed houses is noteworthy. During the period of the present observations, the sections of the city in which the collecting stations were located were sprayed with D.D.T. at an average dosage of 100 mg./sq. ft. in September and February. There were slight reductions in the densities in the first collection made after spraying but the numbers returned to normal in the

subsequent one. For all practical purposes, the indoor spraying with D.D.T. in the dosage employed, had little effect on the population of *Culex fatigans* resting inside houses.

(*b*) *Gardens*: (Tables IV-B and IV-C and Charts 1 and 2).—The Empress Garden and the Government Horticultural Garden at Ganeshkhind were visited

TABLE IV.

Poona City.

Monthly densities of mosquitoes expressed as mean number collected per man-hour; April 1954 *to March* 1955.

(A) **Poona Urban-indoors.**

	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Time spent: man-hours	24	24	24	24	24	24	12	24	24	24	24	36
Culex fatigans ...	8·6	4·7	7·3	17·9	17·2	19·5	19·1	12·9	21·2	36·1	28·8	17·6

(B) **Poona gardens-outdoor shelters.**

Time spent: man-hours	18	24	24	30	24	24	18	24	30	24	24	30
All mosquitoes ...	9·4	16·1	17·1	13·2	15·4	10·1	11·9	9·6	6·1	10·7	10·2	8·6
Aedes alboscutellatus ...	...	...	...	...	...	0·1	...	...	...	...	...	...
,, *jamesi* ...	...	...	...	...	...	0·1	...	...	...	...	...	...
,, *vexans* ...	...	...	...	...	...	2·7	0·4	0·3	0·1	...	*	*
,, *lineatopennis* ...	...	...	...	0·1	0·1	0·1	...	...	*	...	...	...
,, *annulirostris* ...	...	...	*	0·1	0·1	*	...	...	...	*	...	...
,, *thomsoni* ...	...	...	...	...	...	...	0·1	...	...	...	...	...
,, *cogilli* ...	...	...	...	...	*	*	...	...	...	...	...	...
,, *greeni* ...	...	...	...	...	0·3	...	...	...	...	...	...	...
,, *gubernatoris* ...	...	...	*	...	...	...	...	...	...	...	...	...
,, *albopictus* ...	2·7	0·1	0·9	0·5	0·3	0·5	0·9	0·5	0·2	...	*	*
,, *vittatus* ...	...	...	*	0·1	...	...	...	...	...	...	...	...
,, *w-albus* ...	...	...	0·4	0·2	0·3	0·9	0·4	*	0·1	*	*	...
,, sp. indeter ...	...	...	...	...	...	*	...	...	...	...	...	...
Armigeres obturbans ...	...	...	...	...	0·1	0·4	0·4	*	...	...	...	...
Culex bitaeniorhynchus ...	...	...	*	0·2	0·8	0·5	0·5	0·2	0·4	0·5	0·2	0·2
,, *cornutus* ...	...	...	...	0·1	0·1	*	...	...	...	...	...	...

TABLE IV—(*Contd.*).

	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Culex edwardsi ...	...	...	...	...	...	...	...	...	...	*	...	...
" *fatigans* ...	2·3	4·3	2·5	1·6	0·9	0·6	0·2	0·2	2·1	7·5	7·8	4·7
" *fuscocephalus* ...	...	...	...	...	...	...	...	...	...	...	*	...
" *gelidus* ...	...	...	...	0·1	0·1	*	0·4	...	...	...	...	...
" *nimulus* ...	...	...	*	0·1	0·2	0·1	...	0·3	...	*	...	...
" *sonchittatus* ...	0·1	0·3	0·2	0·1	0·1	...	...	...	...	*	0·9	0·6
" *vishnui* ...	0·1	*	0·1	0·5	0·3	0·3	0·3	0·6	0·4	0·5	0·3	0·3
" *vishnuoroi* ...	...	...	...	...	...	...	0·1	0·2	...	...	...	...
" *nigropunctatus*	...	...	...	...	...	...	0·1	0·3	...	...	...	...
" *pallidothorax* ...	...	...	...	0·2	0·7	0·7	0·6	*	0·1	0·1	*	0·3
" *fuscicornis* ...	...	...	...	...	...	...	...	0·1	...	...	...	...
" *minutissimus* ...	...	...	...	0·1	...	...	...	0·1	0·2	...	0·2	0·2
(Leph.) Sp. indeter	...	...	...	...	...	...	...	...	...	*	...	...
" *fuscanus* ...	...	...	...	0·2	0·4	0·2	0·1	0·1	...	...	...	*
" *malayi*	...	...	...	...	...	...	...	...	0·1	0·1	*	...
" *tinsindis* ...	...	...	0·9	1·3	0·2	...	0·4	4·3	0·9	...	...	...
Nicaibia luzonensis	...	...	...	...	...	...	...	...	0·1	0·1	...	...
Mansonia crassipes	0·1	*	0·5	0·1	0·1	0·6	1·2	*	0·1	...	0·1	0·1
" *annulifera*	...	...	...	...	0·1	...	...	...	...	...	...	...
" *uniformis*	...	...	...	...	...	...	0·2	...	...	...	...	...
Uranotænia uranoides var. *serrata* ...	...	...	...	...	...	...	...	0·5	0·1	*	...	...
Uranotænia campestris	...	...	...	...	0·1	...	0·1	...	*	0·5	*	...
" *maculipleura*	2·7	6·7	3·6	1·1	3·3	0·9	3·3	0·8	0·4	0·3	0·2	0·9
" *recondita* ...	1·3	4·7	7·9	6·8	5·8	1·3	2·3	0·8	0·6	0·4	0·1	1·1
Anopheles culicifacies ...	...	...	...	...	0·1	*	0·1	*	*	...	...	...
" *fluviatilis* ...	...	...	...	...	...	...	0·1	0·2	*	0·3	0·1	...
" *hyrcanus* ...	...	...	...	...	...	...	...	0·1	*	...	...	...
" *jamesi* ...	...	...	...	...	...	...	...	...	...	...	*	...
" *tessellatus* ...	...	...	...	*	*	...	...	...	...	...	...	...
" *theobaldi* ...	...	...	...	...	*	...	...	...	...	...	...	...

TABLE IV—(*Contd.*).

(C) **Outdoor biting day.**

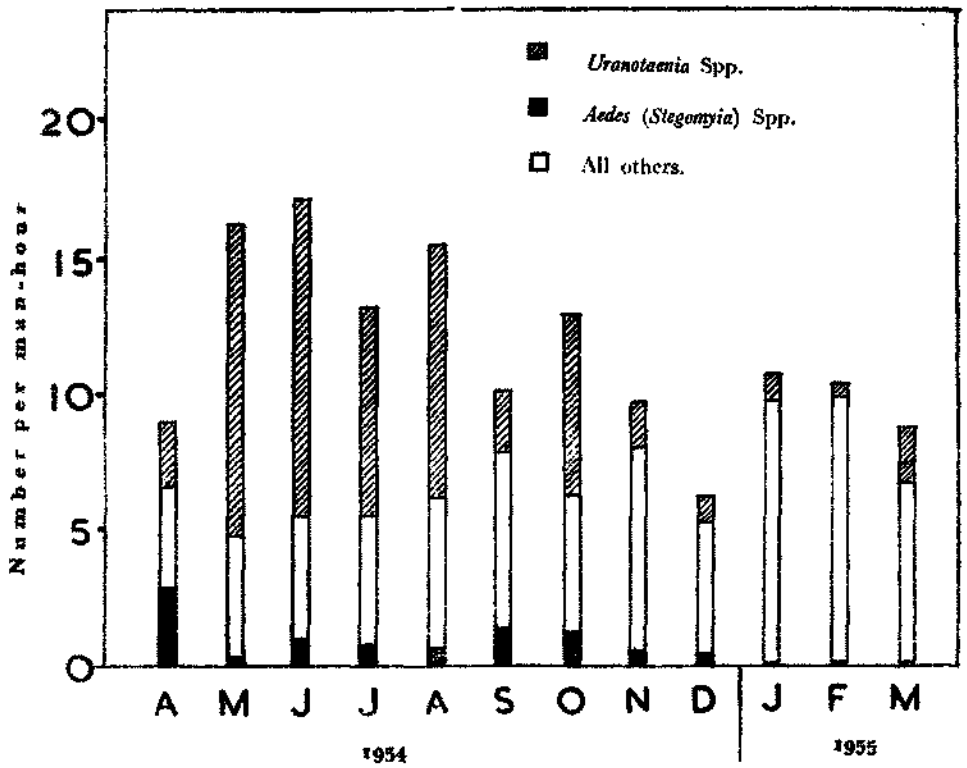
	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Time spent: man-hours	18	24	24	30	24	24	18	24	30	24	24	30
All mosquitoes ...	0·8	0·1	4·3	5·7	4·4	1·7	3·7	1·9	0·2	0·2	0	0·1
Aedes vexans ...	...	...	...	...	...	...	0·1	0·2	...	...	...	...
.. *annulirostris* ..	...	...	...	*	*	*	...	...	...	...	...	...
.. *vogilli* ...	...	...	...	...	0·1	...	...	...	...	...	...	...
.. *greeni* ...	...	...	...	...	0·1	...	...	...	...	...	...	...
.. *albopictus* ...	0·7	*	3·5	3·8	2·3	0·8	2·0	1·5	0·1	0·1	...	0·1
.. *vittatus* ...	...	...	0·1	...	...	*	...	...	...	...	...	...
.. *w-albus* ...	0·1	*	0·7	1·8	1·9	0·8	1·4	*	0·1	*	...	...
Armigeres obturbans ...	...	...	...	...	...	*	0·2	*	...	...	...	...
Tripteroides aranoides var. *serrata* ...	...	...	...	...	...	...	...	*	...	...	...	...
Anopheles culiciformis	...	...	...	...	...	...	...	*	...	...	...	...

(D) **Outdoor biting dusk.**

Time spent: man-hours	12	12	12	12	12	12	6	12	12	12	12	18
All mosquitoes ...	1·5	0·6	3·2	3·4	2·4	0·8	3·3	1·1	0	1·1	0·6	0·6
Aedes vexans ...	...	...	...	...	...	...	...	...	...	0·1	...	...
.. *annulirostris* ...	...	...	0·6	0·1	0·1	...	...	0·1	...	...	...	...
.. *gubernatoris* ...	...	...	0·2	...	...	...	...	...	...	...	...	...
.. *albopictus* ...	1·2	0·2	1·8	2·4	1·5	0·7	3·0	1·0	...	0·6	0·2	0·4
.. *vittatus* ...	...	...	0·1	...	...	...	...	...	...	...	...	...
.. *w-albus* ...	0·3	0·2	0·4	0·2	0·3	0·1	0·2	...	...	...	...	...
Armigeres obturbans ...	...	...	...	...	0·1	...	...	...	...	...	...	...
Culex bitaeniorhynchus ...	...	...	...	...	0·1	...	0·2	...	...	...	...	...
.. *fatigans* ...	...	0·2	0·1	0·8	0·3	...	...	...	...	0·3	0·3	0·2
.. *univittatus* ...	...	...	...	...	...	...	...	...	...	0·1	...	...
.. *vishnui* ...	...	...	...	...	...	...	...	...	...	...	0·1	...
Mansonia uniformis ...	...	...	...	...	...	...	...	...	...	0·1	...	...

*Density of less than 0·04 per man-hour.

CHART 1.

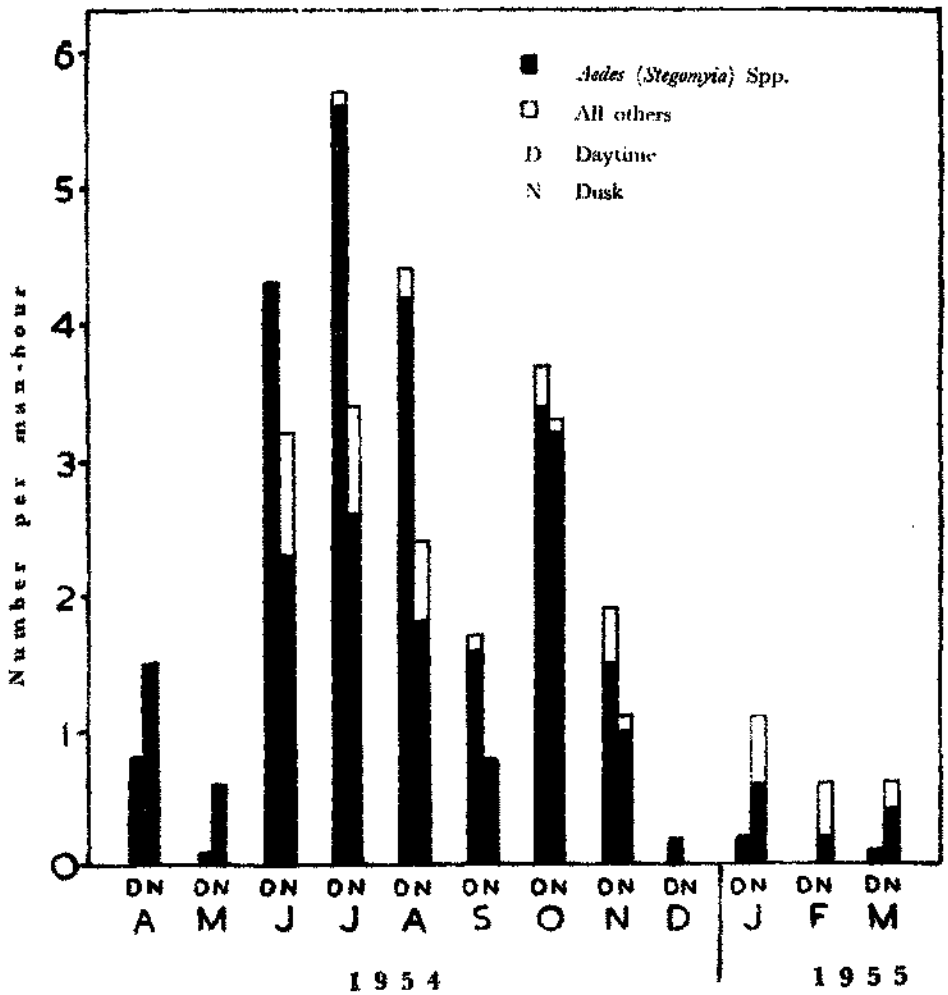


Seasonal prevalence of mosquitoes, expressed as number collected per man-hour, by months, April 1953 to March 1955: Outdoor shelters, Poona Gardens.

once a fortnight. In addition to the collections of biting mosquitoes, the work in the gardens consisted of searches for resting mosquitoes in bushes, mudholes and holes in tree stumps. Two insect collectors spent a total of six man-hours in each garden between 09.00 and 12.00 hours on the appointed day. The collections in the Empress Garden were made in two sections spending exactly half the time in each. One man concentrated in the mudholes, etc., and the other man in bushes. In Ganeshkhind Garden, the collections were made in three sections, spending exactly two man-hours in each. The collections of biting mosquitoes were made both during day and dusk in Empress Garden but only during day at Ganeshkhind Garden. Both the gardens are well wooded with tall trees and are perennially irrigated from the Mutha Canal system. For purposes of this report, collections made in those two gardens have been pooled together.

Mosquitoes resting outdoors.—The species of mosquitoes which were commonly found resting in the gardens were *Aedes albopictus*, *A. w-albus*, *Culex bitæniorhynchus*, *C. fatigans*, *C. univittatus*, *C. vishnui*, *C. pallidothorax*, *Mansonia crassipes*, *Uranotaenia*

CHART 2.



Seasonal prevalence of mosquitoes, expressed as a number collected per man-hour, by months, April 1953 to March 1955: Biting mosquitoes during the day and at dusk, Poona Gardens.

c aculipleura and *U. recondita.* The other species either were present in small numbers or their prevalence was restricted to a few months only.

For purposes of study, the mosquitoes found are considered in three groups, *Aedes* (*Stegomyia*) spp., *Uranotaenia* spp., and all others. Taking all species together, the highest numbers found resting in outdoor shelters were in May and June

and the lowest in February. *Stegomyias* (consisting of *Aedes albopictus* and *A. w-albus*) were collected in outdoor shelters in comparatively smaller numbers than other species of mosquitoes and no marked seasonal trend is noticeable. This is misleading, for, the same species captured while actually biting man (Chart 2), show a distinct seasonal trend with a markedly high prevalence between June and November. The difference arises out of their well-known day-time activity. Unlike most other species of mosquitoes they do not 'rest' in shelters during day time but are out seeking bloodmeals. The *Uranotaenias* were represented by three species, two of which, *U. maculipleura* and *U. recondita*, were consistently found in all months of the year. Their highest numbers were found in May to August. In the third group, including all other mosquitoes, *Culex fatigans* was the commonest species and accounted for the bulk of the mosquitoes collected between January and March when it was most prevalent. Between August and November, it was comparatively scarce. *Culex vishnui* occurred in small numbers throughout the year and there was no marked seasonal variation. A point of interest is the comparative scarcity of members of the subgenera *Lophoceratomyia* and *Mochthogenes* in the two gardens.

Biting outdoors (Chart 2).—The bulk of the biting mosquitoes consisted of the two species of *Stegomyias*. There was a marked seasonal trend. The highest number collected was during the monsoon months, a period when most of the tree holes were filled with water.

Manjri (Table V and Charts 3 and 4).

Indoors.—The mosquitoes collected inside houses in Manjri consisted primarily of *Culex fatigans*, *Anopheles culicifacies* and *A. subpictus*. The few other species of *Culex* and *Anopheles* taken were of no significance. *Culex fatigans* was the most abundant species, and April and May were its peak months of prevalence. The densities of Anopheles were highest in August and September. The low densities of Anopheles in this area is attributable to the D.D.T. indoor spraying carried out as an antimalaria measure.

Resting outdoors.—The mosquitoes resting in outdoor shelters, however, were quite rich in species. Fortyfive species of mosquitoes were found and taking all of them together (Chart 3), the lowest prevalence was found in May and the highest in August. The species collected throughout the year were *Culex bitæniorhynchus*, *C. fatigans*, *C. vishnui*, and *C. fuscanus*. Those which occurred in most months, if not in all, were *Aedes albopictus*, *Culex gelidus*, *C. minutissimus* and *Anopheles culicifacies*. The other species occurred in only some months of the year.

Culex fatigans contributed considerably to the total density and there was no distinct seasonal trend in regard to it. *C. bitæniorhynchus* was much less common and no seasonal trend was noticed with it also, though its highest prevalence was in September. *C. vishnui* was abundant between July and January. *C. fuscanus*, though it occurred throughout the year, occurred in very small numbers except in August.

TABLE V.

Manjri.

Monthly densities of mosquitoes expressed as mean number collected per man-hour; April 1954 to March 1955.

(A) **Indoors.**

	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Time spent: man-hours	6	6	6	6	9	6	6	6	6	8	6	5·8
All mosquitoes ...	49·0	42·5	31·0	12·0	21·1	20·2	12·5	14·3	14·0	19·9	31·2	25·3
Culex bitæniorhynchus	...	...	...	...	...	...	0·2	...	...	0·1	...	...
,, *fatigans* ...	48·3	41·0	29·3	8·8	8·9	8·2	9·0	12·8	10·8	18·1	30·2	23·4
,, *univittatus* ...	...	...	...	...	...	...	...	...	...	...	...	0·2
,, *vishnui* ...	...	0·2	...	...	...	...	...	0·5	0·2	0·1	0·2	0·2
,, *pallidothorax* ...	...	...	...	...	0·1	...	...	...	...	...	...	...
Anopheles culicifacies	0·3	0·3	0·5	1·5	2·0	0·3	1·7	1·0	1·2	0·8	0·2	0·9
,, *fluviatilis* ...	...	...	...	...	0·1	...	...	...	0·8	0·5	0·5	0·2
,, *moghulensis*	...	...	...	...	...	...	...	...	0·3	...	...	...
,, *subpictus* ...	0·3	1·0	1·2	1·7	9·8	11·7	1·7	...	0·2	0·1	0·2	0·3
,, *tessellatus* ...	...	...	...	...	...	...	...	...	...	0·1	...	...
,, *turkhudi* ...	...	...	...	...	0·1	...	...	...	0·5	...	...	0·2
,, *vagus* ...	...	...	...	...	0·1	...	...	...	...	...	...	...

(B) **Outdoor shelters.**

Time spent: man-hours	12	12	12	12	18	18	12	12	11	17	12	8
All mosquitoes ...	8·2	5·8	12·9	14·4	25·3	22·7	20·2	13·3	19·2	15·1	20·8	14·3
Aedes jamesi ...	...	...	...	...	...	...	0·2	0·1	...	...	...	...
,, *vaxans* ...	...	...	...	...	...	0·2	1·1	0·3	0·2	0·1	...	...
,, (*Aedim.*) sp. indeter ...	...	...	...	...	...	...	...	...	...	0·1	...	...
,, *lineatopennis* ...	...	...	...	0·3	0·1	0·3	0·1	...	...	...	...	...
,, *annulirostris* ...	...	...	...	...	0·1	0·3	...	...	...	...	...	...
,, *vogilli* ...	...	...	...	...	0·1	...	...	...	...	...	...	...
,, *albopictus* ...	...	...	1·6	1·7	0·8	0·5	0·8	0·9	0·1	0·2	0·1	...

TABLE V—(*Contd.*).

(B) **Outdoor shelters.**—(*Contd.*)

	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Aedes vittatus ...	...	...	0·2	0·6	...	0·2	...	...	...	...	...	...
,, *w-albus* ...	...	...	0·2	0·9	0·8	0·1	...	...	...	...	...	...
Armigeres obturbans ...	...	...	...	...	...	...	...	0·1	...	...	...	...
Culex bitæniorhynchus	0·2	0·4	0·2	0·8	1·4	4·2	3·3	0·5	1·6	2·5	2·7	3·1
,, *cornutus* ...	...	...	...	...	0·1	0·1	...	...	...	...	...	...
,, *fatigans* ...	7·3	4·7	8·8	5·2	10·7	8·9	5·2	4·2	3·5	4·8	13·0	7·5
,, *fuscocephalus* ...	...	...	...	...	...	...	...	...	...	0·1	0·2	...
,, *gelidus* ...	...	...	...	0·1	0·1	0·3	2·5	0·3	0·1	0·1	...	...
,, *mimulus* ...	...	...	...	0·1	0·1	0·1	...	0·1	...	...	...	...
,, *mimvittatus* ...	0·2	...	...	0·1	...	...	...	...	0·1	0·4	0·9	0·9
,, *nishnui* ...	0·6	0·6	0·3	3·4	4·8	4·6	3·7	3·1	3·8	4·0	2·5	1·6
,, *whitmorei* ...	...	...	...	...	...	...	1·2	1·0	..	0·1	...	...
,, *nigropunctatus* ...	...	...	...	...	...	0·2	...	...	...	...	...	...
,, *pallidothorax* ..	...	...	...	...	0·6	0·7	0·4	0·1	...	...	...	0·1
,, *flavicornis* ...	...	...	...	...	...	...	...	0·1	...	...	...	...
,, *minor* ...	...	...	...	...	...	...	0·1	...	...	...	...	...
,, *minutissimus* ...	...	...	0·1	0·3	...	...	0·2	0·3	3·9	0·3	0·8	0·6
,, (*Lopho.*) sp. indeter ...	...	...	...	...	0·1	...	...	...	...	...	...	...
,, *fuscanus* ...	0·1	...	0·1	0·1	3·6	1·3	0·3	0·1	0·1	0·1	0·1	0·1
,, *raptor* ...	...	...	...	...	0·1	...	...	...	...	...	...	...
,, *pluvialis* ...	...	...	1·1	...	0·1	...	...	0·5	0·1	...	...	...
,, sp. indeter ...	...	...	...	...	...	...	...	...	2·8	0·4	...	...
Ficalbis luzonensis ...	...	...	...	...	...	...	...	0·1	...	0·1	...	...
Mansonia crassipes ...	0·1	...	...	0·1	...	...	...	...	...	...	...	...
Tripteroides aranoides var. *serrata*	...	...	...	...	...	...	...	0·1	...	...	...	...
Uranotaenia recondita ...	...	...	0·2	0.4	0·1	0·1	0·3	0·2	0·5	...	...	...
,, *maculipleura*	...	...	0·2	...	0·1	0·1	...	...	...	...	...	...
Anopheles culicifacies ...	...	...	0·1	...	0·8	0·1	0·4	0·2	0·3	0·5	0·3	0·1

TABLE V—(*Contd.*).

(B) **Outdoor shelter**—(*Contd.*).

	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Anopheles fluviatilis	...	...	...	...	0·1	...	0·1	0·6	1·4	0·9	0·3	0·1
,, *hyrcanus* ...	...	...	...	...	...	...	...	...	0·3	...	...	...
,, *jamesi* ...	...	...	...	...	...	...	...	0·3	0·1	...	0·1	...
,, *jeyporiensis* ...	...	...	...	...	0·1	...	...	...	...	0·1	...	...
,, *maculatus* ...	...	...	...	...	0·1	...	...	...	...	...	...	...
,, *moghulensis*	...	...	...	...	...	...	0·1	...	...	...	...	...
,, *subpictus* ...	...	0·1	...	...	0·3	0·5	0·3	0·1	0·1	...	...	...
,, *tessellatus* ...	...	...	...	...	0·3	0·3	...	0·2	...	0·1	...	...
,, *theobaldi* ...	...	...	...	...	...	...	...	...	...	0·1	...	...
,, *turkhudi* ...	...	...	...	...	...	...	...	...	0·4	0·3	...	...

(C) **Outdoor biting dusk.**

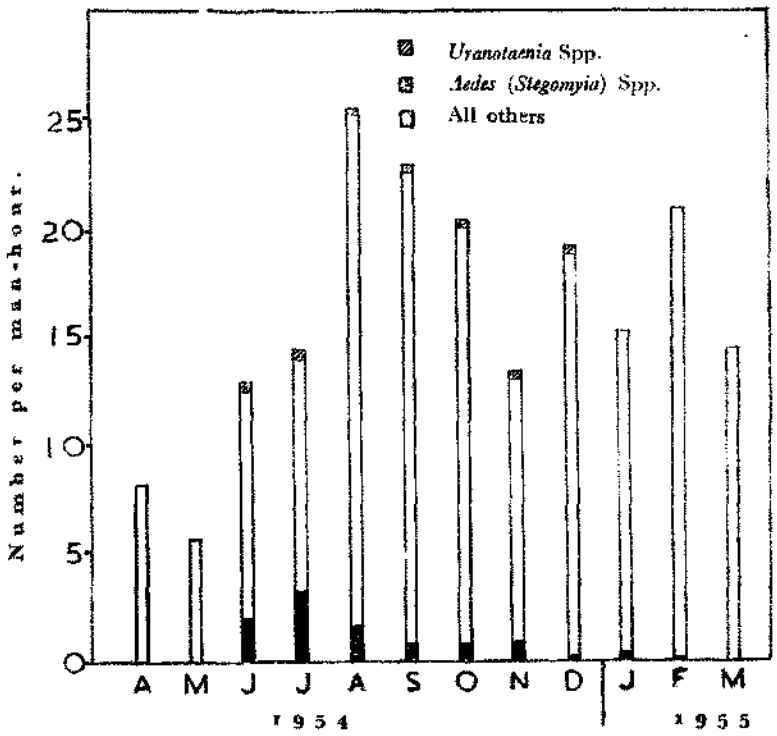
Time spent: man-hours	24	18	24	24	18	24	24	24	24	36	24	18
All mosquitoes ...	0	0	0·1	1·3	0·4	0·4	0·3	0·1	0	0·1	0·3	0·2
Aedes vexans ...	...	...	...	...	...	0·2	...	...	...	...	...	...
,, *lineatopennis* ...	...	...	...	†	0·1	...	...	†	...	...	...	...
,, *annulirostris* ...	...	...	...	†	...	...	...	...	...	...	...	...
,, *albopictus* ...	...	...	†	0·6	...	...	0·2	...	...	†	...	...
,, *vittatus* ...	...	...	†	0·4	0·1	†	...	...	...	...	...	...
,, *w-albus* ...	...	...	...	0·1	0·1	...	...	...	...	...	...	...
Culex bitaeniorhynchus ...	...	...	...	...	...	...	...	...	...	...	†	...
,, *fatigans* ...	...	...	†	0·1	0·3	0·2	0·1	0·1	...	†	0·3	0·2

†Density of less than 0·04 per man-hour.

The small number of *Stegomyias* in this area is worthy of note, and is perhaps because the area is not so well wooded as the gardens in Poona City, and therefore there are few tree holes.

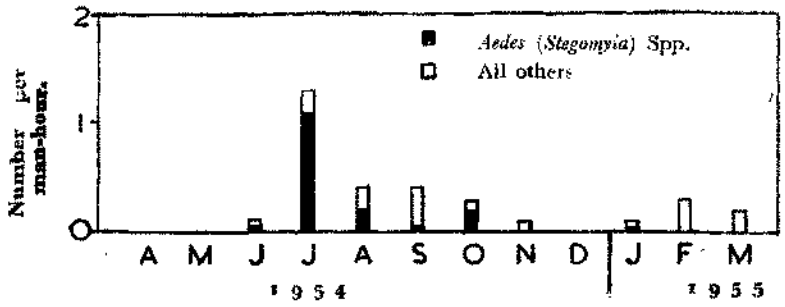
Biting outdoors (Chart 4).—The mosquitoes collected biting man were never high. The highest number was collected in July when the increase was entirely due to *Stegomyias*.

CHART 3.



Seasonal prevalence of mosquitoes, expressed as number collected per man-hour, by months, April 1953 to March 1955: Outdoor shelters, Manjri.

CHART 4.



Seasonal prevalence of mosquitoes, expressed as number collected per man-hour, by months, April 1953 to March 1955: Biting mosquitoes at dusk, Manjri.

Baramati (Table VI and Charts 5 and 6).

Indoors.—Three species of Aedes, five of Culex and eleven of Anopheles were found inside houses. But the bulk of the mosquitoes consisted of *Culex fatigans* which in March rose to the high figure of 64·3 per man-hour. *Anopheles culicifacies*, *A. fluviatilis* and *A. subpictus* were found throughout the year but their densities were not high. They were extremely abundant in this area in the past, i.e., before the advent of the D.D.T. spraying operations in July 1953. The effect of the spraying is reflected by the low densities of all mosquitoes during the monsoon and post-monsoon months, August to December, when they should have really been very high.

TABLE VI.

Baramati.

Monthly densities of mosquitoes expressed as mean number collected per man-hour; April 1954 to March 1955.

(A) **Indoors.**

	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Time spent: man-hours	17	12	12	12	12	12	18	12	11	12	12	11·7
All mosquitoes ...	27·1	20·3	18·5	30·8	13·8	4·8	8·1	7·6	12·3	19·7	44·2	70·4
Aedes vexans ...	...	...	...	...	...	...	0·2	...	...	...	...	...
,, (*Aedim.*) sp. indet.	...	...	...	0·1	...	...	...	...	...	...	...	...
,, *albopictus* ...	0·1	...	...	...	...	...	...	...	...	...	...	...
Culex bitaeniorhynchus ...	0·1	0·1	...	0·1	...	...	...	...	...	...	...	0·1
,, *fatigans* ...	21·3	16·2	9·3	8·2	6·9	3·3	5·0	6·2	8·1	16·6	40·0	64·3
,, *vishnui* ...	0·1	0·1	...	...	...	...	0·2	...	0·1	0·2	...	0.2
,, *univittatus* ...	0·1	...	...	...	...	...	...	...	...	...	...	0·3
,, *fuscanus* ...	...	...	...	...	...	...	...	...	...	...	...	0·2
Anopheles culicifacies ...	4·1	2·2	2·0	5·1	1·3	0·6	1·1	0·3	0·5	0·7	1·0	1·0
,, *fluviatilis* ...	0·2	0·4	0·3	0·7	0·5	0·1	0·4	0·3	1·1	1·3	1·8	1·1
,, *hyrcanus* ...	...	...	...	...	...	...	0·1	...	...	...	...	0·1
,, *jamesi* ...	...	...	...	0·1	...	...	...	...	...	...	0·1	...
,, *moghulensis*	...	...	...	...	...	...	...	0·3	...	...	...	...
,, *stephensi* ...	...	...	...	...	0·1	...	...	...	0·4	0·3	...	0·1
,, *subpictus* ...	1·0	1·2	6·6	14·0	4·8	0·8	1·2	0·4	1·5	0·5	0·8	1·3
,, *tessellatus* ...	...	...	0·3	2·4	0·2	0·1	...	0·1	0·2	0·1	0·3	0·5
,, *turkhudi* ...	0·1	0·1	...	0·2	0·1	...	...	...	0·5	0·1	0·3	1·1
,, *vagus* ...	...	...	...	...	...	...	...	...	...	...	...	0·1

TABLE VI—(*Contd.*).

(B) Outdoor shelters.

	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Time spent: man-hours	18	16	12	12	12	12	18	12	11	10	12	12
All mosquitoes ...	7·3	4·7	12·6	19·8	19·4	20·4	23·3	9·4	9·5	18·1	7·5	5·9
Aedes pipersalatus ...	0·2	...	...	...	...	...	...	...	...	...	...	...
,, *vaxans* ...	...	...	2·9	3·8	3·0	2·8	2·8	1·3	0·8	1·0	0·3	...
,, (*Aedim.*) sp. indet. ...	...	...	0·1	0·2	1·8	1·6	0·9	...	...	...	...	...
,, *lineatopennis* ...	...	...	...	...	...	0·2	...	...	...	...	...	...
,, *albopictus* ...	...	...	...	0·2	...	0·3	...	...	...	...	...	...
Armigeres obturbans ...	...	...	...	...	...	...	0·1	...	...	...	...	...
Culex bitaeniorhynchus ...	0·2	0·4	0·8	1·4	1·4	2·3	2·2	0·4	0·9	1·2	...	0·1
,, *cornutus* ...	...	...	...	...	...	...	0·1	...	0·1	...	...	...
,, *fatigans* ...	6·1	2·9	2·8	2·6	4·3	4·2	3·1	0·6	2·1	9·5	5·1	4·7
,, *gelidus* ...	...	...	0·1	0·4	0·2	0·2	0·4	0·3	0·1	...	...	...
,, *mimulus* ...	...	...	...	...	...	...	†	...	...	...	...	...
,, *univittatus* ...	0·1	0·1	0·2	0·1	0·3	...	...	...	...	0·1	0·3	0·3
,, *vishnui* ...	0·5	1·2	5·5	11·0	8·3	8·2	9·3	5·3	4·1	5·9	1·7	0·8
,, *whitmorei* ...	...	...	...	...	...	...	1·9	...	...	...	...	0·1
,, *nigropunctatus* ...	...	...	...	...	...	...	0·3	...	...	...	...	...
,, *pallidothorax* ...	...	...	...	...	0·1	0·2	0·6	...	...	...	...	...
,, *minutissimus* ...	...	...	...	...	0·1	...	...	...	...	...	...	...
,, *fuscanus* ...	...	...	...	0·1	...	0·3	0·4	0·3	0·1	0·1	...	...
,, *pluvialis* ...	...	...	...	...	0·1	...	0·1	...	...	...	...	...
,, sp. indet. ...	...	...	...	...	...	...	...	0·1	...	...	...	...
Anopheles culicifacies ...	...	...	...	...	...	0·1	...	0·3	0·4	...	0·1	...
,, *fluviatilis* ...	0·2	0·1	...	...	...	...	0·1	0·3	...	0·2	0·1	...
,, *hyrcanus* ...	...	...	...	...	...	...	0·3	0·5	0·5	0·1	...	...
,, *jamesi* ...	...	...	...	...	...	0·1	...	...	...	...	0·1	...
,, *subpictus* ...	...	...	0·1	...	...	0·1	...	...	0·1	...	...	...
,, *tessellatus* ...	...	...	0·2	0·1	...	...	0·8	0·3	...	...	...	...
,, *turkhudi* ...	...	...	...	...	...	...	...	...	0·3	...	...	...

TABLE VI—(*Contd.*).

(C) **Outdoor biting dusk.**

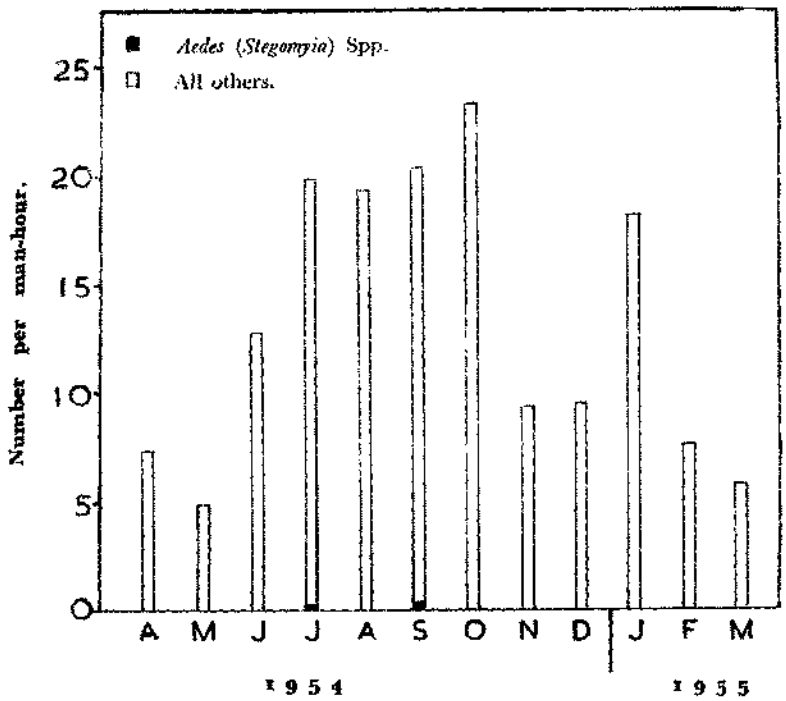
	Apr.	May	Jan.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Time spent: man-hours	72	48	48	48	48	48	72	48	36	48	48	48
All mosquitoes ...	0·1	0·2	0·8	0·6	0·8	0·8	1·1	0·2	0·2	0·1	0·1	0·1
Aedes pipersalatus ...	...	0·1	...	...	...	...	...	...	...	...	...	...
,, *vexans* ...	...	...	0·2	0·1	0·3	0·3	0·2	†	0·1	...	†	...
,, (*Aedim.*) sp. indet. ..	...	...	0·5	0·3	0·4	0·3	0·6	†	...	...	...	...
,, *lineatopennis* ...	...	...	...	...	...	...	†	†	...	...	...	...
,, *scutophagoides* ..	...	...	...	...	...	†	...	...	...	...	...	...
,, *albopictus* ...	†	†	†	0·1	†	...	...	...	...	...	...	...
,, *vittatus* ..	...	...	†	...	...	...	...	...	...	...	...	...
Culex bitæniorhynchus	†	†	...	†	...	0·1	†	...	...	...	...	...
,, *fatigans* ...	0·1	†	...	†	...	†	0·2	†	...	†	...	...
,, *vishnui* ...	†	...	†	0·1	...	0·1	†	...	...	†	...	...
Anopheles culicifacies ...	†	†	...	...	...	†	...	...	...	...	...	...
,, *fluviatilis* ...	...	...	...	...	...	...	...	†	...	...	...	...
,, *hyrcanus*	...	...	...	...	...	...	†	0·1	0·1	†	...	...
,, *tessellatus* ...	...	...	...	...	...	†	...	...	...	...	...	...
Genus and sp. indeter.	...	...	...	...	...	...	†	...	...	...	...	...

† Density of less than 0·04 per man-hour.

Outdoor resting (Chart 5).—The outdoor shelters (in this case the sugarcane fields) were rich both in variety of species of mosquitoes and their numbers collected. Taking all mosquitoes together, there was a general increase from June till October after which there was a gradual fall. Four species of Aedes, one of Armigeres, thirteen of Culex and seven of Anopheles were found resting in the sugarcane fields. The major species among them were: *Aedes vexans*, *Culex bitæniorhynchus*, *C. fatigans* and *C. vishnui*. *Aedes vexans* was common between June and October and apparently the monsoon conditions were favourable to it. The densities of *Culex bitæniorhynchus* were more uniform throughout the year. The densities of *Culex fatigans* were erratic and bore no relation to season. *Culex vishnui* which was the most abundant species resting in the sugarcane gardens was most prevalent from June to January. The total absence of *Uranotaenias** and the negligible numbers of *Stegomyias* in this area, are noteworthy.

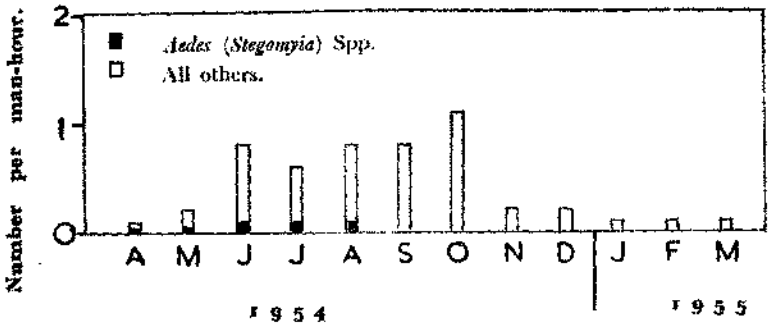
* In subsequent collections made in the mud-holes along streams, *Uranotaenias* have been found in Baramati area also.

CHART 5.



Seasonal prevalence of mosquitoes, expressed as number collected per man-hour by months, April 1953 to March 1955 : Outdoor shelters, Baramati.

CHART 6.



Seasonal prevalence of mosquitoes expressed as number collected per man-hour by Months, April 1953 to March 1955 : Biting Mosquitoes at dusk, Baramati.

Outdoor biting (Chart 6).—The numbers collected biting outdoors at dusk were never high and though 15 species in all were found actually biting man, only *Aedes vexans* and an apparently undescribed species closely related to it accounted for the bulk of them. Highest numbers were found between June and October.

Bhorghat (Table VII and Charts 7 and 8).

Both in variety and numbers, the mosquito fauna of Bhorghat was very rich and surpassed those in all the other field study areas. Fiftysix species belonging to nine genera were collected, taking all types of collections into consideration. Mosquitoes collected *indoors* consisted mainly of *Culex fatigans* and of small numbers of 13 species of Anopheles.

TABLE VII.

Bhorghat.

Monthly densities of mosquitoes expressed as mean number collected per man-hour; April 1954 to March 1955.

(A) **Indoors.**

	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Time spent: man-hours	8	8	8	14	8	8	8	8	12	8	7·6	7·2
All mosquitoes ...	2·4	0·8	0·1	7·4	0·8	0·3	6·0	8·8	8·5	4·6	9·5	14·7
Armigeres obturbans ...	...	...	...	...	...	...	...	0·1	...	...	...	0·1
Culex fatigans ...	1·8	0·8	0·1	3·9	0·5	0·3	1·4	1·1	1·8	2·3	8·2	12·6
,, *vishnui* ...	...	...	...	...	...	...	...	...	0·2	...	...	0·3
,, *khazani* ...	0·3	...	...	...	...	...	...	...	...	...	...	...
,, sp. indet. ...	..	...	...	...	...	...	...	...	...	...	...	0·1
Tripteroides aranoides var. *serrata* ...	...	...	...	...	...	...	...	0·3	...	...	...	...
Uranotænia recondita ...	...	...	...	...	...	...	0·6	0·1	...	0·1	...	...
Anopheles annularis ...	...	...	...	...	...	...	0·1	0·1	0·1	...	...	...
,, *culicifacies* ...	...	...	...	1·4	0·3	...	0·5	0·5	0·8	0·4	...	...
,, *fluviatilis* ...	...	...	...	...	...	...	...	0·8	0·4	0·5	0·3	0·1
,, *jamesi* ...	...	...	...	0·1	...	...	2·3	2·0	0·3	...	0·1	0·6
,, *jeyporiensis* ...	...	...	...	...	...	...	...	0·5	0·3	0·1	0·1	0·1
,, *maculatus* ...	...	...	...	...	...	...	0·1	0·1	0·1	...	...	...
,, *moghulensis*	0·5	...	...	...	...	...	0·6	2·4	4·0	1·1	0.8	0.6
,, *pallidus* ...	...	...	...	0·1	...	...	...	...	...	...	...	...
,, *splendidus* ...	...	...	...	...	...	...	...	...	0·1	...	...	0·1
,, *subpictus* ...	...	...	...	1·3	...	...	0·3	...	...	0·1	...	...
,, *turkhudi* ...	...	...	...	...	...	...	...	0·1	0·3	...	...	...
,, *theobaldi* ...	...	...	...	0·1	...	...	0·1	0·6	0·3	...	...	...
,, *vagus* ...	...	...	...	0·6	...	...	...	...	...	...	...	...

TABLE VII. (*contd.*)

(B) **Outdoor shelters.**

	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Time spent: man-hours	16	14	16	16	16	16	16	16	24	16	16	16
All mosquitoes ...	11	19·4	9·5	5·3	5·3	6·6	18·3	21·9	27·8	41·1	37·3	24·8
Aedes (*Aedim.*) sp. indet.	...	...	...	...	...	...	...	...	0·1	...	...	...
,, *annulirostris* ...	...	...	...	0·1	0·1	...	...	...	...	...	...	...
,, *dissimilis* ...	...	...	...	0·1	...	...	...	...	...	...	...	...
,, *greeni* ...	...	...	...	...	0·1	...	...	...	...	...	...	...
,, *gubernatoris* ...	...	...	...	0·1	...	...	...	...	...	...	...	...
,, *pseudotaeniatus* ...	...	...	...	...	...	...	...	...	...	0·1	...	...
,, *albopictus* ...	0·1	1·4	1·4	0·8	1·0	1·9	1·9	3·5	3·3	1·5	1·6	0·3
,, *nittatus* ...	...	...	...	...	...	...	...	...	0·1	...	0·1	...
,, *w-albus* ...	...	0·1	1·3	3·6	2·6	2·1	1·2	1·1	0·1	0·2	0·1	...
Armigeres obturbans ...	...	...	...	...	...	0.5	0.1	0.1	...	...	...	...
Culex bitaeniorhynchus ...	...	0·1	...	...	0·3	...	0·1	...	†	0·1	0·1	...
,, *cornutus* ...	...	...	...	...	...	...	0·3	...	...	...	...	...
fatigans .	0·3	0·1	...	0·1	...	...	...	...	...	...	0·4	1·1
,, *mimulus* ...	...	...	...	...	0·1	...	...	...	0·2	0·6	0·4	0·1
,, *pishnui* ...	0·1	...	...	...	0·2	...	...	0·1	...	0·4	0·8	0·4
,, *fragilis* ...	0·1	...	...	...	...	...	...	...	...	...	...	...
,, *nigropunctatus*	...	...	...	...	...	...	0·1	0·1	...	...	...	...
,, *pallidothorax* ...	...	...	...	0·1	...	...	0·1	0·3	0·3	2·2	0·8	0·6
,, (*Culic.*) sp. indet. ...	0·3	...	...	...	...	...	0·1	...	...	...	...	...
,, *flavicornis* ...	...	...	...	...	...	...	2·8	2·1	0·9	...	...	...
,, *minor* ...	...	0·3	0·2	...	...	...	...	...	...	...	...	...
,, *minutissimus* ...	...	...	...	...	...	...	...	...	†	...	0·7	0·1
,, (*Loph.*) sp. indet.	1·0	0·2	0·1	...	...	...	...	...	5·2	10·3	11·9	6·3
,, *fumanus* ...	...	...	0·1	...	...	...	...	...	...	0·2	0·2	...
(*Lutzia*) sp. indet.	...	...	...	...	...	...	...	...	†	...	...	...
,, *khazani* ...	0·6	0·6	0·2	...	...	0·3	0·6	1·3	1·9	4·0	4·3	2·1

TABLE VII. (*contd.*)

(B) **Outdoor shelters.** (*Contd.*)

	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Culex pluvialis ...	...	...	...	...	...	0·1	...	...	†	...	...	...
,, *brevipalpis* ...	...	...	...	...	...	...	...	...	...	0·1	1·1	...
,, sub genus and sp. indet.	...	...	...	...	...	...	...	0·8	1·8	3·8	3·3	1·3
Ficalbia luzonensis ..	...	...	...	...	...	...	...	...	...	...	0·1	0·1
Heizmannia viridis ...	...	...	...	...	...	...	...	...	†	...	...	...
Orthopodomyia anophelo-ides	...	...	...	...	...	...	...	...	0·1	0·2	0·2	0·1
Tripteroides affinis ...	...	...	...	...	...	...	...	...	...	...	0·4	0·3
,, *aranoides* var. *serrata*	...	...	...	...	...	...	2·5	4·5	1·9	0·6	...	0·1
Uranotænia campestris	2·6	2·4	0·9	...	...	...	...	...	0·7	2·1	2·6	1·4
,, *maculipleura*	1·8	2·1	1·1	0·1	0·6	1·1	1·8	...	0·5	0·4	0·1	0·2
,, *recondita* ...	5·1	12·0	4·4	0·4	0·4	0·6	5·2	4·1	2·3	2·8	2·8	6·4
,, *stricklandi*	...	...	...	...	...	...	...	4·2	3·9	3·3	3·4	2·4
,, sp. indet. (*x*)	...	...	...	...	...	...	...	...	6·0	8·1	3·3	2·0
Anopheles aitkeni ..	...	...	...	...	...	...	...	...	...	0·1	0·1	...
,, *culicifacies* ...	...	...	...	0·1	...	...	...	...	...	...	...	...
,, *culiciformis*	...	...	...	...	...	...	...	...	...	0·1	0·1	...
,, *jamesi* ..	...	...	...	...	...	...	..	0·1	†	...	...	...
,, *maculatus* ...	...	...	...	...	...	...	...	...	...	...	...	0·1
,, *vagus* ...	...	...	...	0·1	...	...	...	...	...	...	...	...
(C) **Outdoor biting dusk.**												
Time spent: man-hours	24	24	24	12	18	6	24	24	36	24	24	24
All mosquitoes	0·1	4·1	2·1	0·1	0·9	0·5	3·3	5·3	0·7	0·5	0·9	1·1
Aedes annulirostris ...	...	†	†	...	...	...	...	...	...	...	...	...
,, *cogilli* ...	...	...	...	...	0·2	...	...	...	...	...	...	...
,, *greeni* ...	...	...	†	...	...	...	†	...	...	...	...	...
,, *gubernatoris* ...	...	...	0·1	...	0·1	...	...	...	...	...	...	...
,, *niveaus group* ...	...	...	...	0·1	...	...	...	...	...	...	...	...

TABLE VII. (*contd.*)

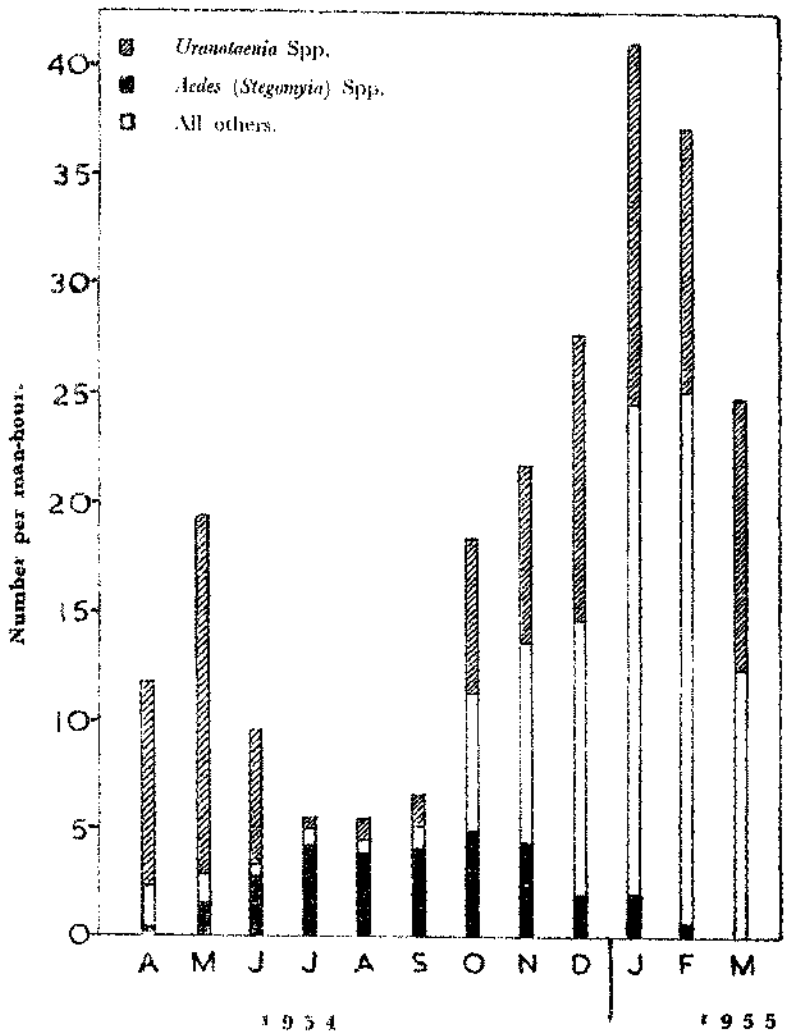
(C) **Outdoor biting dusk.** (*Contd.*)

	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
Aedes pseudotaeniatus ...	...	...	...	...	...	...	†	...	...	...	...	...
,, *albopictus* ...	†	3·3	1·1	...	0·4	0·2	2·5	4·7	0·1	0·1	0·3	†
,, *vittatus* ...	0·1	0·1	0·1	...	...	...	†	†	0·1	0·1	0·2	1·0
,, *w-albus* ...	...	0·6	0·7	...	0·2	0·3	0·5	0·5	0·1	0·1	0·1	...
Culex fatigans ...	...	...	...	...	...	...	...	...	...	†	†	...
,, *vishnui* ...	...	...	...	...	...	...	†	...	...	†	†	...
,, *khazani* ...	...	...	...	...	...	...	...	...	†	...	...	...
Anopheles culciformis ..	...	...	...	...	...	...	...	...	÷	...	...	...
,, *fluviatilis* ...	...	...	...	...	...	...	...	†	...	0·1	...	...
,, *jamesi* ..	...	...	...	...	0·1	...	...	...	...	...	0·1	...
,, *maculatus* ..	...	...	...	...	...	...	0·1	...	0·1	...	0·1	...
,, *moghulensis*	...	...	...	...	...	...	...	...	0·2	÷	0·1	...
,, *rossellatus* ...	...	...	...	...	...	...	†	...	...	...	...	...
,, *theobaldi* ..	...	...	...	...	...	...	...	...	†	...	...	...

†Density of less than 0·04 per man-hour.

Outdoor resting (Chart 7).—The species of mosquitoes collected throughout the year in outdoor shelters in the forest, were *Aedes albopictus*, *A. w-albus*, *Culex khazani*, *Uranotaenia maculipleura* and *U. recondita*. The other species, though sometimes very abundant, were restricted to a few months. The total density of all mosquitoes in outdoor shelters was the lowest in the heavy monsoon months of July, August, September and there was a rapid build up in the post-monsoon months with a peak in January. *Aedes* (*Stegomyia*) spp. were, however, at their highest densities from July to November and in fact they formed the bulk of mosquitoes collected in July, August and September. *Uranotaenias* were abundant in all months of the year except July, August and September. 'All other' mosquitoes accounted for half or more than half of the high density in January, February and March. They consisted of a miscellaneous assortment of species but those which at one time or another occurred in large numbers and significantly contributed to the total density were: *Culex* (*Lophoceratomyia*) spp. (December to March), *Culex khazani* (December to March) and *Tripteroides aranoides* var. *serrata* (October to November). Most of the species mentioned are tree-hole breeders and the conditions immediately after the monsoon were extremely favourable for their breeding.

CHART 7.

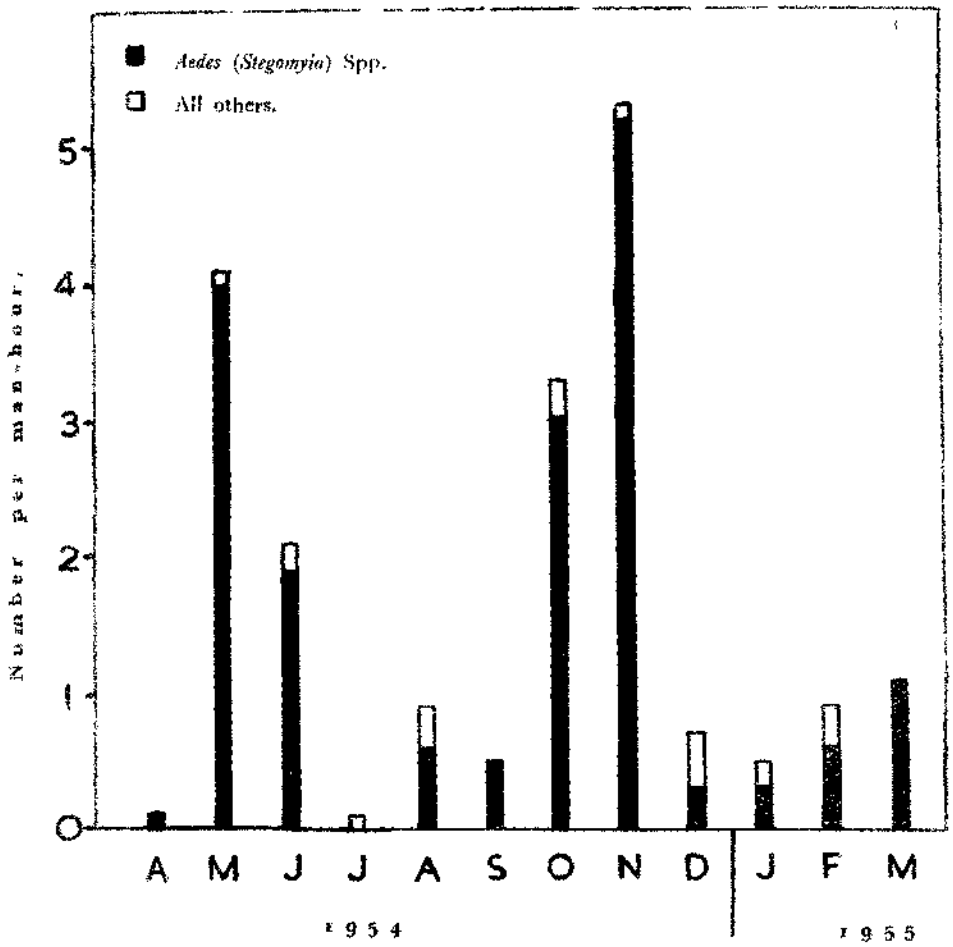


Seasonal prevalence of mosquitoes, expressed as number collected per man-hour, by months, April 1953 to March 1955 : Outdoor shelters, Bhorghat.

The small number of mosquitoes collected resting in outdoor shelters during the months of heavy rainfall, requires comment. The rainfall in this area is heavy and practically continuous from June to September. The routine searches for mosquitoes were made even during those months in spite of the actual rain. There is no doubt that the efficiency of the collecting staff was not at its best when working under such conditions, and the small number collected may partly be attributed to it. But it should also be noted that owing to incessant rain most of the tree holes and ground pools were overflowing and it was also possible that breeding

in such habitats was greatly impaired. In the case of *stegomyias*, the few that were present were noticed and caught because of their predilection for day-time activity. But the other mosquitoes were very difficult to locate.

CHART 8.



Seasonal prevalence of mosquitoes, expressed as number collected per man-hour, by months, April 1953 to March 1955: Biting mosquitoes at dusk, Bhorghat.

Outdoor biting.—The bulk of the mosquitoes collected biting at dusk consisted of *stegomyias*. The curve of their seasonal incidence (Chart 8) does not agree with that of the same species collected resting in outdoor shelters. Very few biting mosquitoes at dusk were collected during July, August and September, the months of the heaviest rainfall. The low number was undoubtedly due to the actual rain which prevented mosquitoes from venturing far out of their shelters and alighting on man. Though no routine collections of biting mosquitoes were

made during day time, the experience was that even during the monsoon months *stegomyias* attacked man provided there was a break in the rain or the rain was reduced to a drizzle.

(3) MOSQUITOES ATTRACTED TO MAN AND OBSERVATIONS ON THEIR BITING ACTIVITY.

In all the four field study areas, collections of mosquitoes attempting to bite man were made routinely in fixed catching stations. In Empress Garden, collections were made fortnightly between 09.00 and 12.00 hours and again on the same day between 18.00 and 21.00 hours. In Ganeshkhind Garden, fortnightly collections were made between 09.00 and 12.00 hours only. In all other stations, collections were made once a fortnight between 18.00 and 21.00 hours.

The procedure adopted in these collections was for two insect collectors to sit quietly either on camp stools or on the ground with both forearms and both legs below the knee fully exposed. They caught every mosquito which alighted on the exposed skins of their bodies. Sometimes when a mosquito alighted on the face or the neck of one insect collector, the other one would collect it. Short culture tubes (100 m.m. by 16 m.m.) were used for this purpose, and the mosquito directly captured in it. Once the collection started, the work went on whether there was rain or not.

These collections were not made with a view to determine the peak hours of mosquito activity but they were made solely with a view to determine the species of mosquitoes which are attracted to man during day time and at dusk. For this reason, no special effort was made to record the exact time at which any species of mosquito was taken, though the total number of mosquitoes of all species taken during the first, second and third hours of each collection were recorded. For the same reason the period of collecton at dusk, viz., 18·00 to 21.00 hours, was not varied from month to month to correspond with the variation in the time of sunset.

Thirtynine, out of eightynine species of mosquitoes found in the district, were actually caught alighting on man. As the mosquitoes were often captured before they had actually bitten the person, one could not be sure whether they would have actually taken a bloodmeal, but the presumption that they alighted on man with the purpose of biting and taking a bloodmeal is strong. Among the thirtynine species actually alighting on man, the following thirtysix were found to bite and to take bloodmeals:—

Aedes pipersalatus, *A. vexans*, *A.* (*Aedimorphus*) species indet., *A. lineatopennis*, *A. annulirostris*, *A. cogilli*, *A. dissimilis*, *A. greeni*, *A. gubernatoris*, *A. pseudotæniatus*, *A. scatophagoides*, *A. albopictus*, *A. vittatus*, *A. w-albus*, *Armigeres obturbans*, *A. magnus*, *Culex bitæniorhynchus*, *C. fatigans*, *C. univittatus*, *C. vishnui*, *C. khazani*, *Mansonia crassipes*, *M. uniformis*, *Anopheles barbirostris*, *A. culicifacies*, *A. moghulensis*, *A. subpictus*, *A. tessellatus*, *A. fluviatilis*, *A. hyrcanus*, *A. jamesi*, *A. karwari*, *A. maculatus*, *A. theobaldi* and *A. vagus*. The four species which alighted on man but were not actually seen to take a bloodmeal, were *Aedes* (*Finlaya*) sp. *niveus* group, *Aedes* (*Finlaya*) sp. indet., *Tripteroides affinis* and a species of undetermined genus.

Of the 5,355 mosquitoes of all species collected alighting on man, the following were taken in fair numbers:—

Aedes vexans, *A.* (*Aedimorphus*) species indet., *A. lineatopennis*, *A. annulirostris*, *A. vogilli*, *A. greeni*, *A. gubernatoris*, *A. albopictus*, *A. vittatus*, *A. w-albus*, *Armigeres obturbans*, *Culex bitæniorhynchus*, *C. fatigans*, *C. vishnui*, *Mansonia crassipes*, *Anopheles hyrcanus* and *A. moghulensis*. The three species of *Aedes* (*Stegomyia*) accounted for 4,693 out of 5,355 mosquitoes collected alighting on man.

It has been the author's experience during these studies, both in the Poona gardens and in Bhorghat, that during the three-hour period of each collection the first hour provided the largest number of mosquitoes and that there was a progressive reduction in the second and third hours, whether the collection was made during day time or dusk. This observation has been made consistently in all months of the year. The consolidated data for the whole year are given in Table VIII. It will, however, be noticed that the mosquitoes of Manjri and Baramati did not follow a similar pattern of behaviour. In Poona gardens and Bhorghat, the bulk of the mosquitoes consisted of *stegomyias* while in Baramati and Manjri it was not so. There is as yet no satisfactory explanation as to why more *stegomyias* were collected during the first hour but one which seems plausible is that the hungry adult *stegomyias*, which are present in any locality, are rapidly attracted to a suitable host as soon as the latter arrives in the locality.

TABLE VIII.

Total number of mosquitoes collected biting man in the first, second and third hour of the three-hour collection period.

Place	Number of collections	Number of mosquitoes				Average number taken per collection			
		First hour	Second hour	Third hour	Total	First hour	Second hour	Third hour	Total
Empress Garden: Day ...	23	86	23	23	132	3·7	1	1	5·7
Empress Garden: Dusk ...	24	107	83	16	206	4·5	3·5	0·7	8·6
Ganeshkhind Garden: Day	19	51	27	11	89	2·7	1·4	0·6	4·7
Manjri: Dusk ...	51	23	48	13	84	0·5	0·9	0·3	1·6
Bhorghat: Dusk ...	44	251	111	14	376	5·7	2·5	0·3	8·5
Baramati: Dusk ...	99	87	122	22	231	0·9	1·2	0·2	2·3

Regarding the activity of *Aedes albopictus* and *A. w-albus*, no difference has been noticed between the numbers collected in day time and dusk made in the same

locality. The data for Empress Garden plotted in Chart 2 indicate that the dusk is as active a period for them as forenoon.

(4) MOSQUITOES ATTRACTED TO BAITS OTHER THAN MAN.

Portable mosquito traps (Ramachandra Rao, *in press*) were used with birds and small mammals as baits to determine the species of mosquitoes attracted to them. They were used on eighty-four occasions between June 1953 and January 1955. They were suspended from trees and on all but seven occasions, they were set up in the evening at 18.00 hours and removed at 06.00 hours the following morning. Mosquitoes were found on fifty-nine occasions and a total of 769 was collected. The baits used were house crows, *mynahs* pigeons, chicken, sparrows, jungle babbler and rats. The data collected are summarised in Table IX. *Culex fatigans* was the species most frequently taken and it was attracted to all the baits used. The other species found were (in the order of frequency of occurrence) *C. bitæniorhynchus*, *Mansonia crassipes*, *C. vishnui*, *C. mimulus* and *Aedes vittatus*. All but six of the 769 mosquitoes were females and most of them had taken fresh bloodmeals, presumably on the baits when collected the following morning.

TABLE IX.

Mosquitoes collected in portable trap with baits, in and around Poona, India. June 1953 *to January* 1955.

Baits used	Number of trials	Mosquitoes													
		All species		*Culex fatigans*		*C. bitænio-rhynchus*		*C. vish-nui*		*C. mimu-lus*		*Man-sonia crassipes*		*Aedes vittatus*	
		T	N	T	N	T	N	T	N	T	N	T	N	T	N
House crows ...	22	19	164	16	122	1	1	...	...	1	1	5	40		...
Pigeons ...	22	14	460	14	439	7	15	1	1	...	...	2	5	...	...
Chickens ...	14	9	41	4	17	4	7	1	1	1	3	1	12	1	1
Mynahs ...	7	5	25	4	24	...	...	...	...	...	...	1	1	...	...
Sparrows ...	2	1	1	...	...	...	...	...	...	...	...	1	1	...	...
Jungle babblers ...	1	1	44	1	44	...	...	...	...	...	...	...	...	...	...
Rats	16	10	34	8	17	2	2	...	...	...	...	2	15	...	...
Total ...	84	59	769	47	663	14	25	2	2	2	4	12	74	1	1

T = times found.
N = number found.

(5) NOTES ON GENERA AND SPECIES OF MOSQUITOES OCCURRING IN POONA DISTRICT.

Genus *Aedes*.

Of the slightly more than 100 species of this genus, which Barraud (1934) recorded in India, only 18 have been found in these studies in Poona District. Two more species which cannot be identified as any one of those previously recorded in India, have also been found, making a total of 20 species taken in the course of these studies. One of the unidentified species belongs to the subgenus *Aedimorphus* and the other to subgenus *Diceromyia*.

SUBGENUS *Aedimorphus*.

The species found are *Aedes alboscutellatus*, *A. jamesi*, *A. pipersalatus*, *A. vexans* and an undetermined species belonging to the group with dark tarsi. Of these *A. jamesi*, *A. pipersalatus* and *A. vexans* are widespread species in India and their occurrence in Poona was to be expected.

Aedes (*Aedimorphus*) *alboscutellatus* is found in Bengal, Bihar, Assam and Burma and it was known in Bombay State hitherto only in Belgaum District. It is a rare species, found resting outdoors in the rock holes.

Aedes jamesi is a rare species found resting in bushes.

Aedes pipersalatus is an uncommon species collected both resting in bushes and while actually biting.

Aedes vexans, a cosmopolitan species, was found in these studies resting in the sugarcane fields and bushes. Many of those collected resting in the sugarcane fields were found to have ingested blood. It bites man readily as seen from the fact that it formed a good proportion of the mosquitoes caught biting at dusk in Baramati. It also bites by day, six specimens having been taken attempting to bite between 09.00 and 12.00 hours in October and November in Ganeshkhind Garden. It was not found in the heavy rainfall area of Bhorghat and it was most prevalent in the irrigated areas. It occurred throughout the year but in greater numbers during and immediately after the monsoon.

Aedes (*Aedimorphus*) spp. indet.—This species has entirely dark tarsi and does not correspond with any species of the group recorded in India. Though less abundant than *Aedes vexans*, it had similar habits and seasonal prevalences and was collected biting man at dusk.

SUBGENUS *Banksinella*.

Aedes (*Banksinella*) *lineatopennis*.—This is the only species of the subgenus recorded in India and is a comparatively rare species in Poona District. It was found resting in the bushes and in sugarcane fields from July to December. It was taken biting man three times, once during the day time and twice at dusk. Barraud (1934) mentions that this species is known to attack man readily at dusk.

SUBGENUS *Christophersiomyia.*

Only three species of this subgenus are found in India and two of them, *Aedes annulirostris* and *A. thomsoni*, have now been found in Poona District.

Aedes (*Christophersiomyia*) *annulirostris.*—This species was hitherto known in India only from the forests of Belgaum and Kanara districts of Bombay State and from Travancore (Iyengar, 1938) and from the type locality in Ceylon. This is an uncommon but not a rare species in Poona District, and has been found resting in the bushes in the forests of Bhorghat and in Poona gardens. It has been taken in all months except February, March and April and it readily bites man, both during the day and after sunset. It has been collected actually biting eight times at dusk and five times during the day at Empress Garden and seven times during day at Ganeshkhind Garden.

Aedes (*Christophersiomyia*) *thomsoni.*—This species is actually more widespread in India than the foregoing one. But it was collected only once in Poona District in Ganeshkhind Garden, resting in bushes, in the month of October.

SUBGENUS *Finlaya.*

Thirtyeight species of this subgenus are recorded in India but more than half of them are found in the Himalayas only. Only six species have been found in Poona District during the present study.

Aedes (*Finlaya*) *cogilli.*—This species was known hitherto only from Kanara and Belgaum districts of Bombay State. It is rare in Poona District but has been found resting outdoors in the bushes and also actually attempting to bite man both during the day (four times) and at dusk (once), all during the monsoon months.

Aedes (*Finlaya*) *dissimilis* var. *karwari* is a common forest species in areas of heavy rainfall. It is rare in Poona District. On one occasion, six females attempted to bite man between 10.30 and 11.45 hours in the forest near Bhimashankar in the month of October.

Aedes (*Finlaya*) *greeni* var. *kanaranus* is a widespread forest-inhabiting species in India. It is rare in Poona District. It was found resting in the forest undergrowths in Bhorghat and in Ganeshkhind Garden and also found actually attempting to bite man at dusk (four times) and during the day (once) in the months of July and August.

Aedes (*Finlaya*) *gubernatoris* is a widespread species, but uncommon in Poona District. It was collected both resting in bushes and actually attempting to bite man at dusk at Empress Garden.

Aedes (*Finlaya*) *pseudotaeniatus* is considered to be one of the commonest species of the subgenus both in the hills and plains of India. It was, however, only rarely found in Poona District. One was taken biting man at dusk in Bhorghat, two were found resting in the forest undergrowths in the same locality and one inside a house in a village near Khadakvasla.

Aedes (*Finlaya*) species belonging to *niveus* group. —A single specimen was collected attempting to bite man at dusk in Bhorghat. Members of this group are difficult to identify.

SUBGENUS *Mucidus.*

Aedes (Mucidus) scatophagoides.—This species, which occurs all over India and tropical Africa, is a comparatively uncommon mosquito in Poona District. It has been found resting in sugarcane fields and only once it was taken biting man at dusk.

SUBGENUS *Stegomyia.*

Of the sixteen species of this important subgenus found in India, only four have been found in Poona District. All the four are widespread species and their occurrence in Poona District is not unexpected.

Aedes aegypti.—Only one male of this species has been collected in Poona District, in a human dwelling in Poona City. Intensive searches for additional specimens both inside and outside houses have been fruitless. After the single male was found in a house in March 1954, twentyfour earthenware pots filled with water were placed in the houses in the neighbourhood, including the particular house in which it was found, periodical examinations of the water in the pots were made for several weeks thereafter but no *Aedes* larvæ were found. Poona is one of the places in India in which *Aedes ægypti* was formerly present. The almost total disappearance of the species from Poona in recent years, is interesting. The reasons for the disappearance are not known but an important contributory factor may be the extensive D.D.T. spraying operations done in the city since 1950.

Aedes albopictus.—This is perhaps the most abundant species of mosquito both in the gardens in Poona City and in the forest regions in the district. It has been found in all places in the district and in all months of the year, the greatest numbers found being during and immediately after the monsoon. It bites man viciously both during the day and at dusk.

The numbers shown as collected while biting in Table III and in Tables IV, V, VI and VII refer only to females. But it should be recorded that whenever fair numbers of females attacked a person, a small number of males also accompanied them and some even alighted on the skin of the collector. This merely indicates that both sexes are active both during the day and at dusk and the males are, perhaps, attracted by the flying females.

Larvæ of *A. albopictus* have been collected several times from tree holes and cut bamboo stumps, both of which are well-known habitats of the species. Dried mud from tree holes has been brought to the laboratory several times and put into water. Imagoes have been reared from the larvæ which hatched. The extreme rapidity with which the life cycle of this species is completed, is worthy of comment. For instance, the following observations were made in June 1953:—

(1) June 1: Mud from a tree hole collected and put into water in the afternoon.
June 4: A well grown larva noticed.
June 5: Pupa formed.
June 6: Adult emerged.

(2) June 4: Mud collected from a tree hole and put into water.
June 6: Many larvæ seen.
June 10: Pupæ formed.
June 11: Adults (eight males and four females) hatched out.

(3) June 9: Mud collected from a cut bamboo and put into water.
June 11: Many larvæ noticed.
June 13: Pupæ formed.
June 16: Four males and six females hatched out.

The development in the first case was very precocious indeed. If the larvæ actually hatched out of the egg on June 1, then the total duration of larval and pupal stages is only five days unless one postulates that the larva had actually hatched out from the egg and was lying dormant in the mud. In the second case, it was only seven days and in the third six days.

A laboratory colony of *A. albopictus* has been successfully established.

This species is difficult to distinguish from a few closely related ones, such as *Aedes pseudalbopictus* and *A. novalbopictus*, without an examination of the male hypophygea. Therefore it is impossible to state that among the thousands of specimens collected and labelled as *Aedes albopictus* there were none of the other two species. But on several occasions, the male hypopygea were examined and found to be those of *A. albopictus*.

Aedes vittatus.—This also is a very widespread species and occurs in all sections of Poona District. But it has never been, except in a few isolated instances, so abundant as *A. albopictus* or *A. w-albus*. It has been collected very actively biting both at day time and dusk.

Aedes w-albus.—This species is quite common and found in all months of the year though less abundant than *A. albopictus* with which it is generally associated. It was not, however, found in the Baramati area. The biting habits of the two species were very similar.

Several attempts to establish a laboratory colony of this species have failed though the methods used were the same as those used for *A. albopictus*.

The larvæ and pupæ of *w-albus* were hitherto unknown. They have been collected and are being described separately (Rajagopalan, 1956).

Genus *Armigeres*.

SUB-GENUS *Armigeres*.

Of the four species recorded in India two, *Armigeres obturbans* and *A. theobaldi*, are now recorded from Poona District.

Armigeres obturbans.—This widespread species was taken in this study only in moderate numbers. It was found in outdoor shelters in Baramati, Bhorghat and Poona gardens in all seasons. It was also collected biting man seven times during day time, and once at dusk in Poona gardens.

Armigeres theobaldi is an uncommon species in India. Only one adult was found in Manjri in September 1953.

SUB-GENUS *Leicesteria.*

Nine species are known in India and only one, *Armigeres magnus*, has been found in Poona. It was found biting at dusk at Empress Garden.

Genus *Culex.*

Species belonging to all subgenera of *Culex* recorded in India, except *Barraudius*, have been found in Poona District. *Barraudius* is recorded within Indian limits in Kashmir only.

SUBGENUS *Culex.*

Of the 22 species in this subgenus, 11 are now recorded from Poona. Among the widespread species, those that were not found were: *C. epidesmus, C. sitiens* and *C. mimeticus. C. fuscitarsis* is recorded by Barraud (1934) as actually occurring in Poona and Lonavla in Poona District but was not collected during the present studies.

Culex bitæniorhynchus is a common species. Though found occasionally inside houses, it is essentially an outdoor rester, found regularly in sugarcane fields both at Baramati and at Manjri and in bushes in the Poona gardens. It bites man both at dusk and during the day but the numbers attracted to man are very small compared to the numbers actually collected resting in the shelters in the locality. It was taken in traps, once baited with a crow, seven times with a pigeon, four times with a chicken and once with a rat. As several of the females found in the traps had fresh blood, it is presumed that they had fed on the bait. The species was found in all months of the year but with an increased prevalence during the monsoon months.

In addition to the typical form of this species, specimens with far fewer pale scales on the wing have been caught. Several such variations in this species are known and at present it is not possible to define them precisely.

Culex cornutus is not a common species and it has been found resting outdoors in sugarcane fields and in bushes in the forest and none has been found biting man. The months in which it has been found are May, July, August, September, October and December.

Culex edwardsi.—Only one specimen of this rare species has been collected in Empress Garden in January. Hitherto it was recorded only in half a dozen places in India, in such widely separated places as Assam, Central India and Coorg.

Culex fatigans.—This ubiquitous mosquito was the most abundant mosquito in all the study areas except Bhorghat. In Poona City it was the only species of mosquito found in the houses, and the routine indoor residual spraying with D.D.T. had little effect on its densities. While its densities were highest indoors, good numbers were also collected in outdoor shelters, in bushes, in sugarcane fields, gardens, etc. No consistent seasonal trends have been noticed in its prevalence. Small numbers of the species have been collected biting man at dusk in all study

areas but the numbers collected are not commensurate with the large numbers actually present in the area. On a few occasions, the females of the species have been collected alighting on man during the day time in shade in Empress Garden and in Ganeshkhind Garden.

In baited traps, good numbers have been collected with house crow, pigeon, chickens, *mynah*, jungle babbler and rat as bait.

Culex fuscocephalus.—This is a very widespread species all over India and South-East Africa but it is never common. Only six specimens have been collected during the present studies, all in Poona City.

Culex gelidus is not a common species in Poona District. It has not been found in Bhorghat area. It has been collected resting in the bushes in Empress Garden and in Ganeshkhind Garden, and in the sugarcane fields at Manjri and Baramati.

Culex minulus is another uncommon species in the district. Small numbers were found resting in outdoor shelters in Bhorghat and in the Poona gardens but none was collected biting man.

Culex univittatus has been collected hitherto from very few places in India and Pakistan. Barraud (1934) records them from one locality in North-West Frontier Province, one in Baluchistan, three in the Punjab and one in Uttar Pradesh. It has, however, a wide distribution in the Mediterranean Region, Africa and Madagascar. Saharanpur in Uttar Pradesh is the easternmost and perhaps the southernmost place in the Indo-Pakistan subcontinent from where this species had so far been recorded. Therefore finding them in Poona District is of considerable interest.

To virologists, this species has a special significance, for recent studies have shown that *C. univittatus* is one of the proved vectors of the West Nile virus in Egypt.

In Poona District, the species can by no means be described as abundant but it is not uncommon and has been collected in small numbers in all months of the year. It has not been found in Bhorghat. It has been found resting in Baramati, Manjri and Poona gardens in bushes and vegetation. Many adults, when caught, had evidence of having taken blood meals and actually one was collected biting man in Empress Garden in January.

Culex vishnui.—This widespread species is one of the common mosquitoes found in Poona District and among the species of Culex it is next in importance only to *C. fatigans*. It was taken in all study areas, both resting outdoors and indoors and on a few occasions captured attempting to bite man at dusk. The number collected biting was, however, very small compared to the large numbers collected resting in the nearby fields and bushes. It was collected only once in a pigeon-baited trap and once in a chicken-baited trap. It has been collected in all months of the year but the highest numbers were between July and October, indicating that the monsoon conditions were favourable for it.

C. vishnui can be separated from two other closely related species, *C. barraudi* and *C. whitei*, only by an examination of male hypopygia. The hypopygia of all males, examined in Poona District, were of the *C. vishnui* type. In 1953, four

females were collected which had a uniformly dark brown scaling on the mesonotum and were, therefore, suspected to be *Culex tritæniorhynchus*, another closely related form. There is however such a high degree of variation in the colouration of the mesonotal scales that it is not advisable to depend entirely upon that character. In all subsequent collections, many doubtful looking males have been examined and they were invariably found to have hypopygia of the *C. vishnui* type. Therefore the occurrence of *C. tritæniorhynchus* in Poona District should be regarded as still doubtful, and even if it does occur, it must be in very small numbers. A thorough study of a large number of males is necessary before the question can be finally decided. *C. tritæniorhynchus* is a species of much importance to virologists because it is strongly suspected to be the vector of Japanese *B* encephalitis in Japan and other Far-Eastern countries.

Culex whitmorei is another widespread species in India which occurs in small numbers in Poona District. This is remarkable when it is known that it occurs in very large numbers in Kanara District and in fact forms the most abundant mosquito species occurring in the forest undergrowth in that region.

SUBGENUS *Culiciomyia*.

Several species of this subgenus are known to occur in India and three of them have been found in Poona District: *Culex fragilis*, *C. nigropunctatus* and *C. pallidothorax*. It may also be mentioned here that one more species, *C. bailyi*, has been collected by the authors in Mahabaleswar (4,500 feet elevation) in the neighbouring Satara District.

Culex fragilis.—This is comparatively a rare species, having been reported hitherto only from Kanara District in Bombay State. One solitary specimen was collected by the authors in Poona District, in an outdoor shelter at Bhorghat.

Culex nigropunctatus is an uncommon species, reported hitherto in peninsular India in only one place in the Western Ghats of Mysore State and one in the northern part of coastal Andhra. Small numbers were found in the Ganeshkhind Garden and in Bhorghat, resting in bushes, immediately after the monsoon.

Culex pallidothorax.—This widespread species has been collected from all sections of Poona District. It occurs in small numbers resting in the shade of bushes and undergrowth in most months of the year with higher densities in the monsoon months. It has often been found with blood in the gut but none has been collected attempting to bite man.

SUBGENUS *Lophoceratomyia*.

The species of this group are extremely difficult to identify. Only the males can be identified with certainty from the shape and arrangement of the special hairs on the antenna and from the hypopygia. The females are sometimes very difficult to distinguish even from those of other related subgenera. Ten species are known in India and of these, four, viz., *Culex flavicornis*, *C. minor*, *C. minutissimus* and *C. plantaginis* were found in Poona District. A fifth species which has a general resemblance to *C. flavicornis*, but with a distinct antennal pattern, has also been found abundantly. It may be one of the 15 species not yet reported from India or an entirely new species.

As the females of the different species cannot always be distinguished with certainty, there is no doubt that there have been some misindentifications. Therefore the total number of each species given should not be considered as very accurate and it is preferable to consider all species of the subgenus together.

All the species rest outdoors in bushes and more often in mudholes and holes and crevices in rocks. Several of the females have been found with blood in their abdomen, but what they feed on, is unknown.

Often, particularly in Bhorghat where they were collected in large numbers, they were found to rest in the holes and crevices of rocks in densely shaded dried-up hill streams, sometimes more than 200 feet from the nearest possible breeding place. Their numbers increased considerably after the monsoon and the highest number collected was in January.

None was collected biting man at any of the stations. This is highly significant when it is realized that good numbers have been found resting in day-time shelters on the same days in the immediate neighbourhood.

SUBGENUS *Lutzia.*

Only two out of the four species known in India have been found in Poona District: *Culex fuscanus* and *C. raptor.*

Culex fuscanus.—Small numbers have been collected practically in all months of the year at one or the other of the study areas. Only one specimen was collected while attempting to bite man.

Culex raptor.—Only one specimen has been collected so far.

SUBGENUS *Mochthogenes.*

Only five species of these mosquitoes have been found in India and three of them, viz., *Culex khazani*, *C. pluvialis* and *C. malayi* have been found in Poona District. Very little has hitherto been known of the habits of these mosquitoes.

Culex khazani.—This attractive looking delicate mosquito with a large velvety black spot on the mesepimeron (likely to be mistaken for *C. nigropunctatus*), occurs fairly commonly in the heavy rainfall zone of Bhorghat. Except for one specimen from Manjri, this species has not been taken from the other areas. This exclusively Indian species had been collected hitherto from Malabar Coast and North Kanara District only.

It has been taken at Bhorghat throughout the year but with a distinct increase in post-monsoon months with a peak in January. Its preferred resting places were mud-holes and crevices in rocks. Females with evidence of fresh blood-meals were taken quite frequently, but what they feed on, is not known. On only one occasion a female alighted on a man, at dusk, presumably for feeding.

Culex malayi is a species more widespread than *C. khazani*, and is found in all parts of India and also in many places in South-East Asia. In Poona area it has been found in all sections excepting Bhorghat (*see* under *C. pluvialis*). Its resting places are similar to those of *C. khazani*, i.e., mud-holes along stream margins.

It has often been found with fresh blood in the stomach, often resting in mud-holes in association with species of *Uranotaenia* but the source of the blood food was not previously known. An observation made by one of the authors (T.R.R.) on February 5, 1955, has provided this information. The notes made on that day read as follows: " At 10.50 a.m. today, one of the insect collectors collected some seven or eight females of *Culex malayi* resting on a creeper, near the water surface of an open step-well, near outdoor catching station number 5 at ninth milestone, Manjri. All had fresh blood, really so fresh that they must have fed only a few minutes previously and a clear fluid was just oozing out from the anus. Two frogs jumped into the water when I approached to investigate. Some ten minutes later, I quietly went down the stone steps and sat quietly on the last but one stone step. Several mosquitoes were flying about near the water surface but did not settle anywhere. In about five minutes, both the frogs returned to the surface and one jumped on to a rock and the other tried to balance itself on floating coconut husks, with only the snout protruding out. Immediately, (within 10 seconds), those mosquitoes alighted on the frog on the rock and one on the exposed snout of the other one. I called an insect collector and just as he came down the steps with his suction tube, the frog jumped back in the water, and mosquitoes started flying. He quickly caught two of the mosquitoes which had been feeding on the frog. Both had fresh blood, but the meal was incomplete as they were disturbed while feeding. Smears of the contents of the gut were made at 12.00 hours. Though very bright red, the blood had already become a gelatious mass at 12.00 hours but nucleated corpuscles were seen. The identification of these mosquitoes were checked and confirmed as *Culex malayi*. This is the first time that the species of this subgenus have been found feeding on frogs ".

Culex pluvialis.—Hitherto this species had been known only from its type locality in Kanara District. That it occurs in Poona District, is now clear. But the females are sometimes very difficult to distinguish from *C. malayi* and there is reason to believe that there have been some misidentifications, and among the several individuals identified as *C. pluvialis* a few may be really *C. malayi*. Therefore the total numbers recorded for *C. pluvialis* may be regarded as only approximate. Its resting places are exactly like those of *C. malayi*.

SUBGENUS *Neoculex*.

Only two species of this subgenus, and belonging to the Protomelanoconian group, are recorded by Barraud (1934) as occurring in India.

Culex brevipalpis, which is a widespread species, has now been found in very small numbers in Bhorghat, resting in the rock holes. Some of the females had traces of blood in their stomachs. The females of this subgenus are known to feed on frogs.

Genus *Ficalbia*.

Mosquitoes of this genus are generally rare and five species are recorded in India. Only one species *F.* (*Etorleptiomyia*) *luzonensis* has been found in Poona District. Hitherto this species was known in South India only from Travancore.

They were rare and all have been collected resting outdoors in bushes. A few had taken blood meals.

Genus *Heizmannia.*

Nine species of this uncommon genus occur in India and one of them *H. viridis* and another undetermined species have been collected from Bhorghat. Little is known of the habits of the members of this genus.

Genus *Mansonia.*

Both the subgenera, viz., *Coquillettidia* and *Mansonioides* are represented in the collections in the Poona District.

Mansonia (*Coquillettidia*) *crassipes.*—It is widely distributed all over India and occurs in small numbers throughout the year resting in the gardens in Poona and Manjri. Eleven females were collected attempting to bite man at dusk in Empress Garden. This is a high proportion considering that only 31 specimens were collected throughout the period of study resting in that garden. *M. crassipes* has also been collected in good numbers in baited traps, four times with crow, twice with pigeon, one each with chicken, *mynah* and sparrow and twice with rats. Apparently, this species has no definite host preferences.

Mansonia (*Mansonioides*) *annulifera* and *M. uniformis* occur in Poona District but are rare.

Genus *Megarhinus.*

Only one species *Megarhinus splendens* was collected in Poona District by the authors, once in Bhimashankar and another time in Bhorghat. This widespread species has been recorded hitherto in the Western Ghats only, south of Sawantwadi. Neither of the two specimens collected were taken as adults, but were reared from pupæ collected in tree holes.

Genus *Tripteroides.*

The five species of this genus recorded in India are all from forest regions and very little is known about their biology. They are not known to take blood-meals. Two of them, *Tripteroides aranoides* var. *serrata* and *T. affinis*, were taken in Poona District, in Bhorghat and in Poona gardens. The latter species is very rare but the former sometimes occurs in fair numbers, specially after the monsoon. They were found to rest in the deep shade in the jungle generally on the vertical damp mud surfaces or on barks of trees, but sometimes also on wet rock surfaces. On one occasion, a female alighted on the body of an insect collector during the day in a Poona garden but it was not observed to attempt to feed.

Genus *Uranotaenia.*

Twentythree species of this genus are recorded from India. Practically nothing is known regarding their biology. Four Indian species, *Uranotaenia*

campestris, *U. recondita*, *U. maculipleura* and *U. stricklandi*, and another species which does not agree with any of the forms described from India, have been collected in Poona District. In point of numbers collected, *Uranotaenias* taken together were next most abundant to *Culex fatigans*.

All the species were characteristically found resting in holes in the mud, made either by crabs or rodents. The holes were either along the margins of the rivers, small streams or channels or ponds. Proximity of water, and dampness of the hole, seemed to be important factors. Very few were collected resting in bushes, or undergrowth and negligible numbers (in Bhorghat) inside houses.

Many of the females are found in nature with blood in their abdomen. Not a single specimen was ever observed even to alight on the body of man, much less to take blood, though large numbers of the species were known to be resting within distance of five to ten feet. *Uranotaenia* spp. are known to suck blood of amphibians and the same habits may be prevalent here also. On two occasions, smears of blood from their guts were made and were found to contain nucleated red cells, but whether amphibian or avian, is unknown.

Uranotaenia campestris.—The form collected is var. *zalena* which has been recorded so far only in Pir Pao, Bombay and Kanara District in Bombay State and in Coorg. It was collected in practically all months of the year though in much smaller numbers than the other species of the genus. In Bhorghat, it was totally absent in the collection made during the monsoon.

Uranotaenia maculipleura.—The collection of this species in good numbers in Poona District is interesting for hitherto it had been reported in India only from Darjeeling District, Bengal. In the Poona gardens it was the most abundant species of *Uranotaenia* while in Bhorghat it yielded first place to *Uranotaenia recondita*. In the Poona gardens, it was more prevalent in the monsoon months than at other times though found in all months of the year.

Uranotaenia recondita.—This has a wider reported distribution in India (not recorded elsewhere) than *U. maculipleura*. In Bombay State, it had been previously recorded from North Kanara District only. In Bhorghat, it was more abundant than other species of the genus. No special seasonal prevalence has been noticed but the highest density found was in May in Bhorghat and in Ganeshkhind Garden, while in Empress Garden higher densities were found in June, July and August.

Uranotaenia stricklandi.—This species again has a very limited distribution in India having been recorded from Darjeeling District of Bengal, Nilgiris in Madras and Coorg. It was found only in Bhorghat in Poona District.

Uranotaenia spp. undetermined.—This is very close to *U. recondita*, but the sternopleura is dark instead of the whole pleura being pale. It occurs in Bhorghat in good numbers. This variety was noticed rather late during the course of the studies, as was also *U. stricklandi*. It is possible that some individuals of these two species may have been misidentified as *U. recondita* or *U. maculipleura*.

Genus *Anopheles*.

Twenty species of Anopheles were collected in the district. The senior author has made several collections of Anopheles in this district since 1944 and

their results, as well as those of others carried out by the Malaria Organisation of Bombay State, have been summarised by Viswanathan (1950). All the species now reported, except *Anopheles aitkeni*, *A. culiciformis* and *A. karwari*, have already been reported by Viswanathan (1950). One species, *Anopheles varuna*, recorded in Poona by Viswanathan (1950) and later by Jacob and Mitra (1952) was not collected in these studies.

The species collected were: *Anopheles aitkeni*, *A. annularis*, *A. barbirostris*, *A. culicifacies*, *A. culiciformis*, *A. fluviatilis*, *A. hyrcanus*, *A. jamesi*, *A. jeyporiensis*, *A. karwari*, *A. maculatus*, *A. moghulensis*, *A. pallidus*, *A. splendidus*, *A. stephensi*, *A. subpictus*, *A. tessellatus*, *A. theobaldi*, *A. turkhudi* and *A. vagus*. Those found actually resting outdoors were *Anopheles aitkeni*, *A. barbirostris*, *A. culicifacies*, *A. culiciformis*, *A. fluviatilis*, *A. hyrcanus*, *A. jamesi*, *A. jeyporiensis*, *A. maculatus*, *A. moghulensis*, *A. pallidus*, *A. splendidus*, *A. subpictus*, *A. tessellatus*, *A. theobaldi*, *A. turkhudi* and *A. vagus*, all in small numbers. Those found biting man were: *Anopheles barbirostris*, *A. culicifacies*, *A. culiciformis*, *A. fluviatilis*, *A. hyrcanus*, *A. jamesi*, *A. karwari*, *A. maculatus*, *A. moghulensis*, *A. subpictus*, *A. tessellatus*, *A. theobaldi* and *A. vagus*.

COMMENTS REGARDING THE IMPORTANCE OF MOSQUITOES IN THE TRANSMISSION OF VIRUSES IN INDIA.

The list of mosquitoes reported in this paper, as occurring in Poona District, contains several which have been implicated as vectors of human virus infections. The role of *Aedes* (*Stegomyia*) spp. in relation to yellow fever and dengue is well known. The distribution of *Aedes aegypti* appears to have undergone a considerable reduction during recent years in India. It was quite common in Poona over a decade ago but has now practically disappeared. How much of this reduction in numbers is due to natural causes and how much to the widespead use of D.D.T. as an indoor residual spray, is not clear.

Among the other mosquitoes, those which are already known or suspected to be responsible for the transmission of viruses in other parts of the world are *Aedes vexans*, *Culex gelidus* and *C. univittatus*. Perphas *C. fatigans* should also be added to the list as it belongs to a complex, the specific identities of whose members such as *C. pipiens*, *C. molestus* and *C. quinquifasciatus* are not yet clearly defined. The virus of western equine encephalitis has recently been recovered from *Aedes vexans* in Minnesota, U.S.A. (Burroughs and Burroughs, 1954). *C. gelidus* is a suspected vector of Japanese *B* encephalitis virus in Malaya (unpublished reports) and *C. univittatus* is known to play a definite part in the transmission of West Nile virus in Egypt (Taylor and Hurlbutt, 1953 and Taylor, Work *et al.*, 1956). *Culex molestus* is reported to have been found naturally infected with West Nile virus in Israel and experimental transmission of the virus has also been reported (Tahori, Sterk and Goldblum, 1955).

Recent work by the Virus Research Centre, Poona (T. H. Work, 1956, *personal communication*) has led to the isolation of several viruses from mosquitoes. A virus belonging to the West Nile—Japanese *B* complex has been isolated in Southern India from *C. vishnui*. An as yet unidentified virus has been recovered from *C. bitaeniorhynchus* from Umbre Village at the foot of the Western Ghats, hardly ten miles from the routine mosquito collection stations of Bhorghat described

earlier in this paper. Sindbis virus has been recovered from a pool of *C. vishnui* collected in Kolaba District in Bombay State and from a mixed pool of *Culex* spp. from North Kanara District, also in Bombay State. At least three other strains of as yet unidentified viruses have been isolated from pools of *C. vishnui* in North Arcot District from Madras State.

In the light of the finding that the blood of the indigenous inhabitants in several parts of Western and Southern India contains neutralizing antibodies to several known neurotropic viruses, particularly against West Nile virus (Smithburn, Kerr and Gatne, 1954) and the recent outbreak of Japanese *B* encephalitis type in South India, (Work and Shah, 1956), these virus isolations from mosquitoes are of considerable interest and lend suport to the view that mosquitoes, particularly *C. vishnui*, may have an important rôle in the transmission of neurotropic viruses in this country.

From April, 1953 to January, 1956, over 2,000 pools including over 1,20,000 individual mosquitoes belonging to more than 40 species collected in Western and Southern India, have been inoculated into white mice and only the virus isolations mentioned above were obtained. The number of pools and the total number of individuals of a few important species inoculated were *C. gelidus* 47 and 1,747, *C. bitæniorhynchus* 63 and 1,747, *C. vishnui* 680 and 52,857 and *C. fatigans* 237 and 9,255, respectively. These figures emphasize the importance of collecting and examining large numbers of mosquitoes in order to determine the rôle of mosquitoes as vectors of viruses in nature.

Detailed information on the distribution, both geographical and seasonal, and the bionomics of culicine mosquitoes in different regions of India, is now a matter of urgent necessity in order to understand further the ecology of arthropod-borne virus diseases.

SUMMARY.

Observations were made on the mosquito fauna of Poona District, Bombay State, India, between April, 1953 and March, 1955 in connection with virological investigations. The studies were particularly directed towards (*a*) determination of the species of mosquitoes occurring in the district, (*b*) determination of their day-time shelters, (*c*) finding out their seasonal prevalences and (*d*) elucidating their biting habits particularly as regards their man biting propensities. Field study areas, representative of three topographical and climatological regions of the district, were selected in which both routine and general collections of mosquitoes, both indoors and outdoors, were made.

Eightynine species of mosquitoes, belonging to the following 11 genera, were found:—

Aedes,	Ficalbia,	Orthopodomyia,
Anopheles,	Heizmannia,	Tripteroides and
Armigeres,	Mansonia,	Uranotænia.
Culex,	Megarhinus,	

Of the 89 species, 24 were recorded for the first time in the district and the specific identities of 11 are still undetermined.

The seasonal prevalences and the day-time resting habits of the mosquitoes in all the study areas have been described.

Forty species of mosquitoes were actually caught alighting on man presumably for feeding and 36 of them were found to bite and take human blood meals. The species which were found to bite man were:—

Aedes pipersalatus, A. vexans, A. (Aedimorphus) species indet., *A. lineatopennis, A. annulirostris, A. cogilli, A. dissimilis, A. greeni, A. gubernatoris, A. pseudotæniatus, A. scatophagoides, A albopictus, A. vittatus, A. w-albus, Armigeres obturbans, A. magnus, Culex bitæniorhynchus, C. fatigans, C. univittatus, C. vishnui, C. khazani, Mansonia crassipes, M. uniformis, Anopheles annularis, A. barbirostris, A. culicifacies, A. moghulensis, A. subpictus, A. tessellatus, A. fluviatilis, A. hyrcanus, A. jamesi, A. karwari, A. maculatus, A. theobaldi* and *A. vagus*. Among the 5,355 mosquitoes of all species collected alighting on man, three species of *Aedes (Stegomyia)* viz., *albopictus, w-albus* and *vittatus* accounted for 4,693.

Detailed notes on the distribution, seasonal prevalence and habits of each of the species of mosquitoes collected, are given.

Brief references are made to some recent isolations of viruses from mosquitoes in India and the importance of mosquitoes in the transmission of viruses in India commented upon.

A direct observation on the feeding of *Culex (Mochthogenes) malayi* on frogs has been made.

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STRUCTURE OF SALIVARY GLANDS IN MOSQUITOES.

A preliminary note.

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HITHERTO the salivary glands of adult mosquitoes have been recognized as paired structures which lie on each side of the oesophagus in the hæmocole. Each gland has been described as trilobed, with a central acinus smaller than the two lateral ones. The glands of either side consist of a cellular part and a central duct which unite to form a common salivary duct that passes forward to open at the base of the hypopharynx. However, deviations from this normal pattern have been mentioned in literature and recently Jensen (1956) has given a brief diagrammatic description of the abnormalities in the salivary glands of *Anopheles albimanus*.

While trying to determine the pattern of abnormalities in the salivary glands of different species of Indian mosquitoes (to be published separately), some important observations have been made which seem to be of great value, as they imply sexual dimorphism among individuals of the same species as well as other characteristics having a bearing on the systematics of mosquitoes.

Glycerine* mounts of the salivary glands of *Anopheles annularis*, *A. culicifacies*, *A. pallidus*, *A. pulcherrimus*, *A. stephensi* and *A. subpictus* revealed that in the case of female specimens, the intra-acinar duct dilates generally in the distal parts of the

* Dissecting out the glands in 0·68 per cent saline and mounting them in glycerine (60 to 70 per cent glycerol), gave better results than using conventional fixatives, stains and other mounting media.

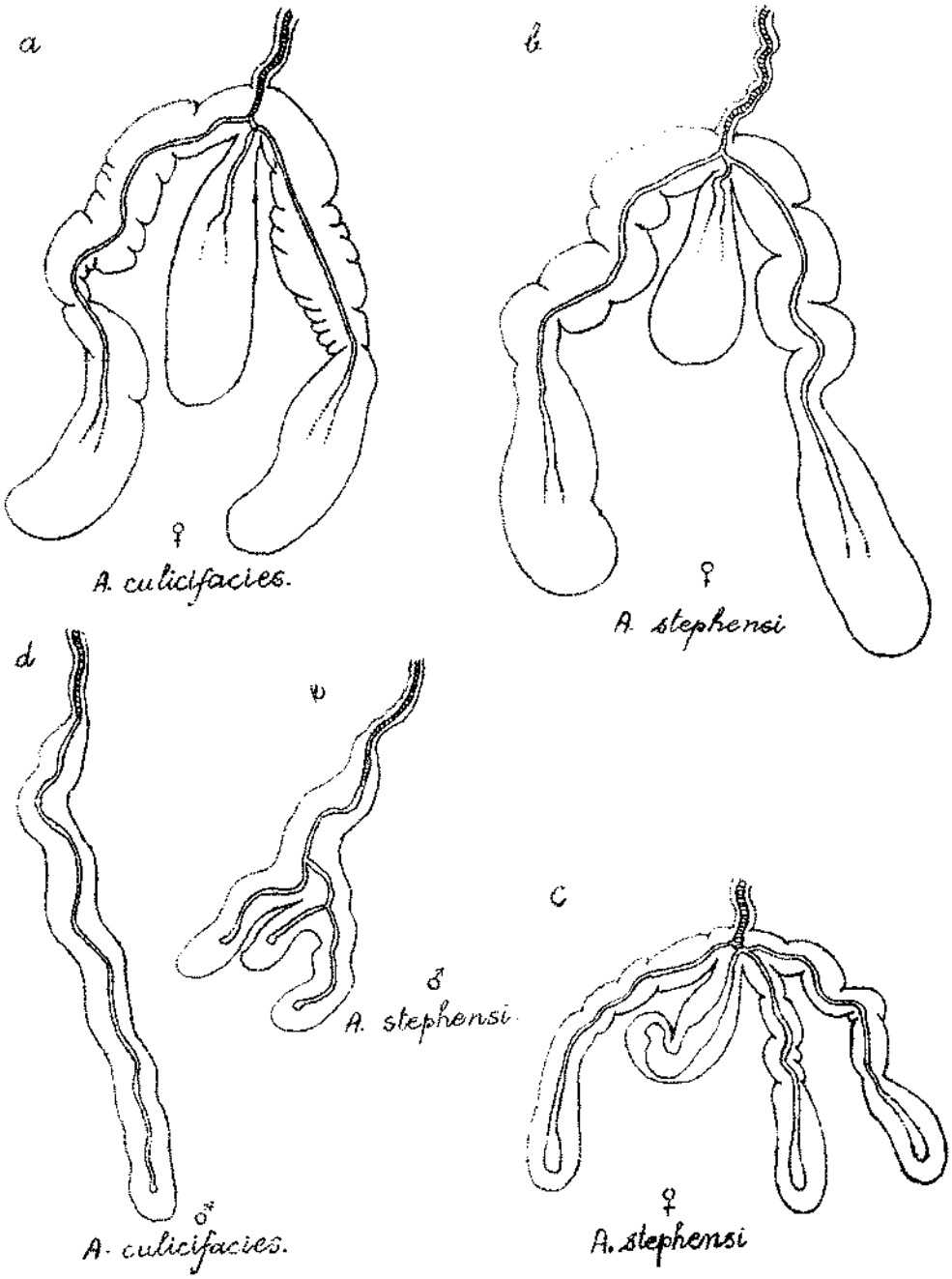
lateral lobes and then becomes invisible. In the central acinus, the duct dilates immediately after entering the lobe (Fig. 1; *a*, *b*). Complete ampulla (dilatation) was observed only in some abnormal glands of *A. annularis*, *A. culicifacies* and *A. stephensi* (Fig. 1; *c*). The ampulla of the intra-acinar duct in anophelines has been mentioned, among others, by Nuttall and Shipley (1903) in their detailed description of the salivary glands, and has been accepted to be present in both sexes of these mosquitoes. However, the present studies have shown that in the case of male anophelines, the intra-acinar duct passes up to the end of the acinus (without forming an ampulla), ending in a pin-head like structure (Fig. 1: *d*, *e*).

Sexual dimorphism has been encountered in the case of laboratory-bred colonies of *Aedes* (*S*) *ægypti* also. The intra-acinar ducts in the males of this species have been found to be similar to those in the salivary glands of male anophelines mentioned above (Fig. 2, *e*). In the case of female specimens, however, it has been noticed that the ducts of the two lateral lobes, after running halfway their length, dilate imperceptibly. But unlike anophelines, the future course of the ducts remains discernible till the tip of the acini where they end abruptly. The duct in the central lobe shows obvious dilatation just after entering the acinus and remains distinctly discernible up to its posterior end (Fig. 2; *c*). No such sexual dimorphism was found in the laboratory-bred colonies of *Culex fatigans*. The intra-acinar ducts in the salivary glands of either sex in this species and the female *Mansonia* (*M*) *uniformis* resemble the ducts in the males of anophelines and *A. ægypti* as described above (Fig. 2; *a*, *b*, *d*).

The arrangement of tænidial rings, surrounding the intra-acinar ducts, has been found to differ in the two tribes. Whereas, in the case of *Anophelini* (Fig. 1) these rings disappear as soon as the duct passes into the acini, in the case of *Culicini* they depict generic and intra-species differences. The tænidial rings in both sexes of *Culex fatigans* surround the ducts throughout their length inside the acini. In the case of male *Aedes ægypti* a similar arrangement is met, but in the female *ægypti* the arrangement is different in that the tænidial rings surround the intra-acinar ducts of the two lateral lobes up to about halfway their length and to a point where the duct becomes slightly enlarged. In the central lobe, these rings disappear just before the point of dilatation of the duct. A similar condition is met with in the female *M.* (*M*) *uniformis* where these rings surround the duct halfway in the lateral lobes and do not at all surround the duct in the central lobe (Fig. 2; *a-e*). Yet another difference between the two sexes in *Aedes ægypti*, was the presence of smaller but equal three acini in the salivary glands of males. The glands in the females present the usual picture of lateral acini being larger than the central one.

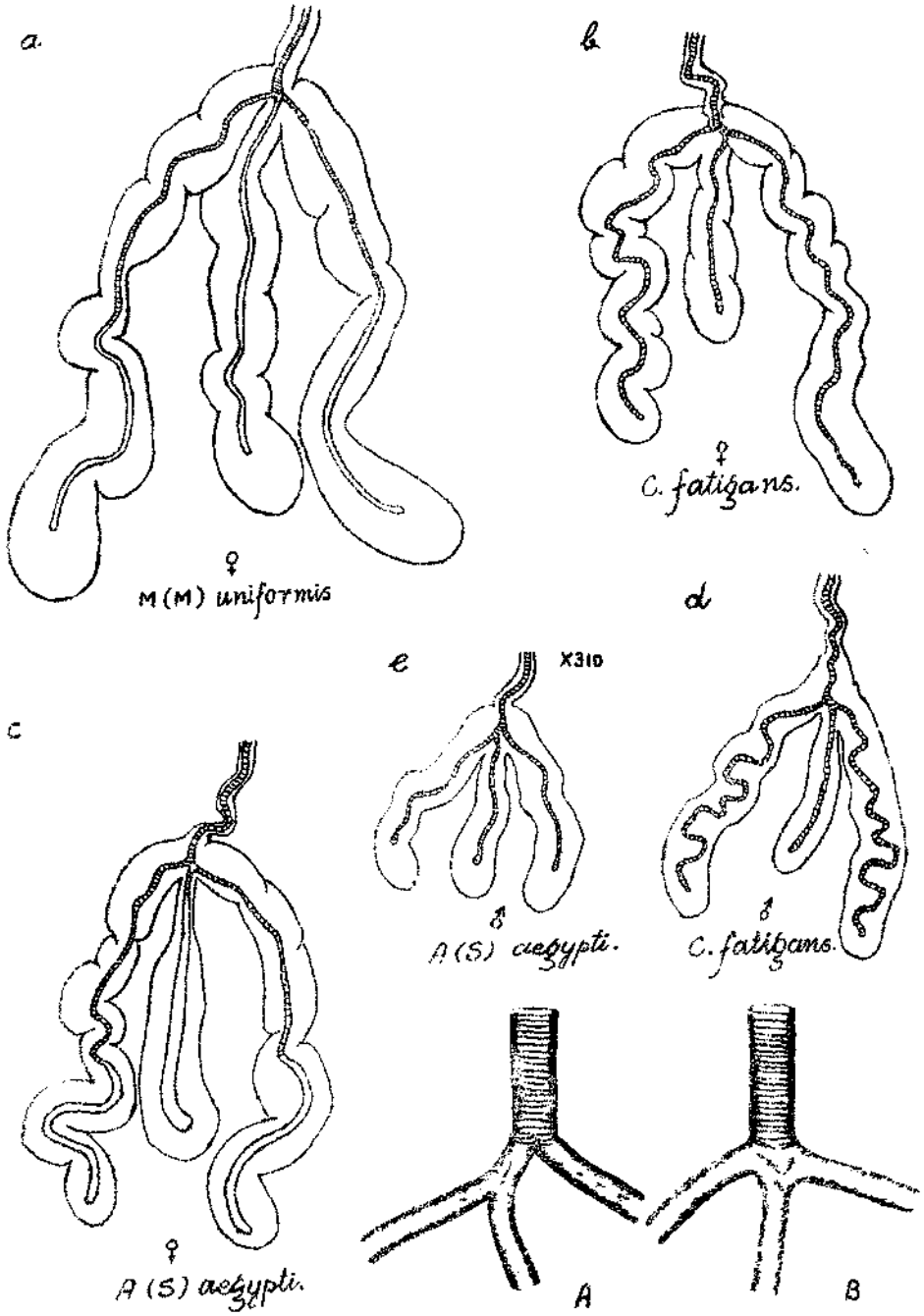
Nuttall and Shipley (1903) observed that " the intra-glandular, or intra-acinar duct, communicate anteriorly with the secondary duct, which is joined at once by the three intra-glandular ducts at one point ". Their diagram of the glands shows the three intra-acinar ducts arising at one point. That this conception is widely held, can be gauged by looking at the diagrams of salivary glands in various text books and literature. Jensen (1956) in the diagram of normal glands of female *A. albimanus* has reported a similar condition. The present studies have shown that in both the tribes (*Anophelini*/*Culicini*) examined, the intra-acinar ducts may

FIG. 1.



Free hand diagrams of the salivary glands of anophelines observed under magnifications '× 310' (a, b, d, e) and '× 225' (c).

FIG. 2.



(a to e).—Free hand diagrams of the salivary glands of culicines observed under '× 310' magnification.

(A, B).—Diagram depicting the mode of furcation of the salivary duct before entering the acini.

arise in a few cases at one point but in the majority of cases it arises at two points as illustrated (Fig. 2; *A, B*). This condition was found both in normal and abnormal glands.

Jensen (1956) has reported only monolobed salivary glands in the males of *A. albimanus*. Present studies, however, have shown that salivary glands in male anophelines may normally be monolobed or multilobed, depending on the species examined (Fig. 1 ; *d, e*). The glands in the case of culicine males were noted normally to be trilobed.

That the observations recorded above were overlooked by earlier workers, is easy to comprehend. Malariologists were mainly concerned with dissecting the female specimens and did have but little opportunity to examine the male salivary glands.

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PREFERENTIAL INDOOR RESTING HABITS OF *A. CULICIFACIES* GILES NEAR DELHI.

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INTRODUCTION.

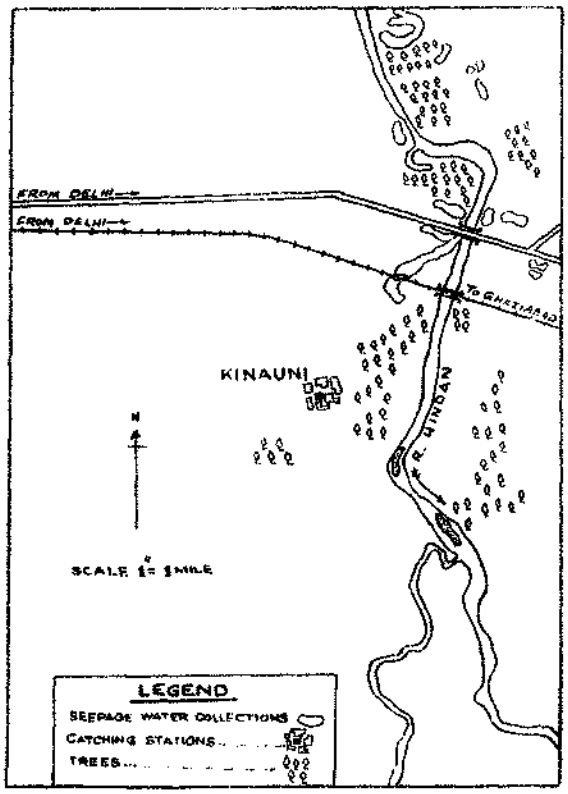
WITH the acceptance of adult mosquito (vector) control as the aim of malaria control programmes, a study of the preferential diurnal resting places of the principal vector species has become imperative. As *A. culicifacies* influences malaria transmission in the greater part of the peninsular India, the present study was undertaken to elucidate the day-time resting habits of this species. In the light of findings presented in this note, it seems that stress has to be laid on insecticidal coverage of certain surfaces inside shelters which has hitherto not been fully appreciated.

METHODS.

Kinauni, an unsprayed village about 13 miles off Delhi near the Hindan River (Map), was selected for observations. The village proper is one mile away from the river and has about 100 huts. Besides the seepage water from the river, the village has some low-lying agricultural lands, among which some are left fallow in turns year after year, which provide the bulk of breeding grounds throughout the year. Some borrowpits, on either side of the road in the vicinity of the village, serve as prolific nursuries for *culicifacies* breeding during the rains and for variable periods afterward.

Mosquitoes were collected from human dwellings and cattlesheds for one hour each during 7 a.m. to 11 a.m. The frequency of catches made per month varied as indicated in Table II.

MAP



The equipment used for catching adult mosquitoes for the present study, was aspirator tubes, flash lights, test tubes and Barraud cages.

There is not much difference in structure between the cattlesheds and human dwellings. Both, as in other rural areas around Delhi, are mud-plastered huts with mud or thatched roof, and with one opening serving as door. Occasionally there may be two such openings without a door of any sort. Cattle are invariably found near human abodes and similarly men are to be found sleeping inside or near cattlesheds and enclosures.

Throughout these observations, the same personnel were employed for making catches and recording the data.

During the period under observation, the annual rainfall averaged 28·86 inches which is just 8·86 inches more than the normal for this area. The average mean temperature varied from 58·2° F. in January to 98·9° F. in June, and the average relative humidity varied from 22·3 per cent in April to 73·2 per cent in September (Table I).

TABLE I.

Meteorological data for Delhi, August 1954 to September 1956.

Months	Years			
	1954	1955	1956	Average
Mean temperature, degrees Fahrenheit.				
January ...	...	57·9	58·5	58·2
February ...	...	63·6	64·0	63·8
March ...	...	77·0	75·8	76·4
April ...	...	80·8	85·6	83·2
May ...	...	85·2	94·5	89·9
June ...	...	95·1	102·7	98·9
July ...	...	89·7	93·7	81·7
August ...	88·5	86·1	91·9	88·8
September ...	86·3	80·8	95·6	87·6
October ...	73·2	76·0	...	74·6
November ...	68·5	67·1	...	67·8
December ...	59·6	60·4	...	60·0
Mean relative humidity, per cent.				
January ...	...	64·4	62·2	63·3
February ...	...	44·4	39·7	42·0
March ...	...	35·9	47·3	41·6
April ...	...	22·3	22·7	22·5
May ...	...	26·4	29·1	27·8
June ...	...	37·8	46·7	42·3
July ...	...	59·1	69·9	64·5
August ...	62·0	68·7	73·5	68·0
September ...	72·8	73·6	54·3	66·9
October ...	45·6	65·8	...	55·7
November ...	44·2	48·9	...	46·6
December ...	56·2	63·4	...	59·8
Total rainfall, inches.				
January ...	...	1·29	1·33	1·31
February ...	...	0·04	0·04	0·04
March ...	...	0·4	0·8	0·60
April ...	...	0·81	0·13	0·47
May ...		0·27	0·0	0·14
June ...	...	2·04	1·12	1·58
July ...	...	4·45	11·28	7·87
August ...	0·58	9·04	3·71	6·11
September ...	2·32	9·8	0·83	4·32
October ...	9·39	3·45	...	6·42
November ...	0·0	0·6	...	0·0
December ...	0·0	0·0	...	0·0
Average annual rainfall				28·86

Table II.

Total number of adult A. culicifacies *collected on different structures inside village dwellings in Kinauni near Delhi (August 1953 to September 1956).*

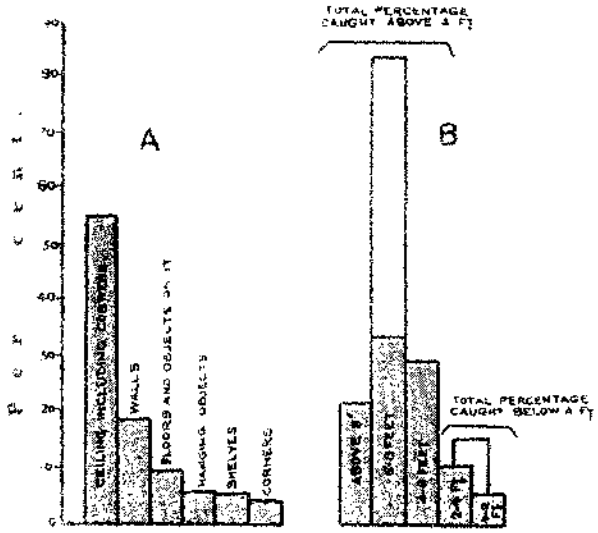
Serial number	Year and month	Number of collections	Total hours	Resting places: Ceiling: Direct	Ceiling: Cobwebs	Walls with-door: Above 6 ft.	Walls with-door: Below 6 ft.	Walls opposite door: Above 6 ft.	Walls opposite door: Below 6 ft.	Side walls: Above 6 ft.	Side walls: Below 6 ft.	Shelves: Above 6 ft.	Shelves: Below 6 ft.	Floor etc.: Direct	Floor etc.: On fire-wood	Floor etc.: On cowdung	Total
	1954																
1.	August ...	4	8	46	28	8	9	...	...	3	7	2	4	5	5	9	126
2.	September ...	10	20	168	75	24	4	7	5	14	29	22	9	19	18	7	391
3.	October ...	8	16	92	42	13	2	7	11	6	7	10	11	17	8	7	233
4.	November ...	3	6	29	15	2	4	2	4	3	5	4	2	4	4	2	80
5.	December ...	2	4	44	31	8	2	4	1	1	1	1	1	1	...	2	97
	1955																
6.	January ...	2	4	22	7	1	1	...	...	1	1	1	1	3	...	2	40
7.	February ...	2	4	24	9	3	...	...	1	2	...	1	...	...	...	...	40
8.	March ...	2	4	10	3	1	2	5	2	3	3	2	2	...	...	...	33
9.	April ...	2	4	27	21	6	3	3	3	6	6	2	2	3	2	...	84
10.	May ...	2	4	17	17	1	...	7	3	3	1	1	1	1	...	...	52
11.	June ...	2	4	17	17	...	...	12	1	1	...	...	...	9	8	...	65
12.	July ...	2	4	16	18	5	...	2	...	...	...	...	...	3	...	1	45
13.	August ...	2	4	39	18	9	3	4	1	8	2	4	...	...	4	4	96
14.	September ...	4	8	98	50	15	20	8	15	13	6	9	9	...	7	2	52
15.	October ...	2	4	42	19	7	5	8	7	5	6	...	3	...	...	...	102
16.	November ...	4	8	179	53	30	44	21	33	29	31	12	23	...	...	1	456
17.	December ...	5	10	193	81	23	25	9	29	12	14	5	11	...	1	...	403
	1956																
18.	January ...	3	6	9	8	...	6	2	4	2	1	...	1	...	...	...	33
19.	February ...	4	8	17	7	1	5	1	3	...	3	...	...	...	...	...	37
20.	March ...	4	8	30	8	1	5	2	5	2	4	...	...	...	...	...	57
21.	April ...	4	8	32	13	1	...	1	1	1	2	...	...	...	...	...	51
22.	May ...	5	10	9	1	...	7	...	5	...	5	...	3		...	...	30
23.	June ...	4	8	...	1	...	3	...	5	...	...	...	...	...	...	...	9
24.	July ...	4	8	9	7	...	2	...	6	...	2	...	...	...	...	...	26
25.	August ...	5	10	45	29	1	4	...	6	...	2	...	...	...	...	...	87
26.	September ...	4	8	44	36	...	8	4	12	...	4	...	...	...	...	...	108
	Total ...	95	190	1258	614	150	164	109	163	115	142	76	83	65	57	37	3033
	Per man-hour			6·6	3·2	0·8	0·9	0·6	0·9	0·6	0·7	0·4	0·4	0·3	0·3	0·2	15·9
	Per cent ...			41·5	20·2	5·3	5·4	3·6	5·3	3·8	4·4	2·5	2·7	2·1	1·9	1·2	99·9

OBSERVATIONS.

Table II gives the number of adult *A. culicifacies* found resting on ceiling and cobwebs hanging from ceiling, the four walls inside the shelters, in shelves, on floor and objects such as firewood, cowdung etc., during the period August 1954 to September 1956. This mosquito was encountered throughout the year for the period mentioned above. During 26 months of this study, 95 collections were made lasting 190 hours during which time 3,033 adults were collected. Unlike the observations made by Ariaratnam (1955) in Ceylon and Rafi (1955) in West Punjab (Pakistan) on the resting habits of *A. culicifacies*, it was found that 61·7 per cent of the mosquitoes rest on ceiling (41·5 per cent on ceiling and 20·2 per cent on cobwebs hanging from the ceiling). Of the remaining, 27·8 per cent were collected on walls, 5·2 per cent on or in the shelves and only 5·2 per cent on the floor and the firewood and cowdung heaps lying on it. Out of those resting on walls, 10·7 per cent were found on walls with door, 8·5 per cent on walls opposite the door (entrance) and another 8·5 per cent on the ramaining two side walls.

For a year, August 1954 to July 1955, the observations included a study of mosquitoes resting on hanging objects and corners (Table III). It was found that only 5·7 per cent of the total catch during this period used hanging objects as resting place. 4·3 per cent were collected in corners, 5·4 per cent in shelves, and 3·1 and 2·1 per cent on firewood and cowdung heaps respectively [Chart 1(*A*)].

CHART 1



Incidence of adult A. culicifacies *at various resting places and heights inside dwellings.*

Table IV gives the breakdown of height inside the two types of dwellings, at which *A. culicifacies* were collected resting from September 1955 to September 1956. It is observed that only 15·7 per cent were collected below four feet level from the

TABLE III

Preferential Indoor resting habits of A. culicifacies *Near Delhi.*

Serial number	Year and month	Number of collections	Total hours	Resting Place: Ceiling		Hanging objects		Walls with door		Walls opposite doors		Side walls		Corners		Shelves		Floor etc.			Total
				Ceiling	Cob-webs	Above 6 ft.	Below 6 ft.	Above 6 ft.	Below 6 ft.	Above 6 ft.	Below 6 ft.	Above 6 ft.	Below 6 ft.	Above 6 ft.	Below 6 ft.	Above 6 ft.	Below 6 ft.	Dir-ect	On fire wood	On cow-dung	
	1954																				
1.	August ...	4	8	46	28	3	2	8	9	0	0	3	7	1	1	2	4	5	5	9	133
2.	September	10	20	168	75	9	6	24	4	7	5	14	19	4	5	22	9	19	18	7	415
3.	October ...	8	16	92	42	13	9	13	2	7	11	6	7	10	6	10	11	17	8	7	271
4.	November	3	6	29	15	5	5	2	4	2	4	3	5	3	2	4	2	4	4	2	95
5.	December	2	4	44	31	4	3	8	2	4	1	1	1	6	2	1	1	1	0	2	112
	1955																				
6.	January ...	2	4	22	7	1	0	1	1	0	0	1	1	1	2	1	1	3	0	2	44
7.	February	2	4	24	9	1	2	3	0	0	1	2	0	1	0	1	0	0	0	0	44
8.	March ...	2	4	10	3	2	1	1	2	5	2	3	3	3	2	2	2	0	0	0	41
9.	April ...	2	4	27	21	3	4	6	3	3	3	6	6	4	3	2	2	3	2	0	98
10.	May ...	2	4	17	17	6	2	1	0	7	3	3	1	1	1	1	1	1	0	0	62
11.	June ...	2	4	17	17	0	0	0	0	12	1	1	0	0	0	0	0	9	9	0	65
12.	July ...	2	4	16	18	0	0	5	0	2	0	0	0	3	0	0	0	3	0	1	48
	Total ...	41	82	512	283	47	34	72	27	49	31	43	50	37	24	46	33	65	45	30	1,428
	Per cent	...	...	35·9	19·8	3·3	2·4	5·0	1·9	3·4	2·2	3·0	3·5	2·6	1·7	3·2	2·3	4·6	3·1	2·1	100·0

floor and 84·3 per cent above this level [Chart 1(*B*)]. No adults of this species were found resting in likely outdoor shelters in spite of repeated searches in this area.

DISCUSSION.

Most observers agree that *A. culicifacies* is a domestic species. As to its prevalence in human dwellings and cattlesheds, the reports are conflicting. In fact, in Indian villages there are no elaborate or separate cattlesheds. Cattle are often found in the proximity of their owners.

Ariaratnam (1955), after analysing the data of *A. culicifacies* catches in Ceylon for a period of one year, concluded that these mosquitoes rest mainly on walls inside a shelter at a height below six feet from the floor. Rafi (1955) observed that *A. culicifacies* and *A. stephensi* in Punjab (Pakistan) show a uniform preference for the entire height of the room. According to this worker, the position occupied by a house-frequenting mosquito is guided by the amount of light, the largest numbers being found where illumination is very low but not dark.

TABLE IV.

Adult A. culicifacies *collected at various heights inside village dwellings in Kinauni, near Delhi.*

Serial number	Year and month	*A. CULICIFACIES* COLLECTED AT DIFFERENET HEIGHTS.					
		0-2 ft.	2-4 ft.	4-6 ft.	6-8 ft.	Over 8 ft.	Total
	1955						
1.	September ...	31	11	72	103	52	269
2.	October ...	9	3	54	23	18	107
3.	November ...	18	42	154	134	94	442
4.	December ...	16	38	88	140	119	401
	1956						
5.	January ...	1	6	10	14	2	33
6.	February ...	0	8	5	13	7	33
7.	March ...	4	6	7	26	12	55
8.	April ...	0	2	8	29	11	50
9.	May ...	0	11	14	4	1	30
10.	June ...	0	4	5	0	0	9
11.	July ..	0	4	8	1	1	14
12.	August ...	2	7	6	6	0	21
13.	September ...	1	10	10	3	1	25
	Total ...	82	152	441	496	318	1,489
	Per cent ...	5·5	10·2	29·6	33·3	21·4	100·0

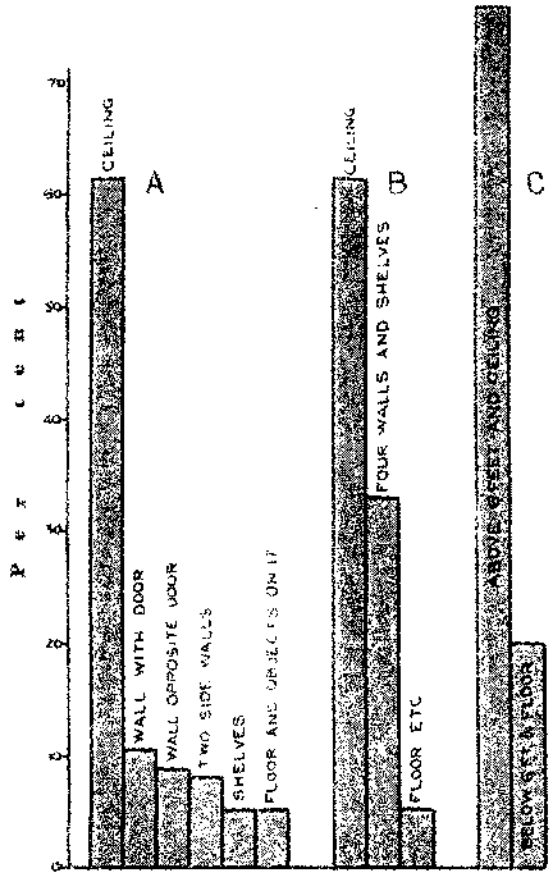
In case of other anophelines, Russell *et al.* (1946) mentioned that *A. fluviatilis* is often found on lower portions of the walls, not more than five feet from the floor. With regard to the preferential indoor resting habits of *A. philippinensis*, there are contradictory reports. Whereas, Iyengar (1944) mentioned that in Bengal this species was found resting on the walls within one and a half feet from the floor, Ganguli (1947) in Bengal-Orissa border found that *A. philippinensis* rested above four feet level and on hanging objects. The later finding has subsequently been supported by other malariologists (Ray, 1955). Deane and Damasceno (1948) found that 90 per cent females of *A. darlingi* and *A. aquasalis* in Brazil rested on the lower two metres (about 6½ feet) of the wall and about 60 per cent in the lowest metre (3·3 feet). Rachou *et al.* (1949), working in the same country, found that the vectors *A.* (*Kortisria*) *cruzii*, and *A.* (*K*) *bellator* showed a general preference for the higher parts of the walls, while *A. darlingi* preferred the middle part of the wall. As far as *A. minimus* is concerned, it has been found to prefer resting on walls of shelters at a level lower than six feet height from the floor. This applies to *A. minimus minimus* in India and Thailand, as well as to its variety *flavirostris* in Philippine Islands.

The present observations clearly indicate that *A. culicifacies* around Delhi prefer to rest on ceiling and on the cobwebs hanging from it and particularly above six feet level from the floor. This assertion is supported by the fact that 61·7 per cent of the mosquitoes collected for this study. (Table II; Chart 2(*A*) were caught resting on ceiling (and cobwebs), and a total of 76·9 per cent on ceiling (including cobwebs) and at and above six feet height [Chart 2 (*C*)]. As already mentioned, out of the remaining 38·3 per cent, the majority of 27·8 per cent were found on walls and only 5·2 per cent each on the floor and objects on it and in the shelves [Chart 2(*B*)].

Muirhead-Thomson (1951) and Rajindar Pal and Sharma (1952) mention that this species shows marked preference for hanging clothes, umbrellas, furniture, firewood, etc. The present findings, however, indicate that such objects provide incidental resting places only. This is in agreement with the observations made by Ariaratnam (1955) in Ceylon. The present studies (Table III) show that only 5·7 per cent of the mosquitoes collected during August 1954 to July 1955 rested on hanging objects.

The resting habits of mosquitoes may be regulated by a complexity of micro-ecological factors, chiefly intensity of light, degree of air movement, texture of resting surface and relative humidity. It has been recorded that indoor resting species may be found in low illuminated spots avoiding both the dark and well-lit places (Muirhead-Thomson, 1941). Shannon (1935) observed that most mosquitoes prefer low illumination while depicting a tendency to avoid complete darkness. Ariaratnam (1955) found that *A. culicifacies* in Ceylon prefers cadjan-walled houses to mud-plastered ones. In India, the ceiling of the village dwellings is dimly illuminated, midway between the darkest and the well-lit parts of the rooms. Also, it is often made of thatch or unpolished wooden boards, giving it a coarse texture. Both these factors may be responsible for high *A. culicifacies* density encountered on the ceilings in the present observations. The corners which were the darkest spots in the dwellings, had only 4·3 per cent of mosquitoes (Table III) and the walls opposite the doors, the most illuminated spot, had only

CHART 2.



Incidence of adult A. culicifacies at various resting places and heights inside dwellings.

5·6 per cent of the resting mosquitoes respectively. It may also be mentioned that the air movement is generally lowest near the ceiling and like light, humidity is also midway between the extremes.

Still more important factor, influencing the preference for resting on ceiling and cobwebs, seems to be an innate behaviouristic tropism wherein *A. culicifacies* has the characteristic to defy gravity (negatively geotropic). No other anopheline exhibits this phenomenon to such a marked degree.

From the present study (Table IV), it seems safe to infer that in Northern India any spray programme that covers the walls above three feet level from the floor and the ceiling with an insecticide deposit, should control malaria transmission to a great extent if *A. culicifacies* happens to be the only vector. Also such a spray programme would prevent wastage of insecticide material in being rubbed off when the rural folk squat and lean against the walls. Besides, the necessity of

removing furniture, cots, and other objects from near the walls will not arise. The cost of labour, insecticide and the time saved thus may be considerable, particularly in times of emergencies and epidemics.

The general practice so far has been to spray walls, ceiling etc., so far as the ordinary spray lance would reach. It is true that most of the ceilings of shelters in rural India may get a residual insecticide deposit in this manner. However, from the results of this study, as discussed above, a stress on ensuring complete coverage of ceiling in shelters, in *A. culicifacies* (vector) region, seems essential. To achieve this, the reach of the average spray lance can be increased by fixing a bamboo to the lance and tying the rubber or plastic hose with twine to the bamboo at a few places depending on the reach desired.

SUMMARY AND CONCLUSIONS.

A. culicifacies found resting inside human dwellings and cattlesheds in Kinauni, a village outside the control area near Delhi, were recorded from August 1954 to September 1956. The observations indicate that:—

1. *A. culicifacies* prefers to rest on ceiling and cobwebs hanging from it than on any other structure inside a dwelling.

2. 84·3 per cent *A. culicifacies* rest above four feet and on ceiling inside a shelter, and 76·9 per cent on ceiling and above six feet level from the floor.

3. Of the four walls, the one with the door showed slightly higher *A. culicifacies* density than any of the other tree walls. The difference, however, does not seem to be of any great significance.

4. Hanging objects, shelves and objects on floor, serve only as incidental resting places for this species.

5. *A. culicifacies* avoids dark places like corners.

6. Low illumination, slightly coarse surface, little or negligible air movement and median humidity may be the cause of high *A. culicifacies* density on ceiling and cobwebs hanging from it. In addition, this species shows marked negative geotropism.

7. It is suggested that in Northern India in areas where *A. culicifacies* happens to be the only vector, a spray programme ensuring complete coverage of the ceiling and of the walls above three feet level from the floor, should control malaria transmission.

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MALARIA AND ITS CONTROL IN AFGHANISTAN (1950-1954).

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INTRODUCTION.

MALARIA and its control in Afghanistan forms an interesting record of the joint efforts of the National Government/World Health Organization and of the United Nations International Children Emergency Fund. No systematic investigations on the malaria problem in Afghanistan were ever attempted before the year 1949, when the World Health Organization offered assistance in the control of malaria and later sponsored investigations by sending World Health Organization teams from time to time.

The entomological and epidemiological investigations were carried out chiefly for determining pre-operation or comparison data or for exploring malarious tracts in the country for expansion of malaria control programme. These data are presented in this paper.

Some information included in this report has already been published (Rao, 1949; Iyengar, 1954) but this report aims at bringing together all available data for easy reference, right from the beginning when both the present authors became associated with the programme.

PHYSIOGRAPHY.

Afghanistan is a land-locked country with its frontiers bordering on the Union of Soviet Russia, Iran, Pakistan, and a narrow strip (Wakhan) extending eastward from the north-east corner to the border of China. The entire length of Afghanistan, as from Wakhan in the north-east to Kohi Malik Siah in the south-west, is about 1,500 kilometers (950 miles), while its width from Zulfkar on the Herat border to Torkham in the Khyber Pass, is nearly 1,000 kilometers (600 miles).

The chief feature in the physiography of the country are the rocky hills, practically without any vegetation. The important mountain range of Hindukush, penetrates Afghanistan in the north-east corner and runs across the country in the south-west direction. It has a curvature with concavity to the south and so opens its arms to the rain bearing winds that sweep up from the Indian Ocean and the Arabian Sea. The range in its north-eastern end has peaks over 20,000 feet with Trichmir reaching 25,263 feet.

There is a net work of big rivers coming down from great heights. The most important river is the Helmand, rising in the central highlands and, after flowing through Hazarjats, comes to Grishk and empties itself in the Lake of Seistan. The Helmand with its tributaries, the Arghestan, the Turanak, and the Arghandab, is considered as the twelfth largest river in Asia. Among other important rivers are Amu, Kundus, Kabul, Harirud, Morghab, Farah Rud and Khash Rud. Rivers flow either to the east or to the west according to their origin from the eastern or western slopes. Those arising from the eastern slopes are mostly the tributaries of River Indus in Pakistan.

Besides the perennial streams, there are several water courses which are liable to be converted into raging torrents after clouds burst in the spring months. They are mere trickles of water at other times.

CLIMATE.

Afghanistan is situated wholly in the north temperate zone between parallels of 29° 30′ and 38° 50′ North and 60° 30′ and 74° 50′ east longitude. The climate in Afghanistan is generally characterised on the one hand by extremes of temperatures—hot summers and rigorous winters—and on the other by scanty rainfall. The rains usually occur during the winter and spring months; the humidity remains low during most of the year. There are, however, five distinct zones of climate of Afghanistan.

(*i*) *Desert climate.*—Of Grishk and deserts of Bakua, Margo, Khash Rud and Seistan.

(*ii*) *Steppe climate.*—Of Kandhar, Maqur, Chaman, Herat and plains of north Afghanistan.

(*iii*) *Subtropical climate.*—Of Jalalabad, Leghman, Khost (with cool winter and few frosts).

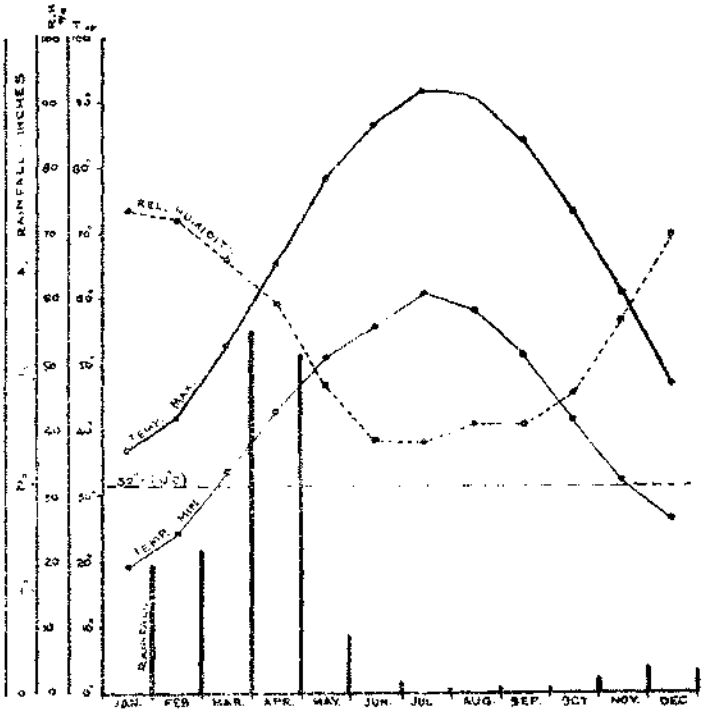
(*iv*) *Temperate climate.*—Of Kabul, Kunar valley, upper Kundus valley.

(*v*) *Extreme Alpine climate.*—In mountains with long severe winters.

The coldest month of the year is "Dalv" (January 21 to February 22) when the lowest temperature in Kabul falls 10 to 20° C. below zero (Chart 1). Uncertainty of rainfall and snowfall, particularly in Kabul area, is often observed. Rainfall averages about 11 inches for the country as a whole with 15 inches in the mountain areas.

CHART 1.

Average monthly maximum and minimum temperatures, relative humidity (Recorded at 7.30 a. m.) and total average rainfall. Years 1944-52. (in Kabul)



PEOPLE.

Afghanistan has an area of approximately 25,300 square miles and a population of about 12 million, giving an average density of 40 per square mile or 18 per square kilometer.

Of the 12,000,000 people in Afghanistan, only some 1,000,000 live in towns and cities like Kabul (300,000) Jalalabad (25,000), Kandhar (80,000). Mazar-i-Shrif (42,000), and Herat (76,000). The rest of the population live in rural areas, out of whom some 2,000,000 are nomads who graze their live stock and raise some grain and vegetables on the higher elevations in summer and migrate to the lower lands of the country or even further down towards Pakistan. There is comparatively a heavy concentration of population in numerous villages situated in the small irrigated valleys of northern, southern and eastern provinces.

Afghans are polite, courteous, religious-minded and hospitable people. Their women-folk observe strict *purdah* (veil). The houses in the rural area are mostly mud structures, rather ill ventilated and ill-lit. The practice of plastering the walls and floors with mud and animal dung, which is so common in India, is unknown in Afghanistan.

AGRICULTURE.

Valleys are very fertile and the chief crops are wheat in winter and rice and corn in summer. In most parts of the country, agricultural activities are carried out at the following times of the year:—

November to May-June—Wheat.

June-July to October—Rice and corn.

Irrigation in Afghanistan is by a system of canals branching from rivers and streams at higher reaches and leading to the fields situated at lower levels.

ANOPHELINE FAUNA.

In all, 16 different species of anophelines were identified and recorded in various parts of Afghanistan during the present investigations.

The anopheline fauna province-wise is given in Table I.

TABLE I.

Species of anophelines *identified in Afghanistan.*

Leghman (Rao, 1949) (Mashraqi Province)	Kundus Khanabad (Kathaghan Province)	Kabul (Ivanger, 1954) (Kabul Province)	Grishk Kandhar (Grishk and Kandhar Provinces)	Herat and Obay (Herat Province)
A. annularis	*A. claviger*	*A. culicifacies*	*A. culicifacies*	*A. superpictus*
A. culicifacies	*A. hyrcanus* var. *pseudopictus*	*A. fluviatilis*	*A. hyrcanus*	...
A. fluviatilis	*A. hyrcanus* var. *sinensis*	*A. stephensi*	*A. maculicolor*	*A. claviger.*
A. maculatus	*A. pulcherrimus*	*A. superpictus*	*A. pulcherrimus*	...
A. moghulensis	*A. superpictus*	*A. subpictus*	*A. stephensi*	...
A. pulcherrimus	..	*A. turkhudi*	*A. superpictus*	...
A. splendidus	...	*A. annularis*	*A. turkhudi*	
A. stephensi	...	...	...	...
A. subpictus	...	...	...	...
A. superpictus	...	...	...	...
A. turkhudi	..	..	..	...
A. vagus	..	...	...	...

(*a*) SEASONAL PREVALENCE.

(*i*) *General.*—In most parts of the country, the presence of anophelines is more or less confined to the period from June to the end of October during which the climatic conditions are usually favourable for their breeding and longevity.

Observations on the seasonal prevalence of anopheline mosquitoes in different areas are recorded as follows:—

(*ii*) *Leghman* (*Eastern Province*).—In Leghman, 12 species of anophelines were found from May to October but in the month of November, *i.e.*, the beginning of the cold season, these species became negligible in numbers.

(*iii*) *Kundus, Khanabad, Talaqan and Kalaizal* (*Kataghan Province*), *years 1950 and 1951.*—In all, five different species of anophelines were found in this area. Their density was built up rapidly from about the first week of July, mostly due to increased breeding output from the ricefields. The peak density reached towards the end of August or first week of September and from then there was a gradual decline and by the end of October it became extremely difficult to get any adult anopheline in this area.

(*iv*) *Kabul* (*Kabul Province*), *year 1952.*—Presence of adult anophelines was first observed during the month of June wherefrom their density progressively increased—reaching the peak between second half of July and throughout August. In the month of October, there was a sudden drop in the density which continued to be low throughout the month.

(*v*) *Kandhar and Grishk* (*Khandar and Grishk Provinces*), *year 1953.*— Out of six species of anophelines encountered from June to October, the principal ones were *A. superpictus* and *A. pulcherrimus.* The peak density of these two species was found to be in the later half of July and throughout August.

(*vi*) *Herat* (*Herat Province*), *year 1954.*—Investigations from July to September were confined to a few places in the valley of Hari Rud River (Obay and Herat). The only adult anopheline detected was *A. superpictus* and its peak prevalence was recorded in the month of August. A few larvæ of *A. claviger* were also found and no adults of this species were encountered.

(*b*) VECTOR

(*i*) *Distribution and sporozoite rates.*—*A. superpictus* is a common vector in the provinces of Kataghan, Kabul, Mashraqi, Kandhar and Grishk. In Mashraqi (Eastern) Province, in addition to *A. superpictus*, *A. culicifacies* is a secondary vector (Map 1).

In all the provinces where investigations were undertaken in Afghanistan, the desnsity of the vector (*A. superpictus*) exceeded that of other anophelines individually during the months of August and September. In Leghman area, the density of the second vector *A. culicifacies* was found to be the highest towards the end of September.

The distribution of vectors in different parts of Afghanistan and the sporozoite rates recorded during the investigations, are given in Table II.

MAP I.

MAP OF AFGHANISTAN

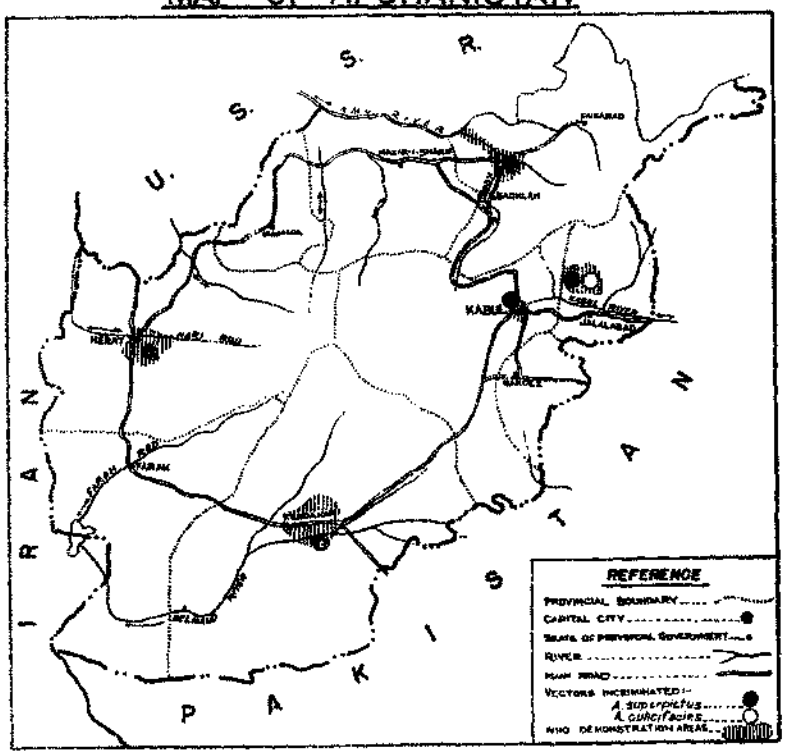


TABLE III.

Distribution of vectors and sporozoite rates.

Year	Province	Vector species	Number dissected	Gland Positive	Sporozoite rate (Per cent)
1949	Mashraqi (Rao, 1949)	(i) *A. superpictus*	768	3	0·4
1949	,, ,,	(ii) *A. culicifacies*	454	2	0·44
1950	Kataghan	(i) *A. superpictus*	1,787	3	0·17
1951	,, ,,	(ii) *A. superpictus*	1,264	1	0·08
1952	Kabul (Iyanger, 1954)	*A. superpictus*	2,059	8	0·39
1953	Kandhar and Grishk	*A. superpictus*	2,898	1	0·034
1954	Herat	*A. superpictus*	1,238	1	0·08

Gland infections in *A. superpictus* were found in different provinces in various months as shown in Table III.

TABLE III.

Gland infections in A. superpictus *in various months.*

Year	Province	Number positive			
		July	August	September	October
1950	Kataghan	Nil	1	1	1
1951		Nil	Nil	1	Nil
1952	Kabul (Iyangar, 1954) ...	3	3	2	Nil
1953	Kandhar and Grishk ...	1	Nil	Nil	Nil
1954	Herat	Nil	Nil	1	Nil

(*ii*) *Feeding and resting habits.*—Regarding the resting habits of *A. superpictus*, this species preferred to rest on the upper parts of the walls or on the ceilings. Adults of this species were collected in greater number in cattlesheds than in houses throughout the season of the prevalence. Forty-two stomach bloods from *A. superpictus* were sent to the Malaria Institute of India, Delhi, for examinations. Out of these, 14 were positive and 28 negatives for human blood.

Attempts were also made to search for *A. superpictus* in hollows or crevices of rocks but none of this species, or even any other anopheline species, were found resting out of doors.

A. culicifacies was also found in very large numbers inside human dwellings and stables during the day.

(*c*) BREEDING HABITS.

Larval catching stations were established in different provinces during the years 1950, 1951, 1952 and 1953 and collections and identification of larvæ were carried out systematically. In terms of breeding places for all anopheline mosquitoes, the following are the places in order of importance where larvæ were found in significant numbers in different areas:—

Ricefields, irrigation channels, river bed pools, rivers and streams, borrow-pits, tanks, reservoirs, wells and plant containers.

In Kundus-Khanabad area, out of the entire collection of larvæ during 1951, 30·0 per cent were from ricefields and 20·6 per cent from the irrigation channels. The collections in the river bed pools and in the river, were estimated as 20·0 per cent out of which the bulk was *A. superpictus*.

A. superpictus.—The breeding places most suited to this species were the pools in the stony beds of the rivers and natural streams, open to sunlight. Other places, next in importance, were the ricefields where they were found during all

stages of growth of the rice plants. The main canals and irrigation channels seldom showed any larvæ of this species.

A. pulcherrimus.—The larvæ of this species were found primarily in ricefields during all stages of growth. In a few areas, the larvæ of this species were collected from the stagnating edges of the river. The irrigation channels and swampy areas were the other sources where these larvæ could be collected.

A. claviger.—This species was found localized to a small pocket of area, namely Ishkamish (Kataghan) and Obay (Herat). The larvæ were collected chiefly from the sparkling spring pools.

A. hyrcanus.—Most of the *A. hyrcanus* larvæ were either from the ricefield or irrigation and field channels. Both varieties *pseudopictus* and *sinensis* were met with. This was confirmed by hatching out larvæ in the laboratory.

A. culicifacies.—This species was found breeding in ricefields, rivers and streams with rocky beds, irrigation channels and swamps.

A. stephensi.—This species was found breeding in river pools, ditches and borrowpits.

A. turkhudi.—The favourite breeding places for this species were river pools and ditches.

A. subpictus.—Larvæ of this species were found in ditches holding turbid water.

A special mention may be made here about the ricefields. These fields are usually flooded and ploughed in the later half of the month of May every year. The transplantation of paddy from the nurseries into the fields usually commences by the first week of June. In the canals and channels which feed the ricefields, an irregular system of intermittency is practised. In the absence of any regulators on the canals, ricefields are profusely irrigated when the canals are open. Harvesting begins from the middle of September and is practically over by the middle of October. Observations on the intensity of breeding of *A. pulcherrimus* and *A. superpictus* in the ricefields, during the growth of plant, indicated that unlike *A. culicifacies* which ceases to breed in ricefields after the plants reach a height of about 12 inches, they breed continuously throughout from transplantation to full maturity of the plants. However, when the plants begin to droop down with the weight of the ear-heads, the bulk of the larvæ are to be seen along the edges of the fields. The ratio of the breeding of *A. superpictus* to *A. pulcherrimus* in the ricefields in some parts of the country (Kataghan area) was found to be 1:7. There seemed to be little doubt that the densities of adult *A. superpictus* in the predominantly ricefield areas, such as in the central section of Kundus Khanabad Valley, were mainly due to their output from the ricefields as hardly any river bed pools could be found in that area.

EPIDEMIOLOGY.

Malaria is endemic in almost all parts of Afghanistan and is chiefly prevalent in the river valleys where rice cultivation is abundant. The endemicity varies from low to high, and hyper-endemic foci of the disease are not uncommon.

Malaria prevalence seems to be associated with the extent of irrigation, particularly of ricefields, and the presence of rivers and streams in the area. The people living away from the rivers and irrigated valleys are invariably found to have little or no incidence of the disease. Such a distribution of the disease is well noticeable in the areas along Hari Rud River in Herat Province, where villages on both sides of the river show much higher spleen rates as compared to those situated high up in the valley and away from the river. The same is true of the population living along Helmand and Arghandab rivers in the provinces of Grishk and Kandhar, respectively. In Kataghan Province, the villages in the vicinity of the rivers Talaqan, Khanabad, Kundus and Oxus showed higher degree of endemicity, as compared to the areas situated away from the rivers.

Small scale house to house malaria morbidity enquiry was undertaken in some parts of the country to get an idea of the total sickness due to malaria. This was rather taxing and not always possible in the orthodox communities who observe strict purdah. However from the limited data obtained in one province (Kataghan), it was found that about 8 per cent of the total population was actually sick during the malaria transmission season.

Malaria morbidity data was also collected from dispensaries and hospitals in Kundus-Khanabad area and in Kabul. In Kundus Khanabad area, there were three public dispensaries and the number of cases of malaria and of other diseases, treated for each month for the years 1947 to 1950, are presented in Appendix I. Similarly dispensary figures of malaria cases from three hospitals in Kabul which were collected from June to October, 1952, are represented in Chart 2. The diagnosis of malaria in the dispensaries and hospitals was entirely on clinical basis and the variations in the number of patients treated, depend on many factors well known to influence such figures. Therefore it is hard to say as to how far these data can be relied upon for any definite conclusions. However, a clue to the general trend of this disease presented by these data is noteworthy. In the Kundus and Khanabad districts, the maximum prevalence of malaria appears to conform practically to four Afghan months namely Asad, Sumbala, Meson and Akrab, *i.e.,* from middle of July to middle of November, after which there is a sharp drop. While in Kabul, as is evident from Chart 2, there is a continued rise in the incidence of disease from July to September after which there is steep fall in the month of October.

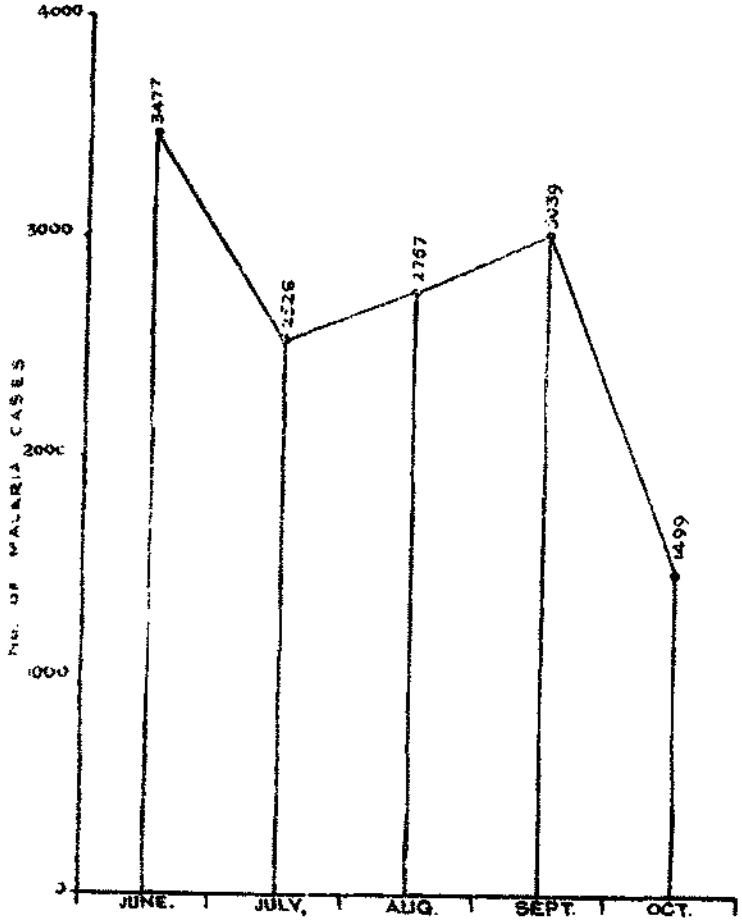
In the absence of satisfactory vital statistics in the country, it is not possible to say anything about the extent of social and economic effects of malaria. It is, however, estimated that out of a total population of 12 millions, about two millions live in malarious tracts.

SPLEEN SURVEY.

Extensive spleen surveys were carried out in the provinces of Kataghan, Kabul, Mashraqi, Gardez Khost, Kandhar, Grishk and Herat. The results of spleen surveys in different parts of this country are given in Appendix III. For this purpose, children of age group 2-9 years, were examined and Hackett's method of classification of spleen enlargement was adopted. On the basis of these

CHART 2.

Monthly malaria cases from 3 hospitals at Kabul, year 1952 (June-October)



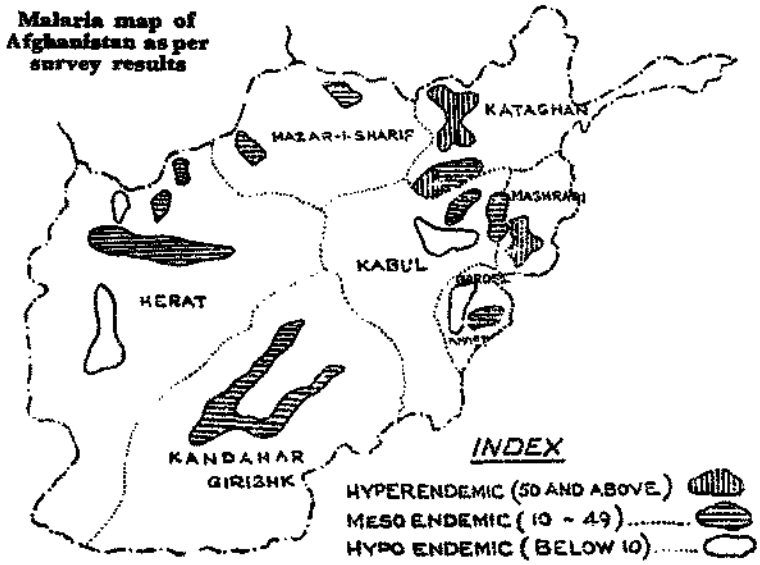
surveys, a map of Afghanistan showing hypoendemic, mesoendemic and hyperendemic malarious tracts has been prepared (Map 2).

(*a*) KATAGHAN PROVINCE.

Spleen surveys were carried out in June-July, 1950. A total of 1,186 children were examined in 24 different localities. The cumulative spleen rate was recorded 44·7 per cent, and the average enlarged spleen was found to vary from 1·3 to 2·8. The spleen rate ranged from 75 per cent in the irrigated section to 10 per cent in some of the barren villages.

In Khanabad Town, and all the villages surrounding it, the spleen rate was to be above 50 per cent. In Kundus area, spleen rate ranged between d 33 per cent.

MAP 2.



(*b*) KABUL PROVINCE.

Spleen surveys in and around Kabul were carried out in March-April, 1952. A total of 1,296 children (2-9 years) were examined. The overall spleen rate was found to be 10·5 per cent and the average enlarged spleen was between 1·9 and 2·5.

During this survey, out of 17 localities visited, 10 showed spleen rates below 10 per cent (hypoendemic) and 7 between 10 and 30·5 per cent (mesoendemic). The high spleen indices of 30·5 per cent and 27·1 per cent were registered in Kalai Hashmat and in Prancha School, respectively.

These surveys were further extended to twenty mile belt around Kabul, and 51 different villages were visited in September-October, 1952, when 2,286 children (2-9 years) were examined and cumulative spleen index was found to be 9·7 per cent with an average enlarged spleen from 1 to 2·7.

In September-October, 1952, Teghab District in Kabul Province was also surveyed, where 426 children were examined and a spleen index of 31·7 per cent was observed with an average enlarged spleen varying from 2·3 to 2·8. This district is about 100 miles away from Kabul in a fertile valley with extensive rice cultivation.

In 1954, spleen surveys in other places in Kabul Province, *i.e.* in Sarai Khoaja, Parwan, Begram, Jabul Saraj and Resa Kohistan were also carried out. In all 3,246 children were examined and all these areas were found to be hypoendemic.

(*c*) MASHRAQI (EASTERN) PROVINCE.

Spleen survey was carried out in December, 1952 in Surkhrud, Beisud, Shevashegi whereby 21 localities were visited and a total of 1,206 children were

examined. The spleen rate was found to be 57·0 per cent and average enlarged spleen varied from 2·1 to 2·4.

(*d*) GARDEZ PROVINCE.

Spleen survey was carried out in the districts of Gardez and Khost in November, 1952. In Khost area, in 41 different localities 1,692 children were examined. The spleen rate and average enlarged spleen were found to be 41·1 and 2·3 per cent respectively.

In Gardez, in 29 localities, 1,435 children were examined. The spleen index was recorded as 7·0 per cent and the average enlarged spleen varied from 1 to 2.

(*e*) KANDHAR AND GRISHK PROVINCES.

Spleen survey was carried out during December, 1952 and January, 1953. In all 1,400 children were examined and the spleen rate worked out as 46·0 per cent. The highest spleen index of 78·6 per cent and the lowest of 12·4 per cent were recorded in the villages of Kalai Bust and Dand, respectively. Average enlarged spleen varied from 1·9 to 2·5.

(*f*) HERAT PROVINCE.

Spleen surveys were carried out in August, 1953 in order to explore new malarious tracts for extending the malaria control operations in the following year. In this province, the districts of Herat, Obay, Anjil, Guzrah, Pushtoon Zurgoon, Kushk and Kalai-Nau were included in the survey. In this connection, 64 different localities were visited and a total of 1,984 children were examined. The spleen rate was recorded as 25·1 per cent, and the average enlarged spleen varied from 1·0 to 3·0.

Out of these 64 localities, 20 showed spleen rates ranging between 32 and 59·7 per cent, 11 between 20 and 25 per cent and the rest below 20 per cent. High incidence of disease was found on either side of the Hari Rud River where rice cultivation was in abundance.

In the same province, some other districts such as Ghorian, Shindand and Farrah were also surveyed during September, 1953. In 20 different localities, 640 children were examined. The spleen rate was recorded as 12 per cent and the average enlarged spleen varied from 1·0 to 1·8.

In 1954, further spleen surveys were undertaken in other areas like Qaysar, Almor, Maymana Town, Pashtoon Kota, Andkhoy, Bilchiragh and Shreen Tegab. A total of 983 children were examined in 19 different localities. A spleen rate of 2·23 per cent was registered and average enlarged spleen varied from 1 to 2.

PARASITE SURVEY.

(*a*) CHILDREN (AGE-GROUP 2-9 YEARS).

The results of blood surveys in various provinces of the country are given in Appendix IV. The parasite rate in children varied in different parts of the country from 1·7 per cent (in Gardez) to 30·1 per cent (in Kandhar Grishk).

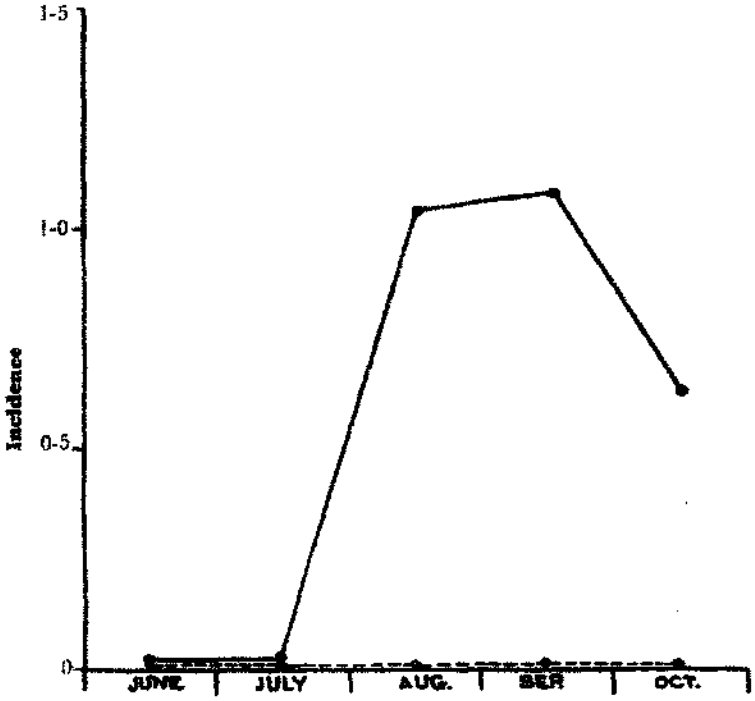
During the course of these surveys, *P. vivax*, *P. facliparum* and *P. malariæ* were met with. *P. malariæ* was almost rare as only one blood smear with this plasmodium was found in Kataghan Province. Rao (1949) has also

reported a few infections with *P. malariæ* in the Mashraqi Province (Leghman) during the year 1949. Mixed infections with *P. vivax* and *P. falciparum* were also noticed during the present surveys.

As a rule, in the provinces of Kataghan, Mashraqi, Gardez, Kandhar, Grishk and Herat, *P. falciparum* was recorded as the most predominant species during the later part and after the malaria transmission season, *i.e.* in the months of August, September, October and November. On the other hand, *P. vivax* was relatively more common in the pre- and early transmission period, *i.e.* in the months of May, June and July. But in Kabul, with a high altitude and severe snow fall for about three months in a year, an exception to this rule was met with. At this place, *P. vivax* was recorded as the most predominant species throughout the malaria season and only stray cases of *P. falciparum* were encountered (Chart 3). Thus

CHART 3.

Monthly incidence of different plasmodial infections among infants in Kabul, Afghanistan, Year 1952.



SPECIES	JUNE	JULY	AUG.	SEP.	OCT.	INDEX
P. VIVAX %	0	0	1·05	1·09	0·79	————
P. FALCIPARUM %	0	0	0	0	0	- - - - - -

malaria in Kabul, which is marked by preponderance of *P. vivax* infections, may safely be categorized as temperate type (Lindberg, 1949).

(*b*) INFANTS (BELOW ONE YEAR).

Collection of infant blood was one of the most difficult aspect of the work of the team due to strict purdah observed by the women folk, and it was not possible to collect adequate blood samples from infants. However, the infant parasite rates which were worked out as a part of the general spleen and blood surveys in the country, are given in Table IV.

TABLE IV.

Results of general infant parasite surveys in Afghanistan.

Province and place	Period	Total infants examined	Positive	Infant parasite rate, (per cent)
Kataghan Province				
Kundes	June-July, 1950 ...	183	6	3
Khanabad	September, 1950 ...	60	1	1·6
Talaqan	October, 1950 ...	170	3	1·8
Kabul Province				
Kabul	March-April, 1952 ...	46	...	0·0
20 miles belt around Kabul	Sept.-Oct., 1952 ...	409	...	0·0
Teghab	Sept.-Oct., 1952 ...	26	...	0·0
Gardez Province				
Khost	November, 1952 ...	53	0	0·0
Gardez	November, 1952 ...	115	0	0·0
Mashraqi Province				
Surkhrud, Beisud and Shevashegi	December, 1952 ...	232	0	14·0
Herat Province				
Obay, Guzrah, Anjil, Pushtoon Zurgoon, Kushk	August, 1953 ...	142	0	0·0

In the face of the high splenic indices recorded, the main feature of the infant parasite rates was that these were rather low.

In order to delimit the period of transmission, systematic monthly infant parasite surveys were carried out in the Kataghan and Kabul provinces and the results thereof, are summarised below with necessary comments:—

(1) Kataghan Province.

(*i*) *First survey—period June-July, 1950.*—One hundred and eighty-three infant blood smears were examined out of which only six were postive. An analysis of these infants, by months of births, are presented below:— i

	Infants born before September 23, 1949.	Infants born after September 23, 1949.
Number examined ...	69	114
Number positive ...	6	Nil

Six infants found infected, out of 69 examined, were born before September 23, 1949 while none of the 114 infants born after that date was found infected. This would suggest that active transmission ceased after the end of September.

(*ii*) *Second survey—period July-August, 1951.*—One hundred and eight infant blood smears were examined, out of which four infants were positive from unsprayed area. Out of the positives, one infant was only six months old and was born in March, 1951. This infant got fever for the first time since its birth, on August 15, 1951.

(*iii*) *Third survey—period September, 1951.*—In all 129 infant blood smears were examined and two infants were found infected from the unsprayed area. An interesting finding was that one of the infants who was found infected on September 19, 1951, was in his eighth month of age. This infant was also examined during the month of July and August with negative results and since then he was kept under observation. It is undoubtedly clear that this infant contracted malaria after August 8, 1951, when his blood was examined last.

(*iv*) *Fourth survey—period October, 1951.*—Two hundred and forty-three infant blood smears were examined. Out of these, eight infants (one from sprayed and seven from unsprayed areas) were found infected and all these infants were born between November 23, 1950 and May 22, 1951, indicating that all infections were probably contracted during the period June to October, 1951.

(2) Kabul.

Five infant parasite surveys were carried out in the months of Jauza (May 23 to June 22), Saratan (June 23 to July 22), Asad (July 23 to August 22), Sumbala (August 23 to September 22) and Meson (September 23 to October 22).

The results of the monthly infant parasite surveys are summarised in Table V.

TABLE V.

Infant parasite surveys—May-October, 1952.

Kabul.

Serial number	Month (Afghan)	Period corresponding to Afghan month in Column 2	Total infant bloods examined	Positive	Species		
					vivax	*falciparum*	*malariæ*
1.	Jauza	May 23-June 22	437	2	2*	Not counted as fresh infections	
2.	Saratan	June 23-July 22	290	...	...	...	...
3.	Asad	July 23-August 22	284	3	3	...	...
4.	Sumbla	August 23-Sept. 22	275	3	3	...	...
5.	Mezan	Sept. 23-Oct. 22	250	2	2	...	...
	Total	May 23-October 22	1,536	10	10	...	...

*(One infant was resident in Kabul for only five days as he came from Kohistan, a place about 50 miles away from Kabul. The other infant got fever within 10 days of its birth. The mother of this infant was a chronic malaria patient with parasite (*P. vivax*) in her blood. This case was probably an instance of interaplacental transmission).

It would be seen from Table V that examination of infant blood, during the months of May, June and July, gave negative results. Only eight fresh malaria infections were detected in course of the five surveys conducted monthly from May 23 to October 22, 1952, and all these positives were found between August 16 and October 15, 1952. Another noteworthy feature was that all these infections were of *Plasmodium vivax*.

TRANSMISSION SEASON.

From observations relating to the seasonal prevalence of the Anopheles (July to October), the height of the vector density (August-September) and the period during which the vector was found infected (July to October), together with the analysis of the positive infants by months of their births, fairly accurate conclusions about the season of transmission of malaria in Afghanistan are possible. In most parts of Afghanistan, the transmission mainly occurs for a short period of about three months, *i.e.*, from the middle of July to the middle of October, with little local variations in this period depending upon the altitude, climate, season of paddy plantation and amount of flow in the rivers and streams in the area. These conclusions are further supported by the studies of the climatic conditions, hospital statistics, and house to house malaria morbidity enquiries undertaken in different parts of the country from time to time and recorded elsewhere.

CONTROL OPERATIONS.

(a) GENERAL.

As already stated, earlier investigations into the malaria problem of the country and its control commenced with the assistance of the World Health Organization in the year 1949. First, a World Health Organization, malaria expert was deputed for a short term of about two months to determine the intensity and distribution of malaria in the country. This was followed by a two-men World Health Organization team for carrying out pilot demonstration of control of malaria by indoor residual D.D.T. spraying. These pilot experiments were conducted in the district of Leghman—Eastern Province—in a small population of about 13,000 in 14 different villages. Later, in the years 1950 and 1951 the World Health Organization gave a full scale demonstration of control of malaria in Kundus-Khanabad area (Kataghan Province) comprising about 810 square miles with a population of about 1,50,000. Thereafter nation-wide antimalaria campaigns with modern methods were organized under malaria control projects sponsored jointly by the Government /World Health Organization/United Nations International Children Emergency Fund.

(b) ORGANIZATION.

The Government established a small scale Malaria Organization towards the end of the year 1948. It consisted of a Malaria President and a small staff to assist him in the office and field work. The first and the only operation launched by this Organization was, at that time, in Pulekhumari—an important industrial town with an area of two and a half square miles and a population of about 8,000.

This organization was later adequately staffed and re-organized with its administrative and technical wings. More technical staff was trained*which was divided amongst fully equipped malaria units for work in the various provinces of the country (Appendix V). The malaria staff was paid adequately as compared to the same category of services in the country for ensuring continuity in the service of the technical personnel.

The total population brought under malaria control by years in Afghanistan is shown in Chart 4.

Province-wise details of malaria control programmes in the country accomplished by the year 1954 are given in Appendix VI.

Analysis of the important operational data of the year 1954 is summarised in Table VI.

During 1951 in Kataghan, the project area was divided for experiments into three main dosage zones to be sprayed with DDT at 2 gm./sq. m., 1 gm./sq. m.—single applications and 1·0 gm./sq. meter in two applications of 0·5 gm. each. Entomological

* Eight Medical Officers and two Entomologists attended a course in malariology held at the Malaria Institute of India, Delhi, either on Government expenses or on World Health Organization regional fellowships.

observations were continued for 18-20 weeks after the spray in the different areas. 1·0 gm./sq. m. application was found as effective as 2 gm./sq. m. for about 12 to 14 weeks after the spraying (Appendix II).

CHART 4.

Population brought under malaria control by years, in Afghanistan

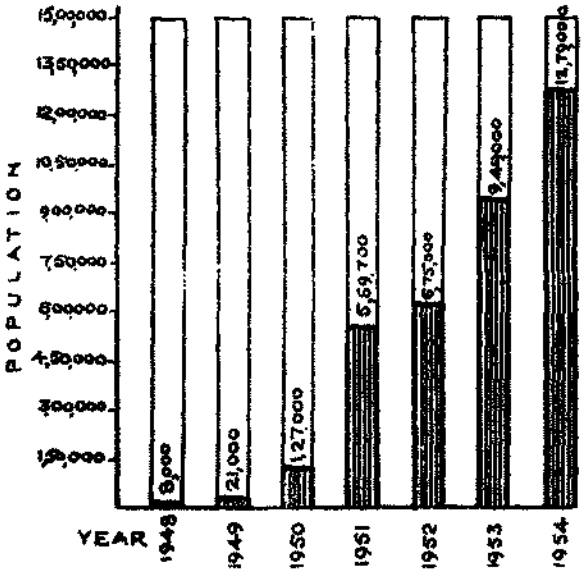


TABLE VI.

Summary of operational data for the year 1954.

	Rural areas	Urban areas
1. Antimalaria measures adopted.	D. D. T. residual indoor spraying	Antilarval measures.
2. Population directly protected.	10,00,000	2,79,000
3. *D.D.T.* dose applied.	1 gm. per sq. metre (45 gm. per capita).	2 oz. per acre water surface.
4. Formulation of D.D.T. used.	50 per cent or 75 per cent water dispersible powder.	D.D.T.-M.K.E.-soap emulsion prepared locally.
5. Cycles of spraying/larviciding.	One cycle annually. (June 1 to July 20 in 45 days).	Weekly application for about 4 months.
6. Cost per capita.	2·7 Afghanis. (0·12 U.S. Dollar).	0·5 Afghanis (0·02 U.S. Dollar).

Note.—23 Afghanis 1 U.S. Dollar.

In view of short transmission season in Afghanistan and on the basis of the critical study of the effects of D.D.T. and cost per capita of the control, only one round of spray of D.D.T. at a dose of 1 gm./sq. meter applied in the shortest time prior to the commencement of the transmission period was considered sufficient.

ASSESSMENT OF RESULTS.

(a) MALARIOMETRIC SURVEYS.

At the expiry of four years' control operations, *i.e.* towards the end of 1953, large scale surveys were undertaken in order to evaluate the various antimalaria campaigns in the country. The total population brought under control during 1953 was 9,49,000 which was increased to 12,79,000 in 1954 (Chart 4). As the World Health Organization team left this country by the end of 1954, large scale evaluation surveys could not be undertaken in that year.

The results, evaluated in 1953, of the various antimalaria campaigns in different provinces, are presented in Table VII and Charts 5, 6, 7 and 8.

TABLE VII.

Spleen and blood surveys, year 1953.

I. KATAGHAN PROVINCE (Chart 5).

(a) **Pulekhumari.**

	October-November 1948 (Pre-operational)	November, 1953 (Post-operational)
Spleen rate (per cent)	76	11
Average enlarged spleen	2·3	1·7
Parasite rate (per cent)	14·2	0·55
Infant parasite rate (per cent)	Not recorded.	0·00

(b) **Kundus and Khanabad.**

	May-June, 1950 (Pre-operational)		November, 1953 (Post-operational)	
	Kundus.	Khanabad.	Kundus.	Khanabad.
Spleen rate (per cent)	30	47·6	15·2	20·5
Average enlarged spleen	1·7	2·3	2·0	2·2
Parasite rate (per cent)	8	9·9	2·5	1·7
Infant parasite rate (per cent)	0·0	3·5	0·0	0·0

TABLE VII.—(*Contd*).

(*c*) **Talaqan, Chardara and Baglan.**

	May-June, 1951 (Pre-operational)			November, 1953 (Post-operational)		
	Talaqan	Chardara	Baglan	Talaqan	Chardara	Baglan
Spleen rate (per cent) ...	60·2	47	74·7	14·6	20·5	24·7
Average enlarged spleen ...	2·4	2	2·4	2·1	2·0	2·3
Parasite rate (per cent) ...	8·1	6	23·5	0·0	5·0	3·3
Infant parasite rate (per cent) ...	17·1	0·0	14·2	0·0	0·0	0·0

2. MASHRAQI (EASTERN) PROVINCE (Chart 6).

(*a*) **Leghman valley.**

	October, 1949 (Rao, 1949)	June, 1951 (Pre-operational)	Nov.-Dec., 1953 (Post-operational)
Spleen rate (per cent) ..	76·2	50·3	9·0
Average enlarged spleen ..	3	2·5	1·7
Parasite rate (per cent) ...	18·5	12·2	0·0
Infant parasite rate (per cent) ..	70·0	14·2	0·0

(*b*) **Shevashegi, Surkhrud, Beisud.**

	Nov.-Dec., 1952. (Pre-operational).			Nov.-Dec., 1953. (Post-operational).		
	Shevashegi	Surkhrud	Beisud	Shevashegi	Surkhrud	Beisud
Spleen rate (per cent) ...	57·45	49·20	58·7	43·1	25·4	36·6
Parasite rate (per cent) ..	21·8	7·5	9·5	5·0	3·4	5·0
Infant parasite rate (per cent) ...	20·6	7·8	1·2	0·0	5·3	2·0

TABLE VII.—(*Contd.*).

3. KANDHAR AND GRISHK PROVINCES (Chart 7).

	Area under control		Comparison area	
	Dec., 1952-January, 1953 (Pre-operational)	October, 1953 (Post-operational)	Dec., 1952-Jaunary, 1953 (Pre-operational)	Oct., 1953 (Post-operational)
Spleen rate (per cent) ...	46	22	15·6	30·6
Parasite rate (per cent) ...	13·1	9·3	8·6	15·3
Infant parasite rate (per cent) ...	10·5	0·0	1·0	10·0

4. TEGHAB AND KHOST (Chart 7 and 8).

	Teghab		Khost		
	Dec., 1952 (Pre-operational)	Nove., 1953 (Post.-operational)	Nov., 1952 (Pre-operational)	Nov., 1953 (Post-operational) 1952	1953
Spleen rate (per cent) ...	31·6	15·4	65·6	41·6	19·5
Parasite rate (per cent) ...	9·1	0·0	not recorded	5·0	3·12
Infant parasite rate (per cent) ...	0·0	0·0	,,	0·0	4·01*

*The spraying operations in the Khost area were scheduled to be closed on July 31, 1953, but the same had to be continued in certain very remote parts of the district till August 10, 1953, on account of various unavoidable reasons. In all, 11 infant bloods were collected from the area, sprayed between August 1 to August 10, and out of these, four bloods were found positive which gave a fairly high parasite rate of 36 per cent. On the other hand, out of 144 infant bloods from the remaining Khost area, sprayed during July, only three were found infected and a low parasite rate of 2·08 per cent was recorded. This finding evidently showed that the transmission of the disease had assumed fairly active proportions in the beginning of August.

5. KABUL PROVINCE (Chart 8).

(*a*) **Kabul.**

	May, 1951 (Pre-operational)	November, 1953 (Post-operational)
Spleen rate (per cent) ...	21	10
Average enlarged spleen ...	2·2	1·1
Parasite rate (per cent) ...	13·4	0·55

TABLE VII.—(*Contd.*).

(*b*) **Sarobi and Nijrab.**

	SAROBI		NIJRAB	
	May-June, 1951 (Pre-operational)	November-December, 1953 (Post-operational)	May-June, 1951 (Pre-operational)	November-Decemer, 1953 (Post-operational)
Spleen rate (per cent) ...	58	10	60.6	27·9
Parasite rate (per cent) ...	22·5	1·6	27.7	0·0
Infant parasite rate (per cent) ...	Not-recorded	0·0	Not-recorded	0·0

CHART 5.

Katagan province
records of spleen indices

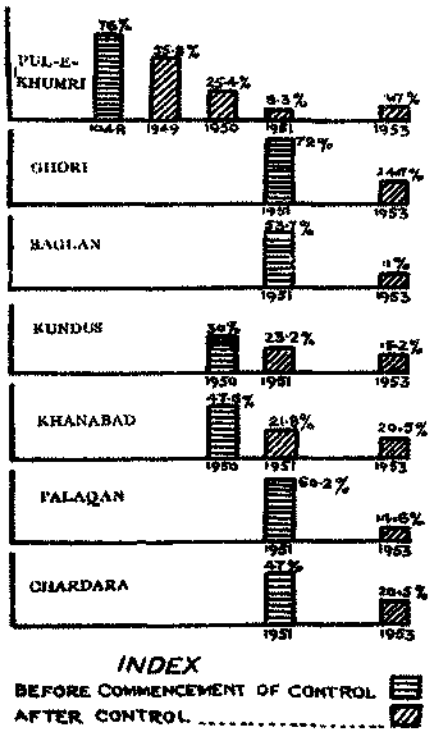


CHART 6.

Mashraqi province

records of spleen indices

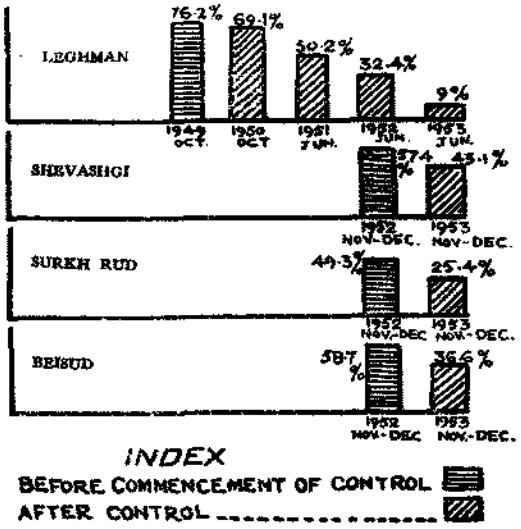


CHART 7.

Kandhar Grishk & Khost Provinces

records of spleen indices

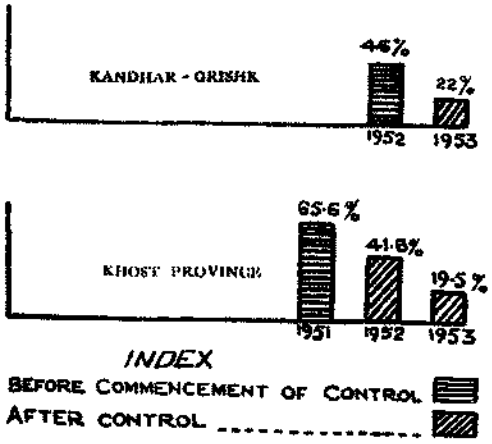
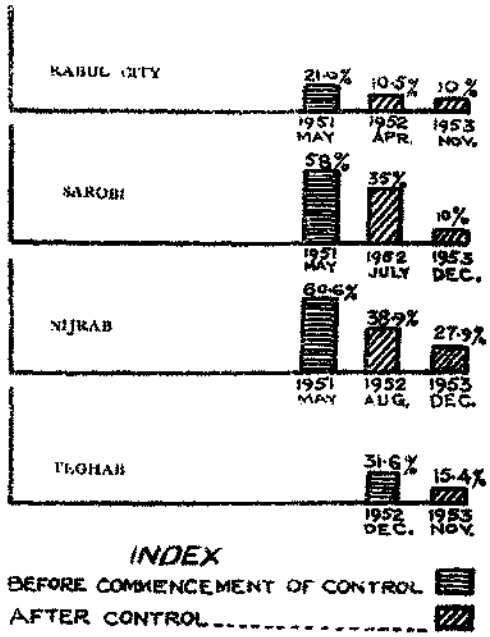


CHART 8.
Kabul province
records of spleen indices



(*b*) IMPROVEMENT IN THE MATERNAL AND CHILD HEALTH.

There are no statistics to prove the improvement in the general health of the mothers and children, but improvement was noticed in certain directions. For instance, from the records of countrywide malariometric surveys carried out in November-December, 1953, it was evident that spleen and parasite rates underwent a significant decrease in children in almost all the areas under control. Also the demand of quinine and other antimalarial drugs, as evidenced from the records of the Health Ministry, decreased considerably in all such areas.

As facilities were available in Kabul for the collection of data of the incidence of malaria among infants, a comparative statement is given in Table VIII.

(*c*) IMPROVEMENTS IN THE ECONOMY—INDUSTRIAL AND AGRICULTURAL.

The impact of malaria control was no less visible on the economy of the areas brought under malaria control. A few concrete instances of the improvement in the industrial and agricultural economy are given here.

In Pulekhumari, the textile mills underwent a sort of industrial revolution with the achievement of a successful control of malaria in that region. Prior to the antimalaria measures, it was a problem to obtain sufficient labour—even on payment of attractive allowances—for operating the mills. The total population of Pulkhumari was 8,000 and daily output of the mills was only 30,000 meters

TABLE VIII.

Cases of malaria among infants in Children Hospital, Kabul, (1951-1953).*

Months	Pre-control	Post-control	
	Year 1951	Year 1952	Year 1953
May ...	...	...	1
June ...	8	...	...
July ...	33	...	1
August ...	18	3	...
September ...	67	3	...
October ...	30	2	...
Total ...	156	8	2

* These figures were very kindly furnished by Dr. V. Lehmus, Pedeatrician, World Health Organization, Kabul.

of cloth. Due to healthy conditions brought about by control of malaria, the population of the place rose to over 20,000 (influx of people from outside) and the output of the mills increased to more than two-fold and plans are in hand to enlarge the factory.

The annual yield of the rice and cotton got practically doubled in the districts of Kundus and Khanabad in the course of four years of malaria control.

As is understood from the Government records, the prices of agricultural land went up manyfold in the districts of Ghori, Kundus and in a number of other areas where malaria was brought under control.

VIEW ABOUT ERADICATION OF MALARIA.

A long term view of the National Malaria Control Programme in the country is extremely hopeful. Antimalaria measures by D.D.T. indoor residual spraying have already become very popular because of the spectacular results and have gone a long way in creating a great fund of goodwill for all kinds of public health work in the rural areas.

Most of the work pertaining to malaria control demonstrations, training of the local nationals, carrying out of basic malaria surveys and establishment of the Afghan Malaria Institute have been completed. A critical review of the whole situation would indicate that:—

(1) The transmission season of malaria all over Afghanistan is of short duration.

(2) One round of D.D.T. indoor residual spraying, at a doze of 1 gm. per square meter, is considered sufficient to intercept the transmission of disease during the short malaria season.

(3) The malariometric indices (Spleen and parasite rates in children and infant parasite rates) in some of the tracts in the country have undergone significant reduction after about four to five years of malaria control operations.

(4) The country is land-locked and is bounded either by high mountains or by vast deserts. The transport facilities are also meagre, which reduce the chances of more frequent exchange of visits between the local nationals and the people living in the bordering countries. The possibilities of importing Anopheles mosquitoes from the neighbouring countries are also quite remote.

(5) *A. superpictus* is the main vector in the country but *A. culicifacies* is also a proven additional vector in the Mashraqi Province. Both these vectors have not yet shown any signs of development of resistance to D.D.T., to which they are equally vulnerable.

To achieve the ultimate goal of eradication of malaria in the circumstances, it is rather imperative to intensify and expand malaria control measures all over the country in the shortest possible time. Alongwith the intensive control measures, suitable steps be taken for the proper investigations of all the reported cases of malaria for giving them radical treatment with 8-amino-quinolines (Primaquin). Thereafter, interruption of these measures by stages be planned under proper suviellance. The needs of the country at the moment, for eradication of the disease, are:—

(1) Firm Governmental budget for malaria control programmes for the next five years.

(2) Adequate supplies of insecticides, equipment and transport.

(3) Sufficient number of local professionals well-versed in the modern techniques of malaria control.

At present, all the aforementioned pre-requisites, for the eradication of malaria, are not difficult to obtain in the country. The Government is apparently conscious of the healthy conditions brought about by malaria control projects in different parts of the country, and is determined to appropriate adequate funds for the eradication of the disease. The supplies of insecticides, equipment and transport are rather generous out of the United Nations International Children Emergency Fund aid. The only problem so far has been the non-availability of well-trained nationals in requisite numbers. To overcome this difficulty, the personnel already trained should on no account be spared and removed from the department for any other duties. The Afghan Malaria Institute, with the help of the World Health Organization, should give priority to training classes especially for the field staff (Malaria Inspectors, etc.) and thus quickly raise the required strength of the field spraying teams.

For purposes of eradication of malaria, a further question may arise as how to deal with the migratory population of 'Nomads' who, during certain part of the year, leave for the plains in the neighbouring country of Pakistan. In the first instance, these 'Nomads' only pass the winter months outside the country and come back before they are subjected to the ravages of actual malaria season

in Pakistan. Secondly, the Government of Afghanistan have undertaken a planned scheme of rehabilitating these 'Nomads' in Helmand Valley, and, as a result, a large population of 'Nomads' have already become static. Thus the problem of 'Nomads', migrating from one place to another, has already been very much solved. Moreover, these 'Nomads' follow certain famous caravan routes for their travel and as such this population can easily be checked for locating reservoirs of infection, and their radical treatment, before they are allowed entry into Afghanistan. It will be highly advantageous to organize radical treatment centres at the borders of the country at a suitable distance from the inhabited areas.

SUMMARY AND CONCLUSIONS.

This paper primarily deals with the entomological and epidemiological data collected during the pre- and post-operational surveys carried out in Afghanistan between the years 1950 and 1954. A nation-wide expansion of malaria control programmes, assessment of the results achieved and the feasibility of launching malaria eradication campaigns in the country, are also discussed.

Distribution of malaria in Afghanistan appears to have a direct relationship with the river valleys where cultivation of rice is common. In areas away from rivers and streams, the incidence of the disease gradually declines so much so that in the remotest places the prevalence of malaria is almost negligible.

The chief vector of malaria in the country is *A. superpictus* with *A. culicifacies* as a secondary vector in the Mashraqi (Eastern) Province. Both these vectors are indoor resters and are equally vulnerable to D.D.T.

Malaria season in Afghanistan is of short duration of about three months, out of which actual peak transmission period does not exceed eight weeks. The transmission of malaria has hence been effectively intercepted by only one round of spraying with D.D.T. at a dose of one gm. per square meter. On an average only 45 gm. of D.D.T. has been spent annually for one person. In other words, with one lb. of technical grade D.D.T., about 10 persons can be protected against malaria in Afghanistan.

For assessing the results, country-wide spleen and blood surveys were undertaken. A spectacular reduction in the spleen indices and infant parasite rates was observed, alongwith a significant fall in the incidence of malaria and concommitantly in the use of the antimalarial drugs. As a consequence, an improvement in the economy, both agricultural and industrial, was noticeable.

A long term view of the malaria control operations, aiming at eradication of disease in a land-locked country like Afghanistan, is extremely hopeful.

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APPENDIX I.

Cases of malaria and of other diseases reported at the dispensaries during the years 1947-1950. Kundus, Khanabad and Talaqan.

Name of the dispensary	Afghan month	Hamal		Saur		Jawza		Saratan		Asad*		Sumbala*		Meson*		Akrab*		Kaus		Jadia		Dalv		Huth	
	Year	Mar. 23 to Apr. 22		Apr. 23 to May 22		May 23 to Jun. 22		Jun. 23 to Jul. 22		Jul. 23 to Aug. 22		Aug. 23 to Sep. 22		Sept. 23 to Oct. 22		Oct. 23 to Nov. 22		Nov. 23 to Dec. 22		Dec. 23 to Jan. 22		Jan. 23 to Feb. 22		Feb. 23 to Mar. 22	
		M	O	M	O	M	O	M	O	M	O	M	O	M	O	M	O	M	O	M	O	M	O	M	O
Kundus	1948	23	129	26	316	54	265	60	233	101	208	311	268	189	215	100	239	79	120	14	52	34	802	42	107
	1949	106	756	48	582	57	903	38	469	50	309	47	262	149	616	150	627	86	607	23	463	14	247	39	662
	1950	49	427	66	750	58	325	...	...	190	920	109	337	191	641	51	462	5	142	10	308	9	236	36	489
Khanabad	1947	98	614	130	1,370	216	1,600	260	1,640	287	1,402	560	1,470	1,700	1,498	2,740	1,160	630	940	430	1,200	172	804	108	670
	1948	85	1,012	160	1,440	260	1,620	275	1,500	299	1,430	624	1,390	1,740	1,539	2,821	974	1,780	876	480	799	184	630	120	726
	1949	60	952	110	1,201	185	1,443	79	929	195	828	341	763	1,224	802	2,260	820	700	723	360	640	105	530	64	670
	1950	68	891	125	1,320	163	1,224	135	896	87	740	65	640	...	...	...	...	...	...	...	...	...	...	...	...
Talaqan	1947	0	164	0	188	25	154	44	151	62	104	775	112	30	125	7	175	0	180	0	175	0	159	0	174
	1948	0	197	0	154	35	178	42	173	47	142	55	143	37	168	11	191	4	189	0	166	0	192	0	200
	1949	0	112	2	112	11	102	26	88	0	0	45	69	36	76	10	108	0	110	0	112	0	113	0	110
	1950	0	320	0	221	30	175	51	149	62	143	73	134	45	169	8	200	0	214	0	208	0	210	0	217

M = Malaria cases. O = Other diseases. *Malaria months.

APPENDIX II.

Summary of adult anopheline catches from the different sprayed areas and from the unsprayed (comparison) areas during the malaria season—Year 1951.

Dosage area and type of stations	Number of stations	Time spent man-hours	Anophelines caught			Density/man-hour		
			A. superpictus	Other species	Total	*A. superpictus*	Other species	All species
I. 2·0 gm./sq. metre dose.								
(a) WETTABLE POWDER.								
Human dwellings sprayed ...	27	64	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
,, ,, unsprayed ...	13	40	113	28	141	2·8	0·7	3·5
Cattlesheds or stables sprayed ...	11	33	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
,, ,, ,, unsprayed ...	9	30	375	66	441	12·5	2·2	14·7
(b) XYLENE-TRITON EMULSION.								
Human dwellings sprayed ..	4	12	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
Cattlesheds or stables sprayed ...	4	12	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
(c) CHECK AREA (UNSPRAYED).								
Human dwellings unsprayed ...	12	36	392	352	744	10·9	9·8	20·6
Cattlesheds or stables unsprayed ...	6	18	588	172	760	32·7	9·6	42·2
II. 1·0 gm./sq. metre dose.								
(a) SINGLE APPLICATION WETTABLE POWDER.								
Human dwellings sprayed ...	25	60	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
,, ,, unsprayed ..	7	23	40	30	70	1·7	1·3	3·4
Cattlesheds or stables sprayed ...	10	18	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
,, ,, ,, unsprayed ...	6	22	73	83	156	3·3	3·8	7·1
(b) TWO APPLICATIONS OF 0·5 GM./SQ. METRE, EACH AT SIX WEEKS INTERVAL, WETTABLE POWDER.								
Human dwellings sprayed ...	10	27	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
,, ,, unsprayed ...	2	6	36	28	64	6·0	4·6	10·6
Cattlesheds or stables sprayed ...	6	12	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
,, ,, ,, unsprayed ...	2	4	75	39	114	18·8	9·8	28·6

APPENDIX II.—(*Contd.*)

Dosage area and type of stations	Number of stations	Time spent man-hours	Anophelines/caught			Density/man-hour		
			A. superpictus	Other species	Total	*A. superpictus*	Other species	All species
(*c*) CHECK AREA (UNSPREAD).								
Human dwellings unsprayed ...	8	24	247	204	451	10·3	8·5	18·8
Cattlesheds or stables unsprayed ...	4	12	311	163	474	25·9	13·6	39·5
III. 0·5 gm./sq. metre dose.								
(*a*) WETTABLE POWDER.								
Human dwellings sprayed ...	6	16	20	11	31	1·2	0·7	1·9
Cattlesheds or stables sprayed ...	3	3	4	8	12	1·3	2·8	4·0
(*b*) CHECK AREA (UNSPRAYED).								
Human dwellings unsprayed ...	4	12	80	77	157	6·7	6·4	13·1
Cattlesheds or stables unsprayed ...	3	4	68	41	109	17·0	10·2	27·2

Note.—D.D.T. 2 gm./sq. metre and 1 gm./sq. metre, respectively, was sprayed during the period May 10 to June 21 and weekly entomological observations were carried out from July to October, 1951.

APPENDIX III.

Spleen surveys.

Serial number	Name of area/places	SPLEENS			
		Number examined	Number positive	Spleen rate, per cent	Average enlarged spleen
	Katagan Province—June and July, 1950.				
	KUNDUS AREA.				
1.	Kundus	213	65	31	1·9
2.	Malargai	56	16	29	1·8
3.	Ludhin	67	16	24	1·8
4.	Chilmzar	16	3	18·8	1·3
5.	Baghmeri	42	14	33	1·6
6.	Gortepa	27	8	30	1·6
	KHANABAD AREA.				
7.	Khanabad	148	116	78	2·2
8.	Chartoot	29 17	17 10	59	2·0
9.	Charsari	19 14	13 9	67	2·1
10.	Choga	28 9	13 6	51	2·5
11.	Khojapista ...	29	21	54	1·9
12.	Janglebashi ..	46	27	59	2·8
13.	Ishantop ...	29	13	48	2·0
14.	Khojabolak ...	33	14	42	2·4
15.	Mullah Ghulam ...	30	21	70	2·7
	Total ...	862	402	46·7	
	OTHER AREAS.				
16.	Larkhabi	16	4	25	1·7
17.	Khamdam	41	16	39	2·0
18.	Chardarah	38	18	47	2·0
19.	Mirzaramzan ...	33	2	6	2·0
20.	Talaqan	70	42	60	2·6
21.	Zirgari	34	16	47	2·1
22.	Salmki	14	2	14·3	1·5
23.	Kalaiizal	24	12	50	1·7
24.	Baglan	56	19	34	1·6
	Total ...	326	131	40·2	

APPENDIX III.—(*Contd.*)

Serial number.	Name of area/places	Spleens			
		Number examined	Number positive	Spleen rate, per cent	Average enlarged spleen
	Katagan Province—May and June, 1951				
1.	Khamdan	45	20	44·4	2·1
2.	Badam Dara ...	48	24	50	2·2
3.	Mughal	10	5	...	...
4.	Zirgiri Nakli ...	33	21	63·6	2·4
5.	Zirgiri	27	17	63·1	2·8
6.	Talukan School ...	56	36	64·2	2·7
7.	Takhta Kuborak ...	27	15	55·5	2·6
8.	Ayandara	33	22	66	2·4
9.	Shoratu	11	4	...	...
10.	Akhtepa (Kalaizal) ...	33	22	66·6	2.5
11.	Tarbooz Guzar ...	57	34	59·6	2·4
12.	Kalaizal (Across the river)	27	17	63	2·1
13.	Jan Garak	26	8	30·7	1·8
14.	Aktash School ...	57	31	54·4	2·8
15.	Aktash Village ...	29	18	62	2·2
16.	Aliabad	31	8	26	1·7
17.	Larkhabi	21	9	42·9	2·0
18.	Charseri	28	15	53·6	2·2
19.	Chardara	39	12	30·8	1·7
	Total ...	638	338	52·2	2·4
	OTHER PLACES.				
20.	Imam Sahib ...	50	7	14	2
21.	Askalan	30	3	10	2·0
22.	Balkh	29	18	62·0	2·3
23.	Mizare-Sharif ...	67	5	7·5	2·2
	Total ...	176	33		

APPENDIX III.—(*Contd.*)

Serial number	Name of area/places	Spleens			
		Number examined	Number positive	Spleen rate, per cent	Average enlarged spleen
	City of Kabul - April, 1952.				
1.	Bagh Alimardan ...	84	14	16·6	2·1
2.	Yaketoot	37	*nil*	...	...
3.	Wazirabad	34	1	3·0	2
4.	Kartewali	23	1	3·0	2
5.	Prancha School ...	129	35	27·1	2·2
6.	Istaqlal School ...	87	3	3·4	1·3
7.	Kartachar	100	7	7·0	2
8.	Nijat School ...	148	8	5·4	2·1
9.	Shar-I-Nau ...	144	5	3·5	2
10.	Kalai Hushmat ...	23	7	30·5	2·3
11.	Shevki	74	14	19	2
12.	Alowdine	54	7	1·3	1·9
13.	Murad Ali ...	76	5	6·5	2·2
14.	Deh Afghanan ...	69	10	14·4	2·5
15.	Sirdaryan Khan ...	70	8	11·4	2·0
16.	Bibimaroo	50	2	4·0	2·0
17.	Tandoor-Sazi ...	91	9	9·8	2·4
	Total ...	1,296	136	10·5	1·9
	In a belt of 20 miles around Kabul City—September and October, 1952.				
1.	Nau Abad-i-Bagrami ...	31	3	9·6	2·0
2.	Kalai Jabar Bagrami ...	31	3	9·6	1·0
3.	Malak Khel Bagrami ...	47	4	8·3	2·2
4.	Butkhak Bagrami ...	53	8	15·9	1·6
5.	Khurd Kabul Bagrami	33	8	24·3	2·2
6.	Kamari Bagrami ...	38	10	26·3	1·9
7.	Shina Bagrami ...	54	7	12·9	2·1
8.	Hussain Khel ...	50	9	18·0	1·3

APPENDIX III.—(*Contd.*)

Serial number	Name of area/places	SPLEENS			
		Number examined	Number positive	Spleen rate, per cent	Average enlarged spleen
9.	Siah Sang	50	13	26·0	1·4
10.	Khawja Chisht ...	52	4	7·6	1·7
11.	Deh Yaya	39	7	17·9	1·4
12.	Bandi Khana Deh Sabz	44	4	9·9	1·7
13.	Karta Char ...	23	5	21·7	2·6
14.	Khawja Rawash ...	42	...	...	...
15.	Tara Khel	35	2	5·7	1·0
16.	Pai Munar	44	2	4·5	1·0
17.	Allo Khel	57	10	7·5	3·0
18.	Bazu Khel	50	...	...	...
19.	Deh Khadai Dad ...	55	10	18·1	2·0
20.	Kabol Bai	18	2	11·1	1·0
21.	Simit Khana ...	8	1	12·5	2·0
22.	Jang Alak	40	7	17·5	1·1
23.	Waisil Abad ...	34	3	8·8	1·3
24.	Agha Ali Shams ...	60	1	1·6	1·0
25.	Kalai Ghaibi ...	43	4	9·7	1·7
26.	Fateh Ullah Khan ...	44	6	13·6	2·0
27.	Chilsatun	68	7	10·2	1·5
28.	Musai	51	5	9·8	2·0
29.	Deh Kalandar ...	48	2	4·1	1·5
30.	Deh Murad Khan ...	41	3	7·3	1·0
31.	Deh Dana	43	3	6·9	1·0
32.	Maktab-Darul Fanun ...	110	9	8·1	1·5
33.	Sarasia	39	4	10·2	1·5
34.	Afshar Teba ...	38	6	15·7	2·0
35.	Kalai Wazir ...	40	3	7·5	1·6
36.	Maktab Ibtadi-Tajruvavi	54	3	5·5	1·3
37.	Maktab-i-Charkala ...	44	3	6·8	1·6

APPENDIX III.—(*Contd.*)

Serial number	Name of area;places	Spleens			
		Number examined	Number positive	Spleen rate, per cent	Average enlarged spleen
38.	Kala Shada ...	42	5	11·9	1·4
39.	Uchi Baghbanan ...	41	0	...	...
40.	Gul Khana ...	46	1	2·1	2·0
41.	Kala-i-Fazil Beg ...	33	1	3·0	1·0
42.	Afshar-i-Nanachi ...	36	1	2·7	2·0
43.	Deh Mozand ...	50	6	12·0	1·3
44.	Kala Jawad ...	33	2	6·0	2·5
45.	Guzar Gah ...	50	3	6·0	1·6
46.	Kala-Ki-Kashif ...	35	1	2·1	2·0
47.	Kala Sher Khan and Char Kala ...	42	1	2·3	1·0
48.	Tangi Saidan ...	60	10	16·6	2·7
49.	Kala Kazi	60	5	8·2	2·5
50.	Baraki	46	3	6·5	1·6
51.	Deh Kepak ...	29	...	...	...
	Total ...	2,286	224	9·7	
	Teghab District, Kabul Province—November, 1952.				
1.	Karya Shinkai ...	41	9	21·95	2·44
2.	Karya Ghazi Khan Khel	40	9	22·5	2·5
3.	Karya Painda Kheil ...	40	9	22·5	2·2
4.	Karya Shalutin ...	35	11	31·42	2·3
5.	Karya Nauroz Kheil ...	62	21	34·0	2·5
6.	Shahi and Seh Pidar ...	32	11	24·6	2·8
7.	Aqa Kheil and Mushwani	38	9	23·6	2·6
8.	Ghani Kheil and Anar Joi	45	17	37·7	2·7
9.	Almas Kheil and Mohib Kheil	9	4	44·4	2·5
10.	Tattar Kheil ...	13	6	46·1	2·3
11.	Shah Kot	26	6	23·0	2·6
12.	Sheikhin	25	12	48·0	2·75
	Total ...	426	124	31·67	2·37

APPENDIX III.—(*Contd.*)

Serial number	Name of area/places	SPLEENS			
		Number examined	Number positive	Spleen rate, per cent	Average enlarged spleen
	Sarai Khoaja—October, 1954.				
1.	Be-zati	33	...	0·0	...
2.	Solaiman	42	3	9·5	1·6
3.	Sahrai-Bezati ...	23	...	0·0	...
4.	Aqua Ali Khoaja ...	24	...	0·0	...
5.	Ojan Shah	34	...	0·0	...
6.	Surkh Beland ...	24	...	0·0	...
7.	Murad Behg ...	64	...	0·0	...
8.	Hadji Behg ...	31	...	0·0	...
9.	Kochgeen ...	31	...	0·0	...
10.	Quala Murad Behg School	35	...	0·0	...
11.	Deh Bala	63	...	0·0	...
12.	Behdak	26	...	0·0	...
13.	Deh Nao	67	...	0·0	...
14.	Ghundab	73	...	0·0	...
15.	Gudara	36	2	5·5	2
16.	Bala Deh	45	14	31·0	2·4
17.	Koaja Mirak ...	34	3	8·8	2
18.	Hum Darah ...	53	2	3·7	1·5
19.	Farza Village School ...	29	1	3·5	2
20.	Zar Garan School ...	33	...	0·0	...
21.	Paen Deh	45	...	0·0	...
22.	Sarai Khoaja ...	41	...	0·0	...
23.	Dubazi Guala Khoaja ...	23	...	0·0	...
24.	Shenjan	35	...	0·0	...
25.	Mareki	33	...	0·0	...
26.	Sarai Khoaja School ...	70	...	0·0	...
27.	Kalakar Village School	35	...	0·0	...
	Total ...	1,082	25	2·3	2·1

APPENDIX III.—(*Contd.*)

Serial number	Name of area/places	Spleens			
		Number examined	Number positive	Spleen rate, per cent	Average enlarged spleen
	Parwan and surroundings—October, 1954.				
1.	Baghe Areque ...	42	1	2·3	1
2.	Quara Qual ...	38	...	0·0	...
3.	Mian Joi	30	1	3·3	1
4.	Karez	30	1	3·3	2
5.	Quara Bagh School ...	88	1	1·1	1
6.	Centre of Charikar ...	67	...	0·0	...
7.	Duland ...	34	...	0·0	...
8.	Bayan	18	...	0·0	...
9.	Khoaja Siaran ...	10	...	0·0	...
10.	Opian	48	...	0·0	...
11.	Mian Shakh ...	61	1	1·6	1
12.	Baba Khel ...	25	...	0·0	...
13.	Pasan	28	...	0·0	...
14.	Leghmani	30	1	3·3	2
15.	Totum dara-ulia ...	44	...	0·0	...
16.	Town dara Sufla ...	44	...	0·0	...
17.	Bayan	41	...	0·0	...
18.	Sadao	32	...	0·0	...
	Total ...	710	6	0·8	1·3
	Bagram and surroundings—October, 1954.				
1.	Ghulam Ali ...	24	...	0·0	...
2.	Mahi Gur	26	...	0·0	...
3.	Dah mullah ...	17	1	6·0	1
4.	Shaka	28	...	0·0	...
5.	Tukhchi and Bhadur Khel	28	...	0·0	...
6.	Yourchi	27	...	0·0	...
7.	Gujar Khel ...	20	...	0·0	...
8.	Khanjai Khel ...	52	...	0·0	...
9.	Quala Beland ...	56	1	1·7	2
10.	Jamshed Khel ...	39	...	0·0	...
11.	Chaekal	33	...	0·0	...
	Total ...	350	2	0·6	1·5

APPENDIX III.—(*Contd.*)

Serial number	Name of area/places	Spleens			
		Number examined	Number positive	Spleen rate, per cent	Average enlarged spleen
	Jabul Saraj—October, 1954.				
1.	Sare Pul	45	...	0·0	...
2.	Ezat Khel	28	...	0·0	...
3.	Deh Nao	16	1	6·2	1
4.	Chainaki	25	...	0·0	...
5.	Sabzi Khel ...	23	...	0·0	...
6.	Sahra	29	...	0·0	...
7.	Shekhan Khel ...	13	...	0·0	...
8.	Hewad Khel ...	38	...	0·0	...
9.	Said Khel	36	...	0·0	...
10.	Akhtajh	28	...	0·0	...
11.	Char Burg	37	1	2·7	1
12.	Munara	45	1	2·2	1
13.	Eshque Ahad ...	29	...	0·0	...
14.	Madad Khel ...	43	...	0·0	...
15.	Deh Bala	52	...	0·0	...
16.	Mir Khan Main Guzar	47	...	0·0	...
17.	Quala Sahra and Pule Matak	32	...	0·0	...
	Total ...	566	3	0·5	1
	Resa Kohistan—October, 1954.				
1.	Nao Abad	41	..	0·0	...
2.	Deh Bali	51	...	0·0	..
3.	Rege Rawan ...	52	1	1·9	1
4.	Murad Khoaja ...	24	...	0·0	...
5.	Jamal Agha Sufli ...	22	...	0·0	...
6.	Mahmood Eraqui ...	23	...	0·0	...
7.	Ali Khel	43	1	2·3	2
8.	Kush Kestan ...	59	2	1·6	2
9.	Jamal Aghaulya ...	56	...	0·0	...
10.	Deh Qurban ...	18	...	0·0	...
11.	Deh Raisan ...	22	1	4·8	1
12.	Ushtar Geram ...	76	...	0·0	...
13.	Dasht	19	1	5·2	...
14.	Kham Zargar ...	32	...	0·0	...
	Total ...	538	6	1·1	1·5

APPENDIX III.—(*Contd.*)

Serial number	Name of area/places	SPLEENS			
		Number examined	Number positive	Spleen rate, per cent	Average enlarged spleen
	Gardez Province—November, 1952.				
	GARDEZ CENTRE				
1.	Gardez School ...	73	2	2·7	1·0
2.	Karya Karshi ...	36	1	11·1	1·2
3.	,, Mailen ...	75	5	6·6	2·0
4.	,, Salu Kheil ...	40	4	10·0	2·0
5.	,, Khawaja Hassan ...	33	3	9·0	1·3
6.	,, Bala Deh ...	36	1	2·8	1·0
7.	,, Ibrahim Kheil ...	42	1	2·3	2·0
8.	,, Davlat Zai ...	50	2	4·0	1·0
9.	,, Aslat Beg ...	18	0	0·0	0·0
10.	,, Sheikhan ...	42	1	2·3	1·0
11.	,, Malik Kheil ...	37	3	8·1	1·3
12.	,, Saidan ...	37	2	5·4	1·0
13.	,, Aban Gran ...	40	1	2·5	1·0
	Total ...	559	29	5·1	1·3
	URGOON AREA				
1.	Urgoon School ...	103	7	6·7	1·2
2.	Anbron	47	3	6·3	1·3
3.	Chana Khawja ...	70	6	8·5	1·8
4.	Balish School ...	89	13	14·6	1·3
5.	Sheikhan	11	1	9·9	1·0
	Total ...	320	30	9·3	1·3
	SAROBI (URBAN AREA)				
1.	Hirzai	30	0	0·0	0·0
2.	Taus Kheil	35	1	2·8	1·0
	Total ...	65	1	1·5	1·0

APPENDIX III.—(*Contd.*)

Serial number	Name of area/places	Spleens			
		Number examined	Number positive	Spleen rate, per cent	Average enlarged spleen
	SAID KARAM AREA.				
1.	Rud Ahmad Zai ...	71	4	5·6	1·2
2.	Maktab Said Karam ...	57	5	8·7	1·6
3.	Kandar Kheil ...	47	1	2·12	1·0
	Total ...	175	10	5·7	1·4
	ZURMAT AREA.				
1.	Zurmat School (Number 2)	40	3	7·5	1·3
2.	Kharshi	82	12	14·6	1·5
3.	Kakali	36	1	2·7	1·0
4.	Zurmat School (Number 1)	87	11	12·6	1·8
5.	Mara Khan School ...	50	4	8·0	1·7
6.	Shaha-ub-Din ...	21	0	0·0	0·0
	Total ...	316	31	9·8	1·6
	KHOST CENTRE.				
1.	Wati Kili	13	6	46·1	3·0
2.	Jangi Kili	27	12	44·4	2·0
3.	Wargha Kili	40	18	45·0	2·2
4.	Mandi Kheil	25	7	28·0	2·1
5.	Dui Kuti	34	9	26·4	1·5
6.	Kalian	21	6	28·5	1·3
7.	Tora Wali	25	7	28·0	1·2
8.	Mardi Kheil	30	10	33·3	2·4
9.	Shakki	42	24	57·1	2·3
10.	Zorian	48	15	31·2	2·0
11.	Mani	35	11	31·4	2·3
12.	Shangi	28	15	53·6	2·8
13.	Derai	15	7	46·6	1·8
14.	Said Amin Shah ...	41	16	39·0	2·5
15.	Kamar Sar	42	20	47·6	2·3
16.	Babar Kheil	47	17	36·1	1·7
17.	Madi Kheil	22	9	40·9	2·3
18.	Ayub Kheil	54	22	40·7	1·6
19.	Mehdi Kheil	68	28	41·1	2·4
20.	Lakhan School ...	80	39	48·7	2·2
21.	Khost School	94	30	31·9	1·7
	Total ...	831	228	39·4	2·1

APPENDIX III.—(*Contd.*)

Serial number	Name of area/places	Spleens			
		Number examined	Number positive	Spleen rate, per cent	Average enlarged spleen
	BAKH AREA.				
1.	Ismail Kheil	41	18	43·9	2·3
2.	Mana Kol	42	23	54·7	2·5
3.	Pahlawan Kheil ...	27	13	48·1	2·6
4.	Nahr	57	21	36·8	2·4
	Total ...	167	75	44·9	2·5
	JAJI MAIDAN AREA.				
1.	Manal Kheil	40	31	77·0	2·9
2.	Ghaza Ghundi ...	16	12	75·0	2·5
3.	Ata Kheil ...	31	20	64·5	2·2
4.	Sor Otki	48	19	39·5	2·2
	Total ...	135	82	60·7	2·5
	ISMAIL KHEIL AREA.				
1.	Mandi Zai School ...	100	24	24·0	1·9
2.	Kaya Shadal ...	37	9	24·3	2·4
3.	Burhan Kheil ...	52	13	25·0	1·8
4.	Dodal	39	9	23·0	2·0
5.	Beg Kala	20	6	30·0	1·6
	Total ...	248	61	24·5	2·0
	TRI ZAI AREA.				
1.	Tri Zai School ...	100	59	59·0	2·7
2.	Ali Sheb	12	9	75·0	2·4
3.	Shapi Kala	23	13	60·8	2·7
4.	Karya Terai	47	31	65·9	1·9
	Total ...	182	113	62·0	2·4
	NADAR SHAH KOT AREA.				
1.	Nadar Shah Kot School ...	80	24	30·0	2·3
2.	Navi Kot	33	7	21·2	1·7
3.	Dakhi	16	6	37·5	2·3
	Total ...	129	37	28·6	2·1

APPENDIX III.—(*Contd.*)

Serial number	Name of area/places	Spleens			
		Number examined	Number postive	Spleen rate, per cent	Average enlarged spleen
	Kandhar-Grishk Provinces—November and December, 1952.				
	ARGHANDAB AREA.				
1.	Kayra Langer ...	43	19	44·1	2·2
2.	Karya Kalacha Hasinoor	30	22	73·3	2·9
3.	Karya Mazra Abbas ...	19	8	42·1	2·1
4.	Sardeh School ...	69	33	47·8	2·4
5.	Sanzari	20	6	30·0	3·0
6.	Kushak School ...	52	19	36·4	2·2
7.	Karya Munara ...	31	21	67·6	2·6
8.	Karya Jalawar ...	32	14	43·7	2·4
9.	Karya Malik Fateh ...	25	12	48·0	2·7
	Total ...	321	145	47·9	2·5
	KISHK NAKHUD AREA.				
1.	Mai Wand	48	31	64·5	2·3
2.	Karya Sardar Gul Mohamad	21	11	52·3	2·5
	Total ...	69	42	60·8	2·4
	DHAND AREA.				
1.	Guzra School ...	52	6	11·5	1·5
2.	Gosh Khana Kakal ...	40	6	15·0	1·6
3.	Alukuzai	15	0	0·0	0·0
4.	Karya Murghan ...	30	2	6·6	1·5
5.	Kokran	35	11	31·4	2·5
6.	Gundi Gan	37	1	2·7	1·0
	Total ...	209	26	12·4	1·9

APPENDIX III.—(*Contd.*)

Serial number	Name of area/places	Spleens			
		Number examined	Number positive	Spleen rate per cent	Average enlarged spleen
	GRISHK AREA.				
1.	Grishk School ...	92	43	46·7	2·2
2.	Karya Tajkan ...	36	18	50·0	2·2
3.	Karya Adam Khan ...	43	21	48·8	2·5
4.	Karya Said Akbar ...	29	15	51·7	2·6
5.	Deh Mozand	31	14	45·1	2·2
6.	Karya Charkha Andas ...	33	18	54·5	2·5
7.	Karya Said Musa ...	25	12	48·0	2·2
8.	Grishk City	45	20	44·4	2·2
9.	Malgir School ...	100	38	38·0	2·3
10.	Nad-I-Ali Kharota ...	128	77	60·1	2·3
	Total ...	562	276	49·1	2·3
	NAWA BARAK ZAI AREA.				
1.	Karya Yasin Khan ...	14	2	14·2	2·0
2.	Karya Mulajuma ...	13	5	38·4	2·0
3.	Karya Fahim Khan ...	11	3	27·2	2·6
4.	Mir Hamza Khan ...	16	3	18·7	2·0
	Total ...	54	13	24·0	2·1
	YAKH CHAL AREA.				
1.	Karya Kharot Nehr Saraj	16	11	68·7	2·2
2.	Karya Barak Zai ...	17	14	82·3	2·5
3.	Karya Mahajrain ...	42	34	80·9	2·5
4.	Sangin School ...	68	30	44·1	2·1
5.	Kasaki Saphla ...	46	27	58·6	2·4
6.	Saqwan Kala ...	26	11	42·6	2·09
7.	Karya Chakha ...	8	5	62·5	2·5
	Total ...	223	132	59·1	2·3

APPENDIX III.—(*Contd.*)

Serial number	Name of area/places	Spleens			
		Number examined	Number positive	Spleen rate, per cent	Average enlarged spleen
	Verdik Loh Garh—March and April, 1953.				
1.	Madan School ...	100	17	17·0	1·9
2.	Karya Rustam Khail Madan	17	2	11·7	1·0
3.	,, Bambai Verdik ...	27	5	18·5	1·4
4.	,, Khaleli Verdik ...	20	2	10·0	1·5
5.	,, Chack Verdik ...	21	2	9·0	1·5
6.	,, Ali Ahmed Verdik	24	2	8·3	1·0
7.	,, Kalai Baba Verdik	23	3	13·0	1·6
8.	,, Rashidan Verdik ...	23	0	0·0	0·0
9.	School Chack Verdik ...	37	2	5·4	2·0
10.	Karya Haider Kheil Verdik	19	0	0·0	0·0
11.	,, Taj Baiki Verdik ...	78	6	7·7	1·4
12.	,, Khamra Khail Verdik	18	3	16·6	1·0
13.	School Saidabad ...	38	2	5·2	2·0
14.	Karya Tamur Kheir, Tangi Verdik ...	35	7	20·0	1·9
15.	,, Pini Sufaid Tangi Verdik ...	12	2	16·6	1·5
16.	,, Kila Amir Verdik ...	23	3.	13·0	1·6
17.	,, Kudi Tangi Verdik	18	4	22·2	1·5
18.	,, Hukumat Kalan Loh Garh	17	1	5·8	1·0
19.	,, Barki Rajan Loh Garh	182	20	10·9	1·4
20.	,, Naib Kheil ,,	25	1	4·0	1·0
21.	,, Kila Nau ,,	63	4	11·1	1·4
22.	,, Nau Shahr Charkh ,,	45	3	6·5	1·0
23.	,, Garm-Abad ,,	35	0	0·0	0·0
24.	,, Pans Ram Marboot Kalan ,,	27	3	11·1	2·0
25.	,, Pad Khawb Rogini ,,	46	7	14·6	1·4

APPENDIX III.—(*Contd.*)

Serial number	Name of area/places	Spleens			
		Number examined	Number positive	Spleen rate, per cent	Average enlarged spleen
26.	Karya Barki Bark Loh Garh	60	6	10·0	2·0
27.	,, Zukum Khail ,,	39	2	5·1	1·5
28.	School Hakumat Kalan ,,	25	1	4·0	1·0
29.	Karya Adhi Syyadan ,,	33	4	15·1	1·0
30.	,, Pad Khawb Shana ,,	39	1	2·4	1·0
31.	,, Mango Khail ,,	39	2	2·5	1·0
32.	,, Doshaband ,,	8	0	0·0	0·0
33.	,, Hisarak ,,	21	0	0·0	0·0
34.	,, Kalangar ,,	34	4	11·7	1·2
35.	,, Saadat Khan ,,	16	1	6·2	2·0
36.	,, Shish Kila ,,	15	1	6·2	1·0
37.	,, Kila Hoofi ,,	23	0	0·0	0·0
38.	,, Kola Nau Arab Khan ,,	18	0	0·0	0·0
39.	,, Koodiveen ,,	14	0	0·0	0·0
40.	,, Mula Rahmat Khan ,,	27	1	3·7	1·0
41.	,, Sayyad Hai ,,	26	2	7·6	1·0
42.	,, Kalangar ,,	26	1	3·7	1·0
43.	,, Shabaki and Kutab Khail ,,	41	2	4·8	1·5
	Total ...	1,477	129	8·75	1·5
	Herat Province—August and September, 1953.				
	OBAY AREA.				
1.	Karya Char Mahal ...	55	19	34·5	2·1
2.	,, Band Banafsh ...	26	10	38·4	2·2
3.	,, Toon	22	...	00·0	0·0
4.	,, Chisat ...	29	14	28·3	2·3
5.	,, Gunabad ...	27	10	37·0	1·6
6.	,, Murkhcha ...	14	7	50·0	1·7
7.	,, Baghal ...	14	1	7·1	2·0
8.	,, Guzrah and Sabiran	61	7	11·4	1·4
9.	,, Sabirah ...	31	7	22·6	2·2
10.	,, Karash ...	10	3	30·0	2·6
11.	,, Sara Siah ...	12	1	8·3	2·0
	Total ...	301	79	26·2	2·2

APPENDIX III.—(*Contd.*)

Serial number	Name of area/places	Spleens			
		Number examined	Number positive	Spleen rate, per cent	Average enlarged spleen
	ANJIL AREA.				
1.	School Kashfi ...	35	16	45·7	1·9
2.	,, Hatili	42	13	30·9	1·8
3.	,, Sarvastan ...	18	4	22·3	2·0
4.	Karya Sarvastan ...	27	7	25·9	1·4
5.	School Maldam ...	52	25	48·07	1·76
6.	,, Abul Walia ...	48	11	22·91	1·26
7.	Karya Buzdan ...	8	3	37·5	1·0
8.	,, Odran ...	26	10	38·4	1·4
9.	,, Gorazan ...	32	13	40·4	2.0
10.	,, Navin Olia ...	59	11	18·6	2·1
11.	,, Turkan ...	25	8	32·0	2·0
12.	,, Navin Sifla ...	42	8	19·04	2·3
13.	,, Jilwarcha ...	40	10	25·0	2·0
14.	,, Ghazan Chashia ...	18	4	22·2	1·7
15.	,, Uqab	12	3	25·0	2·6
16.	,, Karta	14	6	42·8	1·8
17.	,, Khawaja Surma ...	18	4	22·2	1·1
18.	,, Jafari	33	7	21·2	2·0
	Total ..	549	163	29·6	1·8
	HERAT CITY AREA.				
1.	Mavafak School ..	84	5	5·9	2·0
2.	School Safi	81	10	12·3	1·9
3.	,, Jabli	45	5	11·1	1·4
4.	,, Jazai	34	1	2·9	2·0
5.	,, Share Khalil ...	21	...	0·0	0·0
6.	,, Yaka Darkhat ...	15	1	6·6	1·0
	Total ...	280	22	7·86	1·7

APPENDIX III.—(*Contd.*)

Serial number	Name of area/places	Spleens			
		Number examined	Number positive	Spleen rate, per cent	Average enlarged spleen
	KALAINAU AREA.				
1.	Lake Surakh ...	13	1	7·7	3·0
2.	Darab Boom	26	4	15·3	1·5
3.	Makar	31	...	0·0	0·0
4.	Teghab Ismail ...	39	8	20·5	1·8
5.	Markaz Kali Nau ...	78	21	26·9	2·0
6.	Kharistan	15	3	20·0	1·6
7.	Kharistan Koochi ...	20	...	0·0	0·0
8.	Karya Kundi ...	14	...	0·0	0·0
9.	,, Karghi Tu ...	38	5	13·1	3·0
10.	,, Chakar ...	19	4	21·0	1·7
11.	,, Bagak ...	26	4	15·3	1·7
12.	,, Kurukh ...	28	...	0·0	0·0
	Total ...	347	50	14·4	1·9
	GUZRAH AREA.				
1.	School Goshmir ..	77	46	59·7	2·4
2.	,, Ziarat Jah ...	62	6	9·6	1·0
3.	,, Roza Bagh ...	78	27	34·6	2·4
4.	Karya Kurat ...	43	17	39·5	1·8
5.	,, Kurat Sofli ...	28	6	21·4	2·1
6.	School Dadshan ...	39	13	33·3	2·0
7.	,, Sayar Shan ...	28	9	32·1	2·3
	Total ...	355	124	34·9	2·0
	PUSHTON ZARGOON AREA.				
1.	School Pushton Zargoon ...	37	14	37·9	1·2
2.	Karya Balkhian ...	17	7	41·1	1·5
3.	,, Dhe Sair Sad ...	30	15	50·0	2·71
4.	,, Kushak Yazdan ...	23	6	26·0	1·65
5.	,, Khawaja ...	34	18	52·9	1·0
	Total ...	141	60	42·5	2·0

APPENDIX III.—(*Contd.*)

Serial number	Name of area/places	Spleens			
		Number examined	Number positive	Spleen rate, per cent	Average enlarged spleen
	GHORIAN.				
1.	School Ghorian ...	79	11	13·9	1·36
2.	Karya Sarai Asiyab ...	26	1	3·8	1·0
3.	,, Fakdan ...	19	3	15·7	1·33
4.	School Zinda Jan ...	31	...	0·0	0·00
5.	Kalai Reg ...	47	4	8·5	1·2
	Total ...	202	19	9·4	1·3
	SHINDAND.				
1.	School Markaz ...	33	3	9·0	1·3
2.	,, Safan	40	9	22·5	1·66
3.	,, Amarat ...	58	16	27·5	1·87
4.	Karya Farashkhan ...	37	8	21·6	1·62
5.	Ali Abad	32	6	18·7	1·66
6.	Karya Sasian	22	5	22·2	1·2
7.	Kab-Dez	27	0	00·0	0·0
8.	Koshak	23	2	8·6	1·5
9.	Alia Bazar	35	3	8·5	1·6
	Total ...	307	52	16·9	1·6
	FARRAH.				
1.	Karya Bisnau ...	24	1	4·57	1·0
2.	Farrah Markaz ...	20	1	5·0	1·0
3.	Gun Kan	22	...	0·0	0·0
4.	Kabai Rafan ...	31	3	9·6	1·3
5.	Deh Farrah	10	...	0·0	0·0
6.	Dara Abad	24	3	12·5	1·6
	Total ...	131	8	6·1	1·5

APPENDIX III.—(*Contd.*)

Serial number	Name of area/places	Spleens			
		Number examined	Number positive	Spleen rate, per cent	Average enlarged spleen
	Herat, Maymana, Andhkoy, etc. October and November, 1954.				
	QAYSAR AREA.				
1.	Qaysar School ...	64	2	3·12	1
2.	Sar Asyal	44	...	0·0	...
3.	Nowdary	35	...	0·0	...
4.	Koni	25	...	0·0	...
	Total ...	168	2	1·21	1
	ALMOR AREA.				
1.	Almor School ...	70	1	1·42	2
2.	Diwanakhana ...	16	...	0·0	...
	Total ...	86	1	1·16	2
	MAYMANA TOWN.				
1.	Boy School	187	2	1·07	1·5
2.	Girls' School	71	...	0·0	...
	Total ...	258	2	0·77	1·5
	PASHTOON KOTE.				
1.	Afghan Kote	12	...	0·0	...
2.	Tor Pakhtoo	25	...	0·0	...
3.	Onjilat	18	3	16·66	2
4.	Momenabad	20	6	30·0	1·16
	Total ...	75	9	12·0	1·77
	ANDKHOY.				
1.	Andkhoy School ...	122	3	2·46	1·5
	Total ...	122	3	2·46	1·5
	BILCHIRAGH.				
1.	Kawalian	50	1	2·0	2
2.	Tokhlimast	38	1	2·63	2
3.	Nishar	32	1	3·12	2
4.	Bilchiragh	50	1	2·0	2
	Total ...	170	4	2·35	2
	SHREEN TEGAB.				
1.	Islam Kala	35	...	0·0	...
2.	School	69	1	1·44	1
	Total ...	104	1	0·96	1

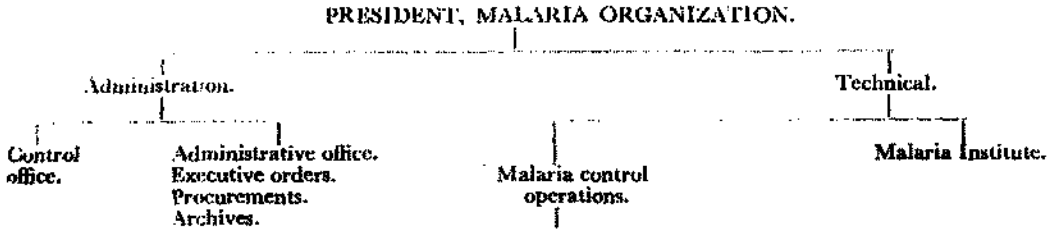
APPENDIX IV.

Blood surveys in Afghanistan: Children 2-9 years.

Serial number	Name of province	Number of localities visited	Sprayed (years) or unsprayed	Period of survey	Total number examined	Total number positive	Species			Parasite rate, per cent
							vivax	*falciparum*	*malariæ*	
1.	Kataghan	24	Unsprayed	June-July, 1950	496	44	34	9	1	8·9
2.	,,	15	1950	October, 1950	424	21	4	17	...	5·0
3.	,,	13	Unsprayed	October, 1950	312	41	11	30	...	13·1
4.	,,	15	1950 and 1951	July-August, 1951	415	10	5	5	...	2·2
5.	,,	19	Unsprayed	July-August, 1951	472	38	29	9	...	8·05
6.	,,	17	1950 and 1951	October, 1951	492	4	2	2	...	0·8
7.	,,	13	1951	October, 1951	372	7	2	5	...	1·9
8.	Kabul	17	Unsprayed	March-April, 1952	506	12	12	...	...	2·3
9.	,,	51	Unsprayed	September-October, 1952	1,364	26	26	...	...	1·8
10.	,,	12	Unsprayed	September-October, 1952	213	18	16	2	...	9·0
11.	Gardez	41	Unsprayed	November, 1952	220	9	6	3	...	4·1
12.	,,	24	Unsprayed	November, 1952	346	6	5	1	...	1·7
13.	Eastern (Masharqi)	21	Unsprayed	December, 1952	1,045	139	59	80	...	13·3
14.	Kandhar	38	Unsprayed	December, 1952 to January, 1953	244	52	22	30	...	30·1
15.	Grishk ,,	30	1953	October, 1953	901	72	28	44	...	8·1
16.	Herat	64	Unsprayed	August, 1953	901	26	14	12	...	2·8
17.	,,	20	Unsprayed	Sepetmber, 1953	149	00	...	...	...	0·0
				Grand Total	8,872	524	275	249	1	

APPENDIX V.

Afghanistan Antimalaria Organization: Year 1954.



	Provinces					
	Mashraqi	Kataghan	Kabul	Gardez and Khost	Kandhar and Grishk	Herat
Staff						
Directors (Medical)	1	1	1	1	1	1
Additional Malariologist ...	...	1	...	...	...	1
Entomologist	...	...	1	...	...	1
Technicians (Paramedical) ...	9	15	10	5	10	15
Transport						
Lorries	2	2	2	1	1	2
Jeeps, station wagons or pick-ups ...	1	2	2	1	2	3

APPENDIX VI.

Programme of antimalaria operations implemented during 1954.

Area of operations	Herat	Kataghan	Kabul	Mashraqi	Kandhar Grishk	Khost	Total
POPULATION PROTECTED	2,50,000	3,18,000	3,35,000	1,36,000	2,00,000	40,000	12,79,000
STAFF ENGAGED							
Doctors (Medical) ...	2	2	1	1	1	1	8
Entomologists ...	1	...	1	...	...	...	2
Technicians ...	16	17	12	10	10	5	70
Clerks	1	1	1	1	1	...	5
Storekeepers ...	1	1	1	1	1	...	5
Drivers	5	4	4	3	3	3	22
TRANSPORT							
Trucks	2	2	2	2	1	1	10
Pick-up	1	1	...	...	1	...	3
Jeeps	2	1	1	1	1	1	7
Station wagons ...	...	...	1	...	...	1	2
SPRAYERS							
Pressure pump, *i.e.*, Hudson	150	...	...	40	100	30	320
Pressure pump, *i.e.*, Lafstrand ...	...	90	...	...	...	...	90
Stirrup pumps ...	...	40	40	35	...	...	115
D.D.T.							
In terms of technical D.D.T. of United Nations International Children Energency Fund, in lbs. ...	41,250	5,280	6,930	11,220	25,080	3,520	93,280
In terms of Technical D.D.T. of Government in lbs.	...	47,520	4,220	6,358	...	...	58,098
Provision for labour *Afghanis*	64,060	93,600	35,000	32,000	42,840	8,000	2,75,700
Petrol (in gallons) for the project	1,500	1,000	200	400	500	200	3,800
Petrol for going to and coming back from the area of operations ...	600	320	...	...	180	120	1,220
Date of commencement of spraying operations	June 1, 1954	June 1, 1954	June 10, 1954*	June 8, 1954	June 5, 1954	June 25, 1954	
Date of completion of spraying operations	July, 25 1954	July 25, 1945	July 25, 1954	July 25, 1954	July 25, 1954	July 25, 1954	

*Larvicidal measures to last from April 10, 1954 to October 15, 1954.

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The following contractions are in use in the *Indian Journal of Malariology*, whether the number to be expressed is one or more :—

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When there are references to two or more papers by the same author published in the same year the letter *a* should be placed immediately after the year in the first case, the letter *b* in the second case, and so on ; e.g. Blank, J. (1936*a*).

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Brit. Med. J.
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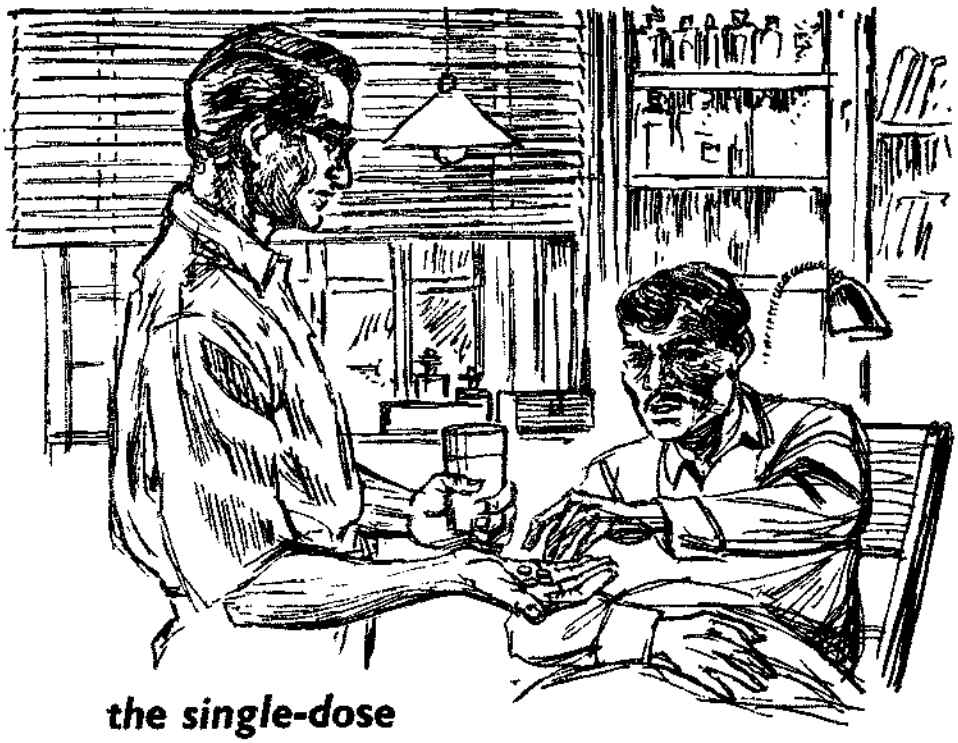
Bull. Soc. Path. Exot.
C. R. Soc. Biol.
Geneesk. Tijd. Ned.-Ind.
Ind. J. Mal.
Ind. J. Med. Res.
J. Mal. Inst. Ind.
Jl. R. A. M. C.
J. Trop. Med. Hyg.
Meded. Volk. Ned.-Ind.
Rec. Mal. Surv. Ind.
Rev. App. Ent.
Riv. Malariol.
Trans. Roy. Soc. Trop. Med. Hyg.
Trop. Dis. Bull.
U.S.A. Pub. Hlth. Rpts.



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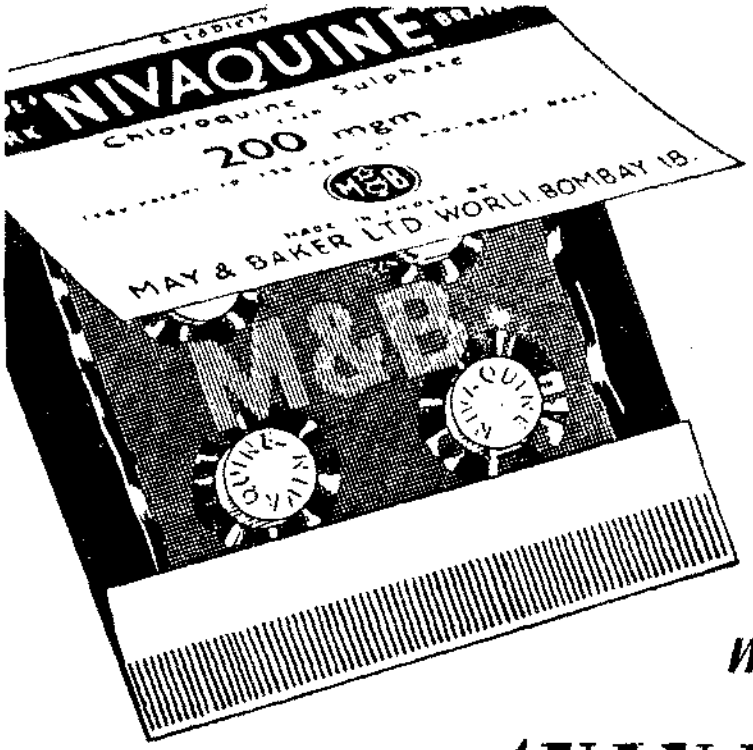

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Indian Journal of Malariology, **11**, 2, June 1957.

FIRST BIRTH CENTENARY OF SIR RONALD ROSS.

MAY 13, 1957 was the first birth centenary of Sir Ronald Ross, one of the greatest benefactors of mankind. It was his epoch-making discovery of the malaria transmission by mosquitoes in 1897-1898 that deprived that deadliest disease in human history of the menace of the mysterious. It is perhaps the greatest tribute to his memory that the fell scourage which started in the unknown past is now in the last stages of its existence, and those who will have the privilege of celebrating his second birth centenary, may have only an academic interest in that disease.

If it is a fact that life survives death, Sir Ronald's soul must be feeling supreme solace at the new hope of realization of his life's dream of freeing the world of the 'million-murdering death' whose 'cunning seeds' a bountiful Destiny had brought within his ken.

India can legitimately claim Sir Ronald as one of her sons, because he was born in this country and it was here that he benefited humanity by his grand discovery. India, therefore, owes to him a national debt and to discharge that debt she is already on high road leading to eradication of malaria from its confines.

Sir Ronald was the type of pioneer— one of the rare personalities who re-shape the contours of life. He had all the hostility to encounter which the great always expect from those who are too small to apprehend their mind and ways. With his patient and laborious application to research work not in a very congenial atmosphere, with the fetters and formalities of service galling him, he stands before us a figure of simple fascination and disquiet. His revolt against the agonies of mankind was unquenchable and his passion for humanity, compelling. He worked on his mosquitoes day after day with unremitting industry and impassioned concentration in the 'cloudy, dull hot' climate of Secunderabad. The afternoons might be drowsy and the cushioned seats quite seductive, but the stern benefactor of humanity had seen the path and trod it with a relentless will. His vigour of mind and body was dedicated to the cause. He used his own money, although he was a poor man, with a family, for assistance in capturing different kinds of mosquitoes and for feeding them on malarious patients. At last, the day of days came. On August 20, 1897 when about to abandon the great quest—discouraged and exhausted—the 'relenting God' placed within his hand 'a wondorous thing'. In the tissues of a 'dapple-winged' mosquito—a female Anopheles—he saw under the cracked eye-piece of his microscope, a dark stain, the 'million murdering' malaria parasite.

Ross was not only a medical research prodigy. His title to rank among the great figures of history would have been as unchallengeable without the splendid chapter of the theory of malaria transmission. He was a great mathematician, a poet, a novelist, a composer of music, and a high class scholar of history, philosophy and classics. The supreme fact about him was that he mingled science and muse in the same breath. He left behind soul-lifting poetry wherein he has conversed with hills, torrents and pastures. There is almost a Wordsworthian touch in his 'patient winds raking up crystals of dry snow' enabling 'sightless mountains, yearning for the sun, to lift their titanic countenances to Heaven's glow'.

England recognized his eminence by decorating him successively with C.B., K.C.B., and K.C.M.G., and electing him a Fellow of the Royal Society. He was awarded in 1902 the Nobel Prize for Medicine in recognition of his great discovery.

Surely will he live in history for ever!

—*Editor.*

Indian Journal of Malariology, **11**, 2, *June 1957*.

STUDIES OF IMMUNITY TO *PLASMODIUM BERGHEI* INFECTION.

I. The transmission of acquired immunity against *P. berghei* from female rats to their offspring.*

BY

P. G. SERGUIEV

AND

N. A. DEMINA.

(*Institute of Malaria, Medical Parasitology and Helminthology, Department of Public Health, Moscow, U.S.S.R.*)

[August 14, 1956.]

Plasmodium berghei strain was received by the authors in October, 1954, through the courtesy of the Director of the Malaria Institute of India, Delhi, Lieut.-Colonel Jaswant Singh. Considering that the reaction to a given strain may somewhat differ in animals of different origin, it was deemed advisable first of all to study the course of infection in the authors' strains of white mice and white rats.

It was found that course of the *P. berghei* infection in the authors' experimental animals did not substantially differ from the pattern described in various publications abroad in white mice (Vincke and Bulke, 1949; Ramakrishnan and Satya Prakash, 1950; Thurston, 1950; Ramakrishnan, Krishnaswami and Satya Prakash, 1951; Mercado and Coatney, 1951; Ramakrishnan, 1952; Greenberg, Nadel and Coatney, 1953; Matiela *et al.*, 1954; Sergent, 1954 and Rodhain, 1954*a*:1954*b*) as well as white rats (Ramakrishnan, Satya Prakash and Krishnaswami, 1951; Ramakrishnan, Satya Prakash, Krishnaswami and Chanan Singh, 1953; Krishnanswami, Satya Prakash and Ramakrishnan, 1953).

Refractory animals were encountered among both mice and rats, some of them being resistant to repeated infections.

The authors ascertained by special experiments that the resistance of white rats to repeated inoculations was not brought about by inapparent infection resulting from a primary or one of the succeeding inoculations.

*Reported at the Conference of the Institute on November 21, 1955.

The infection in mice was, as a rule, fatal. In most of the white rats (17 out of 18 females and 8 out of 9 males), mitigation of the infection was recorded; the acute phase was followed by a latent one. In the latent period, the blood of the rats was infectious; during this period repeated injections did not cause patent parasitæmia.

The principal aim of the authors' investigation consisted in ascertaining whether acquired immunity to *P. berghei* is transmitted from the female to its offspring. Various quantities of parasites were injected to young rats born of females which had been previously infected, and of control females not infected beforehand.

According to published data, young rats of various age-groups manifest a different degree of sensibility to *P. berghei.* According to Raffæle and Baldi, 1950; Galliard and Lapierre, 1951; Fabiani *et al.*, 1951; Hsu and Geiman, 1952; Rodhain, 1954*a*: 1954*b*; Zuckerman and Yeoli, 1954; Bruce-Chwatt, 1954; Galliard *et al.*, 1954; Singer *et al.*, 1955, young rats aged from several days to 2-2 1/2 weeks without exception were susceptible to *P. berghei*, the infection leading to a lethal outcome.

Young rats of a more adult age, from three weeks to two months, are likewise highly susceptible. The course of the infection is, however, milder and in some cases a spontaneous disappearance of the parasites is recorded: the acute phase of the infection being followed by latent one.

It should be borne in mind that in the above-mentioned investigations, the rats were infected with large numbers of parasites. Raffæle and Baldi (1950) did not specify the number of parasites inoculated. For example, in one experiment, Rodhain (1954*a*: 1954*b*) introduced 765,800 parasites into young rats, weighing from 34 to 77 gm. (probably aged one or two months), i.e. about 310,000-465,000 parasites per 20 gm. of weight. In another experiment 1,000,000 parasites were injected into young rats weighing from 25 to 30 gm. Galliard *et al.* (1954) introduced 2,000,000 parasites intra-abdominally or subcutaneously into 13-day old rats. In both cases, acute fatal infections were recorded. Zuckerman and Yoeli (1954) introduced 10,000,000 infected erythrocytes into two-week old rats, and 40,000,000 into 30-day old rats. Bruce-Chwatt (1954) infected young rats aged from 2 to 4 weeks with 1-2-3 million parasites; according to authors' estimates, approximately 1,000,000 parasites per 20 gm. of weight.

It follows from the above data that in all the mentioned experiments, the number of parasites inoculated into the young rats was very large. With such a scale of infection, the individual differences in the susceptibility of young rats to *P. berghei* may not have manifested itself. As a result, the infection should have occurred in every case, which, as a matter of fact, it did.

MATERIAL AND METHOD.

For inoculations, highly parasitized blood was used. The number of erythrocytes in 1 ml. of blood of the donor animals was determined in a haemocytometer and the parasites per 500 erythrecytes were counted in a slide stained with Romanovsky's stain. The blood was diluted with an equal volume of sodium

citrate (3·8 per cent). An amount of sodium chloride solution (0·85 per cent), sufficient to get the requisite number of parasites in 0·1-0·2 ml. was added. In most cases the injection was administered subcutaneously. In some experiments the blood with the parasites was injected intra-abdominally.

When no determination of the exact number of parasites was required, the number of parasites was calculated approximately (from the volume of the introduced blood). The rats subjected to hyperimmunization, received tenfold dose of parasites. The inoculation was repeated two or three times with a month's interval.

The infected female and male rats were isolated from fresh non-infected rats. When the females became pregnant, they were placed in separate cages.

The dates of the births were recorded. A strict record of age of the young rats was kept. In order to determine whether infection can develop in young rats, injected with a small number of parasites, two experiments were carried out.

Young rats aged from 10 to 20 days, born of two previously non-infected females, were used. 100-1,000,000 parasites for each 20 gm. of weight, were injected to the young rats. The results are shown in Table I.

TABLE I.

Infection of young rats by various numbers of parasites.

Number of parasites injected to the young rats per 20 gm. of body weight	Number of rats	Became infected	From the infected rats	
			Died	Killed in a state of agony
100	3	...	...	...
1,000	5	2	1	1
10,000	3	3	2	1
100,000	3	3	1	2
1,000,000	1	1	...	1

1,000 parasites per 20 gm. was the smallest dose resulting in infection. (Six weeks after delivery, two mother rats were infected with *P. berghei*. Both exhibited a typical infection).

The authors performed seven experiments in which a sum total of 78 young rats born of 11 females, were infected (Table II).

The rats were infected at the age of 10 to 20 days with varying numbers of parasites, ranging from 1,000 to 200,000 being injected subcutaneously for every 20 gm. body weight.

Forty young rats were born of females immune to *P. berghei*. In order to achieve hyperimmunization, four of these rats were inoculated before delivery twice or thrice with a large number of parasites at monthly intervals.

TABLE II.

Infection of young rats born of immune or control females.

	Immune rats					Total	Control rats: I group			Total	Control rats: II group			Total
Serial number of rat ...	16	21	24	19	41	...	42	47	48	...	44	45	46	...
Number inoculated ...	21*	7	3	5	4	40	7	3	5	15	4	10	9	23
Number found negative ...	21	7	2	5	4	39	1	...	...	1	2	7	7	16
Number found positive results ...	...	...	1	...	...	1	6	3	5	14	2	3	2	7
Number died ..	...	...	1	...	...	1	2	2	5	9	2	2	2	6
Number killed in a state of agony ...	...	...	...	...	...	...	4	...	...	4	...	...	...	...
Mitigated infection ...	...	...	...	...	...	...	...	1	...	1	...	1	...	1

* From two litters.

Thirty-eight rats were born of control females not infected with *P. berghei* before delivery.

Of the 40 young rats born of immune females and suckled by them (Nos. 16, 21, 24, 19 and 41), only one (No. 59) that was inoculated with 20,000 parasites per 20 gm. of weight (Table III), took the infection.

The results of inoculation of the young rats born of control females and suckled by them, were different. These young rats could be divided into two groups. The rats of the first group turned out to be highly receptive to *P. berghei*. Fourteen out of 15 young rats of this group became infected. Nine died in the acute stage of infection, four were killed in a state of agony and only one exhibited mitigated infection.

The mothers (Nos. 42, 47 and 48) of these young rats, which were infected $1\frac{1}{2}$ months after delivery, proved to be susceptible to *P. berghei*. As a result of being inoculated subcutaneously with 4 to 16 million parasites per 20 gm. body weight (the number authors generally used to infect adult rats), they developed acute infection with a great number of parasites.

Most of the young rats of the second group, 16 out of 23, proved to be resistant to *P. berghei*. Thorough systematic examination of their blood revealed no parasites. Twelve of these young rats were inoculated repeatedly by being injected subcutaneously with 100,000 to 400,000 parasites. This repeated inoculation resulted in infecting only one young rat, the dose being 400,000 parasites. The other seven rats developed an acute infection; six of them died with a large number of parasites in their blood.

TABLE III.

Results of inoculation of young rats infected with various quantities of P. berghei *parasites and born of immune or control females.*

Number o parasites	Rats born of: Immune females					Control females: I group					Control females: II group				
	Total inoculated	Number infected	Of these: Died	Killed	Mitigation	Total inoculated	Number infected	Of these: Died	Killed	Mitigation	Total inoculated	Number infected	Of these: Died	Killed	Mitigation
1,000 ...	...	...	...	...	...	3	2	1	1	...	2	0	...	...	...
5,000 ...	6	0	...	...	...	...	...	...	...	...	...	...	...	...	...
10,000 ...	9	0	...	...	...	10	10	8	1	1	8	2	2	...	...
20,000 ...	4	1	1	...	...	...	...	...	...	...	...	...	...	...	...
50,000 ...	5	0	...	...	...	...	...	...	...	...	...	...	...	...	...
100,000 ...	15	0	...	...	...	2	2	...	2	...	13	5	4	...	1
200,000 ...	1	0	...	...	...	...	...	...	...	...	...	...	...	...	...
Total ...	40	1	1	...	...	15	14	9	4	1	23	7	6	...	1

The females from which the young rats of the second group were born, proved resistant to *P. berghei*. One and a half months after delivery they did not react to the inoculation of 8 to 10 million parasites per 20 gm. body weight; no parasites were found in their blood and an inoculation of their blood to fresh mice was negative. A month later, the rats were inoculated repeatedly. In two of them the inoculation was positive (Nos. 45 and 46), while the third (No. 44) again proved to be resistant.

This series of experiments proves that the susceptibility of young rats to *P. berghei* is determined by the immunity of their mothers.

In order to ascertain the rôle of mother's milk in maintaining immunity acquired from the mother, the authors performed the following experiment.

Eleven young rats were born of the famale No. 16, that turned out to be immune to *P. berghei* as a result of previous infection with this parasite.

Two days after birth, six of the rats were placed together with female No. 43, which had not been infected and which had been delivered of a litter at the same time as female No. 16.

Eight days later, when the 11 young rats were 10 days old, all of them were infected with 5,000 to 50,000 parasites (per 20 gm. weight). Not a single young

rat became infected, though some mice infected at the same time as the rats exhibited a typical acute infection (Table IV).

TABLE IV.

Infection of young rats born of an immune female (No. 16). Five of them were suckled by mother, and six by a non-immune female (No. 43).

Infection	Age	Suckled by female	Number of parasites inoculated	Number of young rats inoculated	Became infected
I	10 days	Immune No. 16	5,000	3	...
			50,000	2	...
		Non-immune No. 43	5,000	3	...
			50,000	3	...
II	26 days	Immune No. 16	10,000	3	..
			50,000	2	...
		Non-immune No. 43	10,000	3	1
			50,000	3	3

Sixteen days later, when the young rats were 26 days old, they were inoculated repeatedly; this time they were inoculated subcutaneously with 10,000 to 50,000 parasites. Not a single young rat fed with the immune mother's milk was infected. Of the six young rats suckled with the control female's milk, four rats became infected: one out of the group that was inoculated with 10,000 parasites and all the rats inoculated with 50,000 parasites (Table IV).

As a result of a test inoculation, 1½ months after delivery, female No. 43 contracted the disease and exhibited a typical infection.

DISCUSSION.

The aim of this investigation was to find out whether acquired immunity to *P. berghei* is transmitted to the offspring.

Acquired immunity being transmitted to the offspring, is known to occur in a number of bacterial and virus infections of man and animals. Measles can be cited as a classical example where the immunity, acquired as a result of the disease, lasts for life. This immunity is transmitted from mother to children. It is known that children born of mothers immune to measles, do not contract measles up to the age of two months. In the subsequent months and up to the age of a year, the susceptibility to measles gradually increases. At the age of a year, the immunity is lost, and then the children become susceptible to the infection. The transmission of acquired immunity is known to occur not only in cases of diseases

of man and animals but also of birds. In the latter case this transmission is effected through the eggs. The transmission of antibodies has, for instance, been proved in Newcastle's disease, *Rous sarcoma*, in some salmonelloses (*S. pillorum*, *S. gallinarum*, *S. typhi*), infectious bronchitis and bird plague. This fact has also been established in cases of encephalitis—western equine (W.E.E.) and St. louis (S.L.E.) (Sooter *et al.*, 1954 and Kissling *et al.*, 1954). It is shown also in spirochaetosis of fowls (Kroo and Orbaneja, 1935).

In the protozoal infections, the fact of transmission of acquired immunity from mother to offspring is described in trypanosomiasis (Kolodny, 1939; Culbertson, 1938: 1939: 1940: 1941). But Werner (1954) could not confirm the data of these investigations. Ossipowsky (1955) established that the young rabbits, born of mothers possessing some degree of resistance to coccidia, were found to have more resistance to this parasite than the offspring of the susceptible rabbits. The problem of transmission of acquired immunity in malaria has not been studied as yet.

Immunity to *P. berghei* in young rats, born of immune females, was studied by Bruce-Chwatt (1954), Terry (1955), Fabiani and Fulchiron (quoted by Fabiani, 1954).

Bruce-Chwatt (1954) infected young rats aged 1, 2 or 3 weeks respectively with 1, 2 or 3 million parasites (according to authors' estimates, approximately with one million parasites per 20 gm. of weight). All the infected young rats contracted the disease (69 born of immune rats and 57 of controls).

All the 37 young rats born of controls and 39 out of the 42 young rats born of immune females, which were infected at the age of 2 or 3 weeks, developed a lethal infection. The young rats born of immune mothers showed a somewhat lower degree of parasitæmia, and a somewhat longer duration of the patent infection than the young rats born of non-infected females. The authors are inclined to regard this fact as a manifestation of immunity obtained by the young rats from their immune mothers.

Young rats were inoculated at the age of four weeks. Out of 20 controls, 19 died; out of 18 born of immune females, nine died. One should, however, bear in mind that at the age of four weeks the young rats already possess some degree of natural immunity to *P. berghei*. This non-specific age resistance to *P. berghei* in young rats, born of immune mothers, was probably more pronounced because it developed against the background of specific immunity.

If this is not the case, then it becomes inexplicable why acquired immunity transmitted by the mother and ineffective at an early age might have manifested itself at a later age.

The authors believe that more definite results could have been obtained in Bruce-Chwatt's experiments if it had been taken into account that in such experiments, the establishment of proper relationships between the intensity of immunity, on the one hand, and the dose of infection, on the other hand, is of decisive importance.

In cases of inoculation of a great number of parasites, the immunity inherited by the young rats from their immune mothers might have proved insufficient to prevent infection, as it was observed in the above experiments.

Terry (1955) points out that he recorded in young rats, born of immune rats, longer incubation period and a somewhat smaller degree of parasitæmia than in controls born of non-infected females. No factual material is cited.

Fabiani (1954) reports that in his experiments with Fulchiton in 1952, he observed mitigated infection in young rats born of immune females, while in control young rats born of control females the infection was lethal.

In another series of experiments, Bruce-Chwatt (1954) studied immunity to *P. berghei* in young rats of immune females and suckled by normal females, and *vice versa.* All the young rats inoculated, when they were two or four weeks old, with approximately 1,000,000 parasites per 20 gm. of weight, contracted the disease and developed an acute infection with a high parasitæmia. The mitigation of the infection was observed in 5 out of 28 young rats. Among them, three out of eight were infected at the age of four weeks, born of a normal female and suckled by an immune female.

As a result of these experiments, Bruce-Chwatt (1954) came to the conclusion that immunity to *P. berghei* is transmitted through the mother's milk. It is, however, quite impossible to understand why this immunity manifests itself to a greater degree at a later age. This proposition of Bruce-Chwatt (1954) is in contradiction with the existing concepts concerning the dynamics of intensity of acquired immunity transmitted from mother to children.

Terry (1955) reports that young rats born of normal females and nursed with the milk of immune females were more resistant to *P. berghei* than in the opposite case. In this case also no factual material has been presented.

The authors observed a more rapid decrease of immunity in young rats, born of an immune female but suckled by a control female as compared with young rats of the same litter, suckled by mother.

The authors approached with caution the problem of the transmission of acquired immunity to the offspring, considering that the intensity of the immunity, transmitted to young rats from immune females, might have been insufficient to withstand the inoculation of a great number of parasites.

As a matter of fact, when inoculating at the most 200,000 parasites per 20 gm. of weight, the authors obtained quite distinct results, which unquestionably bore out the fact that young rats born of immune females possessed a pronounced immunity to *P. berghei.*

Along with this, the authors encountered a very interesting fact, which testifies that young rats may be non-susceptible not only when born of immune females, but also young rats born of females, which at definite stages of their lives are resistant to *P. berghei,* proved non-susceptible to this parasites.

To cite an example, Rats 45 and 46, whose young proved non-susceptible to *P. berghei,* were resistant to the first inoculation made 1 to 5 months after delivery and contracted the disease as a result of the second inoculation a month after the first. Rat 44 did not react to any of these infections. Thus even a state of temporary natural resistance can be transmitted to the offspring. It is not clear, however, which mechanism accounts for this resistance.

Another experiment, in which young rats born of an immune female were suckled by a non-infected female, demonstrated the rôle of mother's milk in maintaining the acquired immunity transmitted from the mother.

CONCLUSIONS.

1. The course of *P. berghei* infection in adult white mice and white rats was similar to the discription given abroad. This similarity makes it possible to compare the results they obtained with the findings of authors' investigations.

2. The resistance of rats at the age of ten to twenty days to *P. berghei*, corresponds with that of their mothers.

3. Young rats born of females possessing acquired immunity exhibited a pronounced immunity to *P. berghei*. Of the 40 young rats inoculated, only one became infected.

4. The young rats born of control females (not infected before delivery), reacted to inoculation in different ways, namely:—

(*a*) young rats born of rats susceptible to *P. berghei* proved to be highly susceptible; of the 15 young rats of this group, 14 became infected.

(*b*) most of the young rats (16 out of 23), born of naturally resistant females, proved non-susceptible to *P. berghei*.

5. Young rats, born of a female immune as a result of disease and suckled by her, remained resistant to *P. berghei* for a longer time than those of the same litter but suckled by a normal female.

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D.D.T. WATER DISPERSIBLE POWDERS : STUDIES ON SUSPENSIBILITY IN RELATION TO ACCELERATED TROPICAL STORAGE TREATMENT.

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WATER dispersible D.D.T. powders are constituted of nominal technical D.D.T. content, 5 to 10 per cent of wetting and dispersing agents and remainder of clay. These ingredients are usually air-milled to a very fine powder which forms a reasonably stable suspension when thoroughly mixed with water. Stability of such suspensions in spraying operations, is an essential pre-requisite for efficient and uniform coverage of the likely mosquito resting surfaces with residual insecticides. Stability of such suspensions depends upon a number of factors, important among which are (*i*) type of clay component; (*ii*) extent of grinding of technical D.D.T.; (*iii*) quality and quantity of the wetting and dispersing agents used and (*iv*) electro-kinetic state of the final suspension (Thomas, 1956; Bami, 1955; Bami and Cheema, 1957). In a good quality water dispersible D.D.T. powder, all these factors are so adjusted that the resultant product displays the desired physical properties, concerning particle size, *p*H and suspensibility. W.H.O. Insecticides Expert Committee (1952) laid down physical and chemical standards for water dispersible D.D.T. powders, which have been further revised (W.H.O. Insecticides Expert Committee, 1956) and also adopted by the Indian Standards Institute* (1955). Extensive quality control work in these laboratories on 50 and 75 per cent D.D.T. dispersible powders from divergent sources (Bami, 1955; 1957*b*) has specially emphasized the importance of suspensibility which

*Both these specifications are almost identical but in case of suspensibility, I.S.I. specifications prescribed a minimum of 50 per cent while W.H.O. specifications allowed a tolerance of ± 10 per cent on the values obtained on account of method of test.

was prone to wide fluctuations and also sensitive to handling, tropical storage, etc. Apart from determination of suspensibility of a product as such, the above specifications also prescribed an accelerated storage pretreatment, according to which the product was kept at a temperature of 53-55° C. and 25 gm. per sq. cm. pressure for 24 hours, prior to suspensibility determination. This treatment is expected to detect possible loss of suspensibility due to physico-chemical changes in the ingredients with prolonged storage, especially under tropical conditions. The present investigations have been designed to study the extent of decrease in suspensibility when 50 and 75 per cent D.D.T. dispersible powders were subjected to accelerated tropical storage pretreatment and its possible relationship to actual loss of suspensibility by storage of the materials under tropical conditions.

EXPERIMENTAL.

Two hundred and five samples of 50 per cent D.D.T. dispersible powder were tested for their suspensibility according to the W.H.O. (1956) specifications method but without accelerated tropical storage pretreatment. The values of D.D.T. in suspension ranged from 45 to 70 per cent. The amount of clay which remained in suspension was also determined in several cases as follows:

Fifty per cent D.D.T. dispersible powder (12·5 gm.) was suspended in 250 ml. of standard hard water and the upper 225 ml. of the suspension withdrawn as specified (W.H.O., 1956; Bami, 1957*b*). The remaining 25 ml. of the sediment and suspension was treated with excess of acetone when D.D.T. dissolved completely. The suspension of clay was filtered, washed with acetone, dried and weighed. Percentage of clay in suspension was calculated from the equation

$$\frac{1000\ (6{\cdot}25-W)}{9\times 6{\cdot}25}$$ Where W = Weight of clay found in 25 ml. of the residue in the cylinder.

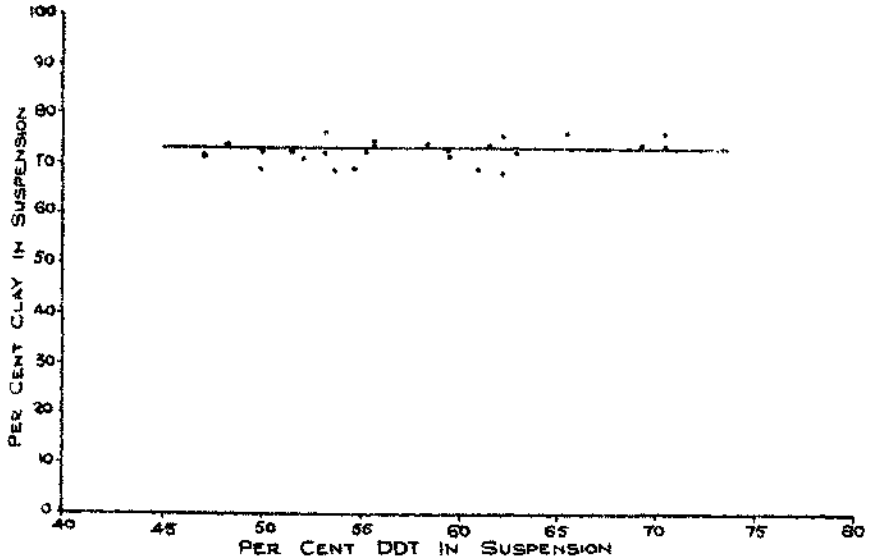
The values of clay percentages in suspension were plotted against different values of suspensibility ranging from 45 to 70 per cent (which actually represent percentage of D.D.T. in suspension) in Graph 1.

The above 50 per cent D.D.T. dispersible powder samples were subjected to accelerated tropical storage treatment (I.S.I. specification, 1955; W.H.O. specification, 1956) and their suspensibility determined subsequently. The fall in the values has been represented in Graph 2. Ten samples of 50 per cent D.D.T. dispersible powder having different suspensibility values according to W.H.O. specifications (1956), were stored for six summer months in the laboratory. A duplicate set of the same samples was also stored at O°C. for the same period. Afterwards both these sets of samples were analysed for their suspensibility and the results in both cases were indentical to the initial, within the limits of experimental variation of ±10 per cent as allowed by W.H.O. specifications (1956).

Seventy samples of imported 75 per cent D.D.T. dispersible powders representing four different manufacturers were tested for their suspensibility, and the values obtained varied from 45 to 75 per cent. These samples were subjected to accelerated tropical storage treatment and the fall in suspensibility values recorded in Graph 3. Effect of actual tropical storage on the suspensibility of 75 per cent

GRAPH 1.

50 per cent D.D.T. dispersible powders. Suspension of clay in relation to D.D.T.



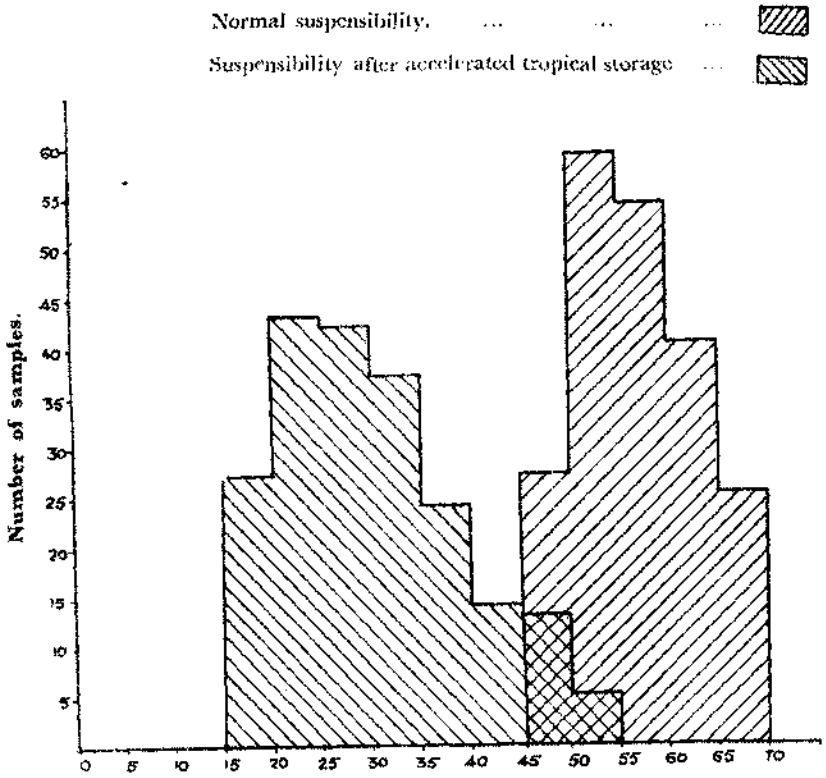
D.D.T. dispersible powders from four suppliers, was previously investigated (Bami, 1957*a*) and the mean suspensibility curves representing five samples of each of the suppliers A to D, are represented in Graph 4. However, the method of analysis, used for suspensibility, was the one prescribed in W.H.O. specifications (1952). In order to compare the previous results, with the present study of the loss of suspensibility due to accelerated tropical storage test in the laboratory, it was necessary that a correlation between the suspensibility values obtained with the previous (W.H.O. specifications, 1952) and the current methods (W.H.O., 1956; I.S.I., 1955) be established. With this object in view, several samples of 75 per cent D.D.T. dispersible powder were tested for their suspensibility according to the previous W.H.O. specifications (1952) and also analysed according to the present method (W.H.O. specification, 1956) without incorporation of the accelerated tropical storage pretreatment. As both the experimental techniques were based upon the principle of sedimentation, the calculated linear relationship was confirmed by similar correlation in experimental values within the accepted limits of experimental variations (Graph 4). Relevant decrease in suspensibility after accelerated tropical storage (Graph 3) could, therefore, be compared broadly with the previous findings, more so when they represented the supplies from the same sources, though not manufactured at the same time.

RESULTS AND DISCUSSION.

Present studies on suspensibility of D.D.T. and clay constituents of 50 per cent D.D.T. dispersible powders, indicated that irrespective of the rate of settling of

GRAPH 2.

50 per cent D.D.T. dispersible powders.

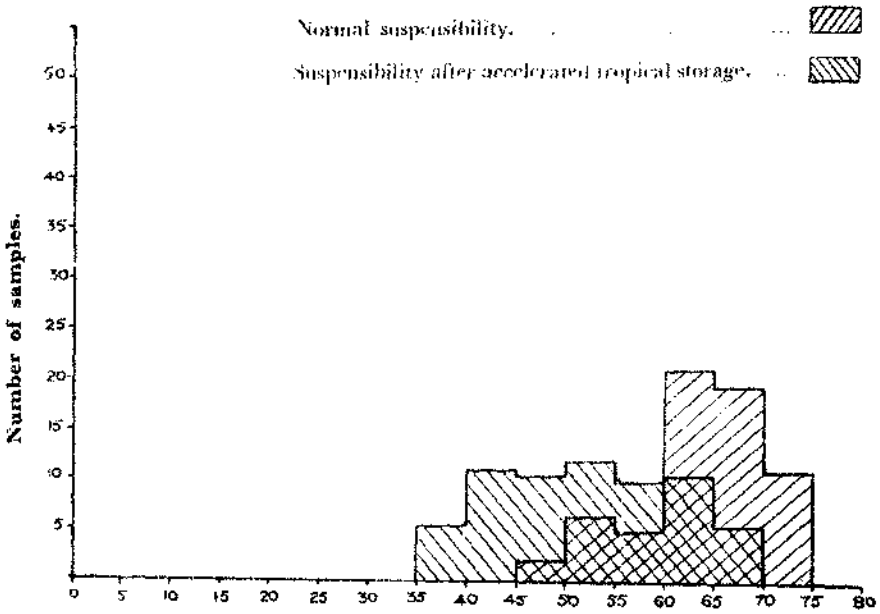


D.D.T. particles, the clay particles settled uniformly and independently. In the present cases, 73 ± 4 per cent of the clay particles remained in suspension while the proportion of D.D.T. particles varied from 45 to 70 per cent (Graph 1). Evidently, apart from the question of purity and fineness of clay, the actual dispersibility was largely governed by the rate of settling of D.D.T. particles. This in turn was dependent upon the fineness of particle size to which D.D.T. is ground, the quality and quantity of dispersing agents used and the total effect of these factors on agglomeration of particles as already investigated (Bami and Cheema, 1957; Thomas, 1956).

From Graph 2, it is evident that suspensibility of 50 per cent D.D.T. dispersible powder samples falls by nearly half on an average when subjected to accelerated tropical storage pretreatment. Due to the same reason, even though 100 per cent of the samples were having a suspensibility of at least 45 per cent, only 9 per cent met the actual W.H.O. specifications (1956) requiring at least 45 per cent of D.D.T. in suspension after accelerated tropical storage pretreatment. Similar studies with 75 per cent D.D.T. dispersible powder (Graph 3), indicated that nearly

GRAPH 3.

75 per cent D.D.T. water dispersible powders (Imported).



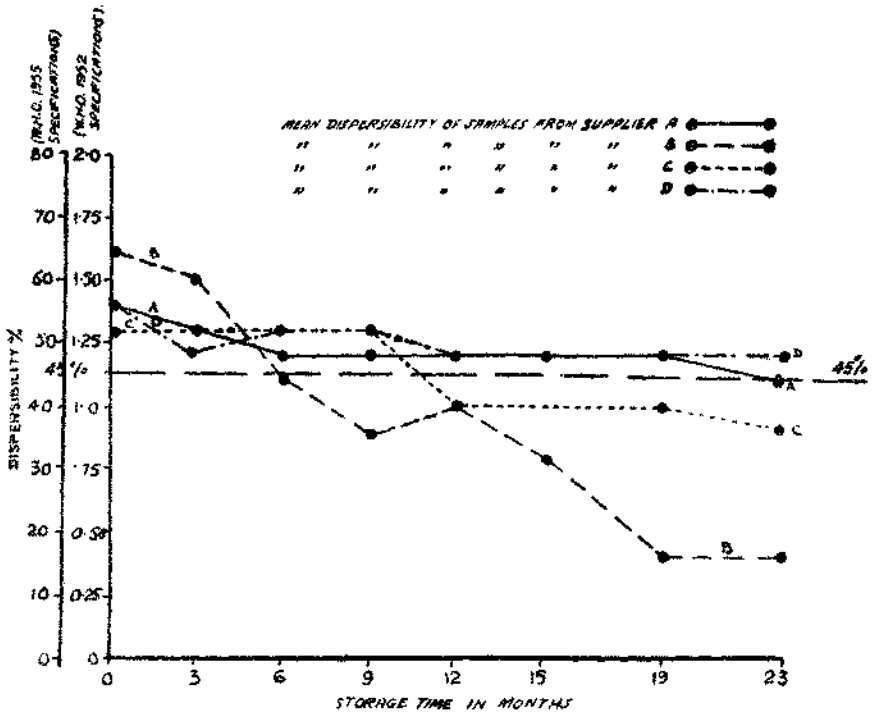
75 per cent of the total samples continued to have a suspensibility value of 45 per cent or more even after the accelerated storage pretreatment. Evidently, with the same range of suspensibility initially, 75 per cent D.D.T. dispersible powders were less susceptible to deterioration of suspensibility. In the case of present 50 per cent D.D.T. dispersible powders, such a behaviour has been associated with relatively coarser particles of D.D.T. and clay and deficient quantities of dispersing agents (Bami and Cheema, 1957). On the other hand, current investigations on 75 per cent D.D.T. dispersible powders have indicated much finer particle size of both clay and D.D.T. constituents and higher percentage of dispersing agents which collectively resist loss in suspension.

Samples of 50 per cent D.D.T. powder continued to retain their original suspensibility after six months tropical storage, and needless to say that keeping them at 0°C. afforded no advantage at all. In the case of 75 per cent D.D.T. dispersible powders, however, a much longer period of observation under actual tropical storage conditions was available for comparison (Graph 4). Products from all the four suppliers (A to D) indicated mean suspensibility values of more than 50 per cent initially according to recent methods (W.H.O., 1956; I.S.I., 1955). In case of suppliers A and D, these values remained above 45 per cent even after 23 months of actual tropical storage while product from C showed slight decrease after a year's storage which was, however, steadily maintained. Amongst the four suppliers, products from B only showed a marked and extensive deterioration in

suspensibility during second year of storage and thus 75 per cent of these products remained up to standards even after 23 months (Graph 4). These results can be broadly compared with those obtained with suspensibility studies involving accelerated tropical storage pretreatment, where three fourth of the total mixed samples continued to meet the W.H.O. specification (1956) along with high initial suspensibilities. It would be fair to conclude therefore that accelerated tropical storage preceding suspensibility analysis, is of practical value in determining the susceptibility of D.D.T. dispersible powders to deterioration under tropical conditions of storage.

GRAPH 4.

Mean dispersibility of imported 75 per cent D.D.T. dispersible powders after normal tropical storage.



SUMMARY.

Two hundred and five samples of 50 per cent D.D.T. dispersible powder and 70 samples of 75 per cent D.D.T. dispersible powder were subjected to suspensibility determinations both before and after acclerated tropical storage treatment as specified (W.H.O. specifications, 1956; I.S.I. specifications, 1955). In the case of 50 per cent D.D.T. dispersible powder suspensibility of clay was consistant at 73 ± 4 per cent. Initially both types of formulations had nearly equal range of suspensibility values, but only 9 per cent of the 50 per cent D.D.T. powders met the specifications

as compared to 75 per cent in the case of 75 per cent D.D.T. formulations. Correlation with fall in suspensibility, after actual tropical storage, indicated good agreement in the latter case. It appears that accelerated tropical storage pretreatment, prior to suspensibility determination, could help to forecast the stability of a product in tropics.

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ON TWO NEW PIROPLASMS FROM THE AFRICAN MONGOOSE, *HELOGALE UNDULATA RUFULA.*

BY

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INTRODUCTION.

THE parasites described in this paper were observed for the first time in April, 1954, when the author was working on the trypanosomes of the African mongoose.

Ten mongooses were kindly sent in April, 1954, by Professor P. C. C. Garnham from Kenya, East Africa, where they live in the burrows of white ants On examining their blood, in thin smears, all the animals were found infected with a small round, ring-like, non-pigmented, intracorpuscular parasite. The number of parasites was fairly low (about one parasite per field). Twelve more mongooses were received in February, 1955, which were also infected with this parasite.

In May, 1956, Dr. R. B. Heisch supplied ten more mongooses, which were examined within a week of their arrival. All the animals harboured the infection, which was variable in intensity; there being 1-2 parasites per field in some, while 2-3 and even 4 parasites per field were present in others.

The spleen of one of the mongooses was removed and on the third day afterwards, its blood was examined to count the number of parasites, which showed a small increase (not more than 2-3 parasites per field). On the sixth day after splenectomy, the animal died due to some unknown cause.

On June 4, 1956, another mongoose was splenectomized. A daily count was made, which showed a steady rise in the number of parasites, division forms and erythrocytes parasitized with more than one parasite. The details of the effect of splenectomy on this mongoose will be descussed later. The result was confirmed when two more mongooses were splenectomized, when they showed a steady rise in the number of parasites.

The first piroplasm from the blood of carnivores, belonging to the family Viverridae, was described by França (1908) from the Egyptian mongoose,

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Herpestes ichneumon, and was called *Piroplasma herpestides*. The most common forms were spherical bodies of 0·5 to 1·0 μ in diameter with crescentic peripheral nuclei. Some round bodies possessed a small nucleus, like a granule on one side, giving the appearance of a 'ring'. Ovoid or piriform individuals not more than 1·5-1·8 μ in length were also seen. Some other interesting forms were paired piriform or cross-shaped bodies, formed by four elongate parasites, 0·5-0·8 μ long, with rod-shaped nuclei directed towards the centre of the cross, the protoplasm occupying the periphery. 'Bacilli-form' parasites were not found. Later the same parasite was redescribed under the name of *Nuttallia herpestides* (Franca, 1910); while Thomson and Robertson (1929) called it *Babesia herpestidis* (=*herpestides*).

Patton (1910), while describing *Piroplasma gibsoni*, recorded a piroplasm, *Piroplasma* sp., from the blood of the Indian mongoose, *Herpestes mungo*. Carpano (1939) referred it to *Nuttallia mungo*; but since this parasite (*Piroplasma* sp.) was not described by Patton (1910), this name *N. mungo* automatically becomes *nomen nudum*.

Leger and Leger (1920) described a piroplasm from the civet cat, *Viverra civetta* in Sénégal. The parasite was round or avoid and did not exceed 1 μ in length. A relatively large nucleus was situated at the periphery. Double infection of the erythrocytes was exceptional. 'Bacilli-form' and 'cross' forms were absent, nevertheless the parasite was called *Nuttallia civettae*.

Bédier (1924) described *N. legeri* from the African mongoose, *Herpestes calera*. The parasite measured 1 to 1·5 μ in length and 5–8 parasites per field were often present. The 'cross' form and 'bacilli-form' parasites were absent. This piroplasm was classified as such due to its small size and round form.

Davis (1929) described *Babesia felis* from the Sudanese wild cat, *Felis ocreata*. It was referred to the genus *Nuttallia* by Carpano (1939). The parasite was small and varied from less than 1 to 2·25 μ in diameter, the majority being about 1·25 μ. The forms were mostly circular ring-shaped and resembled young malaria parasites, although parasites with irregular shapes were commonly seen which were interpreted as resulting from amoeboid movements. The organism divided into four in a cruciform manner.

Wenyon and Hamerton (1930) described *Babesia civettae* Leger and Leger, 1920; from a civet cat, *Viverra civetta* (which died in the Zoological Gardens, London) from West Africa.

Jackson and Dunning (1937) described the first piroplasm pathogenic to the domestic cat in South Africa, which caused biliary fever. Because it was closely related to *Babesia* (=*Nuttallia*) *felis* Davis, 1929, they called it *N. felis* var. *domestica*.

Nuttallia cynicti was described by Neitz (1938) from the yellow mongoose, *Cynictis penicillata* from the Orange Free State, South Africa. The author described round (1·8-2·2 μ), ovoid (1·5 $\times$ 2·2 μ) and large, round (3 to 6 μ) forms.

Heisch (1952) found a genet cat, *Genetta genetta neumanni*, from Kenya, East Africa, infected with two parasites; *Nuttallia garnhami* and *Babesia genettae*. The former parasite, circular or slightly oval, appeared as a tiny ring, 1 to 1·5 μ in diameter. Dividing forms were numerous, consisting of irregular masses of chromatin with slight projections and amoeboid forms with lobose pseudopodia

called "minute octopuses". 'Cross' forms were numerous. A number of minute young forms were also seen which measured about 0·6 μ in diameter and were circular, comma- or peardrop-shaped. The latter parasites, in the earlier stage, were piriform in shape and arranged in pairs; later on, some circular, amoeboid, irregular, oblong, dividing and 'band' forms were seen. The piriform organisms were 2·2-3·2 μ in length and about 1 μ in width. The nucleus was relatively large and multiplication took place by 'budding'.

The description of piroplasm, *Babesia vanhoofi*, from the blood of a mongoose, *Helogale varia* of Katanga, Belgian Congo, was given by de Smet and Lips (1955). Of the nine captured mongooses, four were infected. The parasite, which was rare, occupied the centre of the red blood corpuscle. The liver, spleen, lungs, kidneys and brains were invaded by the parasite. The annulations (1·16-2·23 μ in diamter) were considered to be the young forms which attained the size of 3·4 μ. The pear-shaped individuals were 1·16 μ-3·48 μ long. Blood inoculations were not successful.

MATERIAL AND METHODS.

For the cytological preparations of the parasite, the material consisted of thin blood films which were dried in air, fixed in methyl alcohol for 3-5 minutes and stained for one hour or more with Revector Giemsa stain (Hopkin and Williams Ltd.) at a concentration of forty drops of the stain in 20 c.c. of buffered distilled water, *p*H 7·2-7·4. The films were prepared from the tails of the mongooses. Impression smears of the tissues (liver, kidney and spleen) were made and stained in the same way as the blood films.

For sections, tissues and ticks were fixed in formol-saline or Carnoy's fluid. Sections (8 μ thick) were stained with Maximow's (1924) Azure II eosin and haematoxylin, iron haematoxylin or Giemsa colophonium (method of Shortt and Cooper, 1948).

TWO NEW PIROPLASMS FROM THE AFRICAN MONGOOSE.

One small circular or ovoid, ring-shaped, non-pigmented parasite has always been seen in the blood of all the mongooses. This parasite looked like the so-called *Nuttallia*.

The other, piriform in shape, appeared in the blood of the splenectomized mongoose and could be easily distinguished from the former by its staining reaction and its larger size. It looked like a typical *Babesia*.

COURSE OF NATURAL INFECTION.

The natural infection in all the thirty-two mongooses has been observed over a period of 25 months. Any of these animals examined at any time, irrespective of its period in captivity, was found infected. One pair of mongooses remained alive for over twelve months; on each examination their blood showed the presence of an occasional parasite. Although parasitæmia has not been recorded quantitatively in terms of blood count, a careful search always revealed a 'small' parasite in the erythrocytes.

EXPERIMENTAL INOCULATION.

França (1910) failed to infect a clean animal in a single intraperitoneal inoculation of blood. Davis (1929) was able to infect domestic cats, *Felis domesticus*, with the strain obtained from a wild cat, *F. ocreata*. In this way, he inoculated twenty-two cats and in no case did the infection fail to ensue, but on the other hand, attempts to infect animals other than cats [two dogs, one sheep, one calf, white mice and rats, and gebrils (Sudanese desert rat)], completely failed.

Since the present author could not get mongooses free of infection, this experiment could not be carried out, but six rats (three were less than ten days old) and ten baby mice were inoculated with infected blood on different occasions and kept under close observation for many months, but in no case has infection ensued.

COURSE OF INFECTION AFTER SPLENECTOMY.

The spleen appears to play an important role in protozoal infections. Kikuth (1927) found a heavy infection of *B. rossi*, the presence of which was not suspected before, in an African monkey of the genus *Cercopithecus*.

Davis (1929) found that splenectomy performed at any time of the infection resulted in an intense infection characterized by anæmia and hæmoglobinuria.

Heisch (1952) reported that the blood of the genet cat, after removal of the spleen, was swarming with parasites in different stages of development.

Taliaferro, Cannon and Goodloe (1931) and Taliaferro and Pavlinova (1936), in their work on *Trypanosoma lewisi* and *T. duttoni*, showed that splenectomy, which reduces the functional level of the reticulo-endothelial system of the rat or mouse, may alter the normal course of infection with these organisms.

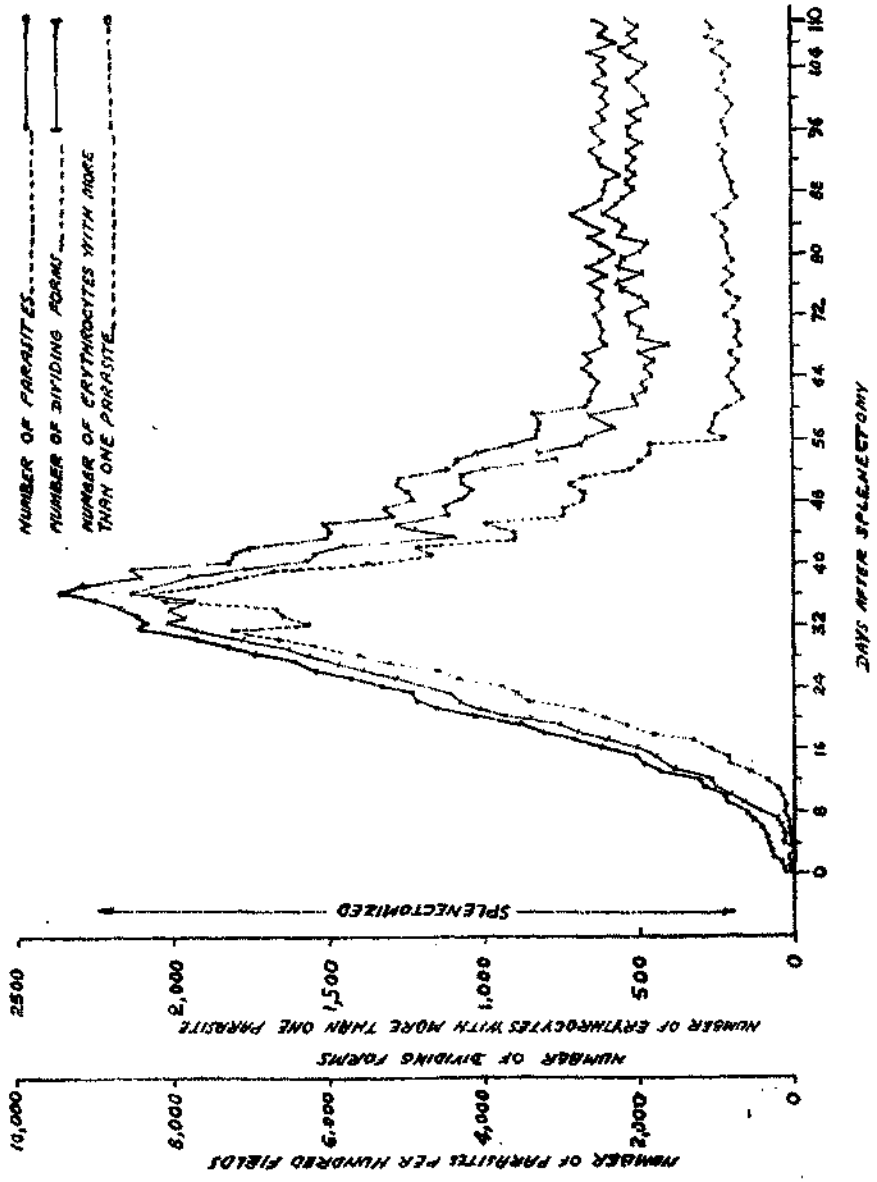
Splenectomy of the vertebrate host has been a well established technique in the study of protozoal infections, and is a standard procedure in all laboratories dealing with malaria in experimental animals. Jaswant Singh, Ray *et al.* (1951) suggested splenectomy in *M. mulatta mulatta* in cases where the malarial infection is chronic and parasites are detected with difficulties. Subsequently, using this technique, Jaswant Singh and Ray (1952) demonstrated differentiation of mixed infections with more than one plasmodia. As a rule, in such cases, one species of parasites predominates over the other, and thereby makes identification difficult, but removal of spleen is followed by high parasitæmia in respect of all the existing species. The same group of workers adopted splenectomy as a standard procedure to determine the effect of an antimalarial in the sterilization of blood-induced infection (Jaswant Singh, Misra *et al.*, 1951).

Grewal (1956*b*) found that splenectomy of rabbits, infected with *T. nabiasi*, increased the multiplication period, which continued for a longer period than usual. The number of parasites per c.mm. of blood was also higher in the splenectomized than in the normal rabbits.

The course of infection in only one splenectomized mongoose has been observed during a period of three months. The degree of infection from the day of splenectomy (June 4, 1956) onwards, with a daily count for parasitæmia, is

CHART 1.

Course of experimental infection of Babesia (Babesiella) henschi *sp. nov. in splenectomised mongoose.*



stated quantitatively in terms of parasites per 100 fields. The blood count was always made with a 1/12 inch objective and a × 10 ocular, the draw-tube of the microscope being at 169 mm. This gave a magnification of 2,000 times. Parasites were always counted from a well-prepared and closely packed film.

BABESIA (*BABESIELLA*) *HEISCHI* SP. NOV.

Just before removal of the spleen, when the first examination was made, there were 136 parasites per 100 fields, out of which 27 were dividing forms, and 4 erythrocytes had more than one parasite. The following day, the number increased up to 186 parasites (29 dividing forms and 7 erythrocytes containing more than one parasite); thereafter, the infection increased progressively (Chart 1) until on July 10, 1956, thirty-six days after splenectomy, parasitæmia was at its peak with 9,420 parasites (8,510 dividing forms and 2,080 erythrocytes with multiple infection). After this, the infection started decreasing and fluctuated between 2,500 and 6,000 parasites per 100 fields with 1,800 to 5,000 dividing forms and 170 to 1,800 erythrocytes with multiple infection.

MORPHOLOGY.

The average diameter of the red blood cells of the mongoose is 6·0-6·5 μ. In dried blood films, stained by Giemsa stain, the typical form of the parasite appears as a small circular ring-like body resembling a young malaria parasite. The dark red-staining chromatin is relatively large and its disposition is usually peripheral (Plate I, Fig. 1, 2, 3); in some, it is in the form of a small granule (Plate I, Fig. 4, 5, 6, 7, 8, 9) when it occupies only a portion of the circumference of the ring. It is never seen projecting externally. In some cases it is dispersed around a varying proportion of the periphery of the parasite (Plate I, Fig. 10, 11, 12, 13, 14), while in other cases the chromatin occupies the whole circumference, when it is difficult to distinguish the cytoplasm (Plate I, Fig. 15). In some minute forms the chromatin occupies the bulk of the parasite and the cytoplasm is barely indicated by staining (Plate I, Fig. 16, 17).

The faint blue-staining cytoplasm is very delicate. The enclosed vacuole varies in size and determines the diameter of the parasite. Due to the presence of a vacuole, the parasite exhibits a variety of shapes. The majority of parasites are *circular*, but some other forms are also present. They are as follows:—

Elongate forms, which are generally thin and much longer than broad. They vary from 1·75 to 3 μ in length and 0·25 to 0·75 μ in the width and have usually terminally placed nuclei (Plate I, Fig. 18, 19);

Rod-shaped or '*bacilli-form*' *individuals* which have the chromatin and cytoplasm more or less in equal quantity (Plate I, Fig. 20);

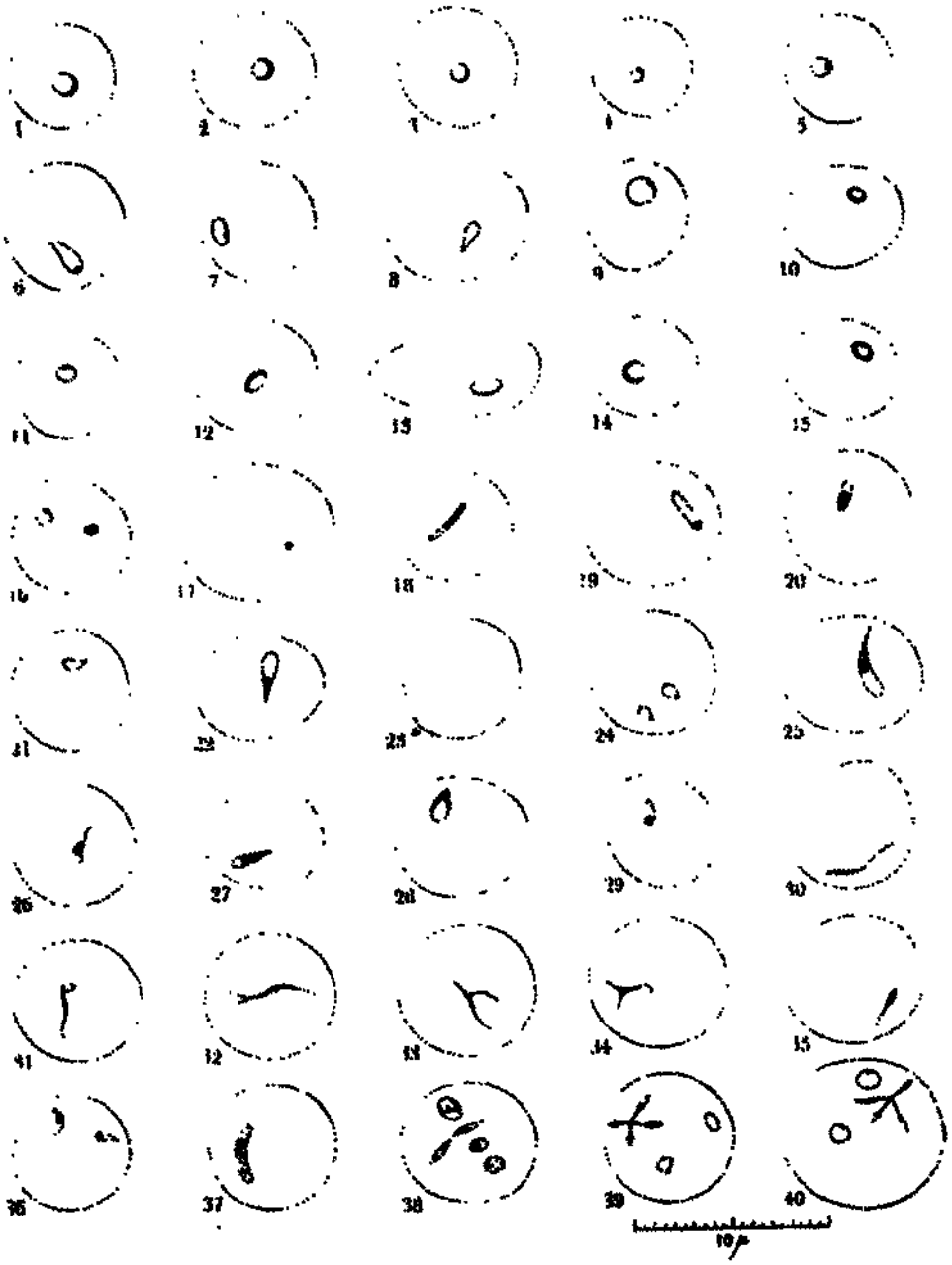
Pear-shaped forms, with variable-sized nucleus, range in size from 0·75 to 2·5 μ in length and 0·5 to 1·5 μ in width (Plate I, Fig. 6, 8, 21, 22, 23, 24);

Spindle-shaped forms ranging from 2 to 4 μ in length and 0·25 to 1·25 μ in width (Plate I, Fig. 25, 26, 27);

There are also some *oval*, *ovoid* or *oblong forms* (Plate I, Fig. 13, 28, 29);

PLATE 1.

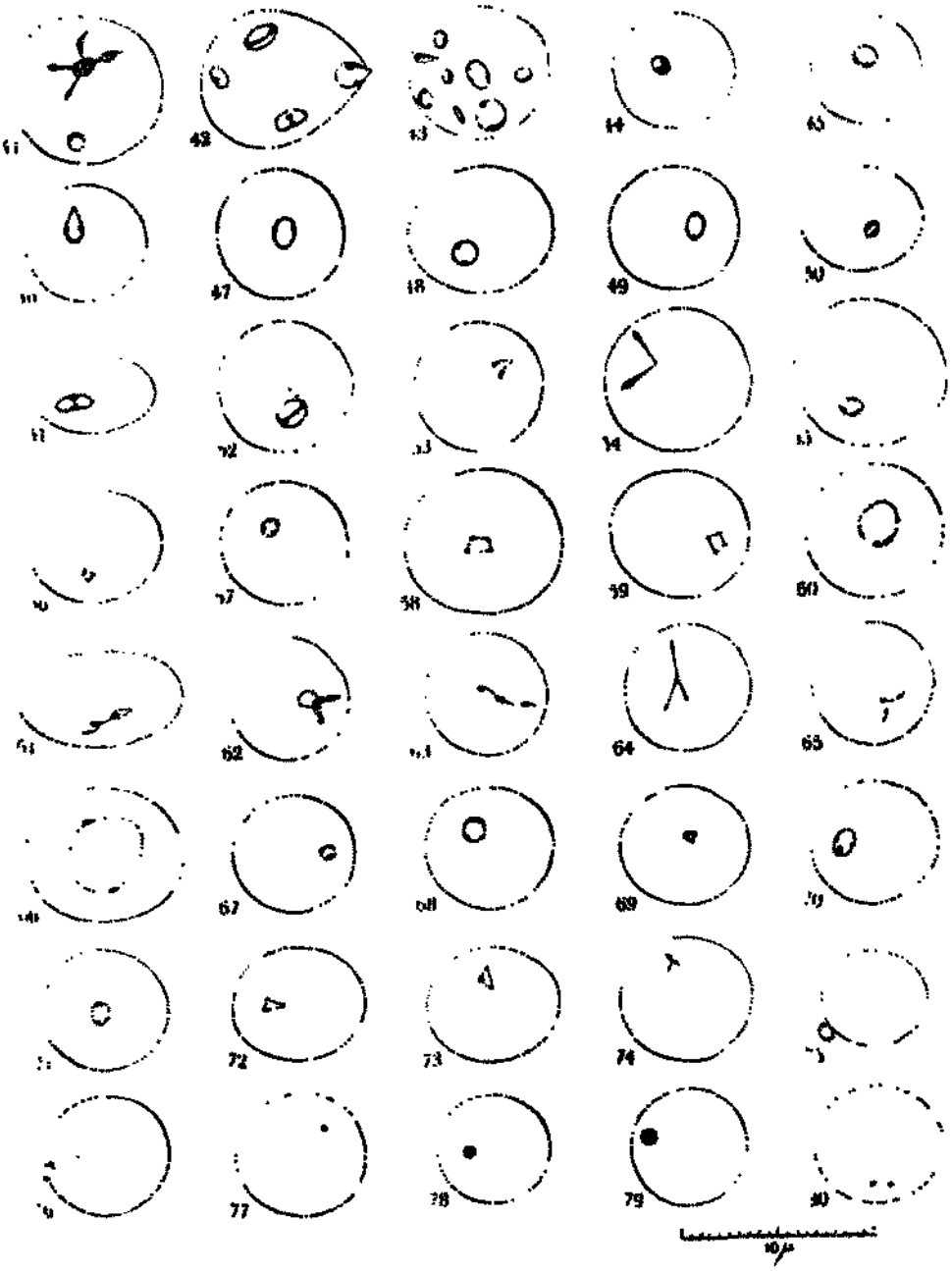
Various forms of *Hucaria* (*Babesiella*) *tatei* sp. nov.



(*Continued*)

Plate 1 (cont.)

Various forms of *Babesia* (*Babesiella*) *bovis* sp. nov.



Some *irregular forms* which appear to have resulted from amoeboid movements of the parasite are also common (Plate I, Fig. 30-34);

Some forms like a *drum-stick* (Plate I, Fig. 35), *comma-* or *peardrop-shaped* or *moonlike forms* (Plate I, Fig. 36), and *Toxoplasma-like forms* (Plate I, Fig. 37) are also found.

MULTIPLE INFECTION.

The parasitized erythrocytes, before splenectomy, usually contained a single parasite; but after removal of the spleen, when parasitæmia increased considerably, 2, 3, 4, 5, 6, 7 or even 8 parasites, representing different stages of development, were observed (Plate I, Fig. 38-43).

DIVISION.

A small granule migrates to each end of an elongated chromatin body and this process is completed when two granules get separated from each other (Plate I, Fig. 44-50). Division forms tend to be H-shaped (Plate I, Fig. 51, 52); V-shaped dividing into two (Plate I, Fig. 53-54); forms with four nuclei (Plate I, Fig. 55-60); 'cross' forms (Plate I, Fig. 39, 40, 41) or forms representing only one arm of the 'cross' (Plate I, Fig. 38, 61). Division can also be interpreted as occurring by 'budding' (Plate I, Fig. 62). There are some other interesting forms of the parasite in division which are shown in Plate I, Fig. 63-65. A form with nuclei situated at a great distance united with a fine thread-like cytoplasm, is seen in Plate I, Fig. 66.

The 'cross' forms which are the characteristics of the genus *Nuttallia* are not very common, unlike the case in *Nuttallia herpestides* França (1910); *N. equi* Nuttall and Strickland (1912), *Babesia felis* Davis (1929); *N. cynicti* Neitz (1938) or *N. garnhami* Heisch (1952). A careful search was required in order to find a typical quadruple cruciform parasite. The arms of these forms radiate from a round central body which is sometimes quite insignificant. The four resultant daughter individuals have a variable arrangement of chromatin and cytoplasm. In some, the chromatin is directed towards the point of origin and the cytoplasm is outside (Plate I, Fig. 39, 40); while in others the reverse is the case (Plate I, Fig. 41).

One very peculiar character of the division forms is that the majority of them have the chromatin divided into three separate blocks, of which two are small and one large (Plate I, Fig. 67-74). It appears that the division of two chromatin bodies into four is not simultaneous. According to Wenyon (1926), the details of the nuclear division and bud formation of the piroplasms have as yet been studied only in dry films, and so little reliance can be placed on the appearance of nuclear changes in such preparations.

As regards the effect of the parasite upon its host, the only evident change was a hypertrophy of the spleen which was considerably enlarged. After splenectomy the signs of anæmia, i.e. diffuse basophilia (polychromasia) of the erythrocytes was a prominent feature. The erythrocytes with multiple infection appeared slightly enlarged (Compare Plate I, Fig. 3-5 with 40-43). The mongoose

did not show any signs of illness throughout the infection, even when parasitæmia was at its peak. The appetite also remained quite normal.

From the rapid multiplication of the parasite after splenectomy, it appears that the daughter individuals escape from the erythrocytes and invade the fresh ones. Nuttall and Strickland (1912) observed the four merozoites of *Nuttallia equi* escaping from the corpuscle without breaking it. The process of their actual invasion could not be seen by the author, although many fresh preparations were examined under phase contrast for quite a long time. It is presumed that the parasite, after escaping from the erythrocyte, moves about in the plasma and invades the fresh corpuscles (Plate I, Fig. 23, 75, 76). Some parasites which do not leave the erythrocytes, undergo division in the same blood cell and so cause multiple infection of the erythrocytes, and parasites representing different stages of development are observed (Plate I, Fig. 38-43).

Some anaplasmoid spherical forms are commonly seen and are easily distinguishable from the piroplasms due to their dense appearances, deep red-staining and sharp outlines. They vary in size from 0·25 to 0·75 μ in diameter and sometimes two or three of these bodies are seen in one erythrocyte (Plate I, Fig. 77-80). There was no change in their number after splenectomy. It is difficult to determine whether they are actual parasites or merely Jolly bodies, which have also been seen by Davis (1929) and Jackson and Dunning (1937) in the blood of cats.

BABESIA (*BABESIA*) *HOAREI* SP. NOV.

Heisch (1952) had observed a large piroplasm, piriform in shape, which multiplied by budding, like a typical *Babesia*, in the blood of a genet cat, after splenectomy. The parasite appeared when the *Nuttallia* infection was at its height.

In the splenectomized mongoose, a *Babesia*-like parasite was observed which differed in its life cycle from *B. genettae* Heisch, 1952. It appeared on June 14, 1956, the tenth day after splenectomy, when the number of parasites per 100 fields was five (Chart 2); out of which one erythrocyte had a single parasite and two had double infection. The infection increased steadily after that, until on June 17 (thirteen days after splenectomy or the fourth day after appearance of the parasite in the blood), the number was the highest with 82 parasites; out of these, twenty-six erythrocytes contained one, sixteen contained two, and six contained four parasites in each. The infection began to fluctuate after this, until on June 30 (twenty-six days after splenectomy or sixteen days after the appearance of the parasite), it could not be seen in the blood. For 16 days it remained absent, until on July 17 (forty-three days after splenectomy or sixteen days after the first disappearance), only two parasites in a single erythrocyte were observed. After this, once again, there was a steady increase, until on July 22 (the sixth day after the second appearance), parasitæmia was at its peak with 88 parasites per 100 fields; out of these, sixteen erythrocytes contained one, seventeen contained two, eight contained four and six contained one parasite in each. Then there was a sudden fall in the number of parasites and none was seen in the blood two days after they had reached the peak. The third relapse occurred on August 1 (the eighth day after the second disappearance) when two parasites in a single erythrocyte were seen. A steady increase occurred once again, until on August 5 (the fourth day after the third

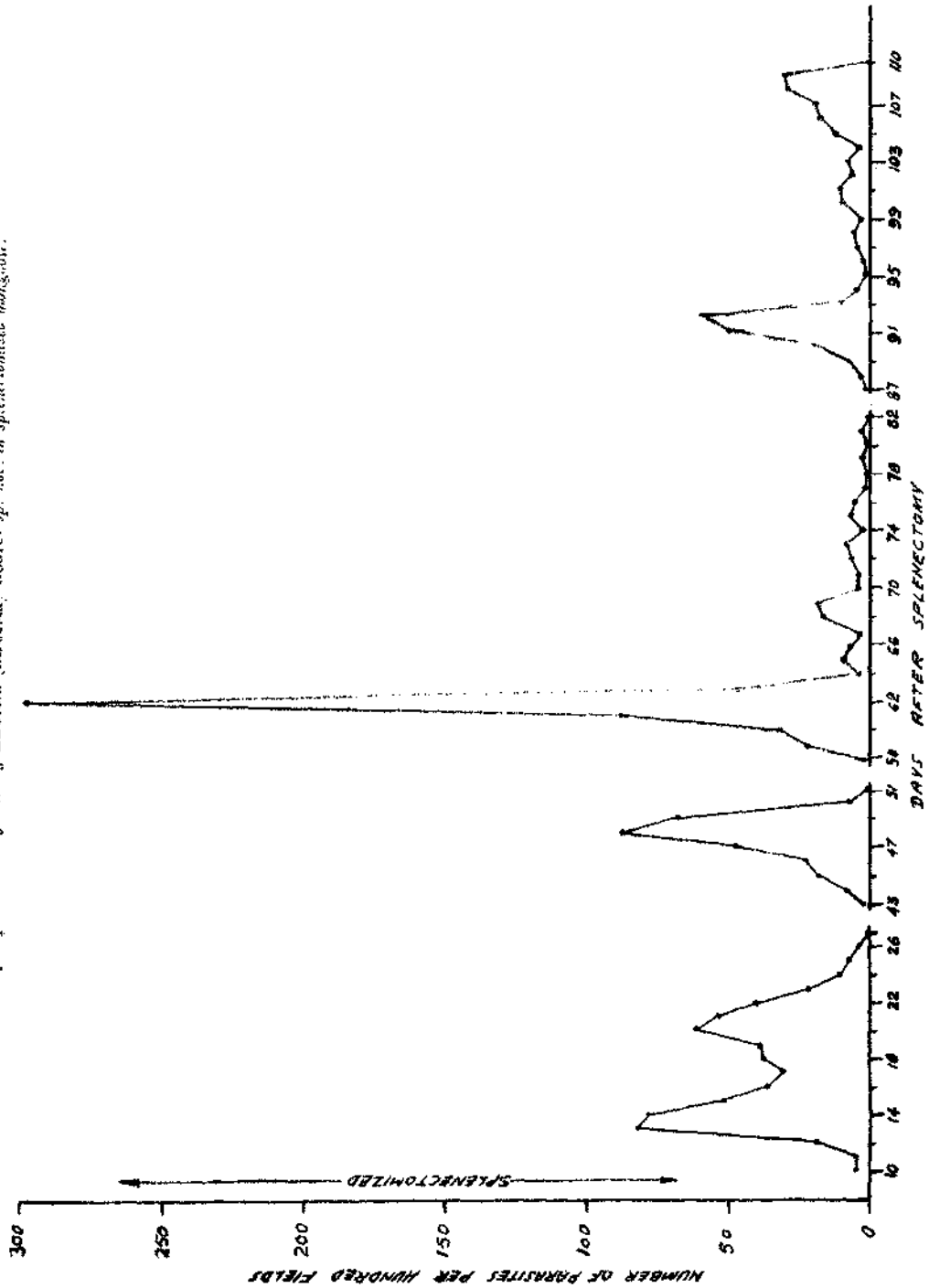


CHART 2.

Course of experimental infection of Babesia (Babesia) bigeminum *sp. nov. in splenectomized monkeys.*

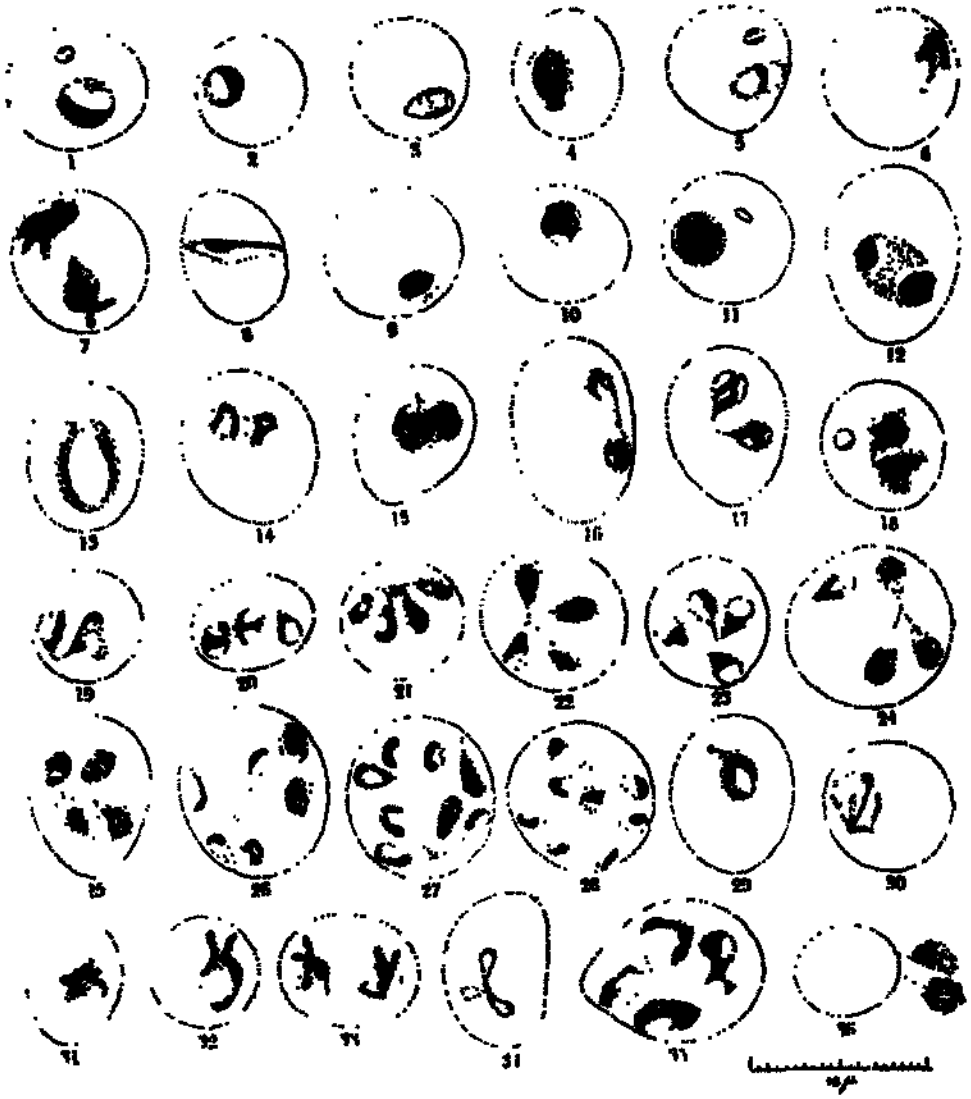
appearance) parasitæmia was at its peak with 298 parasites per 100 fields. Of these, seventy-four erythrocytes contained one, eighty contained two and sixteen contained four parasites in each. Then there was a sudden decline, after which the infection fluctuated until none was seen on August 25, 1956. The fourth relapse was observed on August 30, 1956 (fifth day after the third disappearance), when only two parasites in a single erythrocyte were seen. There was a steady increase, until on September 4 (the sixth day after the fourth appearance) parasitæmia was at its peak with 60 parasites per 100 fields. Of these, twenty-two erythrocytes contained one, thirteen contained two and three contained four parasites in each. Once again, there was a sudden decline and no parasites could be seen after September 8, 1956 (the tenth day after the fourth relapse). The course of infection of this parasite could not be followed after this.

MORPHOLOGY.

The parasites vary in length from 2·25 to 4·8 μ and in breadth from 1·25 to 4·0 μ. The smallest, more or less, piriform individuals (Plate II, Fig. 22-28) measuring 2·25 to 3·75 $\mu \times$ 1·25 to 2·25 μ with an average of 2·98 $\mu \times$ 1·73 μ, appear as a result of division and are arranged in pairs. The so-called adult forms with a single parasite in one erythrocyte are seldom piriform, but are *circular* (Plate II, Fig. 1, 2), *oval* (Plate II, Fig. 3), *elliptical* (Plate II, Fig. 4), *oblong* (Plate II, Fig. 5), *amoeboid* (Plate II, Fig. 6, 7) or *band-forms* stretching across the red cell (Plate II, Fig. 8).

The chromatin body is the most characteristic of this parasite. It is divided into two quite conspicuous portions; the round or oval body, the nucleus which takes a deep-red stain; and the other with no definite shape takes a faint-red stain. It is closely connected with the nucleus, which has no strict rule for its displacement. It may be situated laterally, centrally, anteriorly or posteriorly. There is a central vacuole which is not sharply outlined and is surrounded by a variable quantity of cytoplasm. Occasionally, the blue staining cytoplasm assumes a vacuolated appearance, a structure also noted by Nuttall and Graham Smith (1906) in the case of *Piroplasma* (=*Babesia*) *canis*. Sometimes the faint portion of the chromatin mass stretches across the entire body of the parasite in which case no vacuole is seen (Plate II, Fig. 12, 18). The chromatin body and the cytoplasm are represented in the figures by larger and smaller dots respectively.

Multiplication is by division or budding. The different stages of division are: the nucleus becomes elongated along the periphery of the body (Plate II, Fig. 9), then a constriction is formed in the middle (Plate II, Fig. 10) and the daughter-nuclei become independent (Plate II, Fig. 11, 12). Then the body of the parasite begins to divide (Plate II, Fig. 13, 14) and ultimately two pear-shaped daughter-individuals are formed which remain united with each other by a fine cytoplasmic thread (Plate II, Fig. 15-17). The two parasites proceed to divide again (Plate II, Fig. 18-20) in order to give rise to four piriform daughter-individuals which remain attached to (Plate II, Fig. 21-24) or separate from (Plate II, Fig. 24, 25) each other.



Hoare (1930) found the final formation of four merozoites in *Babesia crocidurae*. Heisch (1952) described the multiplication of *B. genettae* by budding with the formation of four daughter-individuals. De Smet and Lips (1955) described *B. vanhoofi*, as showing pear-shaped couples in the red blood corpuscles, ultimately giving rise to four individuals. On the other hand, Wenyon (1926) reported that in dogs heavily infected with *B. canis*, fifteen or more parasites may be found crowded together in a single blood cell. In this species of *Babesia*, four daughter-individuals start to undergo another division, as a result of which six (Plate II, Fig. 26) and then eight (Plate II, Fig. 27, 28) parasites are present in one erythrocyte. When eight merozoites are present, they may be attached together in groups of four (Plate II, Fig. 27) or may be separated (Plate II, Fig. 27, 28).

Division by budding is also observed and small merozoites can be differentiated by the presence of some chromatin and cytoplasm projecting out of the 'mother' individual (Plate II, Fig. 29-33).

Some peculiar forms show the process of division as well as budding (Plate II, Fig. 34, 35).

The parasites outside the erythrocytes are quite common in a heavier infection and appear to be attached to a fresh cell (Plate II, Fig. 36). Whether or not, they are free swimming forms in the plasma, is difficult to judge.

Mixed infection of the parasites described here is common (Plate II, Fig. 1, 5, 11, 18).

As regards the effect of this parasite upon its host, it is difficult to hold it solely responsible for the enlargement of spleen, because of the mixed infection of the two piroplasms. The anæmia cannot be regarded due to this parasite because even when it is hardly seen in the blood, polychromasia of the erythrocytes remains evident. Blood corpuscles with multiple infection are slightly enlarged (Plate II: Compare Fig. 2-4, 6, 10, 30-32 with 24-28, 35).

CLASSIFICATION AND DISCUSSION.

The classification of the piroplasms of the mongoose is most interesting and important because the two parasites, morphologically different from each other, are present in the same animal. After following their detailed description, it was felt necessary to set out the views of previous authors on the classification of piroplasms with a short note on author's views.

The classification of the various members of the family Babesiidæ (=Piroplasmidæ) Poche, 1913 appears to be in a state of confusion, so it is essential and is a matter of extreme importance that some general agreement, amongst workers in these diseases of animals, be reached. França (1910: 1917: 1918) recognized a number of genera on morphological grounds. A careful systematic study was made by du Toit (1918) who modified França's scheme and retained six genera (*Babesia*, *Nicollia*, *Nuttallia*, *Smithia*, *Rossiella* and *Gonderia*, and two sub-genera, *Babesia* and *Piroplasma* in the genus *Babesia*) in the family Babesiidæ. Wenyon (1926) rejected these generic and sub-generic names of du Toit and also *Microbabesia*, a generic name proposed by Sohns (1918), and *Babesiella*, a sub-genus proposed by Mesnil (1919), on the grounds that all the organisms in this family agree with one another in that they consist of cytoplasm and chromatin and

produce no pigment. He included them in a single genus *Babesia*. Two French authorities on piroplasms, Donatien and Lestoquard (1930) disagreed with Wenyon's rejection of certain genera and recognized genus *Piroplasma* consisting of two sub-genera, *Piroplasma* and *Babesiella*, and genus *Nuttallia*. Thomson and Hall (1933) believed that Wenyon's classification, which is based on essential morphological characteristics, is justified. Sergent *et al.* (1954) recognized the classification of Donatien and Lestoquard (1930). Brumpt (1949) agreed with two more genera : *Achromaticus* Dionisi, 1898 and *Babesiella* (=a sub-genus) Mesnil, 1919. Poisson (1953) followed Brumpt's (1949) classification except that he gave preference to *Microbabesia* Sohns, 1918, which was also recognized by Brumpt (1949) in a foot-note (p. 525). Travassos Santos Dias (1953) in his classification recognized only two genera: *Piroplasma* Patton, 1895 consisting of two sub-genera *Piroplasma* Patton, 1895 and *Microbabesia* Sohns, 1918; and *Nuttalia* França, 1910. Sergent *et al.* (1954) discarded the genus *Babesia* and recognized two genera: *Piroplasma* and *Babesiella*.

The affinities of the two piroplasms of the mongoose described in this paper are as follows:—

The first shows a close resemblance to the piroplasms belonging to the genus *Nuttallia* França, 1910, because it has some rare typical 'cross' forms, but the majority of the parasites during division are pear-shaped and divided into two. The typical 'cross' arrangement during division into four, was believed by França (1910) to be characteristic of the genus *Nuttallia*, but it is of little importance because Leger and Leger (1920) found a piroplasm in the civet cat, *Viverra civetta* which they called *Nuttallia civettae*, though they did not see any 'cross' forms. Bédier (1924) gave the name *N. legeri* to a piroplasm from the mongoose, *Herpestes calera* and wrote " Malgré l'absence de division en croix, nous croyons pouvoir le classer dans le genre *Nuttallia* França, 1909 (=1910), en raison de sa taille exigüe et de sa forme arrondie ".

The second parasite undoubtedly belongs to the genus *Babesia* Starcovici, 1893.

In order to place these two piroplasms in their proper place, a slight modification of Wenyon's (1926) classification has been made:—

Family	Babesiidae Poche, 1913.
Genus	*Babesia* Starcovici, 1893.
Sub-genus	*Babesia* Starcovici, 1893.
Sub -genus	*Babesiella* Mesnil, 1919.

The reasons put forward for this classification are: that the genus *Babesia* Starcovici, 1893 has priority over the genus *Piroplasma* Patton, 1895. As regards the sub-genera, the classification by Sergent *et al.* (1954) and some other authors cannot be recognized because they have raised the sub-genus *Babesiella* Mesnil, 1919 to the rank of a genus, *e.g.*, Carpano (1934) gave the name *Babesiella felis* to a prioplasm of a carnivorous mammal, *Felis concor*, which was not suggested by Mesnil (1919). Donatien and Lestoquard (1930) had exclusively reserved this name for piroplasms of ruminants (ox, sheep and goat). *Microbabesia* Sohns, 1918, according to some workers, claims priority over *Babesiella* Mesnil, 1919 but Sohns (1918) called it a

genus (*Microbabesia divergens*) and not a sub-genus, so it cannot be brought to sub-generic standard.

Once again, preference is given to the sub-genus *Babesia* Starcovici, 1893 to *Piroplasma* Patton, 1895 because of its priority and also because *Piroplasma* is used rather as a vague term and rarely as an accurate name.

The first piroplasm described in this paper is classified as belonging to the genus *Babesia*, sub-genus *Babesiella*. The arrangement of the generic and sub-generic names is adapted from Hoare (1949). This piroplasm of the African mongoose, *Helogale undulata rufula* Peter, 1852, is regarded as a new species, as it occurs in an entirely new type of host, the piroplasms of which have not previously been recorded. The author has much pleasure in naming this piroplasm *Babesia* (*Babesiella*) *heischi* sp. nov. after the name of Dr. R. B. Heisch, who was the first person to see this piroplasm in the blood of these animals.

The second piroplasm belongs to the genus *Bebesia*. As it is different in morphology from the first parasite, it is placed in the subgenus *Babesia*. Its appearance and disappearance from the blood is its most interesting feature. A new specific name is given to it, and it is author's great pleasure to name it *Babesia* (*Babesia*) *hoarei* sp. nov. after the name of Dr. C. A. Hoare, F.R.S.

Data concerning the natural mode of transmission of the parasites have not so far been collected. A careful search for ectoparasites provided the author with five ticks, *Rhipicephalus* sp., which were examined by cutting serial transverse sections. No developmental stages have yet been found.

SUMMARY.

1. Two, non-pigmented, intracorpuscular and non-pathogenic parasites of the African mongoose (Peter's pigmy mongoose), *Helogale undulata rufula*, have been described.

2. One is a small parasite with round, elongate, rod-shaped, 'bacilli-form', spindle-shaped, oval, ovoid, oblong or amoeboid shapes. Division is into two, three and sometimes into four by 'cross' arrangement. This parasite is called *Babesia* (*Babesiella*) *heischi* sp. nov. There was a steady increase in parasitæmia after splenectomy (about 90 parasites per field) after which there was a steady fall. Parasites persisted in the blood for a long time.

3. The other parasite is a circular, oval, elliptical, oblong or amoeboid form which on division into 2, 4, 6 and 8, gives rise to piriform individuals and varies from 2·25 to 3·75 $\mu \times$ 1·25 to 2·25 μ. The parasite appeared in the blood only after splenectomy. Relapses occurred three times. On the first and second appearances, parasitæmia went up to one parasite per field, and on the third appearnace to about three parasites per field. On the first occasion, there was a steady fall with fluctuation in the number of parasites. On the other two occasions, there was a sudden fall. Each time there was a complete disappearance of the parasite from the blood. This parasite is named *Babesia* (*Babesia*) *hoarei* sp. nov.

4. A new classification of the family Babesiidae is proposed.

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INVESTIGATION OF FILARIASIS PROBLEM IN BALLIA TOWN (UTTAR PRADESH).

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[June 18, 1956.]

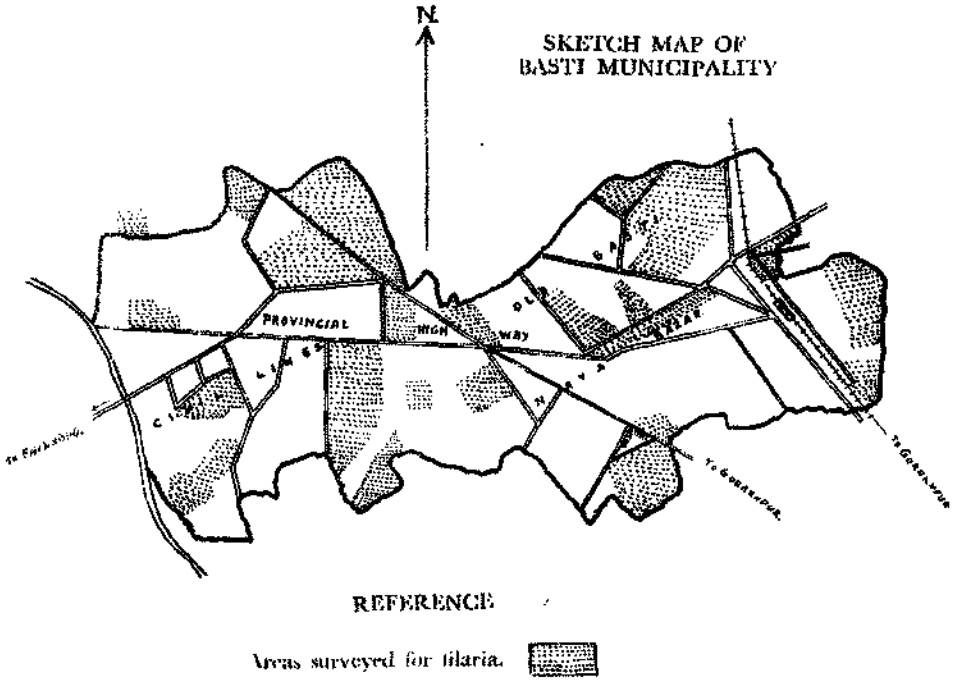
THE National Filaria Control Programme in Uttar Pradesh commenced in the year 1955 by the establishment of three Filaria Survey Units, one at Ghazipur, the other at Jaunpur and the third at Basti and one Filaria Control Unit at Ballia. The present paper deals with the survey work carried out in Ballia Town before the start of the actual control operations.

Ballia is the eastern most district of Uttar Pradesh, bordering on Bihar, separated from the Shahabad District by the River Ganga flowing about two miles south of the district headquarters and Chhupra District by the River Gogra flowing on the north-east. It adjoins the district of Gorakhpur on its north-west and the districts of Azamgarh and Ghazipur on its west and south-west (Map).

The area is deltaic, being situated between the two main rivers of Ganga and Gogra and crossed by many other small rivulets like Sarju and Tons. The sub-soil water level is high. The average annual rainfall is reckoned to be about 41·93 inches, on the basis of the average of the last three years, 1953-55. The rainy season extends from mid-June to the end of August. Road and rail communications exist between Bihar and Ballia and there is a frequent migration of population.

Ballia Municipality is a small town with a population of 30,638. The town is situated on the bank of the River Ganga which is flowing on its south and the

MAP.



Katchamala on its western border. No public utility services have been provided so far, though both the water supply and the surface drainage are under construction. There was no drainage system at the time of survey; waste water being let out from the houses into the open streets and allowed to soak or dry in course of time. Every house drain is thus a potential breeding place for culicine mosquitoes. Railway line passes through the town, dividing it in two halves—northern and southern. There are a large number of borrowpits on the sides of railway tracks. Besides, there is a large pit situated in the heart of the town. A few brick-kilns are situated in the immediate outskirts of the town.

Judging from the number of the educational institutions, literacy rate seems to be fairly high. The town has two degree colleges and a number of high, middle and primary schools. In spite of high rate of literacy, the people are not 'health-conscious'. Although there is a high incidence of disease in the town, very few people know that it is caused by mosquito bites, and still fewer take any preventive measures. The use of mosquito nets is rare.

The filaria survey was conducted in the town from November 1955 to May 5, 1956 between 20·30 and 24·00 hours every night. 3,179 persons, comprising more than 10 per cent of the population, were examined. Advance publicity by cinema shows, lectures and articles in the local press helped a great deal in eliciting public response in spite of the odd hours of the visits.

INFECTION RATE AND AVERAGE INFESTATION.

Out of 3,179 slides examined, 383 were found positive for *W. bancrofti* infection. Five per cent sample of the total slides collected, were got confirmed from the Malaria Institute of India, Delhi. The overall infection rate was found to be 12·05 per cent.

Total Mf. count was 10,899 giving an average infestation rate of 28·19 in 20 c.mm. of blood. The number of microfilariæ, found in one slide, varied from a maximum of 334 to a minimum of one. The youngest children showing infection and disease were 3 and 4 years old, respectively.

In Table I, the infection rate has been shown as found in different age groups.

TABLE I.

Infection rate according to age groups.

Age group	Number examined	Positive	Infection rate per cent
2– 5 years ...	40	2	5
6–10 years ...	175	11	6·3
11–15 years ...	287	35	12·2
16 and above ...	2,677	335	12·6

It will be noted that the infection rate which is high even in the first age-group, shows an increase till the 11-15 years age group is reached. This fits in with the average pattern in other areas in India with *W. bancrofti* (Iyengar, 1938; Jaswant Singh *et al.*, 1956).

Out of the 3,179 persons examined, 1,096 showed disease manifestations, giving a disease rate of 34·47 per cent. The chief symptom was hydrocele. Out of the population showing filarial symptoms, 58·8 per cent presented hydrocele, actual or operated. The disease manifestations in general were as shown in Table II.

TABLE II.

Percentage of the diseased population according to symptoms.

Disease manifestation	Number suffering	Percentage of total diseased
Hydrocele	644	58·8
Either lower extremity	222	20·25
Either upper extremity	147	13·31
Genitals	9	0·82
Chyluria	8	0·73
Fever	66	6·02

An analysis of the incidence of filarial infection in persons with and without manifestations of filarial disease, is given in Table III.

TABLE III.

Incidence of filarial infection.

Category of persons examined	Number of persons examined	Number with micro-filariæ	Percentage positive for microfilariæ
With filarial disease ...	1,096	86	7·81
Without filarial disease	2,083	297	14·20

From Table III, it would be noted that a higher percentage of "positives" are noted in persons without filarial disease, i.e., a carrier state exists.

ENDEMICITY RATE.

In the locality, endemicity rate works out to be 43·81 per cent which is hyperendemic.

ENTOMOLOGICAL SURVEY.

Adult mosquito collections were made from thirty-six fixed catching stations of all types, scattered all over the town. A total of 1,871 *C. fatigans*, 46 Anopheles and 242 Armigeres sp. were collected up to the end of May 1956 in 43 man-hours. Man-hour density for each variety was, 43·51 for *C. fatigans*; 1·07 for Anopheles and 5·62 for Armigeres sp. (Total man-hour collection for all species combined was 50). *C. fatigans* were the most predominant, constituting 86·6 per cent of the total collection. Larval density was 954 per dip using a dipper 4½ inches in diameter and from within four inches of the surface.

DISSECTIONS.

Four hundred and four mosquitoes were dissected; details of the results are given in Table IV.

TABLE IV.

Type and number of mosquitoes collected, dissected and found positive with stage of development.

Type of mosquito	Number collected	Number dissected	NUMBERS POSITIVE FOR LARVÆ:				
			Total	1st stage	2nd stage	3rd stage	4th stage
C. fatigans ...	1,871	365	17	4	4	4	5
Anopheles sp.	46	8	1	...	1*	...	...
Armigeres sp.	242	31	...	...	...	...	...

* Positive Anopheles sp. was *A. splendidus*.

It will thus appear that the vector in this area is *Culex fatigans*, which is prevailing in very high density. 4·6 per cent of *C. fatigans* dissected, were found positive for different stages of development of larvæ. One *A. splendidus* was also found positive, but only sausage shaped larvæ were found in thorax and it could not definitely be established whether it was from human or animal source. In this connection, blood from 17 animals (dogs 9, goats 4, monkey 1, cows 2, bull 1) was also examined, but none was found positive.

SUMMARY.

1. A detailed filarial survey has been carried out in Ballia Town of Uttar Pradesh for the first time.

2. The area is hyperendemic; endemicity rate being 43·81 per cent. The infection is exclusively of *W. bancrofti*. No growth of *Pistia stratiotes* vegetation was observed in the town.

3. The infection rate is 12·05 per cent with an average infestation rate of 28·19 in 20 c.mm. of blood. The disease rate is 34·47 per cent which is fairly high. The chief presenting symptom is hydrocele.

4. The vector species is *Culex fatigans*; all the stages of development of the infective larvæ having been found in it. The mosquito population is very high; man-hour density being 43·51 for *C. fatigans*.

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COMPARATIVE EFFICACY OF 50 PER CENT DIELDRIN-RESIN DISPERSIBLE POWDER AND 50 PER CENT DIELDRIN DISPERSIBLE POWDER.

BY

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[December 12, 1956.]

APPLICATION of suspensions of insecticides like D.D.T., B.H.C. or dieldrin on mud surfaces results in sorption of the active toxicant into the surface and consequent loss of biological activity (Hadaway and Barlow, 1952*a*: 1952*b*; Pal and Sharma, 1952). Considering the seriousness of the problem, particularly in tropical malarious countries, where houses have mud walls, several aspects have been investigated to overcome this handicap. Physically insecticidal formulations behave like pure crystals of these insecticides and it has been observed that coarser particles in these cases would be sorbed to a much lesser degree into the mud surfaces when compared to fine particles. But this approach has limitations. For instance, insecticide particles above 40 microns in diameter are not normally picked up by the mosquitoes and as such coarse particles, even though available on the surface, would have relatively little biological activity. On the other hand, requirements of adequate dispersibility (World Health Organization Specifications, 1956; Indian Standards Institute Specifications, 1955) make it necessary that some of the material should be constituted of superfine particles. For example, D.D.T., B.H.C. and dieldrin dispersible powders meeting the W.H.O. or I.S.I. specifications, have more than 50 per cent of their insecticide contents ground to a particle size of below 10 microns (Bami and Cheema, 1957). Evidently, the advantages offered by fine powders as regards good dispersion and contact toxicity are counter-balanced by extensive sorption of such fine material into the active mud surfaces where it is not available to the insects.

To prevent the sorption of insecticide particles into the mud surfaces and consequent prolongation of their residual effectiveness, various materials like phosphate solutions, urea formaldehyde, resins, etc. have been tried as pretreatments with some success (Hadaway and Barlow, 1952*b*, Macs 1952, Bracey, 1953). There are many practical difficulties associated with this method although theoretically the

approach is sound. Application of high dosage of insecticide on the mud surfaces would lead to superimposition and aggregation of particles and may offset the sorption handicap to some extent. However, in the case of insecticides of high intrinsic toxicity, such as dieldrin, this approach would be uneconomical.

Dieldrin in the form of 50 per cent dispersible powder has been used at dosages of 25 mg. and 50 mg. per sq. ft. in malaria and filaria control programmes, respectively, in India. It has also been found that due to sorption, dieldrin particles of 10-20 micron size have been found to be toxic for less than three days when sprayed on mud surfaces (Hadaway and Barlow, 1952*a*). Considering the fact that 50 per cent dieldrin dispersible powder must be air-milled to a very fine particle size in order to meet the W.H.O. Specifications (1956), the question of loss of active toxicant, which itself is used in low dosage, by surface sorption, is a serious handicap to the use of this material in the tropics where mud houses are the common dwellings.

Shell workers at Woodstock Research Centre in U. K. recently formulated a 50 per cent dieldrin water dispersible powder containing certain resins. It has been claimed that compared to ordinary dieldrin water dispersible powder, sorption of this material into the mud surfaces would be much less. The present investigations were undertaken to study the effectiveness of 50 per cent dieldrin-resin dispersible powder in comparison with ordinary 50 per cent dieldrin dispersible powder, on mud surfaces in the laboratory. The problem has been handled from both chemical and biological angles to elucidate (*a*) amount of active insecticide sorbed and chemical deterioration of both types of formulations on mud surfaces; (*b*) initial and residual toxicity to mosquitoes and flies.

MATERIALS AND METHODS.

Fifty per cent dieldrin dispersible powder and 50 per cent dieldrin-resin dispersible powder as supplied by Messrs. Burmah-Shell Co. were up to W.H.O. Specifications (1956). Composition of resin formulation was not known but it was supposed to contain about 25 per cent of a black resin. Mud panels (6 x 6 inches) were prepared from locally available clay and when completely dry, were sprayed with aqueous suspensions of the above two formulations at the rate of 50 mg. dieldrin per sq. ft.

Biological testing.—Two to three days old laboratory bred mosquitoes (*Culex fatigans*) and flies (*Musca nebulo*) were exposed to the sprayed panels for six and three hours respectively according to technique described by Pal (1951). To get high initial kill and to be able to elucidate difference, if any, in the residual toxicity of the two formulations, insects were exposed to the sprayed surfaces for long periods of three and six hours. In each experiment, three replicates were done and an average percentage mortality after 24 hours, was recorded. Same panels were used for subsequent exposures at weekly intervals till such time as percentage mortality in test insects was less than 50 per cent.

Chemical estimations.—As both the formulations were obtained from a reliable source, estimation of technical dieldrin by total chlorine method (W.H.O. Specification, 1956) suitably modified, was considered adequate. Initially, dieldrin at the

surface of the panels was removed by applying cellophane tape and the tape suitably extracted with benzene. The residue obtained after evaporating the solvent extract, was contaminated with sticking material extracted from the tape itself and estimation of dieldrin by total chlorine method gave very high and inconsistent values. Similar difficulties were also encountered by Alessandrini (1955) in the case of DDT and silicone lubricants were successfully used instead. However, in the present studies the following method worked satisfactorily.

Two panels (total area ½ sq. ft.) sprayed with dieldrin-resin preparation were completely scraped up to a depth of 3 to 5 mm. and the total scraping extracted with benzene for eight hours in a soxhlet extractor. Two more panels, sprayed with the same formulation, were completely covered with cellophane tape and the tape pressed on the entire surface to pick up the particles available at the surface thoroughly. Cellophane tape was gently removed and discarded. Sorbed deildrin in these two panels, was removed by scraping to a depth of 3 to 5 mm. and extracted with benzene as described above. An equal amount of mud sample obtained from an unsprayed panel was extracted similarly to provide the blank reading. Benzene extracts in all cases were evaporated and the residues analysed for their dieldrin content (W.H.O. Specifications, 1956; Bami, 1957).

The above analytical procedure was duplicated in the case of panels sprayed with ordinary 50 per cent dieldrin dispersible powder. Estimations of both the above formulations were repeated at weekly intervals using a fresh set of eight sprayed panels. The results for dieldrin, available at the surface of the panels, were obtained by difference in total and sorbed dieldrin in each case (Tables I and II).

TABLE I.

Fifty per cent dieldrin dispersible powder sprayed on mud panels.

Time in weeks	Dieldrin estimated in mg. per sq. ft.			Per cent mortality in *M. nebulo*	Per cent mortality in *C. fatigans*
	Total (A)	Sorbed (B)	At surface (A-B)		
I	64·6	55·0	9·6	62·0	35·0
II	72·8	*	...	61·3	43·3
III	35·0	26·6	8·4	42·6	36·6
IV	26·2	*	.	38·6	26·6
V	26·6	22·4	4·2	33·3	10·0
VI	18·4	16·4	2·0	28·0	13·3

* Not estimated.

RESULTS AND DISCUSSION.

In Tables I and II are summarized the chemical and biological data in respect of ordinary dieldrin dispersible powder and dieldrin—resin formulation

respectively. In the case of ordinary dieldrin water dispersible powder, even to start with, 24 hours mortality in *C. fatigans* was less than 50 per cent. In the case of *M. nebulo*, however, percentage mortality was above 50 per cent for the first two weeks only. In respect of both the test insects, mortality figures became low gradually during the subsequent period of observation. Compared to the above results, dieldrin-resin dispersible powder not only produced a higher initial kill in the test insects, but the residual toxicity also lasted for longer periods of five and three weeks for flies and mosquitoes respectively.

TABLE II.

Fifty per cent dieldrin-resin dispersible powder sprayed on mud panels.

Time in weeks	Dieldrin estimated in mg. per sq. ft.			Per cent mortality in *M. nebulo*	Per cent mortality in *C. fatigans*
	Total (A)	Sorbed (B)	At surface (A-B)		
I	62·8	43·6	19·2	92·0	65·0
II	54·0	46·0	8·0	73·3	66·6
III	38·8	33·6	5·2	66·6	60·0
IV	28·6	*	...	65·3	36·6
V	24·2	*	...	56·0	33·3
VI	27·2	21·6	5·6	45·3	33·3

* Not estimated.

These biological observations are also supported by the results of chemical analysis. The insecticide present on the surface initially was found to be more in the case of dieldrin—resin formulation when compared to that of ordinary dieldrin formulation.

From the above preliminary observations, it appears that sorption of dieldrin particles into the mud surfaces has been retarded to some extent by the incorporation of resins, with consequent increase in its biological activity. However, better performance of the resin formulation could only be elicited by exposing the test insects to the sprayed surfaces for long periods of three to six hours. This superiority of dieldrin-resin formulation would, therefore, require detailed laboratory and field investigations.

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STUDIES ON PARTICLE SIZE DISTRIBUTION IN RELATION TO SUSPENSIBILITY

Part I. 50 per cent DDT water dispersible powders.

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DDT WATER dispersible powders consist of technical DDT, clay, wetting and dispersing agents. All these ingredients are pulverised together in a grinding mill to a suitable particle size and the resultant powder can be formulated to a relatively stable suspension in water. Products displaying poor suspensibility, would cause rapid settling of DDT particles and spraying of such a suspension would offer inadequate and uneven application of the active ingredient. Evidently, apart from the quality of DDT contained in a formulation, the most important single physical characteristic of a satisfactory material would be its suspension power. Several methods have been devised to determine the suspensibility of DDT powders but the latest are those detailed in World Health Organization Specifications (1956) and also adopted in the current Indian Standards Institute Specifications (1955).

During the course of determining dispersibility of large number of 50 per cent DDT dispersible powder samples, wide variations were observed between samples from different manufacturers, and within the samples from the same manufacturer which are normally supposed to have identical composition and grinding. It was also observed that accelerated tropical storage treatment, prior to suspensibility determination according to specifications, caused varying degree of loss in suspensibility which was not always proportionate to the initial dispersibility of the products. In order to have a better insight into some of these observations, the present study on the relationship of particle size with suspensibility was undertaken in the case of four samples, three of which (A, B and C) represented one manufacturer while sample D was from a different source. Samples A to D had successively increasing suspensibility in water and all these samples were first

completely analysed according to I.S.I. Specifications (1955) and their composition and other physical characteristics determined. For the determination of particle size distribution, Andreasen's sedimentation method, as adopted by Thomas (1956), was further improved to suit the present requirements. Particle size of clay and DDT, both before and after accelerated tropical storage test (I.S.I. Specifications, 1955), was determined in all cases. In these investigations, the identity of dispersing agents used in different formulations was, however, not known and they have been dealt with in a quantitative manner. These investigations were aimed to determine the relationship of particle size with dispersing agents and suspensibility as well as to study the effect of accelerated tropical storage pre-treatment on the above factors.

MATERIALS AND METHODS.

Composition of each of the four samples (A to D) was determined by extracting a weighed amount of the sample with benzene and subsequently with hot water and alcohol. Loss of weight at each stage accounted for DDT and dispersing agents, respectively, while unextracted material was considered to be inert clay. The DDT content of these samples was also confirmed by hydrolysable chlorine method (Bami, 1957) and there was fair agreement in both these values. The clay, washed free from DDT and dispersing agents, was used for determination of particle size of pure clay separately.

Complete physical and chemical characteristics of all formulations, were determined prior as well as subsequent to accelerated tropical storage test according to I.S.I. Specifications. However, acidity and alkalinity of the products was checked with an electrical *p*H meter.

Principle of the method.—The particle size distribution was studied by the sedimentation method applying the Stoke's law. A one per cent w/v, well dispersed, suspension of the powder was allowed to settle under quiescent conditions and a 25 ml. sample was drawn from a fixed level in the suspension. This procedure was repeated at specific intervals of time. The samples drawn were analysed for their DDT and clay contents.

Since the height (h) of the suspension in the settling column and the time (t) of settling were known, the corresponding particle size could be calculated by Stoke's formula.

$$dt = \left\{ \frac{18\eta\, Vt}{(\delta - \rho)\, g} \right\}^{\frac{1}{2}} \qquad \text{(i)}$$

The above equation could be simplified to give the particle size directly in microns.

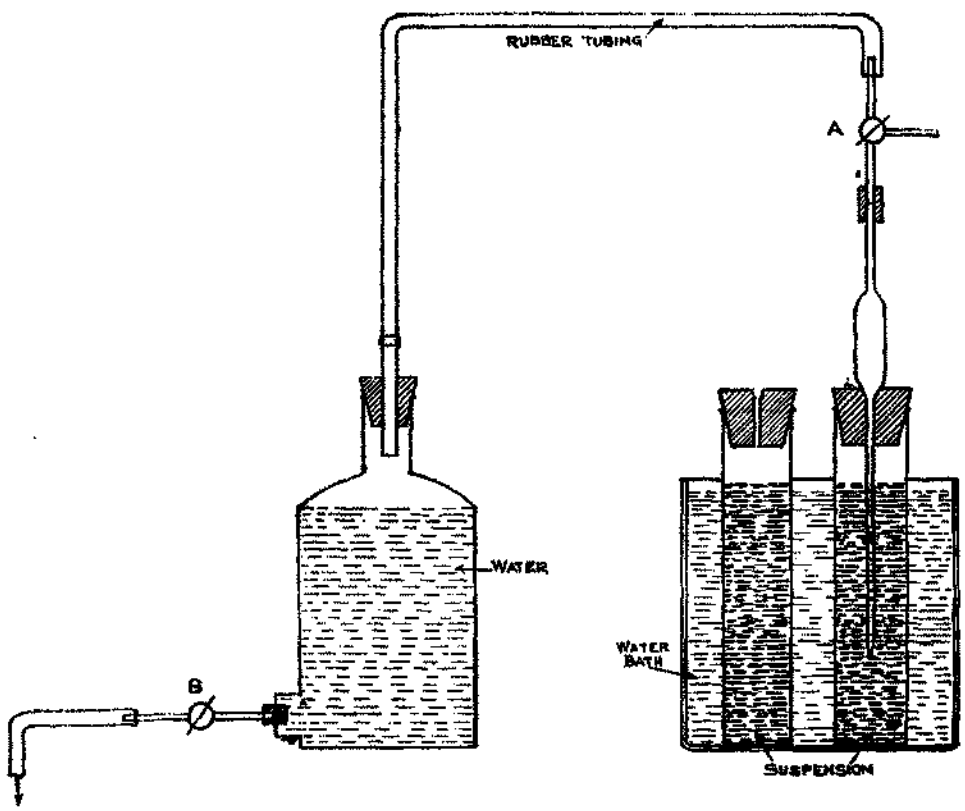
$$dt\ (\text{microns}) = 175 \left\{ \frac{\eta\, Vt}{\delta - \rho} \right\}^{\frac{1}{2}} \qquad \text{(ii)}$$

where η, δ and ρ are, respectively, the viscosity of the liquid medium and densities of the material and the liquid. The velocity of all Vt (=h/t) is given in cm/min. The samples drawn after the interval of time (t) would contain particles smaller than size dt where dt can be calculated from equation. The

data is plotted as cumulative percentage weight of sedimentation against ' equivalent Stoke's diameter dt '.

Apparatus.—A sketch of the apparatus used is shown in Figure 1. It consisted of a tall one litre measuring cylinder used as a sedimentation vessel and an aspirator bottle used as a suction device. A 25 ml. sampling pipette supported vertically was placed in the cylinder. In order to draw samples from a fixed level of the suspension column, the stem of the pipette was marked at a distance of 18 cm. from the lower tip of the pipette. The sampling pieptte was attached to the top of the bottle through a three way tap (A) which could be opened to the air or to the aspirator. The rate of suction was controlled by flow of water from bottom, tap B. The suction was adjusted in these cases to fill the pipette up to 25 ml. mark in 20 ± 1 seconds. The sample in the pipette was emptied into a tared beaker by means of Tap A. The sedimentation cylinders in duplicate were placed in a water bath maintained at 30 ± 1°C. and shielded from draughts and vibrations.

FIG. 1.



Experimental procedure.—10 gm. of the sample was weighed in a 250 ml. beaker and made into a paste with distilled water and allowed to stand for 15 minutes.

More water was added and the whole material completely transferred to a one litre cylinder. The cylinder was filled up to 1000 ml. mark with distilled water and placed in the water bath maintained at 30±1°C. to attain thermal equilibrium. The suspension was uniformly dispersed by removing the cylinder from the water-bath and the surface was cleared of any froth immediately by gentle sucking, taking care not to disturb the suspension. 25 ml. of the suspension was removed from the depth of 18 cm. after an interval of 2 minutes using the suction pipette. Samples were similarly drawn after the intervals of 7, 15, 30, 60, 90 and 120 minutes.

The total of seven samples drawn were dried on a water bath and then kept in an oven for 24 hours at 70°C. The tared containers were cooled, weighed and this offered the total weight of the solid materials including clay, DDT and dispersing agents. The DDT content of each of the above seven samples was determined chemically by hydrolysable chlorine method, suitably modified (I.S.I. Specifications, 1955; Bami, 1957). The clay content was calculated by subtracting the dispersing agent (previously determined) and DDT content from the total dry weight of the sample. Tests were carried out in duplication in all cases.

For particle size distribution of pure clay, a suspension containing 0·5 per cent (w/v) clay, was prepared in distilled water containing about 0·1 per cent of a dispersing agent like triton × 100. Samples were drawn in a similar manner, and weight of the clay in all the samples was determined by simple drying.

The maximum diameter of DDT and clay particles present at depth 'h' below the surface (t) minutes after the commencement of settling, can be calculated by applying equation (*ii*). The percentage of DDT and clay under size dt. at each of the seven sampling times, is given by the percentage weight of the material in each homogenous system =

$$\frac{\text{Wt. of material in 25 ml. sample} \times 100}{\text{Initial content of material per 25 ml. suspension}}$$

RESULTS.

The chemical and physical characteristic of all the four samples are given in Table I while the particle size distribution of DDT and clay constituents of these powders, with and without accelerated tropical storage, are given in Graphs 1-4. It is evident that DDT fractions are generally much coarser than clay. This difference of particle size distribution between DDT and clay is more marked below five and above 30 microns size. For instance, in A, B and C about 60 per cent of clay particles are below five microns and about 2 to 8 per cent above 30 microns. Only 15 per cent of DDT particles are below five microns in A, B and C and 30 per cent above 30 microns in A and B and 12 per cent in the case of C. The clay particles in D are similar in size to clay particles in A, B and C while 30 per cent of DDT particles in D are below five microns and 10 per cent above 30 microns size. The upper limit of particle size in clays is about 35 microns and that of DDT in all cases is about 75 microns. Evidently, Sample C showed much better pulverisation of DDT than in case of Samples A and B while in case of Sample D, the grinding of DDT was even superior to that of Sample C.

TABLE I.

Analysis report on 50 per cent DDT dispersible powder samples.

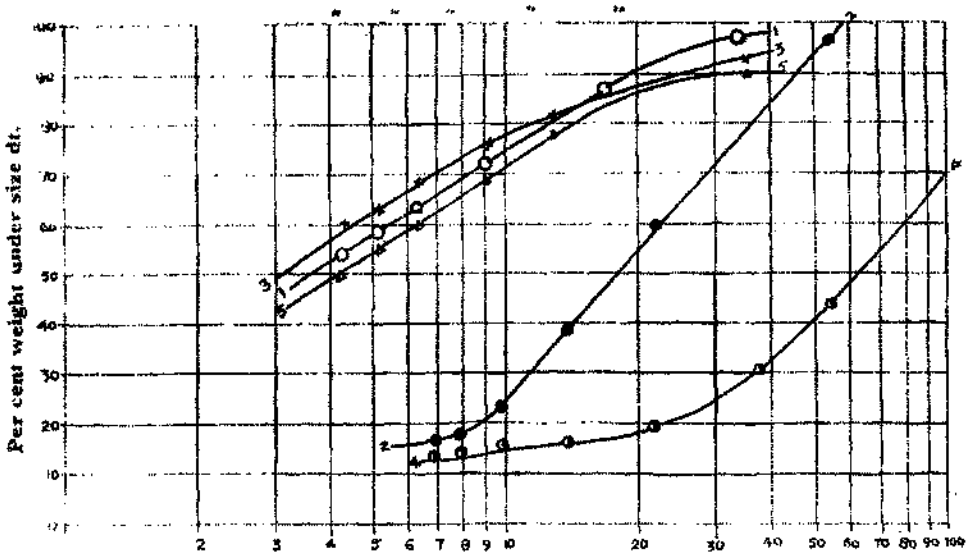
Property	SAMPLES			
	A	B	C	D
1. *Composition*—				
(*a*) DDT content (per cent) ...	54·1	49·75	49·9	48·9
(*b*) Clay content (per cent)	40·5	45·00	43·8	41·7
(*c*) Dispersing agents (per cent) ...	5·4	5·25	7·3	9·4
2. *Physical properties*: (I.S.I. Specifications)—				
(*a*) Dispersibility (per cent)	27·3	33·1	58·2	64·8
(*b*) Dispersibility after accelerated storage (per cent)	15·5	22·7	28·9	55·6
(*c*) Passing 200 mesh sieve 75 microns (per cent)	97·5	98·2	98·5	100·0
(*d*) Passing 200 mesh sieve 75 microns (after accelerated tropical storage) (per cent)	82·0	92·4	92·7	99·4
(*e*) pH	7·3	7·4	7·4	9·8
(*f*) pH after accelerated tropical storage	7·1	7·2	6·9	9·2
3. *Physical properties of the constituents*—				
(*a*) Density of clay	2·45	2·45	2·45	2·45
(*b*) Density of DDT	1·54	1·54	1·54	1·54
(*c*) Viscosity of 1 per cent suspension of the powder at 30°C. (in poises) ...	0·007	0·007	0·007	0·008

Particle size distribution of Samples A, B, C and D, which have been subjected to accelerated tropical storage, has shown that clay constituent of the powders remained unaffected to a large extent. On the other hand, DDT in Samples A, B and C showed a marked increase in particle size due to agglomeration of different particles, especially DDT. In the case of Sample D, however, the agglomeration was very slight. None of the samples, A, B and C, met the I.S.I. Specifications (1955) for dispersibility, which required at least 50 per cent of the DDT in suspension after accelerated tropical storage. Sample B offered 50 per cent DDT in suspension initially but the same determination, after accelerated tropical storage, dropped the value to 29 per cent which is far below the relevant I.S.I. requirements. The fall in dispersibility was adequately supported by appreciable agglomeration of DDT particles (Graph 2). Sample C, although having finer DDT particles when compared to those of A and B, also lost

its suspension powder after accelerated tropical storage due to agglomeration and as such did not meet the I.S.I. Specifications (1955). Sample D was however up to I.S.I. Specifications in all respects. It contained more dispersing agents, finer grinding of DDT when compared to others and showed only a slight degree of agglomeration which supported other findings.

GRAPH 1.

50 per cent DDT dispersible powder Sample 'A'



1. Pure clay.
2. DDT in the formulation.
3. Clay in the formulation.
4. DDT after accelerated tropical storage.
5. Clay after accelerated tropical storage.

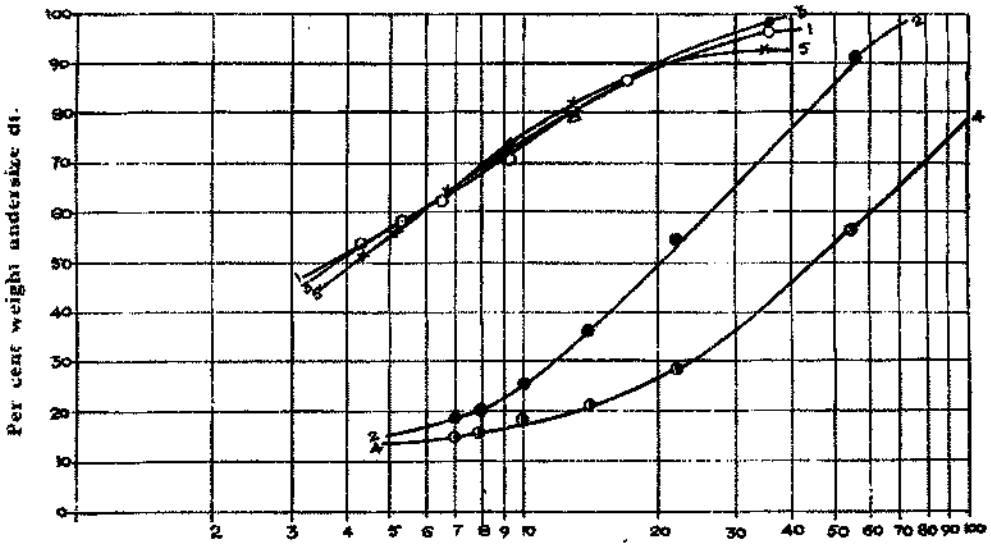
From Graphs 1 to 4, it could be deduced that 100 per cent of products A, B, C and D should pass through 75 microns seive which was proved by actual seive analysis (Table I). The effect of accelerated tropical storage test on the particle size of DDT in all the four cases, was also proportionately accounted for by similar sieve analysis results. Only Sample D met the specifications requiring at least 97 per cent of the material to pass 75 microns sieve subsequent to accelerated tropical storage treatment. *p*H in the case of A, B and C were nearly the same before and after accelerated tropical storage. However, in case of formulation D, the *p*H was slightly higher but within the specification limits.

Particle size distribution of pure clays, was studied in all cases and the results are given along with other curves in Graphs 1 to 4. In the case of Samples A, B and C, pure clay particles gave almost identical values as the formulated products,

while in case of Sample D, it appeared that superfine clay particles were agglomerated to a slightly larger extent.

GRAPH 2.

50 per cent DDT dispersible powder Sample 'B'



1. Pure clay.
2. DDT in the formulation.
3. Clay in the formulation.
4. DDT after accelerated tropical storage.
5. Clay after accelerated tropical storage.

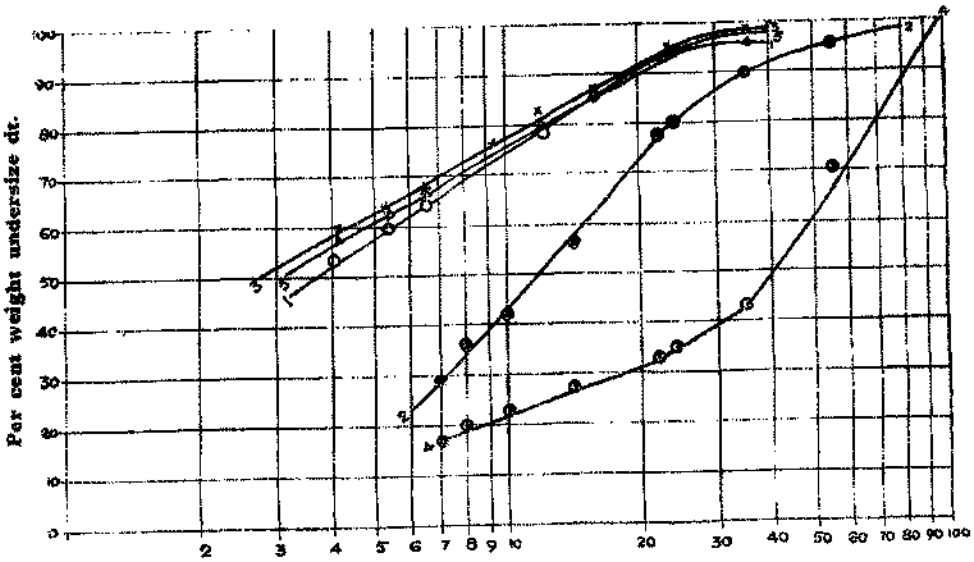
DISCUSSION.

Determination of particle size by sedimentation analysis method required that (*a*) the sample is efficiently dispersed and falls freely; (*b*) Stoke's law is applicable to the system under study. In the present studies, the particle size was required to be studied in relation to I.S.I. specifications for suspensibility and the field performance of the powder. Vigorous mechanical shaking with a view to determine the ultimate particle size as advocated by Amstein and Scott (1951), was, therefore, dispensed with. On the other hand, one per cent (w/v) suspension was shaken by hand according to I.S.I. specifications in order to have the necessary correlation with the other analytical data (Table I) obtained, according to the above specifications. Free settling of suspended particles is largely dependant upon concentration of the suspension and in this connection several workers have observed that in concentrations up to one per cent (w/v) there is negligible interference in the free falling of the various particles (Amestein and Scott, 1951; Jarrett and Heywood, 1954; Thomas, 1956). As such, the present series of

experiments were conducted at one per cent (w/v) concentration and at a temperature of 30±1°C. because it has been found that between 10 and 30° C., this method of analysis would hold good at any given temperature (Thomas, 1956). Sampling was started two minutes after the initial shaking in view of the experience of other workers (Jarrett and Heywood, 1954; Thomas, 1956) who observed that particles would not attain their steady terminal velocities immediately after the settling has begun.

GRAPH 3.

50 per cent DDT dispersible powder Sample 'C'

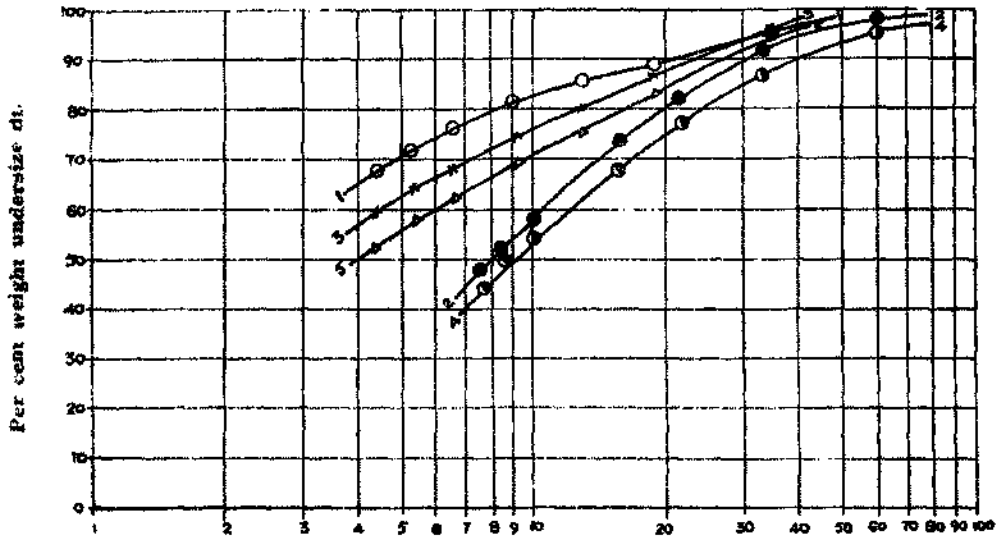


1. Pure clay.
2. DDT in the formulation.
3. Clay in the formulation.
4. DDT after accelerated tropical storage.
5. Clay after accelerated tropical storage.

Stoke's diameter dt values for a given particle may be defined as diameter of a sphere of the same density, and terminal velocity. However, in the present experiments, the DDT particles may be agglomerated with clay and as such dt values may not be strictly applicable to them. However, the method duplicates the field conditions and when it is said that 50 per cent DDT particles in a powder are below 10 microns size, it is meant that 50 per cent of DDT particles settle in the same manner as spherical DDT particles of less than 10 microns diameter would have. These results have been best interpreted by plotting them as per cent DDT or clay under size dt (in microns) on logarithmic graph paper. Although the clays have followed the usual linear pattern, DDT patterns, more so after accelerated tropical storage treatment, have not been so (Graphs 1 to 4).

GRAPH 4.

50 per cent DDT dispersible powder Sample 'D'



1. Pure clay.
2. DDT in the formulation.
3. Clay in the formulation.
4. DDT after accelerated tropical storage.
5. Clay after accelerated tropical storage.

The suspensibility of the water dispersible powders is largely governed by the fineness to which DDT along with clay and dispersing agents has been ground, the quality and quantity of dispersing agents used and electrokinetic state of the suspension. Same type of clay dispersing and auxiliary agents will usually confer the same *p*H values to powders produced in a standard manner and suspensibility of such powders would then depend largely on the fineness of clay and DDT particles. Comparison between Samples A, B and C (Graphs 1 to 3 and Table I) shows that *p*H remaining the same, a sample with progressively finer DDT particles gave better suspensibility. However, initial satisfactoriness of the material may not be the only criteria to ensure stability of the product on prolonged storage, specially under tropical conditions (Bami *at al.*, 1957). Consequently, W.H.O. specifications had introduced accelerated tropical storage test to precede the actual suspensibility determination, in order to stimulate the normal exposure of the material to tropical heat and pressure. The ingredients must withstand this test and still meet the requirements of minimum of 45 per cent of DDT (minimum 50 per cent in case of I.S.I. specifications) being in suspension. In samples, A, B and C, the *p*H remained the same after accelerated tropical storage but the particles of DDT and clay agglomerated to form heavier particles successively displaying poor suspensibility (Table I).

In the case of sample "D", which met I.S.I. specifications, it was evident that suspensibility is not only being governed by fineness of DDT particles (more than 50 per cent below 10 micron size) but also by larger amounts of dispersing agents when compared to those of Samples A, B and C. It can be concluded that having achieved the required degree of grinding of DDT in a powder (at least 50 per cent below 10 micron size), the quality and quantity of dispersing agents is the second most important factor which should be so adjusted that these materials themselves are not prone to deteriorate under tropical conditions (as judged by accelerated tropical storage pre-treatment) and are highly efficient in ultimately dispersing the powder particles.

It is not possible to draw any definite conclusion from these studies, about the effect of *p*H on suspensibility. However, a slightly alkaline *p*H (as in the case of Sample D) generally appears to be more helpful. As the I.S.I. method for the determination of dispersibility and the present particle size determination technique are based upon the same principle and employ nearly similar experimental techniques, a linear correlation has been observed between suspensibility (I.S.I. specification values in Table I) and percentage of DDT particles below 10 microns size which took half an hour to settle.

SUMMARY.

Four samples of 50 per cent DDT dispersible powders were analysed according to I.S.I. Specifications (1955), and particle size of their DDT and clay constituents was determined by Andreasen's (1928) sedimentation method suitably modified.

Clay was superfine in all the cases (about 75 per cent below 10 micron size) and remained largely unaffected. Samples A, B and C did not meet the I.S.I. specifications for suspensibility due to relatively coarser grinding of DDT and quantity and quality of dispersing agents used which caused excessive agglomeration of DDT and clay particles when the samples were subjected to accelerated tropical storage pre-treatment as required by these specifications. Sample D came up to the standards due to finer grinding of DDT (above 50 per cent below 10 micron size) and quality and quantity (10 per cent w/v basis) of dispersing agents, both of which offset the agglomeration loss and effectively dispersed the particles.

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DESCRIPTION OF A HITHERTO UNKNOWN STRUCTURE IN THE MOUTH PARTS OF MOSQUITOES.

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[March 13, 1957.]

AMONGST insects, the habit of blood sucking is a regular feature of *Anoplura*, *Siphonaptera*, and some families of the two orders *Diptera* and *Hemiptera*. In these groups of insects, as well as those non-blood sucking ones which intake liquid diet for their subsistance, the whole head capsule and the generalized gnathal appendages* have become modified into what popularly is described as piercing-sucking type. These specialized mouth parts are characterized by a long tubular, usually jointed beak (*Hemiptera*) or non-jointed proboscis (*Culicidae:Diptera*), enclosing several needle-like bristles commonly known as stylets. The outer tube is formed by the gutter-shaped labium, which is simply a protective structure for the other parts and has little to do with piercing or sucking processes. The mandibles and maxillæ, some times supplemented by or replaced by the labrum-epipharynx, are greatly elongated structures which serve for piercing the skin and also as the food channel through which the liquid food is drawn up. In some cases (*Culicidae*) hypopharynx is also modified into a stylet and contains salivary channel which opens at its tip.

Several structural variations are to be met with in the piercing-sucking type of mouth parts. These differences concern the number of functional stylets, the degree of development and specialization of the mouth appendages, and the structures involved in the formation of food and salivary channels. For instance, in the bed-bug (*Cimicidae:Hemiptera*), the labrum is a short flap that covers the groove in the labium toward the base of the latter. The labium forms the jointed beak. There are no palps and the piercing and sucking apparatus is formed by

* The labrum, epipharynx, two mandibles, two maxillae, hypopharynx, and the labium.

four stylets, two mandibles and two maxillæ. The latter are partly fused to form the food and the salivary channels (Fig. 1). The hypopharynx is reduced and

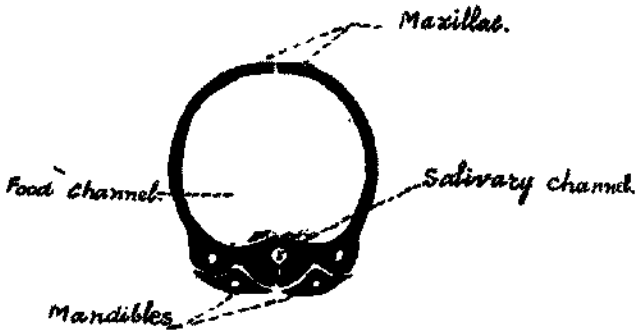


FIG. 1. Mouth parts of bed bug. Cross-section through the feeding bristles of *Cimex lectularius*. (*Enlarged from Kemper*, 1932).

its tip projects into the proximal part of the beak between the bases of the maxillary bristles. It bears the salivary opening, the secretion passing into the salivary channel formed by the maxillæ. The feeding equipment of the true-lice (*Anoplura*) is a highly specialized piercing and sucking mechanism the morphology of which is not definitely known. According to Vogel (1921), there are three superposed stylets which are believed to represent the interlocked maxillæ, the hypopharynx and the labium. The maxillæ form the food channel (Fig. 2), the hypopharynx

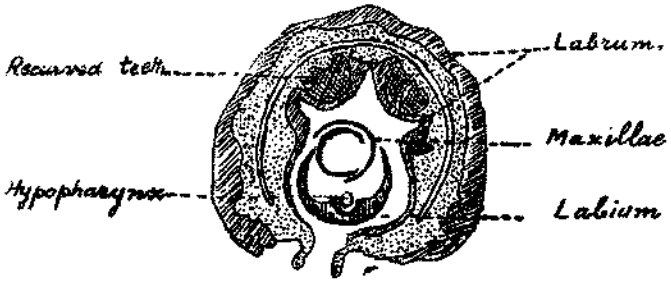


FIG. 2. Mouth parts of *Anoplura*. Section through the labium and piercing sytlets of *Pediculus vestimenti*. (*Enlarged from Vogel*, 1921).

bears the salivary opening and the ventral most stylet, labium, ends in three sharp-pointed, serrtæ lobes, forming the piercing organ of the louse. The mandibles are reduced or absent and no palps are present. Amongst the *Siphonapterous* type (fleas) there are three stylets, two mandibles and one labrum-epipharynx, which pierce the skin and enter the wound. The food channel is formed by the labrum and the apposition of the caudal edges of the mandibles. The latter also forms the salivary channel (Fig. 3). The maxillæ are short structures, each bearing a long segmented palpus. The labium is likewise short with a pair of segmented palpi. The short hypopharynx projects into the proximal end of the food canal between the bases of the mandibles.

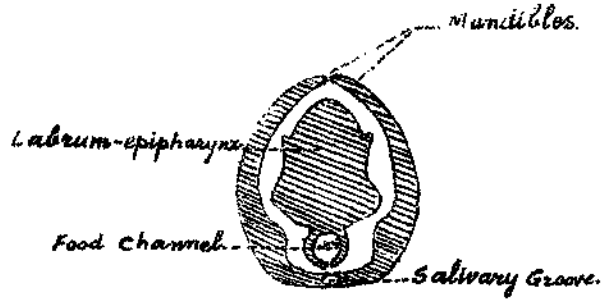


FIG. 3. Mouth parts of flea. Cross-section through stylets depicting the food and salivary channels. (*From description by Patton and Cragg as adopted by Metcalf, Flint and Metcalf,* 1951 --*Enlarged*).

In mosquitoes, and other piercing-sucking *Diptera*, there are six stylets, namely, the labrum-epipharynx, two mandibles, two maxillæ, and the hypopharynx. The food channel is formed by the labrum-epipharynx and the hypopharynx bears the salivary channel (Fig. 4A). The labium forms the tubular sheath, proboscis, around the six stylets and takes no part in feeding. Arising at the base of the maxillæ there are two long slender, jointed palps which lie on either side of the proboscis.

For suction of blood, the skin has to be pierced and the structures forming the food and the salivary channels have to be at level and apposed within the wound to be able to form an efficient sucking system. While the stylets lie within the labium they form a fascicle. But soon after they leave the labium, which folds on itself and acts as a lever to force the stylets against the tissue and ensure thereby piercing of the skin, they still seem to be held close together. This would seem essential for the sucking process, as already mentioned, particularly when two opposite stylets form the food and the salivary channel (*Hemiptera*). In case of Hemiptera, the four stylets have protractor and retractor muscles and, as shown by Weber (1928: 1930), pierce the food tissue by a series of individual jabs. First one mandible thrusts against the tissue followed by its counterpart till they meet at a point underneath the tissue and then the two maxillæ are lowered together; they lie between the two mandibular bristles. In case of mosquitoes, however, the individual stylets show feeble retractile and protractile movement and the whole stylet-complex is applied against the skin puncturing the same by a strong downward or forward thrust of the body .The labium seems to hold the piercing stylets together only at the labellar end, leaving them un-sheathed throughout their length underneath the skin. The exposed stylets during the stab remain so closely applied to each other as to give the impression of being a single rod or needle. The bundle is flexible and the penetrating part of it, within the host tissue, is capable of bending in all directions untill a capillary is pierced or an extravascular pool of blood is encountered (Gordon and Lumsden, 1939). Griffith and Gordon (1952) have even succeeded in demonstrating the flexibility and power of orientation of the tip of the stylet-bundle within the tissue of a live rodent in the process of feeding. It is surprising that during so much strain and stress, the stylets remain firmly adherent to each other without any sign of splitting apart. The phenomenon of stylets

remaining in a close fascicle has also been demonstrated with plant feeders in which case the stylet-bundle traverses all directions beneath the epidermis till the insect strikes at a vein, carrying the sap.

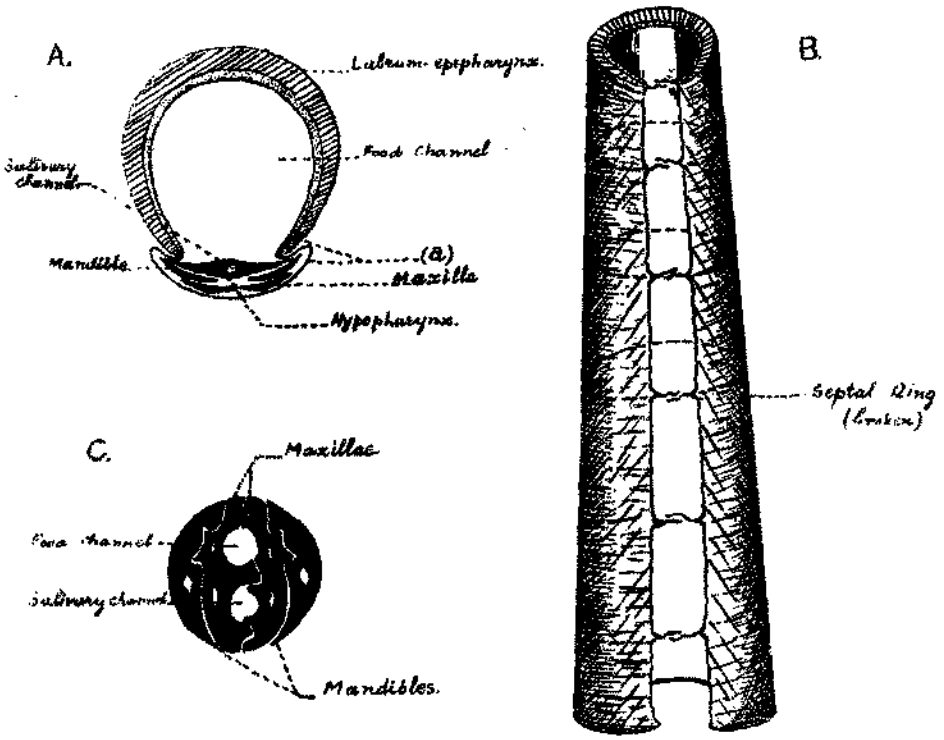
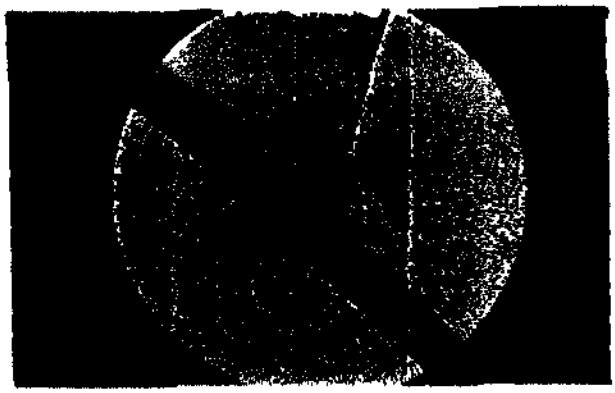


FIG. 4. *A, B*) Mouth parts of mosquito, (diagrammatic). *A.* Cross-section through the stylet-bundle depicting the food and salivary channels. Note the membranous ring (*a*) holding the bristles against the gutter-like opening (food-channel) of labrum-epipharynx. *B.* Ventral aspect of a portion of labrum-epipharynx after removal of enclosed stylets. Note the broken membranous septal rings.

(*C*) Mouth parts of squash bug. Section through the beak of *Anasa tristis* depicting bristle fascicle. (*Enlarged from* Tower, 1914).

In mosquitoes, so far, the consensus of opinion has been that the stylets are probably held together by the surface tension of the fluid in which they remain bathed, the fluid being the salivary secretion passing through the hypopharynx to the tip of the stylet bundle. But this explanation has never afforded full conviction. In case of other insects, however, evidence has been furnished about the existence of structural modifications by which the stylets are held togther. Tower (1914) working on the mouth parts of squash bug (*Anasa tristis*) observed that maxillary bristles are fluted and interlocked so as to form two tubes, the food channel and the salivary canal (Fig. 4 *C*). The mandibles which lie outside, seem to fit in the outer grooves of the maxillæ. The expanded heads of the ridges within the grooves, prevent stylets from splitting apart during the force applied in the act of piercing the skin. Because of the presence of protractor and retractor muscles, the stylets can slide on each other up to a distance equal to the length

Plate III.



Microphotographs of a section of labrum-epipharynx of *[illegible]* showing the presence of septal membranous bands. (For description see text.)

of the muscle. Towers' (1914) work is the only satisfactory explanation about the stylets holding together outside the labium. It is believed that similar mechanisms, in varying degrees, exist in other *Hemiptera* also. In these insects the food and the salivary channels lie side by side, separated merely by a median ridge and groove on the inner surfaces of the maxillæ. This permits wetting of the stylets by the salivary secretion which may be helping to hold them together in addition to the locking arrangement mentioned above. In mosquitoes, however, the salivary channel is quite apart from the food channel, being present medially within the hypopharynx. The salivary fluid can wet the stylet-bundle only at the tip. The adhesion of the stylets within the labium and through their entire length within the body tissues of host due to the salivary secretion does not seem logical. Furthermore, the stylets when dissected in salt solution seem to be non-wettable and difficult to split apart. If the stylet-bundle is severed from the head of a freshly killed mosquito and placed on a slide in a drop of water and covered with a cover slip, any amount of pressure applied on the latter, seldom succeeds in separating the stylets from the labrum-epipharynx. This character of mouth parts necessitated the present study which has brought to light the existence of membranous chitinous bands or rings at varying intervals on the inner surface of the labrum-epipharynx (Fig. 4*B* and Plate III). These rings seem to serve the function of keeping the hypopharynx, mandibles and maxillæ firmly applied against the lips of the ventral gutter-like opening of labrum-epipharynx, forming a tubular channel through which blood and other fluids are drawn up into the buccal cavity at the time of feeding.

The presence of these rings has been observed in either sex of *Aedes ægypti*, *Anopheles annularis*, *A. culicifacies*, *A. filipinæ*, *A. mangyanus**, *A. minimus flavirostris**, *A. stephensi*, *A. subpictus*, and *Culex fatigans*. It is very likely that these rings exist throughout the family *Culicidæ* and may occur in other groups of Diptera with a proboscis. Only future work will confirm this.

To know whether the number of rings on the labrum-epipharynx is specific, attemps were made to count them in the following mosquitoes.—

TABLE I.

The incidence of septal rings in labrum-epipharynx of mosquitoes.

Species	Sex	Number of rings (Range)	Species average
A. ægypti ...	Female	19–29	24·45
	Male	26–33	29·5
A. annularis ..	Female	11–24	15·70
A. culicifacies ...	Female	10–22	17·68
A. stephensi ...	Female	11–21	16·2
	Male	15–20	16·83
A. subpictus ...	Female	10–19	13·90

* Not occurring in Indian area.

Because of the great range between the minimum and maximum number of rings present in any one species, the figures as indicated above are overlapping. Therefore the number of rings cannot be considered to be a specific character. From the specimens examined, it seems that the number (range) is not constant even for the two sexes of same species.

That earlier workers overlooked existence of these structures in either sex of the mosquitoes, may be due to the fact that these rings snap when passed through clearing agents (oils, xylol, etc.). Or they might have been regarded as artifacts in the form of interruptions in the continuity of the fluid in labrum-epipharynx. It is not possible to observe these rings in the conventional mounting media due to the ill effects of passage through clearing agents and effects of the medium itself (chloral-gum). However, when the proboscis is severed and mounted in glycerine (75 per cent glycerine, 25 per cent water), preferably after mascerating it in KOH, these rings are visible even under low power (×50) of a compound microscope. These rings can also be observed in simple water mounts. Passing the mouth parts through various alcohol grades does not injure these unpigmented chitinous membranous rings.

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AQUATIC VEGETATION OF BOKARO RESERVOIR IN RELATION TO ANOPHELINE MOSQUITO LARVÆ AND ITS CONTROL BY APPLICATION OF HERBICIDES.

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[October 3, 1955.]

INTRODUCTION.

AMONG the various reservoirs of the Damodar Valley, Bokaro and Durgapur barrage ponds will have a constant pool-level nearly throughout the year since the fluctuation of a wide range would not be apparent due to the particular nature of the barrages. This would result in an excellent zonation and abundance of herbaceous plants. At Bokaro (with mean rainfall 40·19 inches; relative humidity 69·6 per cent; maximum temperature 89·9°F. and minimum temperature 64·9°F. for the period 1951-54), the water-level management is strictly in conformity with the needs of the Thermal Power House and the fluctuation level is about 1·5 ft. The soil of the submerged shore-line and the paddy fields (about 50 acres in area) is very favourable for vegetation and provides a good field for colonization of aquatic and marsh species. This is in contrast to that at Konar Reservoir, which is a seasonal fluctuating pond with the shore-line almost rocky and still unsuitable for vegetation except for some terrestrial woody and a few herbaceous species within a few patches of the soft-soil fluctuation zone.

At Bokaro, most of the fluctuation zone (Fig. 1) remains under water for nearly the whole year and it may be conveniently divided into two sections: one which is only submerged while the water-level goes up to RL 742-43 (Stations 2,3,4, 8) during and after rains; and the other which has a major portion submerged below the normal operational level, RL 743 of the reservoir (Stations 5,6,7, 1)—most of the latter is usually exposed when the water-level recedes and touches RL 739.

With their rich aquatic flora, these zones have created problem for anopheline-breeding places. Seven species of anophelines have been collected from here and of the probable vectors, *A. culicifacies* has the highest breeding potential (Table I).

TABLE I.

Incidence of anopheline larvæ in Bokaro Reservoir and fluctuation zones, July 1953 to June 1954.

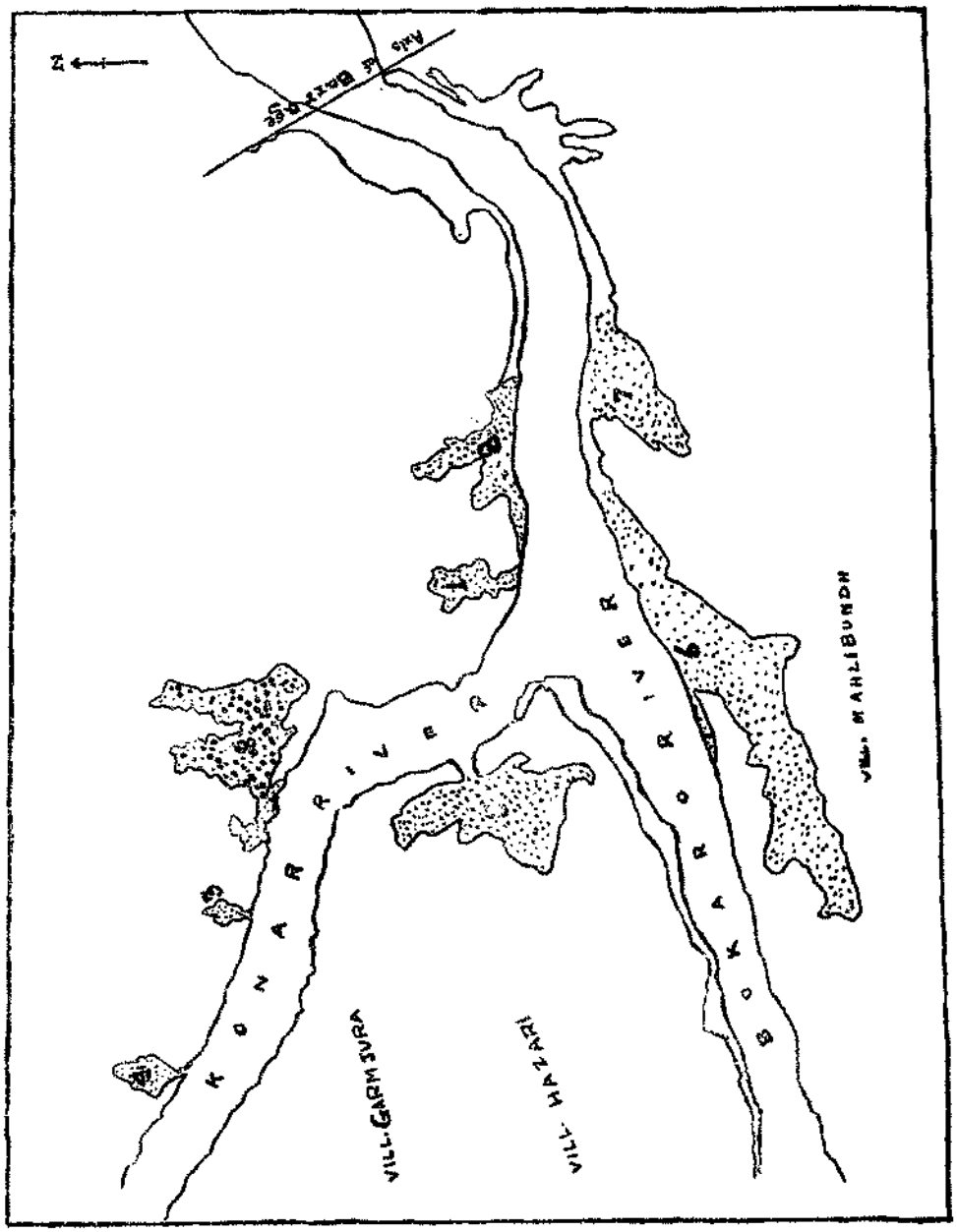
Species	Location	
	Reservoir (per cent)	Fluctuation zone (per cent)
annularis	21·6	22·1
culicifacies	38·5	30·1
fluviatilis	0·64	0
hyrcanus	5·1	8·0
pallidus	1·9	0.8
subpictus	31·4	39·0
vagus	0·86	0

Whereas the conventional larvicidal practices are being followed since the reservoir was completed, the rapid growth of the aquatic flora in the reservoir demands a vegetative growth control programme and that is how the present investigation took shape. In this study, a census of aquatic vegetation of Bokaro Reservoir is presented with a description of mechanical and herbicidal control of some dominant plant species, intimately associated with anopheline-breeding.

VEGETATION OF ANOPHELINE BREEDING PLACES AND ITS CONTROL.

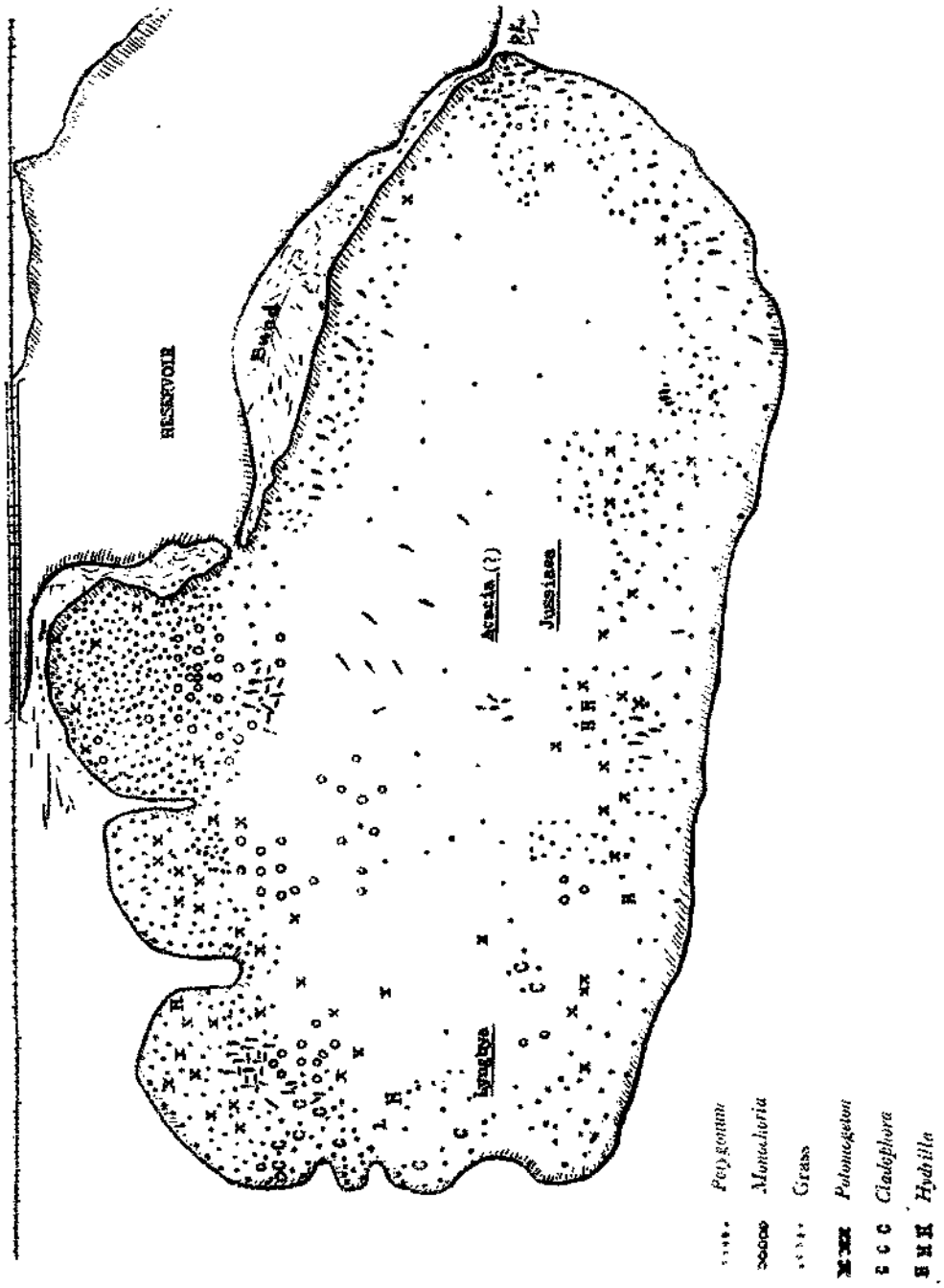
Aquatic and marsh plants constitute a major concern in any antimalaria campaign since they are intimately associated with ecology of anopheline larvæ and provide both shelter and food to the larvæ. If these sources of protection to the larvæ could be removed or controlled, the problem of malaria control within the reservoirs could be practically solved (Hinman, 1938). The plant association at Station 5-7 (Figs. 1 and 2) approach nearly the *flexuous type* (Neogy and Kachroo, 1956). They show dominance of *Polygonum* followed by *Monochoria*, floating *Potomogeton* and a host of other minor associations; from the macroscopic algal association *Cladophora* and *Lyngbya* were prevalent in March-April and were replaced by *Spirogyra* in May-June. *Zygnema* dominated at Station 5 from March-May; and from the microscopical side, desmids and diatoms were abundant. At Station 1, vegetation was a blend of *naked-erect leafy-submerged type* (Fig. 3) with *Scirpus* and grasses having an upper hand as emergent flora, and *Chara*, *Hydrilla* and *Najas* as submerged flora; associated with common *Jussiaea*, *Potomogeton*, *Marsilea* (the latter two rare in May-July). At Stations 2 and 8, *Polygonum*, grasses and *Scirpus* dominated respectively and at Stations 6 and 7 it was *Polygonum*, *Potomogeton* and *Monochoria* (May–July period). Table II gives the incidence of the various plants during March-July, 1955.

FIG. 1.



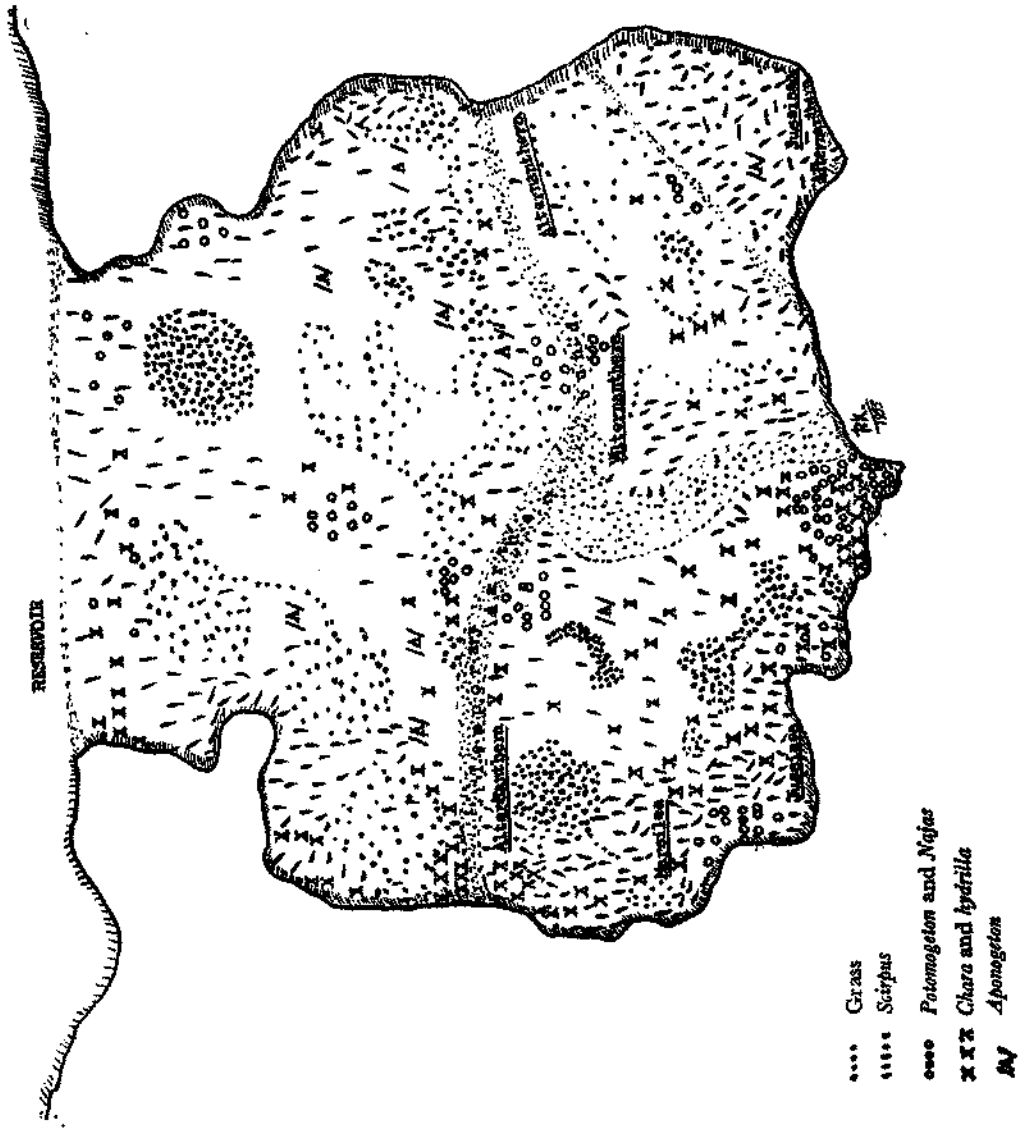
1. Bokaro Reservoir, showing various breeding stations.

FIG. 2.



Station 5, showing incidence of vegetation (April-June, 1935).

FIG. 3.



Station 1, showing incidence of vegetation (April-June, 1955).

TABLE II.

Incidence of various plants during March-July, 1955, in the fluctuation zones of Bokaro Reservoir.

Family	Species	Incidence	Numerical value of symbol*
Acanthaceae	*Alternanthera sessilis* L.	C	50–100
	Hygrophila spinosa T. Anders.	V.C.	100–200
Compositae	*Causulia axillaris* Roxb.	C	50–100
	Eclipta alba Hassk.	N.C.	15–50
	Grangea madaraspatana Poit	N.C.	15– 50
	Sphaeranthus indicus L.	N.C.	15– 50
Convolvulaceae	*Evolvulus nummularius* L.	V.C.	100–200
Euphorbiaceae	*Croton sparsiflorus* Morong	C (mostly along shore-line)	50–100
	Euphorbia thymifolia Burm.	R	5– 15
	Phyllanthus simplex Retz.	R	5– 15
Gramineae	*Ergrostris brachyphylla* Stapf.	N.C.	15– 50
Cyperaceae	*Kyllinga monocephala* Casp.	N.C.	15– 50
Hydrocharideae	*Hydrilla verticillata* Casp.	N	200
Labiatae	*Ocimum canum* Simo	N.C.	15– 50
Leguminosae	*Desmodium triflorum* DC.	N.C.	15– 50
Naiadaceae	*Aponogeton monostachyon* L.	V.C.	100–200
	Najas foveolata A. Br.	N	200
	Potomogeton crispus L.	N	200
Onagraceae	*Jussiaea repens* L.	C	50–100
Polygonaceae	*Polygonum tomeniosum* Willd.	N	200
Pontederiaceae	*Monochoria vaginalis* Presl.	V.C.	100–200
Papaveraceae	*Argemone mexicana* L.	N.C. (along shore-line)	15– 50
Rubiaceae	*Oldenlandia heynei* Br.	N.C.	15– 50

C=Common R=Rare V.C.=Very common N=Numerous N.C.—Not common.

*This is based on a census of species of the whole fluctuation area and calculated at 45×45 ft. area-wise.

Aquatic growth control is primarily directed against *Polygonum* and *Scirpus*. It is, however, difficult to control such species as *Polygonum* by water-level management only; as long as portions of its stem protrude above the water surface, it will continue to grow and extend the potential mosquito-breeding area and also shade-out the relatively less objectionable emergent species and submerged ones. Therefore, for a better maintenance of the fluctuation zone they have to receive a special attention. The two usual methods of mechanical and herbicidal measures were tried and are described below.

CONTROL OF VEGETATION.

MECHANICAL.

Water-level management would be the best method to control most of the aquatic vegetation as practised in Tennessee Valley reservoirs (Hinman and Hess, 1949) but the constant pool-level at Bokaro would not justify such a practice. The other methods would be direct attack on the plants or filling up the undesirable spots. The latter is found to be convenient and economical at Bokaro. The filling up is to be methodical so that there is no scope for some areas to be inundated again and develop into troublesome spots. After completing the filling-up operation, attention has to be diverted towards the shore-line where no weeds would be allowed to colonize.

HERBICIDAL.

Chemicals used in plant control include ammonium sulphate, sodium arsenate, sodium chlorate and 2,4 -dichlorophenoxyacetic acid (and related chemicals, like methoxone, 2, 4, 5-T etc.). Hall and Hess (1950) remark that a majority of species studied by them were found to be very susceptible to 2, 4-D formulations after full summer foliage was reached until autumn. Penfound (1953) states that " Up to the present time, no chemicals have proven practical in control of submerged plants ". However, it was probably Kraus (1941) who first proposed the utilization of growth inhibiting properties of growth regulating substances in connexion with weed control; the activities of their herbicidal properties were demonstrated by Zimmerman and Hitchcock (1942). Whereas herbicides have been used rather extensively for controlling terrestrial weeds, little data is available about their efficacy on aquatic and marsh species; except possibly those of Hall and Hess (1947:1950), Kar (1947), Uhala *et al.* (1949), Surber (1949) and Hitchcock *et al.* (1949:1950). The present studies on the assessment of efficacy of herbicides in controlling aquatic vegetation under local field conditions at Bokaro, are hoped to bridge the gap to some extent.

MATERIAL AND METHODS.

Three herbicides were selected for trials. Two of these *i.e.*, dicotox (ethyl ester of 2, 4-D) and chloroxone (80 per cent sodium salt of 2, 4-D) were compounds belonging to the series of chemicals with 2, 4-dichlorophenoxyacetic acid as main component. These compounds act like hormones to produce abnormal growth

resulting in bending, twisting and gall-formation of weeds and other plants. They are absorbed by leaves and are translocated through the plant to cause death of the entire plant; they are selective and destroy broad leaved weeds. The third herbicide selected was copper sulphate.

The efficacy of these compounds was gauged by the changes in mean curvature of petiole, leaves, stems and branches; colour of stems, leaves and stipules; and some associated characters (Appendices I-V).

The plants were sprayed in their natural habitats. Stations 1 and 5 provided most congenial plots for the application of herbicides since the dominant species occurred here in pure patches (Figs. 2 and 3) and as such the results of the spray could be later easily compared with the neighbouring untreated vegetative zones. The spraying was done in a zig-zag-reversible manner so that each plant could be equally sprayed.

OBSERVATIONS.

The effects of the three herbicides may be treated separately.

Dicotox.—Six fluid oz. of the chemical were applied in three gallons of water per 45×45 ft. area. This application gave good results against *Polygonum* but was not effective on *Scirpus*. Therefore, the quantity was doubled making it twelve oz. in three gallons per 45×45 ft. area. Table III lists the time required for obtaining a kill of the various species in the fluctuation zone of Bokaro Reservoir against which the chemical was tried (*see* also Appendices I and II).

TABLE III.

Duration of kill of some aquatic plants growing in the fluctuation zones of Bokaro Reservoir after spraying with a double dose of aqueous solution of dicotox.

Species	Duration of kill	Remarks
Monochoria vaginalis ...	6 weeks	Effects distinct after 24 hours.
Polygonum tomentosum ...	6 ,,	Effects visible after 24 hours; bud formation after 3 to 4 weeks.
Scirpus articulatus ...	5 ,,	Effects distinct after 7 days.
Sphaeranthus indicus ...	3-4 ,,	Effects distinct after 12 hours.
"Grass" ...	*Nil*	No effect.
Lantana sps. ...	*Nil*	Five per cent leaves showed effect after 24 hours.

This study showed that only the double dose was efficacious and that too between four to six weeks and that the secondary bud formation in *Polygonum* necessitated a second treatment after three to four weeks, preferably with the same dose of herbicide. It may be mentioned here that *Polygonum* flourished luxuriantly in the semiaquatic and terrestrial habitats at Bokaro; therefore, it is the most dangerous species from the stand point of malaria as it greatly helps in breeding of larvæ.

When the plant is sprayed, the hormone-herbicide sets in quick physiological disturbances which cause rapid division of cells and finally their eruption and death. This might result in quick rejuvenation of the dormant bud-initials which escape the herbicide. Thus, it becomes necessary to apply a second treatment three to four weeks after the first treatment so as to eliminate all vestiges of vegetative activity. It is imperative that this process be preceded by maintenance of water-level at or below RL 737-740 so as to expose both the submerged vegetation as well as the basal portions of the emergent flora.

Chloroxone.—This herbicide was applied in two concentrations at the rate of 2½ and 5 lbs. in 20 gallons of water per acre area.

At lower concentration, it was sprayed on *Alternanthera sessilis, Causulia axillaris, Jussiaea repens, Kyllinga monocephala, Monochoria vaginalis, Polygonum tomentosum, Scirpus articulatus*, grasses and a few more weeds. The initial effects of the herbicidal action were visible after 2 (Section A), 3 (Section B), 5 (Section C), or 8 hours (Section D); killing the species within 120 hours to four weeks (Appendix III).

Table IV gives variation in the initial action of this compound. The two-hour group is represented by floating and more delicate species where the inflow of the herbicide was rapid because of no mechanical (morphological) barriers;

TABLE IV.

Chloroxone (2½ lbs/acre): time required for the first visible effects and percentage number of petioles showing effects within one week at Station I (Plot A).

Description	Plants considered					
	Alternanthera	Grass	*Jussiaea*	*Monochoria*	*Polygonum*	*Scirpus*
Time required for the first appearance of epinasty (in hours)	2	8	2	3	3	5
Percentage of petioles showing epinasty after 24 hours (in numbers) ...	30	0–1·3	80	100	25	0–5·3
Percentage number of leaves showing no green after:						
24 hours ...	66	*Nil*	80	75	35	*Nil*
48 ,, ...	82	5	80	75	35	30
72 ,, ...	98–100	5·9	90	...	40	33
96 ,, ...	Dead	5·9	90	90	...	33
120 ,, ...	...	5·9	Dead	100, all half dead†	70	50
144 ,, ...	...	5·9‡	...	*	70*	50*

*=Died within 4 weeks. †=Some plants died within 2 weeks. ‡=Revived later.

the three-hour group is mostly semiaquatic (though *Monochoria* was leading a pure aquatic-life) and some species like *Polygonum* and *Kyllinga* have mechanical barriers as well; the five-hour group has radical leaves with lamina radial and sunken stomata; the anatomy of the grass leaf justifies the inactivity of the weed-kill.

In *Jussiaea* and *Alternanthera*, chloroxone had a double action: it paralyzed the lamina and the amount of spray falling on water or on stems and adventitious roots, pararalysed the latter as well, thus minimizing the time for total kill. The more or less uniform deflection of the various parts concerned, after the initial effect and 24 hours, is natural (Appendix III), as once the lamina starts drying up the absence of any physiological activity is evident and the petiole can have no more eflection. When, however, the lamina is heavier as in *Polygonum* or *Monochoria*, the deflection continues till the tip of the leaf meets the surface after forming a bend in *Monochoria* or rolls inwards and dries up in *Polygonum*.

When sprayed at the rate of five pounds per acre against *Desmodium triflorum*, *Eclipta alba*, *Oldenlandia heynei*, *Phyllanthus simplex* and *Rolygonum tomentosum*, all but *Polygonum* were killed within 120 hours (Table V). Further work was confined to studies on *Polygonum*, (*i*) on a patch near the shore-line with nearly terrestrial conditions and (*ii*) on a semisubmerged patch. The initial effects were marked within three hours. On the land the stems bent through 75 to 90° after 48 hours and most of the leaves fell off within 120 hours (Plate IV, Fig. *A*, *B*) and the well sprayed plants died within two weeks. Those in water, took about four weeks to die and did not show a marked bend of the stem; they developed secondary buds during the second week after treatment (Appendix IV).

It is evident that the absorption of the herbicide was more rapid in plants on land than in those in water as shown by the mean petiole curvatures (Chart 1) at various intervals. The rapid inflow in land plants, results in their early death. In water, the contact of the spray may not be complete and this resulted in the formation of fresh buds.

Copper sulphate.—Aqueous solution of copper sulphate was applied at the rate of 8 gm. in ten gallons of water per acre area.

The treatment proved efficacious against *Jussiaea repens*, *Monochoria vaginalis*, floating *Cladophora glomerata* but could not eliminate *Polygonum*, grasses and submerged *Chara*, *Spirogyra* and *Najas* (Table V). The initial effects were apparent within 4 to 48 hours. *Monochoria* showed marked deflection of its petiole (Plate V, Fig. *A*, *B*), the submerged algæ could not be affected even though a higher dose (16 gm. in 10 gallons per acre) was employed. The effects after treatment on a few plants at Station 1, Plot B, are presented in Appendix V.

It is noted that in this case the initial action is less pronounced, *e.g.* the petiole in *Monochoria* shows just a deflection of 10 to 30°; in *Jussiaea* 0 to 30° and in *Scirpus* about 10° (that too in only 50 per cent of cases); nor is there any gradual change in colouration of leaves etc. This is obvious when one considers that it is a non-hormone herbicide. Thus, the rapid fall shown by the petiole in *Monochoria*, 24 hours after treatment, is due to drying up of the lamina *rather* than by application of the herbicide (copper sulphate).

PLATE IV.

A

B

FIG. 4. *Polysaccum crassipes*: *A*, untreated; *B*, 48 hours after treatment with chloroxone (5 lbs. per acre).

PLATE V

A.

B.

FIG. 1. *Vallisneria spiralis*. (*A*) untreated; (*B*) 48 hours after treatment with copper sulphate (3 gm. per acre).

TABLE V.

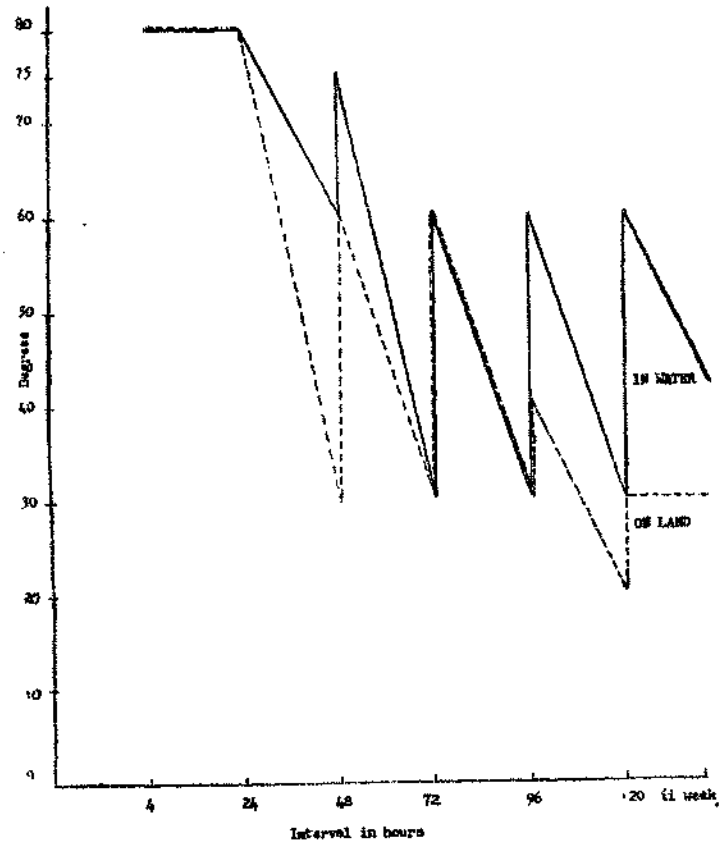
Time for total kill of herbaceous and semi-woody weeds found in the fluctuation zones of the Bokaro Reservoir after spraying with aqueous solutions of chloroxone and copper sulphate.

Species	Chloroxone (concentration)		Copper sulphate
	Lower	Higher	
A. Species showing initial effects after 2 hours:			
Alternanthera sessilis	120 hours	...	...
Desmodium triflorum	...	96 hours	...
Jussiaea repens	120 hours	...	...
Oldenlandia heynei	...	120 hours	...
B. Species showing initial effects after 3 hours.			
Causulia axillaris	120 hours	...	...
Eclipta alba	...	120 hours	...
Grangea madaraspatana	144 hours	120 hours	...
Kyllinga monocephala	120 hours	...	...
Monochoria vaginalis	2 weeks	...	...
Polygonum tomentosum	22 days (22 per cent)	15-20 days	No effect
Sphaeranthus indicus	120 hours	...	...
Phyllanhtus simplex	...	120 hours	...
C. Species showing initial effects after 4 hours.			
Cladophora glomerata	...	...	7 days
Jussiaea repens	...	...	1½ weeks
Monochoria vaginalis	...	...	1½ ,,
Scirpus articulatus	...	...	4 weeks
D. Species showing initial effects after 5 hours.			
Scirpus articulatus	22 days	...	...
E. Species not killed by either.			
Ergrostris sps.	No effect	No effect	No effect
"Grasses"	Revived after 24 hours	,, ,,	,, ,,
Chara, Najas	No effect	,, ,,	,, ,,

DISCUSSION.

Like other herbicides with 2, 4-D as main component, dicotox and chloroxone are essentially effective while the plants are in their meristematic condition as this is the most congenial phase for the stimulating activity of the herbicide to cause depletion of the reserves to a large degree. According to Frazier (1943) and Baker *et al.* (1944), this depletion is important in a consideration of weed control.

CHART 1.

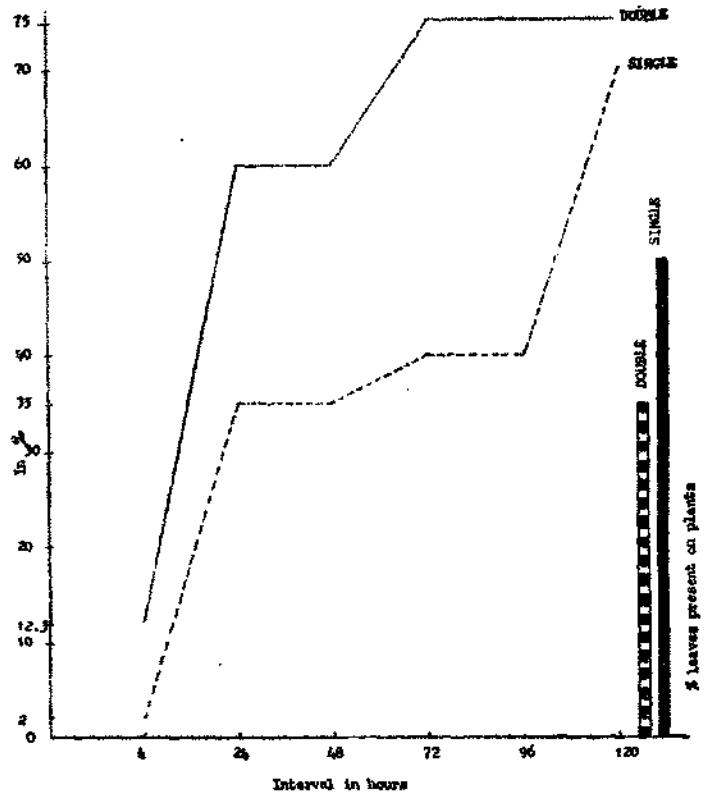


Mean petiole curvatures in *Polygonum* (in water and on land) after treatment with chloroxone (5 lbs. per acre).

The time during which the initial effects of chloroxone (2½ lbs. per acre) become apparent (Table IV), is directly related to the morphology of the species under consideration and probably it also accounts for the degree of variation shown by different species in percentage number of petioles showing epinasty after 24 hours or percentage number of leaves showing no-green between 24 to 144 hours.

Thus, in *Polygonum* the changes in the no-green are not so rapid as in *Jussiaea*, *Alternanthera* or *Monochoria*. *Scirpus* keeps nearly the same speed. But grasses do not seem to be affected at all (Table V). This agrees with observations of Marth and Mitchell (1944) and Hamner and Tukey (1944).

CHART 2.



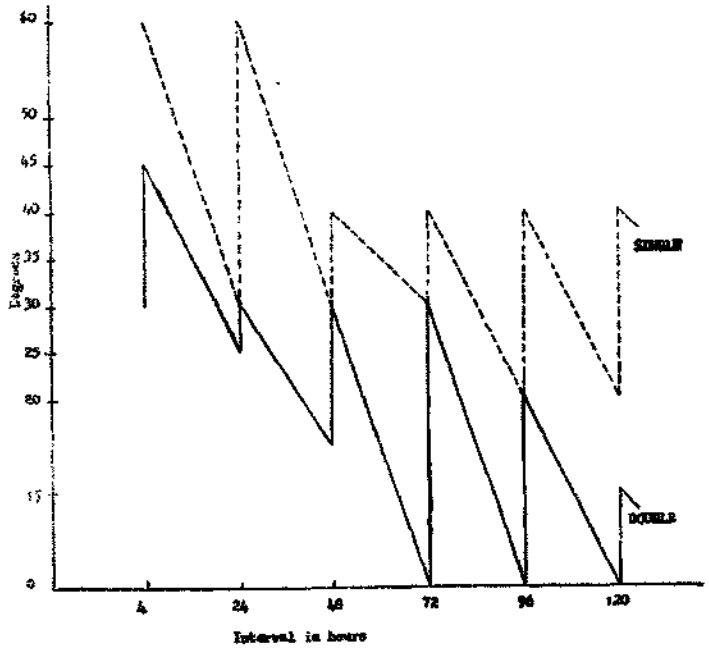
Number of leaves showing no-green after treatment with single and double doses of chloroxone and of those present on plants of *Polygonum* after 120 hours (in percentage).

A higher dose (5 lbs. per acre) was more efficacious and this is illustrated by the percentage of leaves showing no-green, percentage of leaves present on plants after 120 hours (Chart 2) and the mean leaf curvatures (Chart 3) after treatment with a single and a double dose. Further the duration of the kill is also nearly half in double dose-sprayed plants. It implies, therefore, that in order to eliminate the harder species, whether in vegetative or reproductive phases, it is desirable to apply a higher dose (during the draw-down periods).

Copper sulphate, a non-hormone type of herbicide, produced only localized superficial burning effects which could later extend through the plant to some

distance from the point of contact—this is in accord with the effects of sodium chlorate, sodium thiocyanate and some other arsenic compounds (Marth and Mitchell, 1944). Thus, even a week after the first treatment, the petioles retained their greenish hue and only the lamina showed signs of local death, turning white-greenish and shrivelling-up. In the case of chloroxone, the leaves showed curling up immediately after treatment, followed by change of colouration from greenish yellow —yellowish green—light brown—brown and finally drying up and falling off—illustrating a physiological sequence expected from a hormone.

CHART 3.



Leaf curvatures in *Polygonum* after treatment with a single and a double dose of chloroxone.

Further ineffectiveness of copper sulphate is illustrated by a comparison of the initial deflections caused in the petioles of the various species sprayed with it and chloroxone under similar conditions (Table VI).

Thimann (1947) states that stomata do not play a major part in penetration of the spray but certainly provides the most rapid path of entry and claims that the negative results of Weaver and DeRose (1946) agree with his results. During the present studies, it was found that stomata were the main channels for the inflow of the hormone-herbicides; an appraisal of this problem and related physiological processes is under study.

TABLE VI.

Initial deflection in petioles after treatment with a low concentration of chloroxone and copper sulphate.

Plants	Deflection (°)	
	Chloroxone	Copper sulphate
Jussiaea repens	20-40	10-30
Monochoria vaginalis	50	10-30
Scirpus articulatus	10-20	0-10 (only in 50 per cent cases)

SUMMARY.

Bokaro Reservoir is a constant pool-level pond with a draw-down of about 1·5 ft. The fluctuation zone is rich in aquatic and marsh plants, showing at some places a *flexuous* type and at some other places *naked-erect leafy-submerged* type of vegetation, the dominant species being *Polygonum tomentosum, Scirpus articulatus, Potomogeton crispus, Najas foveolata, Hydrilla verticillata, Monochoria vaginalis* and *Aponogeton monostachyon* respectively; among algæ, diatoms, *Chara zeylanica, Cladophora glomerata, Zygnema indica* var. *damodari* and *Spirogyra* sps. This rich vegetation makes the fluctuation zone a congenial habitat for breeding of about seven anopheline species, the most common being *A. culicifacies, A. subpictus* and *A. annularis*, respectively.

Mechanical control of aquatic vegetative growth was achieved by filling up the breeding spots, where possible; and for the rest application of herbicides was tried. At the rate of 6 oz. per 45 sq. ft. area dicotox was ineffective against *Polygonum* and *Scirpus*, whereas at 12 oz. per 45 sq. ft. area, it killed *Polygonum tomentosum, Monochoria vaginalis, Sphearanthus indicus* and *Scirpus articulatus* within 3 to 6 weeks. Two and a half pounds per acre application of chloroxone eliminated *Jussiaea repens, Alternanthera sessilis, Causulia axillaris, Kyllinga monocephala, Sphaeranthus indicus* (120 hours); *Grangea madaraspatana* (144 hours); *Monochoria vaginalis* (14 days); *Scirpus articulatus* (22 days) and *Polygonum tomentosum* (30 days) within 120 hours to 4 weeks. Five lbs. per acre of the same killed *Desmodium triflorum* (96 hours); *Oldenlandia heynei, Eclipta alba, Grangea madaraspatana, Phyllanthus simples* (120 hours); and *Polygonum tomentosum* (15 to 20 days) within 96 hours to 20 days. Eight grammes per acre of copper sulphate eliminated *Monochoria vaginalis* and *Jussiaea repens* (1½weeks), *Scirpus articulatus* (4 weeks) and floating *Cladophora glomerata* within 7 days. Grasses and the submerged flora were unaffected and proved resistant to all weed-killers.

Secondary buds appeared on *Polygonum* 2-3 weeks after first treatment with dicotox and chloroxone and necessitated a second treatment. It is suggested that the vegetative phase of the vegetation and the period of draw-down in the reservoir are the best periods for such operations.

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Appendix I.

Effects of dicotox (12 oz. per 45 sq. ft. area) on polygonum, 6 weeks after treatment.

(Station 5).

Parts of plant considered	Before treatment	After treatment				
		24 hours	First week	Fourth week	Fifth week	Sixth week
Mean curvature: stem ...	65–75°	35–48°	nearly 10-20°, horizontal with soil	Emergent completely dead, hard	...	...
branch ...	110–130°	no change	no change	no change	no change	no change
leaf ...	60–75°	30-45°	10–40°	10—40°	all defoliated	...
Colour:						
stem ...	green-light green	light green-light brown	more yellowish, light brown	black-violet-brown; submerged greenish, nearly fresh	same; adventitious buds initial stage on submerged nodes (3 per cent)	stems forming a black-brown heap, adventitious buds sprout young shoots (10 per cent)
ochrea ...	greenish-yellow to light-brown	light brown-brown	brown	torn off and fallen	...	...
lamina ...	green	wilting, in 30 per cent tips brownish	rolled inwards and upwards, 50 per cent non-green, yellowish; 60 per cent drying up	faintly light brown, dried up or defoliated	absent	...

Appendix II.

Effects of dicotox (12 ozs. per 45 sq. ft. area) on Scirpus and grass, six weeks after treatment.

(Station 1).

Parts of plant considered	Before treatment	After treatment				
		24 hours	First week	Fourth week	Fifth week	Sixth week
			Scirpus articulatus			
Mean leaf curvature	80–90°	effect indistinct	60–75°	signs of decay set in	Dead	...
Colour:						
lamina ...	deep green	no effect	yellowish	yellowish-golden yellow		
			" Grass "			
Mean stem curvature	75–80°	no effect	same	same	same	same
Mean leaf curvature	80-99°	,,	,,	,,	,,	,,

Appendix III.

Effect of chloroxone (2½ lbs. per acre) on some plants, up to 120 hours after treatment.

Station (1, Plot A), Bokaro Reservoir.

Parts of plant considered	Before treatment	After treatment					
		2 hours	24 hours	48 hours	72 hours	96 hours	120 hours
Jussiaea repens							
Mean curvature branch ...	110–130°	180°	180°	180°	180°	180°	...
,, ,, petiole ...	60–80°	40°	40°	40°	40°	40°	...
Colour: stem ...	greenish pink-pink	same	yellowish green	yellowish	brown (plant sinking)	brown (withered)	...
leaf ...	dark green (often greenish pink)	same	20 per cent only greenish	same	10 per cent only green, rest pinkish dirty white	...	dead
Alternanthera sessilis							
Mean curvature branch ...	140–160°	140°	180°	180°	180°	180°	...
,, ,, leaf ...	60– 80°	50°	50°	50°	50°	50°	...
Colour: stem ...	greenish-pink-green	same	yellowish	yellowish-light brown	same	...	...
leaf ...	dark green	same	66 per cent non-green	82 per cent non-green	92 per cent non-green	dead	...
Monochoria vaginalis							
		3 hours			120 hours	Second week	
Mean curvature lamina ...	50°	30°	same, rolled inwards	same	30°	...	...
,, ,, petiole ...	100°	150°	150°	same	170°	...	...
Colour: lamina ...	greenish	same (tips shrivelled up)	white-brownish	same	sunk below water level	dead	...

APPENDIX III.—(*Contd.*)

Parts of plant considered		Before treatment	After treatment					
			2 hours	24 hours	48 hours	72 hours	96 hours	120 hours
Cassulia axillaris								
Mean curvature	leaf ...	50–80°	150°	175°	175°	175–180°	...	...
Colour:	lamina ...	greenish-yellow	same	tips burnt-black margins rolled.	same	violet-brown, curled inwards	...	...
	stem ...	yellowish-green	same	yellowshih	yellowish-violet	violet-black	dead	...
Polygonum tomentosum								
Mean curvature	stem ...	65–80°	same	65°	same	45°	same	...
,, ,,	branch ...	70–80°	same	50–60°	same	same	same	...
,, ,,	leaf ...	30-(50)-80°	60°	30-60°	30–40°	20–40°	same	...
Colour:	stem ...	green-greenish yellow	same	yellowish, 2 per cent with tips whitish, 5 per cent margin rolled inwards	golden yellow 35 per cent; 9·5 per cent margin rolled inwards	35 per cent golden yellow	70 per cent same, 20 per cent defoliated	..
	ochrea ...	yellowish green	same	light brown	brown, 50 per cent fallen off	same	same	...
Kyllinga monocephala								
Mean curvature	leaf ...	45–80°	...	...	...	15–20°	...	...
,, ,,	peduncle ...	80–110°	...	...	...	45–158°	...	...
Colour:	leaf ...	greenish	...	...	...	light brown,	dead	...

APPENDIX III—(*Contd.*)

Parts of plant considered	Before treatment	After treatment					
		2 hours	24 hours	48 hours	72 hours	96 hours	120 hours
Sphaeranthus indicus							
Mean curvature branch ...	60–75°	same	60°	same	same	...	...
,, ,, leaf ...	30–45°	same (7 per cent effected)	same	50 per cent effected, 20°	10–20°	...	...
Colour: stem ...	dirty grey-greenish	same	light brown	brown- black	brown-black	...	...
leaf ...	grey-greenish	same	sod-light brown	light brown	same, dead	...	...
Scirpus articulatus							
		5 hours					
Mean curvature leaf ...	90–100°	80°	same	50–60°	50 per cent dead	...	...
Colour: leaf ...	deep green	50 per cent yellowish	same, tips dried up	white yellowish	nearing death	(died within a month)	...

Appendix IV.

Effects of chloroxone (5 lbs. per acre) on Polygonum, two weeks after treatment.

(Station 5).

Parts considered	Before treatment	After treatment					
		3 hours	24 hours	48 hours	72 hours	96 to 120 hours	Second week
		Polygonum tomentosum (aquatic)					
Mean curvature stem ...	160-180°	same	same	same	135-110°	125°	100°
,, ,, petiole ...	80°	same	same	60-75°	30-60°	same	40°
,, ,, lamina ...	30-(50)-80°	same (15° in 15 per cent)	30-40°	15-25°	15°	10°	same
Colour: stem ...	yellowish green	light brown	same	yellowish	light brown	same	brown except in basal region.
lamina ...	green	same	10 per cent golden yellow	22 per cent same	light brown	22 per cent same	yellow green-brownish
		Polygonum tomentosum (terrestrial)					
Mean curvature stem ...	90°	same	same	75°	same	20-30°	same
,, ,, petiole ...	80°	same	same	30-60°	same	30-40-30°	same
,, ,, lamina ...	30-(40)-80°	30-45° (12·3 per cent tips effected with 15-25°)	25-30°	15-30°	same	10-20-0°	same
Colour: stem ...	yellowish green to light brown	same	60 per cent light brown	same	yellowish	light brown	dark brown
lamina ...	green	same	15·5 per cent golden yellow	golden yellow-yellow (5:5)	75 per cent golden-yellow	brownish	same 14 per cent plants dead

Appendix V.

Effects of copper sulphate on some plants, 1½ weeks after treatment.

(Station 1, Plot B).

Parts considered	Before treatment	After treatment			
		4 hours	24 hours	72 hours	1½ weeks
		Monochoria vaginalis			
Mean curvature petiole ...	100–120°	130°	135–150°	168–180°	same
Mean curvature lamina ...	20–40°	30–30°	0–5°	sinking	
Colour leaf ...	greenish	5 per cent yellowish tips.	10 per cent whitish-green-tips, mostly dried up.	light-green to dirty white	Dead
		Jussiaea repens			
Mean curvature branch ...	100°	180°	180°	180°	180°
Mean curvature petiole ...	80–30°	30–30°	same	30–50°	same
Colour: stem	nearly pinkish-green	same	same	brown-violet brown	same
lamina ...	pinkish-green	pinkish	same	pinkish-brown	dead
		Scirpus articulatus			
Mean curvature leaf ...	100–110°	100° (in 50 per cent cases)	same	same	...
Colour: leaf ...	deep green	same	same	50 per cent yellow	same, expected to die within a month
		"Grass"			
Mean curvature stem ...	75–90°	50–65°	65°	recovered	no effect
Mean curvature leaf ...	60–80°	same	60°	,,	,,

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

XXIV. Selection of a chloroquine* resistant strain.

BY

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[February 4, 1957.]

A SEVEN hundred and fifty fold resistance of a strain of the parasite to sulphadiazine was described by Krishnaswami *et al.* (1954) who reviewed the literature to date on the resistance of various plasmodia to different antimalarials both in the laboratory and the field. The present study reports the selection of a strain resistant to chloroquine 200 times the dose required by the parent strain to be cleared from the peripheral blood temporarily.

MATERIAL AND METHODS.

The adult albino mice used in the study were obtained from the colony maintained at the Institute. No choice was exercised with regard to the sex of the animals. The parent strain of the parasite has been maintained by rat to rat passage since 1952 and had undergone 266 passages when taken over in mice for the present experiments. The route of inoculation was always intra-peritoneal and the dose was 5×10^6 parasites every time.

The technique of isolating the resistant strain and determination of the degree of resistance was as described previously in similar studies mentioned above, on the parasite with regard to sulphadiazine. The administration of the drug for the selection of the resistant strain, however, was not oral, but parenteral, once a day as described by Jaswant Singh *et al.* (1954).

*The proprietary preparation used was RESOCHIN, Bayer. Each ampule contained 5 c.c. of 5 per cent solution containing 0·25 gm. of 7-chloro-4-(4-diethylamino-1-methylbutylamine) quinoline-diphosphate corresponding to 0·15 gm. resochin (chloroquine) base.

A five per cent resochin solution in an ampule was suitably diluted with normal saline to provide the requisite dose and when higher doses were used, the five per cent solution was concentrated by gentle heating.

The minimum effective dose of resochin to produce a Class II effect, namely clearance of parasitæmia from the peripheral blood at least for three consecutive days (Ramakrishnan *et al.*, 1951), was found to be 0·1 mg. per 20 gm. mouse weight twice a day for three days administered orally (total dose 30 mg./kg., unpublished report). As already stated, for purposes of selection of the resistant strain, the drug was administered parenterally once a day. The dose with which the commencement of exposure was made, was 2·5 mg./kg. which was twelve times less than the minimum total dose required by mouth to clear the parasitæmia for three days. Thirty three passages of the strain were made in the course of seven months, the dose of the drug being progressively increased (Table I).

TABLE I.

Protocols for the selection of resochin resistant strain of P. berghei.

Serial passage number	Mouse number	Date of passage	Details of drug administration		Time taken in days
			Dose administered mg./20gm.	Number of times	
0	2087	1956 June 1	·05	One	...
			·075	One	...
1	2107	June 13	·15	One	12
2	2141	June 26	...	...	13
3	2144	June 29	·2	One	3
4	2147	July 4	·2	Two	5
5	2172	July 17	...	...	13
6	2178	July 20	...	...	3
7	2179	July 24	...	...	4
8	2189	July 26	...	...	2
9	2192	July 28	...	...	2
10	2203	July 30	...	...	2
11	2206	August 1	·2	One	2
12	2213	August 7	·2	One	6
13	2225	August 14	·2	One	7
14	2233	August 17	·2	One	3
15	2241	August 23	·2, ·4	One, One	6
16	2258	September 1	·4	One	9

(*Contd.*)

TABLE I.—(*Concld.*)

Serial passage number	Mouse number	Date of passage	Details of drug administration		Time taken in days
			Dose administered mg./20gm.	Number of times	
		1956			
17	2296	September 11	·4	One	10
18	2311	September 18	·4	One	7
19	2325	September 21	·4	One	3
20	2344	September 26	·4	One	5
21	2370	October 3	·8	One	7
22	2399	October 9	·8	One	6
23	2416	October 15	...	...	6
24	2439	October 20	·8	One	5
25	2455	October 23	1	One	3
26	2493	October 27	...	...	4
27	2513	October 31	1·2	Four	4
28	2554	November 12	...	...	12
29	2571	November 16	...	...	4
30	2578	November 19	1·3	One	3
31	2603	November 25	1·2	One	6
32	2629	November 30	...	...	5
33	2651	December 5	...	...	5
				Total	187 days

In the first three consecutive passages the dose of the drug was increased for each passage. Subsequently, the same dose was administered for several consecutive passages before it was increased. Very occasionally the drug was administered to the same animals twice and sometimes the drug was witheld from a few. The reason for this was that, in the absence of any previous successful record in the literature of a selection of a strain of Plasmodium, resistant to chloroquine, there was a constant apprehension that the strain may be lost by repeated exposure to higher doses of the drug. Nevertheless, after completing the passage from one animal (donor) to the other, the donor was given the drug and ensured that the parasite could still tolerate the drug. For every passage more than one animal was used in order to safeguard the strain from being lost due to death of any one animal.

RESULTS.

At the end of thirty-three passages when a single dose of the drug given parenterally was 60 mg./kg., i. e,. 1·2 mg./20 gm. (Table I), the dose was further increased in the subsequent passages to 75 mg./kg. when it was found that a hundred per cent of the animals so treated died, presumably due to toxicity of the drug. It was confirmed as shown in Table II that the dose, 75 mg./kg., administered intraperitoneally, was lethal to one hundred per cent of the mice.

TABLE II.

The result of a single intraperitoneal injection of 1·5 mg./20 gm. (75 mg./kg.) per mouse.

Number of animals treated	Infected or normal	Result	Interval between drug administration and death
6	Infected	Death	Within 2 hours
6	Normal	Death	Within 2 hours

The M.L.D. 100 of chloroquine administered intraperitoneally to mice would appear to be between 60 and 75 mg./kg. It was obvious that any further attempts to select a strain more resistant than that tolerating 60 mg. of chloroquine per kg. body weight of the host, would be difficult.

In order to assess the degree of resistance of the experimental strain to chloroquine, six animals were inoculated with 5×10^6 parasites each from Mouse 2651 (Table III). Three of them were given 15 mg. and the remaining three 20 mg. per 20 gm. body weight by mouth twice daily for three consecutive days commencing from the first day when the parasitæmia was 200 or more per 10,000 erythrocytes. The progress of infection in three animals is shown in Table III.

Five of the animals, three treated with 4500 mg./kg. (total dose) and two treated with 6000 mg./kg. (total dose) died within nine days from the commencement of treatment. One animal treated with the higher dose survived. In all animals, the parasitæmia declined and in two of them the peripheral blood became negative to parasites. In the animal that survived, after five consecutive days of sub-patency, the parasites became patent again and progressively increased. It was obvious that the experimental strain showed considerable tolerance to chloroquine compared to the parent strain. The degree of resistance was somewhere between 150 and 200 fold. But since five out of six animals died, the test was repeated (Table IV). At the same time, for purposes of comparison six animals were inoculated with the parent strain and treated with the previously determined M.E.D. (Table V). The previous results were confirmed. M.L.D. 100 for chloroquine administered to mice orally would appear to be between the above two dosage schedules, namely, 4500 and 6000 mg./kg.

TABLE III.

Course of infection in animals inoculated with the experimental strain from Mouse 2651 (Table I) and treated with resochin orally.

Mouse number	Dose administered	Parasites per 10,000 erythrocytes from the first day of drugging															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
2658	A	528	228	328	120	8	D	...	...	...	...	...	...	...	...	...	...
2659	B	360	288	296	80	2	4	D	...	...	...	...	...	...	...	...	...
2660	A	366	240	496	196	28	D	...	...	...	...	...	...	...	...	...	...
2661	B	408	208	228	120	40	8	D	...	...	...	...	...	...	...	...	...
2709	B	360	328	88	24	8	N	N	N	N	N	P	12	120	155	548	1436
2711	A	320	328	92	56	P	N	N	N	N	D	...	...	...	...	...	...

A=15 mg./20 gm. body weight twice daily by mouth for three days (total dose 4500 mg./kg.).
B=20 mg./20 gm. body weight twice daily by mouth for three days. (total dose 6000 mg./kg.)
D=Dead.
N=No parasites seen in 100 oil immersion fields.
P=1 parasite in 100 oil immersion fields of thin smears.

TABLE IV.

Course of infection in animals inoculated with experimental strain and treated with resochin orally.

Mouse number	Dose administered	Parasites per 10,000 erythrocytes from first day of drugging												
		1	2	3	4	5	6	7	8	9	10	11	12	13
2775	B	644	368	112	118	64	40	16	3	N	N	P	3	...
2776	A	760	486	336	142	D	...	...	...	...	...	...	...	...
2778	B	488	448	248	84	20 D	...	...	...	...	...	...	...	...
2779	B	768	416	128 D	...	...	...	...	...	...	...	...	...	...
2780	A	728	704	384	158	D	...	...	...	...	...	...	...	...
2781	B	412	220	304	226	84	26	P D	...	...	...	...	...	...

A=15 mg./20 gm. body weight twice daily by mouth for three days (total dose 4500 mg./kg.).
B=20 mg./20 gm. body weight twice daily by mouth for three days (total dose 6000 mg./kg.).
D=Dead.
P=1 parasite in 100 oil immersion fields in thin smears.
N=No parasite seen in 100 oil immersion fields.

TABLE V.

Course of infection in animals inoculated with parent strain (R-3639) and treated with resochin orally.

Mouse number	Dose administered	Parasites per 10,000 erythrocytes from first day of drugging											
		1	2	3	4	5	6	7	8	9	10	11	12
2801	C ...	212	64	2	N	N	N	N	N	16	88	...	...
2802	—do.— ...	208	128	4	N	N	N	N	104	336	374	...	...
2803	—do.— ...	304	72	8	N	N	N	N	N	N	20	...	...
2804	—do.— ...	224	52	6	N	N	N	N	P	2	56	...	...
2805	—do.— ...	216	284	84	N	P	N	N	4	96	1630	...	...
2806	—do.— ...	388	312	18	6	N	N	N	P	8	...	...	...

P = 1 parasite in 100 oil immersion fields in thin smear.
C = 0·1 mg./20 gms./bodyweight twice a day for three days or total dose of 30 mg./kg.
N = No parasite seen in 100 oil immersion fields.

DISCUSSION.

The first successful attempt at selecting a strain of any Plasmodium, resistant to chloroquine, has been recently reported by Ray *et al.* (1956). In discussing their result, the authors point out that previous attempts by several workers in respect of different plasmodia were unsuccessful. In their opinion, the success was due to (1) the commencing dose of the drug to which the parasites were exposed, was small, namely, 0·1 mg. per kg. body weight of chick and (2) the fact that care was taken gradually to increase the dose so that at no time did the parasites disappear from the peripheral blood completely. In the present experiments, however, the procedure adopted to select the strain, resistant to chloroquine, cannot be considered in any way dissimilar to that adopted previsouly for the selection of a sulphadiazine resistant strain by oral (Krishnaswami *et al.*, 1954) or intra-peritoneal administration of the drug (Jaswant Singh *et al.*, 1954).

From the results of the present work, it is clear that it has been possible experimentally to select a strain of *P. berghei* 200 fold resistant to chloroquine compared to the parent strain. In view of the unsuccessful attempts by Schmidt *et al.* (1949) with *P. cynomolgi* and Cooper *et al.* (1950) with *P. vivax* chesson strain, Bishop and McConnachie (1952) with *P. gallinaceum*, Thompson (1948) with *P. lophurae* and the first and the single successful attempt so far by Ray *et al.* (1956) at producing a three fold resistant strain of *P. gallinaceum*, the present results would appear to be very unusual. Whether this exceptional behaviour on the part of *P. berghei*, with reference to chloroquine, is due to any intrinsic difference in physiology

of *P. berghei* from that of other malaria parasites, or due to the difference in the metabolism of the drug in mice as compared to other hosts, nothing can be said from the present experiments.

Josephson *et al.* (1953) while reporting their results on the *in vitro* action of several antimalarials, have classified the drugs into two groups. One group represented by the 4-aminoquinolines, is capable of killing the parasite at any stage in its development (in the erythrocyte) and the other represented by biguanides capable of killing only the dividing forms. They also found a correlation of such a classification with two other characteristics of the drugs, namely, that the former acted *in vitro* and against which it was difficult or impossible to select a resistant strain of malaria parasites and the latter do not act *in vitro* (because they act only through their metabloites and against which it is comparatively easy to select resistant strains of malaria parasites. Instances are many where strains resistant to a very high degree to the latter group of drugs, have been selected. In view of the present results where a comparatively high degree of resistance to chloroquine is exhibited by the experimental strain of *berghei*, the classification referred to above would appear to require re-examination.

The relative susceptibilities of *P. berghei* to several drugs are reproduced below:

Minimum effective dose of some antimalarials against P. breghei *in mice**.

Drug	Mg./kg. body weight of mice		
	Class I	Class II	Class III
Quinine ...	1,200	4,800	6,000
Sulphadiazine ...	1·4	6·0	60·0
Daraprim ...	0·00005	0·0005	4·0
Paludrine ...	2·5	20·0	125·0
Metabolite 'A' ...	0·1	25·0	100·0
Metabolite 'B' ...	0·005	0·05	3·0
Resochin† ...	10·0	30·0	Undetermined

*Reproduced from Krishnaswami *et al.*, 1953. †Unpublished results.

With the exception of quinine, *P. berghei* would appear to be least susceptible to resochin of all the other drugs. It is generally known that the 4-aminoquinolines acts on all stages of the parasites in erythrocytes and *berghei* would appear to be no exception to the rule.

A possible reason for the several unsuccessful attempts to select a resistant strain of different plasmodia to choloroquine may appear to be due to the prolonged action of the drug as it gets fixed in tissues in different proportions and, therefore, the complete elimination from the body is slower than in the case of other drugs. As a result, it would appear that a certain proportion of the dose administered will act cumulatively with the drug already present in the tissues. If this cumulative action be the reason responsible for the unsuccessful attempts, it would follow that the pharmacology of the drug in mice may be radically different to

that in other animals. The metabolism of chloroquine is probably different in the monkey, dog and rat (Finlay, 1951). It is conceivable that the proportion of fixation of drugs in the different tissues also, is different in mice as compared to man, dog, monkey and rat in which animals, the pharmacology has been investigated. In the rat, the relative concentrations of chloroquine in plasma, red cells and whole blood demonstrate a limited localization in red cells and a greater localization in leucocytes (Finlay, 1951).

In general, the 4-aminoquionlines persist in the body for a longer time than other antimalarials (Finlay, 1951). Such studies seem to have been compared to concentrations of the drugs in man and a few other animals but not the mouse. Perhaps, the rate of degradation and elimiation of the drug in mice is more rapid than in other animals.

A daily oral dose of 400 mg. per kg. body weight is said to constitute the M.L.D. 100 for mice (Finlay, 1951). As has been stated earlier, in the present experiments it was found that a dose between 4,500 mg./kg. and 6,000 mg./kg. administered orally and a single dose of 75 mg. per kg. administered intraperitoneally, killed all the mice. It is not surprising that a smaller single perenteral lethal dose is considerably less than the required dose for similar effect orally. Nevertheless, the experimental strain would appear to be resistant to a dose of the drug almost lethal to the host. Beyond this limit, any attempt to select a strain with a higher degree of resistance would obviously be fruitless.

SUMMARY.

The successful selection of a strain of *P. berghei*, resistant to chloroquine (Resochin), requiring 200 times the dose to clear the parent strain from the peripheral blood of mice, is described.

In view of the numerous unsuccessful attempts by others to select a chloroquine resistant strain of different plasmodia, the present result is unusual. Whether this could be attributed to a difference in the physiology of the parasite or to that in the pharmacology of the drug in mice, has been discussed.

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

XXV. The role of casein diet on the acquired immunity of mice infected with *P. berghei.**

BY

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[March 7, 1957.]

RAMAKRISHNAN (1954) dealing with the effects of different qualities of diet and their influence on the infection in P. *berghei* on rats, concluded that a balanced lacto-vegetarian diet was found to favour the host in the development of immunity against the infection. The present experiments were planned specifically to assess the role of casein on the acquired immunity to malaria in mice.

METHODS AND MATERIAL.

Three experiments were carried out involving in all 78† albino mice. The first investigation was in the nature of a preliminary experiment. The second and third were similar to each other in all respects and were in the nature of replicates.

In the first experiment, thirteen animals, each twelve weeks old, were divided into three groups, the first two on experimental diets consisted of five each and the remaining three were the control group of animals. There were two males and three females in each of the experimental group, while in the control group there were two females and one male.

In each of the second and third experiments, 30 animals (15 males and 15 females) were used. They were all twelve weeks old as in the case of the first series. They were divided into three groups of ten each.

The number of different (balanced) diets in each of the experiments was three, namely, (1) one that contained a high proportion of casein, (2) modified basal diet without casein, (3) colony diet which is in routine use for the maintenance of the colony and contains a certain proportion of milk.

* This paper forms a part of a thesis for a doctorate degree of the Punjab University.

† All the mice used in the study were from the colony maintained at the Malaria Institute of India, Delhi.

The food for each animal was supplied daily in an aluminium cup, and was in excess of requirement during 24 hours. The experimental animals were preconditioned for a period of four weeks prior to inoculation. Each animal was weighed twice weekly and the average daily food consumption per group was determined by weighing the unconsumed food every morning. The composition and calorific values of each of the diets are given in Tables I and II.

TABLE I.

Composition of experimental and control diets.

Ingredients	Basal diet, parts per 100 gm. (not used experimentally)	Modified basal diet without casein, parts per 100 gms.	Casein diet, parts per 100 gms.	Colony diet parts per 100 gms.
Unpolished rice flour	80·0	83·32	61·60	...
Dried brewers yeast	8·0	8·34	6·16	1·90
Casein (Merck)	4·0	0·00	24·60	...
Steenbock's salt mixture No. 40 ...	4·0	4·17	3·82	...
Shark Liver oil	4·0	4·17	3·82	1·90
Whole wheat flour	...	...	...	66·66
Skimmed milk powder ...	...	...	...	15·23
Pea-nut kernel	...	...	...	7·51
Calcium carbonate	...	...	...	0·95
Table salt	...	...	...	0·95
Bengal gram	...	...	...	4·90

NOTE.—Stocks of modified basal diet without casein, casein diet and colony diet were prepared every two or three weeks and stored dry. Each animal on any one of the two experimental diets was given one drop each of adoxolin and wheat germ oil every week. Bengal gram in the proportion of 5 gm. per 100 gm. of the rest of the diet, was soaked in water over night and given to the animals on colony diet.

TABLE II.

Per cent composition of proximate principles and calorific values per 100 gm. of different diets (dry).

Diet	Protein	Carbohydrate	Fat	Calorific value
Modified basal diet without casein ...	13·0	64·51	10·73	375·6
Casein diet ...	31·0	51·95	6·61	375·4
Colony diet ...	17·1	61·8	8·6	372·9

All the animals in each experiment, were inoculated intraperitoneally with one million parasites from a single donor. Details of procedure were as described by Ramakrishnan and Satya Prakash (1950*a*).

Thin smears from each of the animals, air dried and stained by J.S.B. technique, were examined daily till the parasites were not patent in the blood. More than one hundred fields were examined before a slide was declared negative. When positive, the number of parasites per 10,000 erythrocytes were enumerated (Ramakrishnan *et al.*, 1953). The criteria for assessment of results were the same as the above authors described with the addition that the average day of death for each group, was considered. The spleen volume was determined in every case as described by Ramakrishnan (1952).

RESULTS.

Average quantities of daily consumption of the different diets per animal are shown in Table III. The only diet which was consumed in increased quantities by the animals in all the three experiments, was the modified basal diet. In the case of the casein and colony diets in all the three experiments, the quantity consumed towards the end of the experiment, was less than in the initial stages.

TABLE III.

Average daily food consumption in gm./mice of different diet.

Period	Modified basal diet without casein			Casein diet			Colony diet			Remarks
Number of experiments	Expt. I	Expt. II	Expt. III	Expt. I	Expt. II	Expt. III	Expt. I	Expt. II	Expt. III	
Period of pre-conditioning (4 weeks) ...	3·9	2·28	2·49	3·1	2·38	2·49	Not recorded	2·02	1·66	...
Period of patent infection (4 weeks) ...	4·3	2·36	2·52	2·8	2·04	2·02	2·9	1·99	1·50	...

TABLE IV.

Weight progress of mice on different diets.

Diet Group	Number of animals used			Average weight in gm. at									Remarks
				Commencement			Post-inoculation			Termination			
Number of experiments	Expt. I	Expt. II	Expt. III	Expt. I	Expt. II	Expt. III	Expt. I	Expt. II	Expt. III	Expt. I	Expt. II	Expt. III	
Modified basal diet without casein ...	5	10	9*	36	25·1	26·6	41	25·5	27·4	36	24·5	24·4	*One animal refractory
Casein diet ...	5	9*	10	38	25·3	28·2	38	24·5	29·8	32	24·2	28·4	*One animal refractory
Colony diet ...	3	8*	10	45	23·3	25·8	42	25·1	26·7	37	24·1	25·6	*Two animals refractory

The trend in weight changes of animals was similar with respect to all diets; there was an increase in weight during the earlier period of infection and a decrease during the later period. Towards the end of the observation, the weights were similar to those at commencement (Table IV).

The indices of the course of infection, and the immunity acquired by the animals on the different diets, are shown in Table V. From the point of view of requirements of the parasites and their growth, it would appear that the casein diet was better than the others. In two, out of the three experiments, the average daily and peak parasitæmia were the highest in animals on casein diet. Further, this diet contributed to the highest average parasitic load per animal consistently in all the three experiments.

In so far as the average day of peak parasitæmia is concerned, it was postponed the longest in the animals on casein diet, consistently in all the three experiments. These latter two indices can be considered both from the point of view of the parasite and that of the host. The greater the parasitæmia, the stronger is the antigenic stimulus. The greater the antigenic stimulus, other conditions remaining favourable to the host, the stronger is the immunity acquired.

It would be seen that the average day of death was postponed to a later day consistently in the three experiments in animals on casein diet as compared to those on other diets. The average spleen volume was also consistently larger in the case of animals on casein diet than in the case of animals on other diets.

The observed values for the average peak and daily parasitæmia, the average survival period and the average spleen volume as stated above, were found to be greater in animals fed on a diet rich in casein compared to those in animals fed on the modified basal diet or the colony diet. The differences between the indices in the different groups of animals, fed on the three diets, were submitted to the 't' test to determine if the differences are statistically significant (Fisher, 1934). The details of the analysis with respect to each of the indices mentioned above, are given in Table VI.

The difference between the observed value for peak parasitæmia in animals fed on casein diet and that on colony diet, was found to be statistically significant in two out of the three experiments. The significance in one case was at one per cent level while in the other at five per cent level. Similar degrees of significance were found between the difference between the observed values of the index in animals fed on casein diet and those on the modified basal diet.

The observed values for the average daily parasitæmia in animals fed on casein diet were found to be significantly higher than those in animals fed (*a*) on colony diet and (*b*) on the modified basal diet, without casein, in two out of three experiments.

The period of survival, however, was not found to be significantly different in animals fed on casein diet to that in animals fed on (*a*) colony diet and (*b*) the modified basal diet, without casein. Nevertheless, the lack of statistical significance of this particular observed value, would not appear to effect the general conclusion that casein diet favours the parasite growth as well as host tolerance to the parasites which in animals fed on the diet multiplied most.

TABLE V.

Different indices of the course of infection in animals fed on the three types of diets.

1	2			3						4			5			6			7			8
DIET GROUP	NUMBER OF ANIMALS			AVERAGE PARASITÆMIA PER 10,000 ERYTHROCYTES						AVERAGE DAY OF PEAK PARASITÆMIA FROM FIRST DAY OF PATENCY			AVERAGE DAY OF DEATH FROM 'O' DAY OF INOCULATION			AVERAGE SPLEEN VOLUME IN C. C.			AVERAGE TOTAL PARASITÆMIA PER 10,000 ERYTHROCYTES (i. e. PRODUCT OF 3A × 5)			Remarks
				3-A Daily			3-B Peak															
Number of experiments	Experiment			Experiment			Experiment			Experiment			Experiment			Experiment			Experiment			
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	
Modified basal diet, without casein ...	5	10	9*	960·7	811·0	868·9	3155	2002·8	1615·5	10·8	17·3	16·02	15·0	18·8	17·8	0·65	0·31	0·40	14410·5	15246	15466·4	*One animal refractory
Casein ...	5	9*	10	823·7	1374·4	1632·7	2838	4451·1	5541·8	19·4	19·8	19·3	23·6	20·3	18·2	0·68	0·51	0·60	19439·3	27900	829715·1	*One animal refractory
Colony ...	3	8*	10	1080·3	676·6	880·7	3180	1619·5	1750·8	10·3	14·0	15·3	14·3	14·5	16·1	0·66	0·34	0·44	15448·2	9810·7	314179·3	*Two animals refractory

TABLE VI.

Details of the statistical analysis of the differences between the indices observed in groups of animals on the different diets.

Description of experiment	Experiment number	Mean values		't'	d.f.	Remarks
		Experimental	Control			
1. Peak parasitæmia.						
Casein diet versus Colony diet	1.	2838	3180	0·3445	6	Not significant
	2.	4451·1	1619·5	3·8087	15	Significant at 1 per cent
	3.	5541·8	1750·8	2·5574	18	Significant at 5 per cent
Casein diet versus Modified basal diet without casein.	1.	2838	3155	0·2106	8	Not significant
	2.	4451·1	2002·8	3·2801	17	Significant at 1 per cent
	3.	5541·8	1615·5	2·6311	17	Significant at 5 per cent
2. Average daily parasitæmia.						
Casein diet versus Colony diet	1.	823·7	1080·3	0·5646	6	Not significant
	2.	1374·4	676·6	3·8601	15	Significant at 1 per cent
	3.	1632·7	880·7	2·6183	18	Significant at 5 per cent
Casein diet versus Modified basal diet without casein.	1.	823·7	960·7	0·4417	8	Not significant
	2.	1374·4	811	3·2809	17	Significant at 1 per cent
	3.	1632·7	868·9	2·4868	17	Significant at 5 per cent
3. Period of survival.						
Casein diet versus Colony diet	1.	23·6	14·3	2·7678	6	Significant at 5 per cent
	2.	20·33	14·5	1·4759	15	Not significant
	3.	18·2	16·1	1·1351	18	,, ,,
Casein diet versus Modified basal diet without casein.	1.	23·6	15	2·0525	8	,, ,,
	2.	20·33	18·8	0·4135	17	,, ,,
	3.	18·2	17·8	0·1012	17	,, ,,

(Contd.)

TABLE VI.—(*Concld.*)

Description of experiment	Experiment number	Mean values		't'	d.f.	Remarks
		Experimental	Control			
		4. Spleen volume.				
Casein diet versus Colony diet	1.	0·68	0·66	0·1428	6	Not significant
	2.	0·505	0·34	2·6984	15	Significant at 5 per cent
	3.	0·6	0·44	2·1621	18	Significant at 5 per cent
Casein diet versus Modified basal diet, without casein.	1.	0·68	0·65	0·1685	8	Not significant
	2.	0·505	0·31	3·7735	17	Significant at 1 per cent
	3.	0·6	0·4	2·7027	17	Significant at 5 per cent

't'=''t'' values. d.f.=Degree of freedom.

The observed values for the average enlarged spleen are found to be significantly greater in casein diet than those in animals fed on (*a*) colony diet, as well as (*b*) modified basal diet without casein, in two out of three experiments.

DISCUSSION.

It appeared from the results that casein diet provided the host as well as the parasite with all the nourishment required by them. But compared to other diets, casein was the only one which caused the infected animals to survive the longest despite the infection in them being the most severe. As stated already, the observed value for the average period of survival of the group of animals fed on casein diet was, however, not found to be significantly greater than that of the other two groups fed on colony and modified basal diets without casein, respectively. The more important observation would appear to be that despite significantly greater values for peak and daily parasitæmias, the animals fed on casein diet did not die earlier than animals fed on the other two diets. Logically, the higher peak and daily parasitæmias could be expected to have been fatal to the animals at an earlier period of the infection. The inference that casein diet enhances host tolerance, is further supported by the significantly higher observed values for the average spleen volume in this group of animals compared to that of the other two groups. The indications were that casein diet facilitated the defence machanism of the host to acquire a greater degree of immunity. Of course, in the case of mice extremely susceptible to *P. berghei* the question of a sufficient degree of immunity to recover from an untreated infection, does not arise (Ramakrishnan and Satya Prakash, 1950*b*).

Antitoxic effects of immunity are often assumed in malaria on account of a higher pyrogenic threshold of parasites in immune hosts (Vishwanathan, 1951). It will continue to be an assumption till there is proof forthcoming for the existence of an antigenic toxin in the red cell and for the parasite debris including the hemozoin pigment liberated in the blood at the time of plasmodial segmentation. A support for the speculation on the existence of an antitoxic factor of immunity in malaria, was furnished by Jaswant Singh and Harwant Singh (1940) in their work on passive immunity in *Plsamodium knowlesi* malaria of monkeys. Eight infected monkeys were administered immune serum; four of these died in a period similar to controls, but the remaining four animals survived for a considerably longer period with patent infection. It would appear that the results of the present investigation confirm the existence of an anti-toxic immunity factor.

The acquired immunity as measured by the average spleen volume was found to be a little more in casein group of animals than compared to others. If it is conceded that immunity to malaria consists of different antiparasitic and anti-toxic factors (Vishwanathan, 1951), then the explanation is that casein diet afforded the mice a greater capacity to acquire anti-toxic factor primarily, which saved the animals from mortality for a longer period.

Discussing cellular and humoral factors of acquired immunity to malaria, Mullingan, Sommerville and Swaminath (1940) concluded that, the factors developed to the extent necessary, to supplement or re-inforce natural immunity and they were, therefore, more highly developed in moderately susceptible than in resistant hosts. In the case of extremely susceptible hosts such as monkeys (in this case mice), they observed that death occurred before immunity, sufficient for recovery, was acquired.

SUMMARY.

It is observed that casein diet provided the host as well as the parasite with all the nourishment required. And compared to two other diets, casein was the one which enabled the infected mice to survive longest in spite of the infection being severest. It is, therefore, concluded that milk protein (casein) seemed to increase the capacity of mice to acquire a comparatively greater tolerance to malaria.

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STUDIES ON THE SUSCEPTIBILITY OF *A. SUBPICTUS* GRASSI, 1899 TO CHLORINATED HYDROCARBON INSECTICIDES IN A DELHI STATE VILLAGE*.

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[February 12, 1957.]

INTRODUCTION.

STUDIES on the susceptibility of *A. subpictus* to chlorinated hydrocarbon insecticides were undertaken in a Delhi State village consequent to the failure of DDT residual spray to produce an appreciable decline in its anopheline population. This was noted during the routine evaluation of various insecticides as residual sprays in the field against mosquitoes in a number of villages of Delhi and Uttar Pradesh.

Village Jwalaheri in Delhi State had been sprayed with DDT since 1947. Details of the spraying operations are shown in Table I. In July, 1956, it was sprayed at the rate of 100 mg. DDT per sq. ft. Contrary to expectations, there was little decline in mosquito density even for 48 hours after insecticidal application. The catches of adult mosquitoes revealed that *A. subpictus* was the most dominant species (95 per cent or over).

Preliminary studies on the susceptibility of this species to DDT, indicated development of high tolerance (Sharma and Krishnamurthy, 1957). Details of further studies which have been carried out are reported here.

SUSCEPTIBILITY OF *A. SUBPICTUS* ADULTS TO DDT, GAMMA BHC AND DIELDRIN.

For assessing the susceptibility of mosquitoes to DDT, gamma BHC and dieldrin, technique recommended by W.H.O. (World Health Organization

*This work was carried out under the auspices of "General Behaviour of Vector Species of Anophelines, their Resistance to Insecticides etc." an enquiry financed by the Indian Council of Medical Research.

Tech. Rep. Ser. 80, 1954) was adopted*. For this purpose, mosquitoes were collected from the sprayed village Jwalaheri and for comparison, from a nearby unsprayed village Arthala (Meerut District Uttar Pradesh). World Health Organization technique was slightly modified in respect of time of exposure of test insects to insecticidal deposits. The period of exposure was increased from one to 24 hours for mosquitoes (*A. subpictus*) collected from the DDT sprayed village to obtain mortalities within the critical range. There was no mortality in the test insects after 24 hours, with one and two hours exposure to the insecticidal deposits. Each test was performed with 4-5 concentrations of insecticides, six tubes being used for each concentration and in each tube six mosquitoes were exposed. The tests were replicated six times. To avoid high mortality in transportation, the tests were carried out in the village site. In all cases, active gravid female mosquitoes were used.

TABLE I.

Details regarding DDT spraying of village Jwalaheri, Delhi State.

Date of spraying	Dosage mg. DDT per sq. ft.	Total dosage per year mg. DDT per sq. ft.
August 26, 1947 ...	25	25
July 29, 1948 ...	50	100
September 20, 1948	50	
August 4, 1949 ...	50	75
September 20, 1949	25	
August 10, 1950 ...	50	75
September 20, 1950	25	
July 31, 1951 ...	50	100
September 20, 1951	50	
July 21, 1952 ...	50	100
September 25, 1952	50	
August 7, 1953 ...	100	200
September 21, 1953	100	
July 20, 1954 ...	100	200
September 28, 1954	100	
August 8, 1955 ...	100	200
October 4, 1955 ...	100	
August 27, 1956 ...	100	100

The results of the experiments were subjected to probit analysis and the median lethal concentrations (MLC) were estimated by graphical process (Finney, 1952). MLC figures in respect of the different insecticides, temperature and humidity during the period of observation and other experimental details from the DDT sprayed and unsprayed villages are recorded in Tables II and III, respectively.

*For preparation of insecticidal solutions in white oil, technical DDT, technical dieldrin and lindane were used.

TABLE II.

Details regarding susceptibility tests of DDT resistant A. subpictus *adults from Village Jwalaheri, Delhi State*.*

(All tests were performed with active gravid female mosquitoes within the range of maximum temperature 22·2°C.-28·8°C., minimum temperature 20·5°C.-25·5°C. and relative humidity 87 per cent to 95 per cent).

Insecticides	Concentration of insecticide in oil solution (per cent)	Total number of insects used	Number of insects found dead after 24 hours	Percentage mortality (corrected)	Median lethal concentration (per cent)
DDT ...	1·0	216	45	15·76	
	2·0	216	84	34·98	3·1×24†
	3·0	216	104	44·83	
	4·0	216	122	53·69	
	5·0	212	169	78·42	
	Control	216	13	...	
Gamma BHC ...	0·00625	216	21	7·59	
	0·0125	216	77	34·13	0·014×1‡
	0·025	216	194	89·57	
	0·05	216	202	93·37	
	Control	216	5		
Dieldrin ...	0·0625	216	35	15·81	
	0·125	216	107	49·31	
	0·25	216	139	64·19	0·16×1‡
	0·5	216	203	93·95	
	Control	216	1	...	

MLC figures for *A. subpictus* from the DDT sprayed village for DDT, gamma BHC and dieldrin were found to be 3·1×24†, 0·014×1‡, 0·16×1‡ respectively. MLC figures for mosquitoes from the unsprayed village were 0·28×1‡, 0·023×1‡, 0·25×1‡ for DDT, gamma BHC and deildrin, respectively. The data obtained indicated a high tolerance to DDT exhibited by *A. subpictus* collected from village Jwalaheri, but no cross resistance to either gamma BHC or dieldrin.

*Period of observation was from September 29, 1956 to October 16, 1956.
†Mortality in insects was recorded after 24 hours exposure of the insects to insecticidal deposits.
‡Mortality in insects was recorded after 24 hours with one hour exposure of the insects to insecticidal deposits.

TABLE III.

Details regarding the susceptibility tests of normal A. subpictus *adults from unsprayed Village Arthala (Meerut District) Uttar Pradesh*.*

(All tests were performed with active gravid mosquitoes within the range of maximum temperature 22·2°C-31·1°C., minimum temperature 21·1°C.-26.6°C. and relative humidity 90 per cent to 100 per cent).

Insecticides	Concentration of insecticide in oil solution (per cent)	Total number of insects used	Number of insects found dead after 24 hours	Percentage mortality (corrected)	Median lethal concentration (per cent)
DDT ...	Control	216	4	...	
	0·125	216	56	24·53	
	0·25	216	126	57·54	0·28 × 1†
	0·5	216	154	70·76	
	1·0	216	182	83·96	
	2·0	216	204	94·33	
Gamma BHC ...	Control	324	3	...	
	0·00625	324	48	14·01	
	0·0125	324	101	30·52	0·023 × 1†
	0·025	324	204	62·61	
	0·05	324	235	72·27	
Dieldrin ...	Control	252	2	...	
	0·0625	216	27	11·80	
	0·125	252	74	28·80	0·25 × 1†
	0·25	252	138	54·40	
	0·5	252	169	66·80	

*Period of observation was September 17, 1956 to October 7, 1956.
†Mortality in insects was recorded after 24 hours with one hour exposure of the insects to the insecticidal deposits.

SUSCEPTIBILITY OF *A. SUBPICTUS* LARVÆ TO DDT.

In order to assess the susceptibility of larvæ to DDT, tests were performed with fourth instar larvæ raised from eggs laid by gravid mosquitoes collected from the DDT sprayed and unsprayed villages. The following technique was adopted.

Serial concentrations of DDT in absolute alcohol containing 0·01, 0·02, 0·04, 0·08 and 0·16 per cent DDT, were prepared. The requisite quantity of these concentrations, was introduced in beakers each containing 100 c.c. of water. Five such beakers were used for each dosage (ppm) of insecticide and the tests were replicated four to seven times. Ten fourth-instar larvæ were introduced in each beaker and a small quantity of yeast was added to serve as food for the larvæ. The mortality was recorded after 24 hours. Larvæ which pupated during the period of observation, were not taken into consideration in calculating the mortalities. In control, the larvæ were exposed to equivalent alcoholic solution in water without the insecticide. The amount of alcohol added was same as in the case of highest dosage of insecticide.

The experimental details of the susceptibility tests of *A. subpictus* larvæ from the sprayed and unsprayed villages, such as concentrations of DDT, temperature of water during tests etc. are given in Tables IV and V, respectively. The mortalities of larvæ from the sprayed village, as observed from the data, varied very widely and were inconsistent. Therefore, the degree of resistance of larvæ to DDT could not be assessed quantitatively. However, larvæ from the sprayed village showed definite increase in their tolerance to DDT as compared to those from the unsprayed village. Increase in tolerance, exhibited by the larvæ from the sprayed village, did not appear to be of the same order as that seen in the case of adult *A. subpictus*. This aspect of the problem requires further investigations.

TABLE IV.

Details of experiments regarding the susceptibility of A. subpictus *larvæ to DDT from the DDT sprayed village Jwalaheri (Delhi State).*

Serial number	Concentrations of DDT in alcohol (ppm)	Period of observation	Temperature of water during test C°	Total number of larvæ used for each concentration	Number of larvæ found dead after 24 hours	Percentage mortality
1	Control	November 29, 1956 to December 21, 1956	18 to 21	230	9	3·91
2	0·25	,, ,, ,,	18 to 21	222	66	27·2
3	0·5	,, ,, ,,	18 to 21	283	120	42·4
4	1·0	,, ,, ,,	18 to 21	283	130	45·7
5	2·0	,, ,, ,,	15 to 21·5	220	56	25·5
6	4·0	,, ,, ,,	16 to 21·5	130	51	39·2
7	6·0	,, ,, ,,	19·5 to 21·5	120	55	45·8
8	8·0	,, ,, ,,	19·5 to 21·5	120	46	38·3

TABLE V.

Details of experiments regarding the susceptibility of A. subpictus *larvæ to DDT from the unprayed village Arthala (Meerut District, Uttar Pradesh).*

Serial number	Concentration of DDT in alcohol (ppm)	Period of observation	Temperature of water during test C°	Total number of larvæ used for each concentration	Number of larvæ found dead after 24 hours	Percentage mortality
1	Control	November 11-23, 1956	19 to 21	319	5	1·56
2	0·25	,, ,, ,,	19 to 21	334	164	49·10
3	0·5	,, ,, ,,	19 to 21	332	198	59·64
4	1·0	,, ,, ,,	19 to 21	323	231	71·51
5	1·5	,, ,, ,,	19 to 21	326	260	79·75
6	2·0	,, ,, ,,	19 to 21	329	278	84·50

DISCUSSION.

Instances of insecticide resistance in anopheline mosquitoes have been recorded from a number of countries. However, in India, resistance has been reported only in *A. stephensi* larvæ in Erode, Madras State, where DDT had been used as a larvicide since 1947 (Rajagopalan *et al.*, 1956). In the case of adult mosquitoes, only *Culex fatigans* was reported to have developed resistance to DDT in some States* (Rajindar Pal *et al.*, 1952; Krishnan, 1956). It was considered that as DDT has not been used extensively as a larvicide in India and since in most areas malaria transmission is seasonal and the application of DDT is restricted to certain months of the year only, in the absence of continuous selection pressure of the insecticide on the total insect population, anopheline mosquitoes may not acquire any significant tolerance. But the observations of high resistance to DDT exhibited by *A. subpictus* adults from the DDT sprayed village Jwalaheri, as well as appreciable tolerance to DDT shown by the larvæ where the insecticide was used only as an adulticide, are significant. A redeeming feature, however, is that DDT resistant *A. subpictus* has been found to be as susceptible to gamma BHC and dieldrin as normal *A. subpictus* and should be possible to control by the use of these insecticides. This finding is in conformity with the obseravtions of Davidson (1955*a*:1955*b*) who in the case of *A. sundaicus* and *A. stephensi* in Indonesia and Saudi Arabia, respectively, recorded that the DDT resistant mosquitoes showed normal susceptibility to gamma BHC and dieldrin.

*Technical Report of the Scientific Advisory Board of the Indian Council of Medical Research for the year 1955.

SUMMARY AND CONCLUSIONS.

Studies on the susceptibility of *A. subpictus* adults to chlorinated hydrocarbon insecticides and *A. subpictus* larvæ to DDT from a Delhi State village, sprayed with DDT since 1947, are recorded.

A. subpictus adults were found to be highly resistant to DDT, but they did not show any cross resistance to gamma BHC and dieldrin.

Fourth-instar larvæ, obtained from the eggs of resistant *A. subpictus*, also showed appreciable tolerance to DDT.

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STUDIES ON NURI STRAIN OF *P. KNOWLESI.*

Part XIV. Pathological processes in the liver*.

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[May 3, 1957.]

INVESTIGATIONS on the liver changes in malaria had been taken up by many workers. Although damage to the parenchyma cells in ordinary cases of malaria was not given any credence, most workers agreed from autopsy findings that in extremely severe cases of *P. falciparum* in human subjects, all stages of parenchyma damage like swelling with granular cytoplasm, fatty changes and necrosis were to be found (Dudgeon and Clark, 1917; Klotz, 1929; De, 1938; Maegraith, 1948). However, detailed studies at different stages of acute infection are not complete. Since such investigations on human subjects had not been easy, much of the information available at present is derived from experimental studies in simian malaria (Taliaferro and Cannon, 1936; Taliaferro and Mulligan, 1937; Bhaskar Menon, 1938 and others). Though most of such studies had been restricted to the changes in the cells of the reticulo-endothelial system and the origin and function of the macrophages, mention had also been made of changes in the parenchyma cells. These were 'cloudy swelling', fatty changes and even necrosis of cells round the central hepatic veins in fatal *P. knowlesi* infection. However, Bhaskar Menon (1938) was unable to observe any such necrotic changes in a small series of six monkeys. Subsequently, Rigdon and Stratman-Thomas (1942) demonstrated all stages of parenchyma cell damage in *M. mulatta* infected with a strain of *P. knowlesi* originally isolated by Sinton and Mulligan (1932). Similar findings were reported by Ray (1954) who had observed during preliminary studies that the Nuri strain of *P. knowlesi* was extremely virulent to *M. mulatta* and that fatty changes and centrilobular necrosis of the liver were common in this type of infection. The necrotic changes were most marked when cell infection attained 70 per cent or over.

In the present paper, the author records the findings on a series of detailed investigations on this aspect carried out at the laboratories of the Malaria Institute

*The subject matter of this paper formed part of a thesis entitled "Experimental studies on the liver of *M. mulatta mulatta* infected with simian plasmodia".

of India. Besides biopsy of the liver at various stages of investigations, the course and intensity of infection were also studied. In addition, detailed hæmatological studies were undertaken (to be published in detail elsewhere) for proper appraisal of the type and degree of liver injuries encountered. Attempts were also made to throw some light on the pathogenesis of such liver injuries, a subject still in the realm of speculation.

MATERIALS AND METHODS.

Host.—Fifty wild caught tuberculin negative *M. mulatta mulatta* were utilized for these studies. In addition, liver materials were studied from 29 normal monkeys for comparison purpose during the present observations. Prior to inoculation, all were subjected to detailed hæmatological studies as well as repeated blood examinations for any blood protozoa. Further, the animals were kept on standard diet for at least two to three weeks and body-weight taken regularly till the weight and hæmoglobin level became more or less constant.

Parasite.—A recently isolated strain (Nuri) of *P. knowlesi* (Jaswant Singh, Ray and Nair, 1953; Edeson and Davey, 1953) maintained in these laboratories by serial blood passage, was used for these investigations. The strain had been found to be highly virulent, and irrespective of the dose or route of inoculation all animals succumbed within a period of four to seven days once the patent infection was established.

For infective inoculation, a dose of 5×10^6 parasitized cells per kg. was introduced through the dorsal leg vein, a standard procedure adopted in the laboratories of this Institute for various chemotherapeutic and parasitological investigations.

Blood smears were stained with J.S.B. (Jaswant Singh and Bhattacharji, 1944). For parasite count, an Ehrlich's eyepiece, adjusted to include 100 erythrocytes per field of the oil immersion lens, was used and 100 such fields were examined.

Biopsy.—When liver materials were required during the early or later stages of infection, tissues were removed by laparotomy instead of needle biopsy. Such materials were obtained from various lobes of the liver by rotation; 'Kemithal' was used for anæsthesia and 'gelfoam' applied to the cut surfaces for hæmostatic purpose. During the terminal phase, pieces of liver were removed just prior to or immediately after the death of the animal.

PREPARATION OF TISSUE.

The tissues were sliced into small pieces and fixed in formol saline and kept for 24 to 48 hours. They were then washed in running water and transferred to acetone for 12 to 24 hours. After three such transfers, they were placed in benzene for clearing. Subsequently, the materials were kept in paraffin benzene mixture (50 per cent of each). Three such changes were made varying from 1/2 to 12 hours (depending on the size of the tissues). They were then removed to paraffin (melting point 55°-57°C.) in a paraffin bath (electrically operated) adjusted to

58 to 60°C. Subsequenty, the tissues were removed and placed at the bottom of Leuckhart's imbedding boxes and paraffin poured from the top. The paraffin was then cooled rapidly with ice water. Sections 3 to 5 μ were cut in a Leitz microtome for H.E. stain. Formol fixed frozen sections were cut (10 to 15 μ) in a Sartorius-werk microtome).

STAINS AND STAINING.

The paraffin sections were stained with Ehrlich's hæmatoxylin (Gatenby and Beams, 1950) and counterstained with eosin. A mixture of Sudan III and IV (Key and Whitehead, 1944) or Sudan black was used for frozen sections for demonstration of fat.

RESULTS.

(A) PARASITOLOGICAL FINDINGS.

Detailed observations relating to the course and intensity of *P. knowlesi* infection were made in 32 out of 50 monkeys inoculated. In 12 of them, patent infection was established within a few hours while in the remainder the same was observed by Day 1 (day of inoculation being zero). Thereafter the picture appeared to be more or less similar. In 19 animals (about 60 per cent), the peak parasitæmia was established on Day 5, while in 7, it was on Day 4, and in the rest on Day 6. All animals died within a few hours after the peak infection. In the first group, the percentage (average) of erythrocytic invasion was 0·01, 0·83, 10·26, 42·22 and 83·31 from Day 1 to Day 5, respectively. As compared to cell infection on Day 2, the proportion of rise in parasitæmia from Days 3 to 5 was 12·3, 50·8 and 100 fold. Taking all animals in all the groups into consideration, the intensity of infection at peak varied from 48 to 96 per cent, though in 30 out of 32 animals it was over 60 per cent. Thus, it would be observed that the rise in parasitæmia was almost meteoric, particularly noticeable during the terminal phase (*i.e.* after the last sporulation).

(B) HISTOPATHOLOGICAL FINDINGS.

During the present investigations, though the main attention was focussed towards the liver parenchyma, changes in the cells of the reticulo-endothelial system were not overlooked. In this connection, it may be mentioned again that the specimens of liver were collected at various stages of infection. For this, liver biopsies were made in five monkeys when the cell infection was between 0·3 to 10 per cent (early stage). Similar observations were made in nine others when cell infection had attained between 28 to 53 per cent (later stage). Materials from 20 other monkeys were collected at the terminal stage (57 to 96 per cent) either prior to or just after death. Some of these were also utilized for parasitological investigations indicated earlier.

Side by side, biopsy specimens were studied from 29 normal monkeys for comparison (both during the present investigations as well as for various other investigations not included in the present paper).

(i) Comparison series.

The liver lobule consisted of columns of cells which radiated from the central veins. The columns were separated by irregular or tortuous sinusoids. The liver cells appeared to be similar to those seen in human specimens. As a rule, the cells contained one or two nuclei with prominent nucleoli. The appearance of the central hepatic vein, the portal tract with its vessels and ducts, appeared to be similar to those seen in human liver (Plate VI, Fig. 1).

In the sinusoids, there were cells of the reticulo-endothelial system termed as littoral cells*. As a rule, they appeared to be flattened, lining the walls of the sinusoids.

(ii) Experimental series.

(*a*) *Early stage of infection.*—In all specimens, the lobular architecture was well maintained. There was no apparent parenchyma damage except in one specimen (with 10 per cent cell infection) which showed some degree of swelling of the cells. The central hepatic and the portal veins were normal in appearance. Most of the sinusoids appeared to be normal. Inside the sinusoids, blood elements, a few parasitized cells and occasional swollen Kupffer cells containing malarial pigments, could be observed.

(*b*) *The later stages.*—The lobular architecture was well preserved in all cases. But in three out of nine specimens (with cell infection 38, 46 and 53 per cent), there was necrosis of the parenchyma cells in some (but not all) of the lobules and these were confined to the groups surrounding the central hepatic veins (Plate VI, Fig. 2). There were also fatty changes of the parenchyma cells and the distribution was mainly of diffuse type. In all cases, the central hepatic veins were dilated and in some in which necrotic changes could be seen, they were widely so. Occasionally there was a fair number of polymorphonuclear cells, seen at places in the area of necrosis.

Most of the sinusoids were widely dilated and contained numerous parasitized cells, parasites, pigments, blood elements. Besides, there were Kupffer cells which were swollen and loaded with parasitized cells, pigments, etc.

In the other six specimens (cell infection varying from 28 to 40 per cent), no necrosis could be observed but swelling of the parenchyma cells and fatty changes of diffuse type were the main features. The central hepatic veins were, as a rule, dilated. The sinusoidal ' packing ' was similar to those seen in the other three specimens.

(*c*) *The terminal stage.*—All stages of degeneration of the parenchyma were evident. In most of the stages, the lesion appeared to be highly accentuated. Specimens obtained from well advanced cases (having cell infection of 70 per cent or over) showed the following characteristic features.

*During their studies in simian malaria, Taliaferro and Mulligan (1937) had drawn distinctions amongst these cells. Those which were quiescent and not phagocytic, were termed as ' littoral ' as opposed to the stellate cells of Kupffer (the liver macrophages) which are actively phagocytic. During the present studies, the same nomenclature have been used to distinguish between quiescent and phagocytic cells.

PLATE [illegible].

FIG. 1. Liver section showing normal lobular pattern.

FIG. 2. Liver section showing centrilobular necrosis.

FIG. 3. Liver section showing severe degree of necrosis with fragmentation of cells.

FIG. 1. Section of liver showing heavily packed sinusoids.

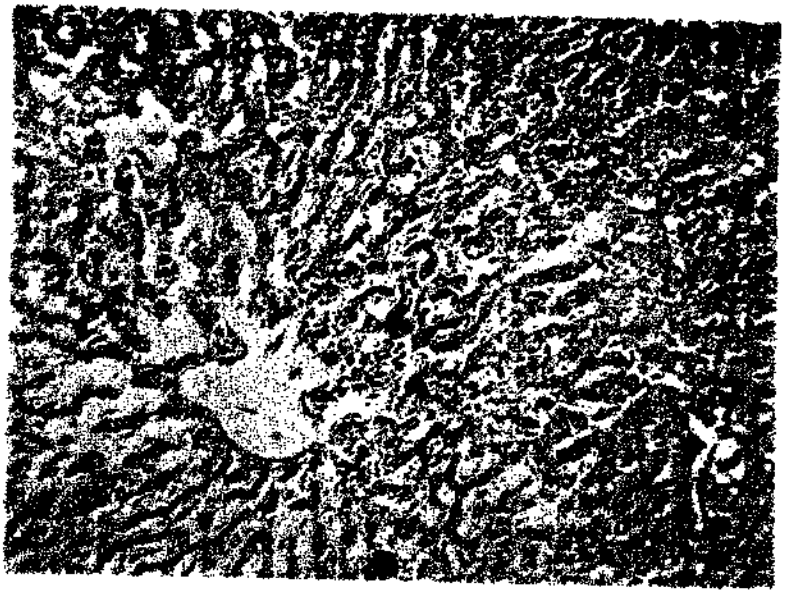

FIG. 2. Liver section showing centrilobular necrosis and dilation and rupture of the central hepatic veins.

The lobular pattern was completely disrupted and in such cases the central hepatic veins could be identified with difficulty and then too only at places. The necrotic changes were extensive and had spread up to or well beyond the midzonal regions. Occasionally the necrosis was so severe that it was only round the periphery of the lobule groups of apparently normal cells in single rows, or in small columns could be seen round the portal tracts. The parenchyma cell damage consisted of early to severe degree of fatty changes to necrosis of cells, and complete fragmentation (Plate VI, Fig. 3).

The sinusoids appeared to be completely packed with parasitized cells, conglomerated erythrocytes ('sludge') pigments, enormously swollen with Kupffer cells, etc. (Plate VII, Fig. 1). The appearance suggested complete mechanical blockage of the sinusoids.

Where the central hepatic veins could be identified, they were found to be widely dilated and their walls were often ruptured (Plate VII, Fig. 2). In some instances, the portal veins or the radicular portal veins in the portal tracts, seemed to be widely dilated.

The overall picture was one of acute cell degeneration, diffuse in character, which had altered the vascular pattern so that the central hepatic veins were not always identifiable. Where this could be done, the central veins were found to be dilated or ruptured. The sinusoids were packed heavily with Kupffer cells, parasites, pigments etc., and these appeared to form a barrier to the normal flow of blood from the portal veins to the central hepatic veins. Occasionally the portal veins were also found to be dilated.

DISCUSSION.

From the data presented, it would be evident that the infection due to the Nuri strain of *P. knowlesi*, was extremely severe and that in almost all cases death was inevitable. The day to day rise in parasitæmia was found to be phenomenal, particularly during the terminal stage when the parasitic density reached as high as 60 to 80 per cent in most of the cases. In this respect, the picture appeared to be similar to that observed in *P. gallinaceum* infection in chicks (Jaswant Singh, Basu and Ray, 1952) and in *P. berghei* in mice (Ramakrishnan and Satya Prakash, 1950).

Such a heavy infection led to destruction of a large number of erythrocytes and consequently the fall in hæmoglobin was extremely rapid. During the terminal stage, reduction in hæmoglobin level was by 46 per cent as demonstrated previously by Ray (1955). The overall picture, therefore, showed rapid development of severe degree of anæmia following heavy erythrocytic invasion.

As to the gross liver changes, it had been demonstrated earlier by Ray and Nair (1954) that in *P. knowlesi* infection the liver was enlarged to the extent of 45 per cent. Apparently, this enlargement was mostly due to congestion.

Histological findings indicated clearly that significant lesions like fatty changes and necrosis, were common in *P.knowlesi* infection and the degree of lesion, particularly the necrotic changes, was proportional to the intensity of infection. Fatty changes were detectable early and were of such a diffuse nature that it was

often difficult to locate the primary origin. However, in several specimens in which necrosis was of acute nature, fatty changes of parenchyma cells could be found round the portal tracts. As to the necrosis of the cells, it may be mentioned that such changes appeared during the later stage of infection though it was not a constant feature until the density of parasites had reached near about 60 per cent. Where necrosis appeared early, the centrilobular distribution could be well demonstrated. In some well-advanced cases, the same could also be observed even though it had spread up to or beyond the midzonal region. In a few cases, however, necrosis was of such a severe degree that the lobular structure was lost and the central veins could hardly be identified.

While much attention had been paid in the past on the pathogenesis of liver injuries like centrilobular necrosis, fatty changes were considered to be merely the earlier stages of the same pathological processes caused by the same stimuli.

In view of this, the observations reported by Ray (1954) should be of interest as it was shown that transfusion of whole blood or even washed cells from normal monkeys (but not plasma), prevented necrotic changes though it could not prevent fatty changes. In all these specimens, sinusoidal ' packings ' were found to be as complete as seen in untreated specimens. In other words, the cells round the central hepatic veins were benefited in spite of sinusoidal blockage. These observations, coupled with the phenomenon of dilation and sometimes of rupture of the central hepatic veins in untreated animals, would appear to be the main objections against the theory of sinusoidal blockage as a factor responsible for such necrosis. Because, firstly, such a blockage would lead to collapse of the central veins rather than dilatation. Secondly, a blockage in the sinusoids would automatically make blood transfusion infructuous.

Further, it may be observed that although there was severe degree of anæmia during the terminal phase, *per se* this could hardly be considered as a factor for necrotic changes as no necrosis could be seen in *P.cynomolgi* infetion in which anæmia was observed to be much more severe, nor in the animals which were bled anæmic (Ray, 1955).

It is, therefore, likely that centrilobular necrosis is perhaps caused by the action of a sphincter mechanism in the hepatic venous tree as postulated by Maegraith *et al.*, (1947). Further, humoral or reflex mechanism might be involved in setting the constrictor mechanism into action (Maegraith, 1948). Under such circumstances, one of the possibilities could be that anoxæmia, due to breakdown of large number of erythrocytesd uring sporulation, was the stimulant for sphincter action. As such, prevention of necrosis by transfusion could be achieved merely by combating such anoxæmia (Ray, 1955). In addition, factors like metabolic products of sporulation may also be involved in the process as was suggested by Maegraith (1948). Thus, it would appear that several factors may be involved in the pathogenesis of centrilobular necrosis. Similar indications are there for the genesis of fatty changes as it was shown that transfusion could not prevent fatty changes whereas choline minimized such pathological processes. In this connection, recent findings on the effect of *P. knowlesi* infection in sympathectomized monkeys, as reported by Ray and Sharma (1957), would appear to be highly interesting. Equally

surprising are the changes in the liver observed after inoculation of plasma from infected animals to normal ones (Ray, 1957).

Obviously, further studies would be indicated to unravel the mysteries of fatty changes as well as of centrilobular necrosis in *P. knowlesi* infection which usher in such dramatic episodes during the final phase.

SUMMARY.

Fifty *M. mulatta mulatta* were used in studying the effect of *P. knowlesi* infection on the liver.

For proper appraisal, parasitological studies as well as observations on the blood picture (to be reported elsewhere) were taken up. It was observed that the rise in infection was almost meteoric. *Pari pasu* there was a rapid fall in hæmoglobin indicating the development of severe degree of anæmia.

While fatty changes appeared fairly early, necrosis could be seen at about 40 per cent cell infection. However, the necrotic changes were not of such a constant nature as fatty changes until the parasitic density attained was 60 per cent or over. The fatty changes were of diffuse nature or sometimes of peripheral distribution. The necrosis was invariably of centrilobular type.

Certain points have been discussed on the pathogenesis of liver injuries of the types mentioned above. Further, some light has been thrown as to the lines on which future researches should be channelized.

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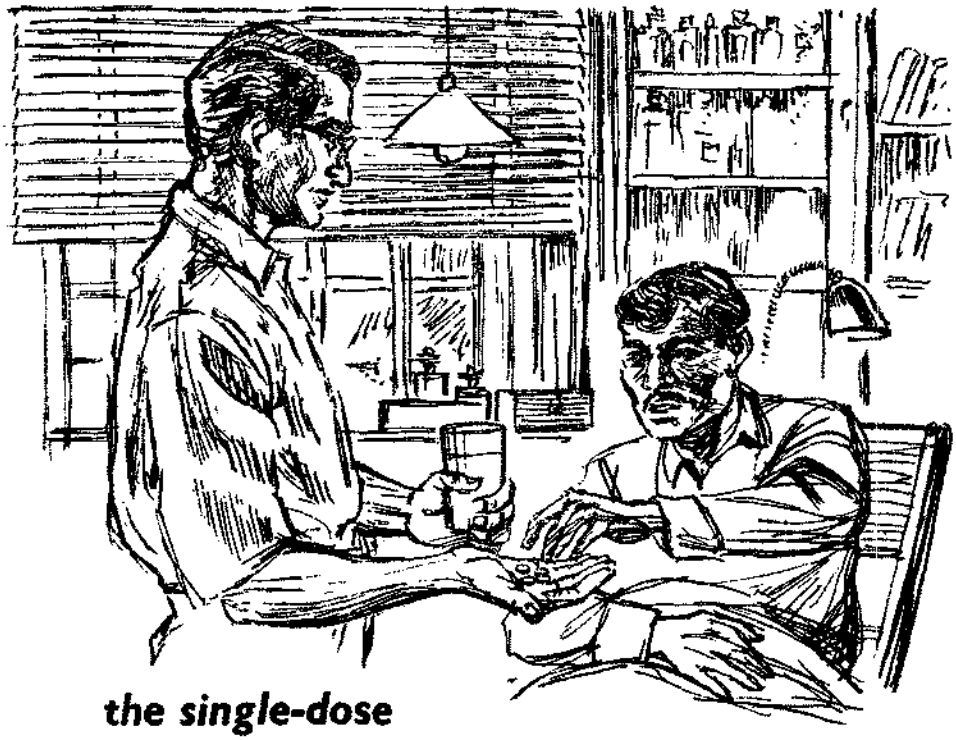

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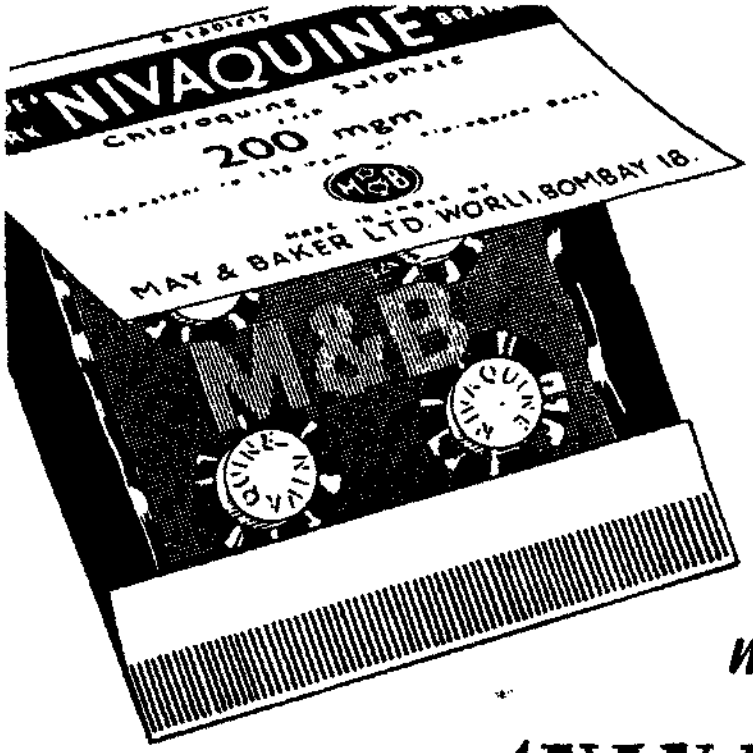

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DETECTION OF MALARIA PARASITES BY CONCENTRATION METHOD.

BY

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AND

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[May 8, 1957.]

For diagnosis of malaria, examination of thick and thin smears of blood, preferably on the same slide, has been recommended as the standard procedure by Christophers *et al.* (1936). Subsequently, much emphasis was placed on the examination of thick films for rapid diagnosis of malaria (Field, 1948; Walker, 1948; Ray, 1949; Jaswant Singh, 1950). Although the actual density at which parasites should become patent in a thick film preparation has not been clearly defined, diagnosis can be established quite often by examination of thick films. However, in some cases, particularly in *P. falciparum* infection, such examinations may have to be repeated. It is perhaps for this group of cases that the concentration method had been attempted by several workers. Some of the techniques involved, would appear to be complicated, like those described by Berliner *et al.* (1948), Christophers and Fulton (1938) or the ones advocated by Ferrebee and Gieman (1946) for which bovine albumin is necessary. Quantity of blood required is large and repeated centrifugations are necessary. But the procedure adopted by Halawani and Sobky (1939) would appear to be simple and involves the use of 0·5 c.c. of blood centrifuged with 10 c.c. of one per cent acetic acid (or formalin) solution for 10 minutes.

The present report records an attempt to follow the same technique adopted by the last group of workers, with a view to exploring the possibilities of application of the procedure for general use in cases where thick film may not have revealed any parasite although clinically diagnosed as malarial infection.

MATERIALS AND METHODS.

One hundred *M. mulatta mulatta*, experimentally inoculated (by blood passage) with the three simian plasmodia (*P. knowlesi*, *P. cynomolgi* and *P. inui*) for routine strain maintenance, were utilized for the purpose. The dose of inoculum for each plasmodium was 5×10^6 parasitized cells per kg. of the body weight; a routine procedure adopted in these laboratories.

For concentration method, 0·5 c.c. blood was taken from the dorsal leg vein of each monkey four hours after inoculation and once a day thereafter. The blood was immediately centrifuged with 10 c.c. of one per cent acetic acid for 10 minutes at 1,500 r.p.m. A small drop was pipetted out from under the whitish layer and was placed on the centre of a clean slide and spread out with the corner of a spreader in the same way as is usually done in respect of thick films. Ordinary thick and thin films were also made for comparison purposes. Both types of films were

TABLE I.

Parasite count in thick smears made by ordinary thick drop method and concentration method.

Monkey number	Parasite count per 100 fields of ordinary thick smears	Parasite count per 100 fields of thick smears made by concentration method	Ratio
7369	7	34	1 : 4·8
7377	8	22	1 : 2·7
7371	3	42	1 : 14
7381	14	45	1 : 3·2
7384	2	12	1 : 6
7372	1	6	1 : 6
7369	440	1540	1 : 3·3
7372	15	140	1 : 9·3
7384	52	250	1 : 4·8
7366	6	24	1 : 4
7376	2	10	1 : 5
7390	1	6	1 : 6
7368	20	72	1 : 3·6
7394	1	4	1 : 4
7418	1	3	1 : 3
7394	25	75	1 : 3
7412	12	38	1 : 3·2
7373	6	15	1 : 2·5
7410	9	25	1 : 2·8
7420	2	12	1 : 6
7421	1	5	1 : 5
7415	6	50	1 : 8·3
7422	7	50	1 : 7·1
7438	3	16	1 : 5·3
7445	4	15	1 : 3·7
7432	70	220	1 : 3·1
7579	20	108	1 : 5·4
7553	200	750	1 : 3·75
7339	2	10	1 : 5
6849	50	180	1 : 3·6
7461	21	130	1 : 6·2
7565	15	55	1 : 3·7
7583	10	45	1 : 4·5
6375	120	800	1 : 6·7
6516	8	35	1 : 4·4
7566	4	9	1 : 2·2
7370	70	340	1 : 4·8
7339	3	12	1 : 4
7591	1	8	1 : 8
7251	103	750	1 : 7·3
7028	20	170	1 : 8·5
5912	90	320	1 : 3·5

stained with J.S.B. stain (Jaswant Singh and Bhattacharji, 1944). In the case of thick smears, one hundred microscopic fields were examined to determine the density of parasites.

RESULTS.

Of the 100 monkeys examined, in 60 plasmodial infection was detected by the concentration method, whereas parasites could be found in ordinary thick smear examination in 42 cases only. Further, it would be observed from Table I that amongst the monkeys found positive in both the methods, the density of parasites in the concentration method was about 2·2 to 14 times more than that observed in the ordinary thick film preparation.

CONCLUSION.

It would, therefore, be obvious that the concentration method has definitely some advantage over the ordinary thick film preparation in that at least parasites were detectable in 18 per cent cases more which would have been missed under ordinary circumstances. Further, since the concentration method revealed somewhat higher density of parasites (where they were present), the search would appear to be less-time consuming than that in an ordinary thick film. The present findings also confirm the observation previously made by Halawani and Sobky (1939).

It is, therefore, suggested that where facilities exist the above technique should be followed in all suspected cases of malaria when ordinary blood examinations fail to reveal parasites. For this 0·5 c.c. blood should be collected from the median cubital vein with a hypodermic syringe under aseptic precautions and processed in the same way as described earlier.

How far the above technique could be made available under field conditions, particularly in a large scale control or perhaps eradication programme, and whether any further modification could be achieved to make it more applicable for such conditions, are under investigation.

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Indian Journal of Malariology, **11**, 3, *September 1957*.

ASSOCIATION OF *CHARA ZEYLANICA* WILLD. WITH ANOPHELINE BREEDING WITHIN DAMODAR-EDEN CANAL AREA OF WEST BENGAL.

BY

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AND

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[October 2, 1956.]

INTRODUCTION.

CABALLERO (1919) noted that *Chara foetida* caused the death of mosquito larvæ by asphyxiation and it led to a suggestion that charophytes might greatly assist in solving mosquito problem. MacGregor (1924) in his tests with *Chara hispida* on the development of mosquito larvæ noted its futility against anophelines and again emphasized its futility in 1928 (Quoted by Hamlyn-Harris, 1928). Howard (1926) communicated that Tarnagradski working in Northern Caucasus, found that many of the breeding places of *Anopheles maculipennis* were heavily overgrown with *Chara*. In the same year, the Malaria Commission in Spain also reported the ineffectiveness of *Chara*. Blow (1927) noted that neither charophytes nor even the glucocides prepared from the dried plants, had any larvicidal action. Hamlyn-Harris (1928:1932) observed that characeæ did not themselves possess any larvicidal properties (using *Chara fragilis* and *C. fatigans* in his 1932 experiments). Matheson and Hinman (1929) reported that *Chara fragilis* prevented mosquito breeding partly due to excessive amount of oxygen given off by the plant (see also Matheson and Hinman, 1928); in 1930 they observed that they were completely convinced that *Chara* had a marked lethal effect on mosquito larvæ.* The subject seems to have lost interest in the later years. The present investigation is an incidental one. Thus recently, while analyzing the association of some aquatic plants with anopheline breeding, it was possible to study the incidence of

*Buhot (1926) even recorded the related genus *Nitella* inhibiting breeding but this presumption was upset when during the same year Hamlyn-Harris (1927) found no less than three species of mosquitoes breeding side by side with *Nitella*.

anopheline breeding with *Chara*, in nature, in artificial *Chara* ponds and under laboratory conditions by determining the rate of emergence over vegetative and reproductive *Chara*.

NATURAL HABITAT OF *CHARA*.

Two species of *Chara*, C. *zeylanica* Willd. and *C. fragilis* Desv., are very common in the lower Damodar Valley. The former is noted to be nearly restricted to ponds and the latter to slow-flowing temporary canals and usually occurring in pure colonies.

TABLE I.

Association of Chara zeylanica-Jussiaea repens *with some algæ at pond Mèuè (Chotkhand) for September-November, 1955.*

Algal species	September	October	November
Chalmydomonas braunii	C	C	NC
Sphaerella lacustris	R(?)	O	O
Chlorococum humicolum	NC	C	?
Scenedesmus quadricauda	NC	NC	R
Pediastrum tetras	NC	NC	NC-R
Tetraspora species	VC	NC	?
Cladophora glomerata	NC	C	T
Oedogonium deceipiens	C	C	NC-R
Phycopeltis epiphyton	NC	C	NC-R
Spirogyra neglecta	C	NC	NC-R
Closterium moniliferum	C	NC	NC-C
Cosmarium granatum	C	C	C
C. reniforme	C	NC	NC-R
Staurastrum dejectum	C	C	NC
Phacus pleuronectes	R	R	?
Euglena viridis	NC	R-O	R-T
Merismopedia tenuissima	?	NC	C
Cylindrospermum species	C	R	O
Oscillatoria sancta	NC	NC	R
Diatoms	VC-N	N	C

T=trace (1-5); R=rare (5-10); NC=not common (10-50); C=Common (50-100); VC=Very common (100-200); N=numerous (over 200) (Numerical value within brackets)

C. zeylanica nearly always occurs in clear water ponds and in association with other submerged aquatic plants such as *Najas minor*, *Hydrilla verticillata*, *Lagarosiphon roxburghii*, *Ottelia* etc.; pure colonies are not uncommon. They occur along shallow water banks of ponds; the emergent-submerged plant commonly associated is *Marsilea* and of the other associations, the floating *Jussiaea repens* and *Alternanthera sessilis* were most common.

In pond Mènè (Chotkhand), it was possible to study its intimate association with *Jussiaea repens* and some algæ for September-November period (Table I). (By the first week of December, due to a great fall in water level, the *Chara*-corner was exposed, and exterminated).

ANOPHELINE BREEDING IN *CHARA*-PONDS.

Breeding in *Chara*-ponds was studied in (*i*) artificial breeding pots with diverse submerged plants and (*ii*) from some selected corners of ponds with pure or mixed *Chara* populations (the associates not more than one species). During a comparative study of breeding in the former case, each week the eggs and larvæ were collected and identified so that the pots were clear and ready to receive another laying-in of eggs for the next week. In case of natural ponds, each sample represented total for 25 standard dips; using a frying pan (eight inches diameter, two inches depth) for collection.

(*i*) *Artificial breeding pots.*—*Chara zeylanica*, *Hydrilla verticillata* and *Ottelia alismoides* were grown separately in earthen pots, and for comparison four more such artificial pools were provided individually with *Limnanthemum indicum*, *Jussiaea repens*, *Azolla pinnata* and *Salvinia natans*. All the seven artificial breeding pots were, as far as possible, undisturbed and kept in open (Plate VIII).

During March, breeding was quite encouraging in all pools but it fell gradually till May, later showing absolutely no breeding. From March to May, among the submerged plants, *Ottelia* showed maximum breeding, *Hydrilla* coming next, and *Chara* showing the least breeding (Graph 1). The absence of *A. vagus* from *Chara*-pools is interesting but not significant. Table II gives comparative breeding in all the artificial breeding pots for the period March-July, 1955.

(*ii*) *Ponds.*—In nature, breeding was observed at Chotkhand in pond Mènè from a clearly demarcated corner with a pure patch of *Chara* and at Burdwan from a way-side depressed land having *Chara-Cladophora* association restricted towards one of the banks. The incidence at each place is shown in Tables III and IV.

It is seen that *A. hyrcanus* is more commonly associated with *Chara* at Chotkhand while at Burdwan *A. annularis* is the only associate. The latter is interesting as the same species showed dominance in artificial *Chara*-pools kept in the laboratory (about 200 yards away from this depressed land). It is possible, *Cladophora* has not to do much with this association (as illustrated in Table IV) as the laboratory pools were completely bereft with it.

However, at a place where *Chara* formed a dominant submerged associate of a floating-mat species, *Jussiaea repens*, the anopheline breeding was more representative: *A. annularis*, *A. philippinensis*, *A. hyrcanus* and *A. barbirostris*.

TABLE II.

Comparative anopheline breeding in artificial breeding pools for March-July, 1955.

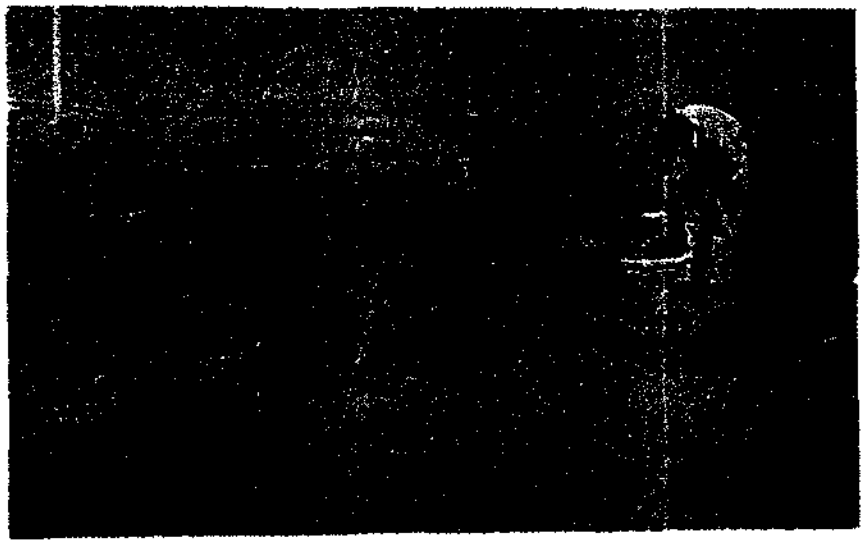
Vegetation	Monthly incidence of anophelines				
	March		April		May-July
Submerged:					
Chara zeylanica ...	*A. subpictus*	1	*A. annularis*	1	Nil
Ottelia alasmoides ...	*A. subpictus*	66	*A. subpictus*	22	Nil
	A. vagus	12	*A. vagus*	2	
Hydrilla verticillata ...	*A. annularis*	5	Nil		Nil
	A. subpictus	11			
	A. vagus	2			
Floating:					
Limnanthemum indicum ...	*A. annularis*	3			
	A. subpictus	2	Nil		Nil
Jussiaea repens ...	*A. subpictus*	1	*A. annularis*	1	Nil
Azolla pinnata ...	*A. vagus*	2			
	A. subpictus	3	Nil		Nil
Salvinia natans ...	*A. aconitus*	8	Nil		Nil

TABLE III.

Anophelines associated with Chara *in pond M'enè for September-November, 1955.*

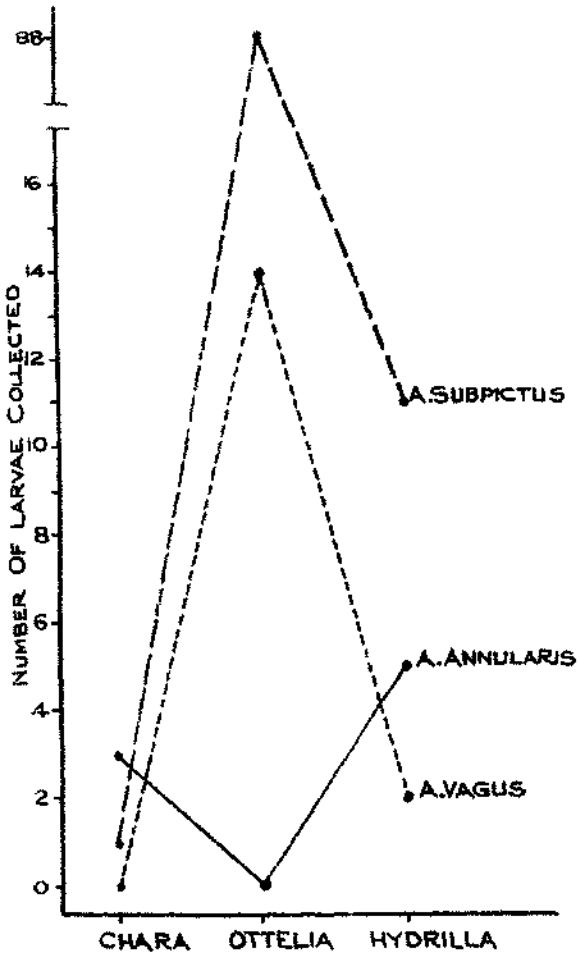
Date of observation	Species of anophelines and their incidence		Plant cover
September 20, 1955	*A. annularis*	1	Dense
	A. hyrcanus	6	
September 28, 1955	*A. hyrcanus*	6	Dense
October 7, 1955 ...	*A. hyrcanus*	2	Dense
October 17, 1955 ...	*A. hyrcanus*	4	Dense
October 21, 1955 ..	Nil		Dried up

PLATE VIII.



Position of the artificial breeding pots in open. 1. mixed flora, 2. *Limnanthemum*, 3. *Chara*, 4. *Jussiaea*, 5. *Ottelia*, 6. *Azolla*, 7. *Hydrilla*, 8. *Salvinia*, 9. *Eichornia* (1 and 9 not considered). The measurements given are in feet and inches.

GRAPH 1.



Comparative breeding in artificial breeding pots, each with a submerged plant species, under open air conditions (March-May, 1955).

TABLE IV.

Anophelines associated with Chara-Cladophora *association during July-September, 1955.*

Date of observation	Species of anophelines and their incidence		Plant cover
July 26, 1955 ...	Nil		Dense
August 4, 1955 ...	*A. annularis*	4	Dense
August 11, 1955 ...	*A. annularis*	6	Dense
August 18, 1955 ...	Nil		Dense
August 25, 1955 ...	*A. annularis*	2	Dense
September 3, 1955 ...	Nil		Dense
September 13, 1955	Nil		Dense
September 19, 1955	*A. annularis*	1	Medium

RATE OF EMERGENCE OF ANOPHELINES OVER *CHARA*.

For this experimental study, vegetative and reproductive *Chara zeylanica* was grown in glass jars filled with ordinary tap water (with unavoidable slight dilution of ferric compounds) and each jar was covered with a mosquito netting. No controls were kept. Later, an equal number of eggs or first-stage larvæ was introduced in each. Enough space was provided to facilitate movement of the adults to be emerged. *A. philippinensis* and *A. subpictus* were selected for this study and the results are shown in Tables V and VI.

From these experiments, it is evident that *Chara* does not in itself possess any lethal action on anopheline larvæ of this region (nor on *Culex* or *Mansonoides*). The poor rate of emergence, though marked, was also noted in cultures of other algæ used in determination of rate of emergence of some other anophelines at the same time and place (Neogy and Kachroo, 1956). However, in the reproductive *Chara* the emergence rate was very poor and this is hard to explain, since all conditions were similar. The second marked point is that the emergence of the adults seems to be associated with a fall in *p*H and temperature. Further studies are needed to assess its significance as it involves biologic and organic factors, not under the scope of the present note.

DISCUSSION.

The death of mosquito larvæ in *Chara* has been ascribed to asphyxiation (Caballero, 1919) or due to excessive amount of oxygen given off by the plant which might inhibit, decay and oxidize organic substances in solution, thereby making this type of food unavailable and further dissolved oxygen may interfere with digestive processes (Matheson and Hinman, 1929).

TABLE V.

Rate of emergence of A. subpictus *in* Chara zeylanica.

Experiment	Date of observation	Eggs/larvæ used	Emergence of adults	Sign of larvæ	pH	Temperature °C.
				(A) Chara, vegetative		
I	March 17, 1955	10 (First stage)			7·8	35
	March 24, 1955		1	Active for the whole period.		
	March 26, 1955		4			
	March 30, 1955		5		Emergence: 100 per cent	
II	March 22, 1955	10 (First stage)				
	March 25, 1955		2	Active for 4 days; doubtful later.	7·8	35
	March 30, 1955	(2 pupæ dead)	6		Emergence: 80 per cent	
III	April 27, 1955	25 (First stage)			7	34
	April 28, 1955		0 (1 larvæ dead)	Active for 6 days: doubtful later.		
	May 3, 1955		1		7·5	29
	May 7, 1955		(5 pupæ)			
	May 11, 1955		4			
	May 13, 1955	(14 larvæ dead)	0		Emergence: 24 per cent	
IV	October 10, 1955	30 eggs			7	30
	October 20, 1955	(30 larvæ)	0	Active throughout.		27
	October 30, 1955	(20 larvæ dead)	6			27·5
	November 1, 1955	(Rest larvæ dead)	0		Emergence: 20 per cent	
				(B) Chara, reproductive		
V	April 27, 1955	25 (First stage)			7	33·5
	April 28, 1955	(15 larvæ dead)	0	Active for 6 days; doubtful later.		
	May 3, 1955	(7 larvæ dead)	0		6·5	29
	May 7, 1955		2		Emergence: 8 per cent	

The non-lethal action of *Chara* has been deduced from the experimental studies presented here. Mainly the rate of emergence in *Chara*-ponds or the presence of mosquito larvæ over it have been observed. However, the problem demands a biologic approach: *Chara* is a much branched alga and has a specialised structure. It provides substratum for a number of epiphytic diatoms and desmids etc.; a number of filamentous algæ get themselves entangled within it. This association in nature is unavoidable and unfortunately overlooked by most of those who ascribed it to be inhibitory. This association makes it useful to larvæ in two ways: as providing protection against their enemies and providing least surface disturbances; and as the store-house of algal food. It is now a more or less a decided

fact that larvæ live mainly, if not exclusively, on phytoplanktonic food (Neogy and Kachroo, 1956). It may be mentioned here that even *Chara* itself, during its reproductive stages, provides food for larvæ by contributing its oogonia (the presence of which is not uncommon within the guts).

TABLE VI.

Rate of emergence of A. philippinensis *in* Chara zeylanica (*vegetative*).

Experiment	Date of observation	Eggs/larvæ used	Emergence of adults	pH	Temperature°C.
I	September 20, 1955	20 eggs		7	30
	September 22, 1955	20 larvæ (Second stage)			29
	October 3, 1955	7 dead	(2 pupæ)		26
	October 5, 1955		6		28
	October 7, 1955		1		29
	October 10, 1955		(3 pupæ)		
	October 11, 1955		3	?	?
	October 13, 1955	Rest larvæ dead	3		28 Emergence: 65 per cent

The question of excessive oxygen produced by *Chara* is out of question when the formation of the same by such plants as *Hydrilla verticillata*, which has absolutely no inhibitory action against anophelines or culicines, is considered. Besides, the addition of dry extract of *Chara zeylanica* did not in any way retard the emergence of adults in some of the above experiments.

SUMMARY.

Chara zeylanica does not inhibit anopheline breeding as determined by a study of breeding in (*i*) artificial breeding pots with submerged *Chara*, *Ottelia*, *Hydrilla*; (*ii*) in natural ponds and (*iii*) by studying the rate of emergence of *A. subpictus* and *A. philippinensis* over *Chara*.

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A NOTE ON THE DEGREE OF SUSCEPTIBILITY OF *A. CULICIFACIES* TO D.D.T. IN SOME PARTS OF BOMBAY STATE.

BY

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AND

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[May 23, 1957.]

THE object of this note is to record the results of experiments carried out to determine the degree of susceptibility exhibited by *Anopheles culicifacies* Giles, in some parts of Bombay State. Some of the experiments were carried out in areas in which D.D.T. spraying had not yet been done while others in areas in which D.D.T. spraying had been in progress for a varying number of years ranging from one to eight.

MATERIAL AND METHODS.

The tests were carried out in accordance with the standard procedures recommended by the Expert Committee on Malaria of the World Health Organization (Technical Report Series No. 80, 1954) except that D.D.T. para-para (MP 108°C.) recrystallized in the National Chemical Laboratory, Poona, was utilized in most of the experiments. D.D.T. (Technical), supplied specially for the purpose by the Malaria Institute of India, was used in the remaining experiments. Due allowances for the higher mortalities obtained with D.D.T. para-para have to be made in interpreting the present results and comparing them with those obtained by others. The test insects utilized were all wild-caught females of *A. culicifacies* which had taken full blood meals the previous night. Collections were made in human dwellings and cattlesheds in the selected villages between 08.00 and 11.00 hours and the exposures were completed generally before 13.00 hours and in a few cases before 17.00 hours on the same day. In the initial stages of the experiments, it was noticed that heavy mortalities of mosquitoes were occurring even in the controls not exposed to D.D.T. but it was soon discovered that the high mortalities were due to unsuitability of recovery cages. By the simple expedient of lining the cylindrical metal wire recovery cages (height 4 inches, diameter 2½ inches) by a clean sheet of white paper, the heavy control mortalities

were eliminated. In the controls, mosquitoes were exposed to filter papers which had been impregnated with white oil and ethylene dichloride mixture only, without D.D.T.

In each experiment, six mosquitoes were exposed and a minimum of six replications were made with each concentration. In a very few cases, seven to eight replications were made.

RESULTS.

The percentage mortalities obtained (after applying Abbott's formula) by exposure of *A. culicifacies* females to the different concentrations of p,p'-D.D.T., are presented in Table I. Similar percentages obtained with D.D.T. (Technical) are presented in Table II. In all cases, the mortalities were recorded twentyfour hours after the completion of the one-hour exposure. The mortalities recorded at the several concentrations are also plotted in Charts 1 and 2 for para-para and technical D.D.T., respectively. As could be expected the p,p'-D.D.T. gave slightly higher kills than technical D.D.T. at the same exposure times.

The data have been subjected to probit analysis. The median lethal concentrations as well as the regression equations of log concentration-probit kill, were determined. The localities from which these insects were collected and tested, whether they were sprayed or not, if sprayed the number of years they were under D.D.T. spray, and the median lethal concentrations recorded, etc., are stated in Tables I and II. It will be noticed that with p,p'-D.D.T., the median lethal concentrations for *A. culicifacies* were the lowest, ranging from 0·24 to 0·38 per cent in the three unsprayed areas. In the sprayed areas, the median lethal concentrations ranged from 0·41 to 0·54 per cent. In the case of D.D.T. (Technical), no experiments were done in unsprayed areas and the median lethal concentrations in the four sprayed localities studied ranged between 0·50 and 0·60 per cent. The slightly higher M.L.C. in the sprayed places, than in the unsprayed ones, may suggest that there was some degree of lower susceptibility in the sprayed localities. Statistical significance has, however, not been found for the difference.

In order to study whether there is any significant relationship between the median lethal concentrations obtained and the number of years the area had been under D.D.T. spray, the data in respect of tests with p,p'-D.D.T. have been plotted in Chart 3 and a regression line drawn. The equation for the regression line is $y=0{\cdot}372+0{\cdot}0131X$ (y=M.L.C. expressed as D.D.T. per cent; X=Number of years). The observed regression co-efficient does not differ significantly from zero at the five per cent level of probability ($P=>0{\cdot}05$). The co-efficient of correlation was $+0{\cdot}441$ and was also not significant. For strict evaluation, Chart 3 is based only on the estimations made with p,p'-D.D.T. It would not have been correct to plot the data for D.D.T. (Technical) on the same chart (Chart 3) along with those for p,p'-D.D.T., although from a rough study made from the combined data it was seen that the slope of the line would not have materially changed even if such data had been included. It will, therefore, be seen that though the regression line is found to have a slightly ascending slope with increase in the number of years of spraying, the slope is of no significance and there is, therefore, no indication that the degree of susceptibility has undergone any change.

TABLE I.

Percentage mortality (adjusted after applying Abbot's formula) in A. culicifacies *females (engorged with blood) recorded at the end of twentyfour hours reckoned from the end of the one hour exposure period.*

(The figures represent the average of six replications, each replication consisting of six mosquitoes.)

p, p'—D.D.T.

Place	Month of experiment	Month and year when D.D.T. spraying was started	Concentrations of D.D.T. used (per cent)									Controls. No D.D.T. but white oil only	M.L.C. as determined by probit analysis (per cent)	Log concentration × 100·0 — probit kill regression equation
			0·0625	0·125	0·25	0·375	0·5	0·75	1·0	1·5	2·0			
Poona District, Baramati.	July, 1955	June, 1953	3	2	18	...	32	62	81	97	100	7	0·535	$Y = -2{\cdot}930 + 2{\cdot}907x$
Poona District, Dhond.	August, 1955	Unprayed	15	18	52	72	77	83	96	96	100	19	0·240	$Y = -0{\cdot}588 + 2{\cdot}348x$
Dharwar District, Bellary.	September, 1955	Unsprayed	15	8	15	...	46	81	91	100	100	0	0·379	$Y = -1{\cdot}560 + 2{\cdot}546x$
Bijapur District, Haldur.	October, 1955	July, 1949	0	14	40	40	69	90	87	100	100	2	0·472	$Y = -3{\cdot}152 + 3{\cdot}049x$
Kolaba District, Indapur.	November, 1955	June, 1953	0	16	20	34	56	88	80	100	94	0	0·410	$Y = -1{\cdot}008 + 2{\cdot}330x$
Kolaba District, Dasgaon.	November, 1955	Unsprayed	0	6	19	64	72	89	90	91	100	0	0·365	$Y = -3{\cdot}418 + 3{\cdot}285x$
Thana District, Revti.	July, 1956	July, 1949	...	17	14	...	46	...	91	...	100	0	0·412	$Y = -2{\cdot}491 + 2{\cdot}865x$
Poona District, Manjri.	September, 1956	June, 1953	0	5	16	45	58	74	81	97	100	3	0·453	$Y = -3{\cdot}430 + 3{\cdot}174x$

NOTE.—Approximate direct distances from Poona City — Baramati 50 miles, Dhond 45 miles, Bellary 300 miles, Haldur 270 miles, Indapur 80 miles, Dasgaon 100 miles, Revti 70 miles, Manjri 13 miles.

TABLE II.

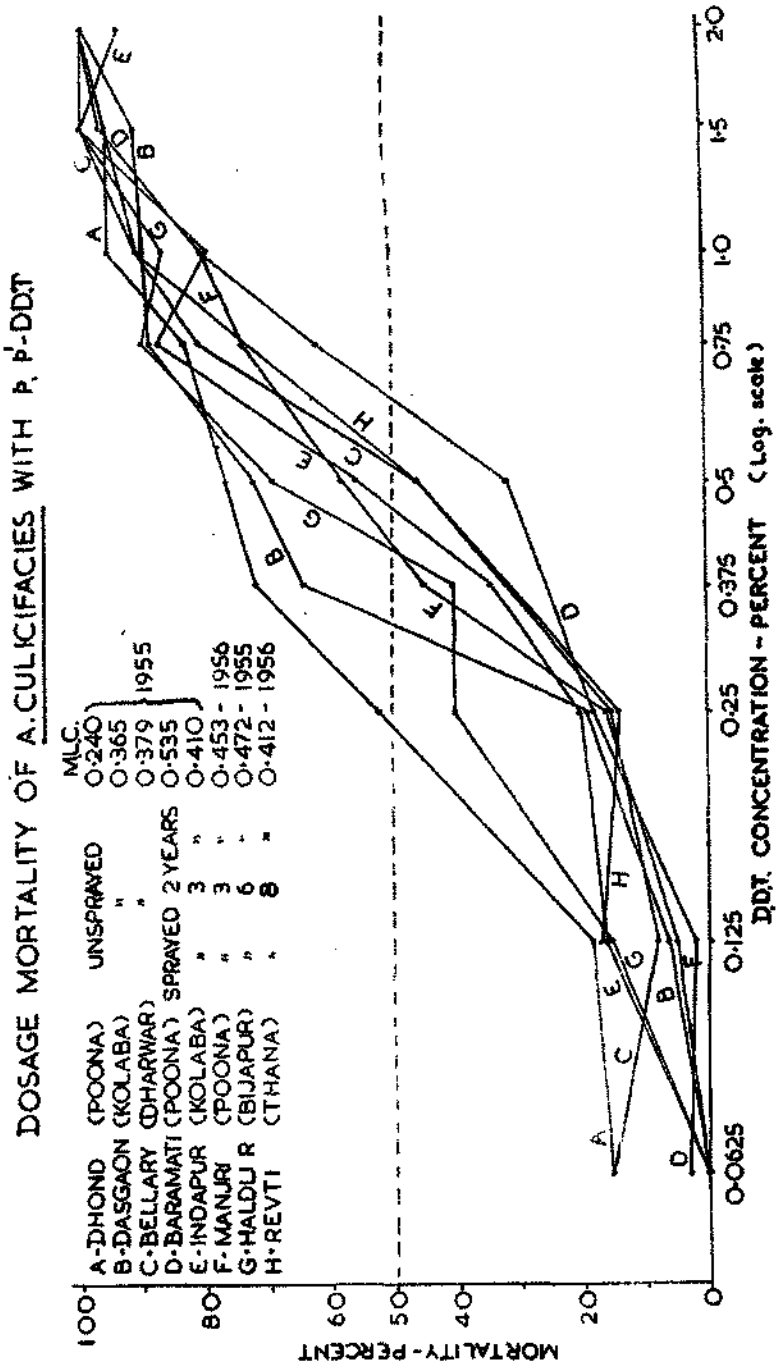
Percentage mortality (adjusted after applying Abbott's formula) in A. culicifacies *females (engorged with blood) recorded at the end of twentyfour hours reckoned from the end of the one hour exposure period.*

(The figures represent average of six replications, each replication consisting of six mosquitoes.)
Technical D.D.T. (from the Malaria Institute of India.)

Place	Month of experiment	Month and year when D.D.T. spraying was started	Concentrations of D.D.T. used (per cent)									Controls. No D.D.T. but white oil only	M.L.C. as determined by probit analysis (per cent)	Log concentration × 1000 — probit kill regression equation
			0·0625	0·125	0·25	0·375	0·5	0·75	1·0	1·5	2·0			
Thana District, Revti	July, 1956	July, 1949	0	6	9	33	38	72	61	100	94	0	0·571	$Y = -3{\cdot}170 + 2{\cdot}964x$
Poona District, Manjri.	August, 1956	June, 1953	0	3	17	39	53	59	67	94	100	0	0·536	$Y = -2{\cdot}763 + 2{\cdot}845x$
Panchmahals District, Khodwa.	September, 1956	July, 1950	8	21	28	19	43	61	70	88	93	0	0·501	$Y = -0{\cdot}175 + 1{\cdot}787x$
Poona District, Dhond.	October, 1956	July, 1956	4	7	10	30	43	56	72	89	88	0	0·598	$Y = -1{\cdot}538 + 2{\cdot}354x$

NOTE.—Approximate distance from Poona–Khodwa 300 miles.

CHART I.


DOSAGE MORTALITY OF A. CULICIFACIES WITH P, P'-DDT
A-DHOND (POONA) UNSPRAYED MLC. 0·240
B-DASGAON (KOLABA) " 0·365
C-BELLARY (DHARWAR) " 0·379 1955
D-BARAMATI (POONA) SPRAYED 2 YEARS 0·535
E-INDAPUR (KOLABA) " 3 " 0·410
F-MANJRI (POONA) " 3 " 0·453 - 1956
G-HALDU R (BIJAPUR) " 6 " 0·472 - 1955
H-REVTI (THANA) " 8 " 0·412 - 1956
MORTALITY - PERCENT
100
80
60
50
40
20
0
0·0625
0·125
0·25
0·375
0·5
0·75
1·0
1·5
2·0
D.D.T. CONCENTRATION - PERCENT (Log. scale)

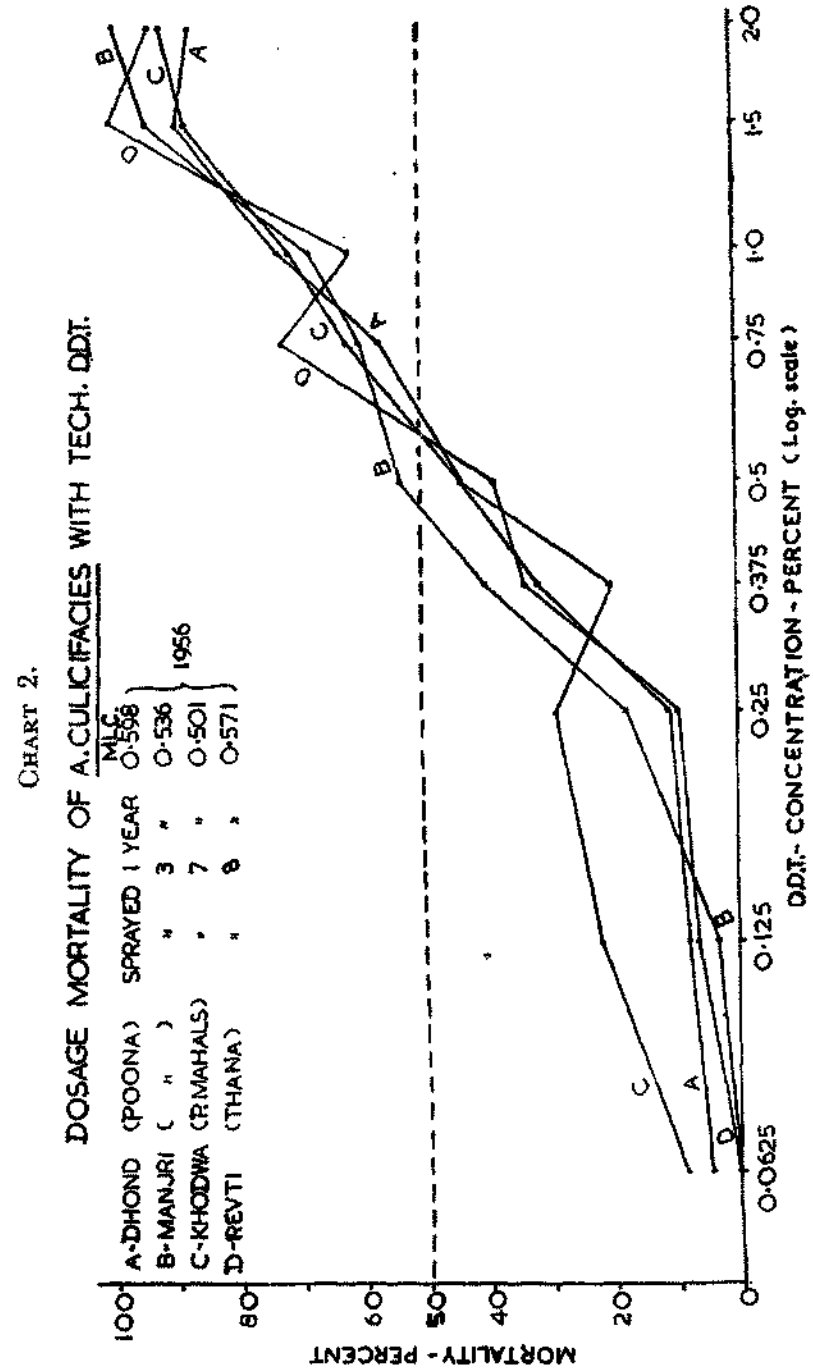
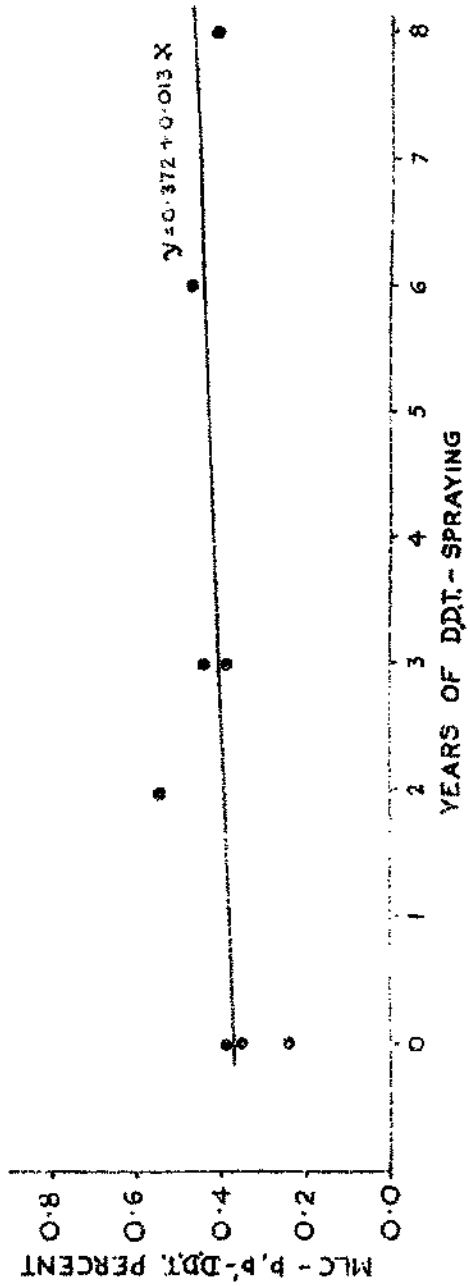


CHART 2.
DOSAGE MORTALITY OF A. CULICIFACIES WITH TECH. D.D.T.
A-DHOND (POONA) SPRAYED 1 YEAR M.L.C. 0·568
B-MANJRI (") " 3 " 0·536
C-KHODWA (R.MAHALS) " 7 " 0·501
D-REVTI (THANA) " 8 " 0·571
1956
MORTALITY - PERCENT
100
80
60
50
40
20
0
0·0625
0·125
0·25
0·375
0·5
0·75
1·0
1·5
2·0
D.D.T.- CONCENTRATION - PERCENT (Log. scale)

CHART 3.


A.CULICIFACIES
y = 0·372 + 0·013 X
MLC - p,p'-DDT PERCENT
0·0
0·2
0·4
0·6
0·8
0
1
2
3
4
5
6
7
8
YEARS OF D.D.T.- SPRAYING

DISCUSSION.

These studies have shown that in widely separated localities in Bombay State, there has been no significant increase in the median lethal concentrations of D.D.T. for *A. culicifacies* though the localities have been sprayed for a number of years varying from one to eight.

Whether or not *A. culicifacies* in Bombay State would in future lose its susceptibility to D.D.T., is at present only a matter for surmise. It would be improper to utilize the regression line drawn in Chart 3 by extrapolation to determine the number of years which would be required for the mosquitoes to show a significant reduction in susceptibility. If the angle of the slope continues to remain the same as at present, the median lethal concentration would still be below one per cent at the end of even forty years. But theoretically there is nothing to indicate that the slope would continue to have the same properties in later years as in the earlier years of exposure to D.D.T.

The data now recorded show that selective pressure, if any, to reduce susceptibility has not been active so far in these areas where the tests have been carried out. Whether it is due to the fact that the spraying is being carried out only during a short duration each year or whether it is due to an innate biological characteristic of the insect, cannot yet be stated. One should also not overlook the theoretical possibility that though the regression line now obtained in Chart 3 does not show any significant loss of susceptibility even over a period of eight years, such a loss may still occur in a population suddenly by a mutation. The mutations may be due to natural causes unconnected with D.D.T. spraying or may be the result of D.D.T. itself though there is no proved instance of such a kind till now. Laven (1956) for instance has successfully produced mutants in *Culex pipiens* by X-ray irradiation, though they are not mutations having any relationship to the resistance problem. While these remote theoretical possibilities should not be overlooked, the very fact that no lowering of susceptibility has occurred even after eight years of spraying, is suggestive that the species is innately incapable of becoming resistant. If the resistant biotypes were already there for pre-selection, they would have by this time become perceptible and established themselves. However, the need for a constant vigil is stressed in order to recognize any sudden appearance of ineffectiveness of D.D.T. in any locality so that it could be dealt with promptly.

The experimental evidence now obtained for the continued susceptibility of *A. culicifacies* to D.D.T., is also fully supported by the observations made in the field. Even in areas where D.D.T. spraying has been carried out for a number of years, up to 10 years as in the case of Dharwar District, D.D.T. spraying continues to have the same effect on *A. culicifacies* populations as before. It has been extremely difficult to collect an adequate number of *A. culicifacies* adults for these experiments in the sprayed areas while the neighbouring unsprayed areas have not generally presented any such difficulty. This continued low density of *A. culicifacies* in sprayed houses is itself an indication that the adults are being killed by the residual deposit of D.D.T. on the walls of the houses.

It would be interesting to compare the M.L.Cs. obtained by the authors with those determined for other anophelines by the use of the same techniques. Wharton (1955) found an M.L.C. of 0·41 per cent for *A. maculatus* in

Malaya and 1·0 per cent and 0·7 per cent for *A. atroparvus* and *A. quadrimaculatus*, respectively, in London. Busvine (1956) has found an M.L.C. of 0·95 per cent with susceptible *A. gambiæ* from Nigeria while Elliott and Ramakrishna (1956) have shown that in the same locality after spraying the M.L.C. for *A. gambiæ* had risen to 1·6 per cent, indicating the develpoment of resistance. Halcrow (quoted by Busvine, 1956) has found for *A. gambiæ* in Mauritius an M.L.C. of 0·7 per cent. Recently, Davidson (1956) has found an M.L.C. of 0·6 per cent D.D.T. for strains of *A. gambiæ* which are susceptible as well as resistant to dieldrin in Nigeria. In Greece where anophelines were first reported to have developed resistance to D.D.T., Livadas (1955) reports M.L.Cs. of 1·4 per cent and 2·75 per cent for *A. sacharovi*. The M.L.Cs. found for *A. culicifacies* in Bombay State are therefore considerably on the lower side, even allowing for the fact that p,p'-D.D.T. had been used.

Recently, Sharma *et al.* (1957) have carried out a number of tests on *A. annularis*, *A. culicifacies*, *A. fluviatilis* and *A. subpictus* in parts of Rajasthan, Uttar Pradesh and Delhi State and have found M.L.Cs. of 0·19 and 0·43 per cent (D.D.T. Technical) for *A. culicifacies* in unsprayed villages. For *A. annularis*, the figure is 0·56 per cent, for *A. fluviatilis* 0·30 per cent and for *A. subpictus* 0·28 per cent. In certain sprayed areas of Delhi State, the M.L.Cs. for *A. subpictus* have been found to be as high as 3·1 and 2·2 per cent even on an exposure for 24 hours instead of the usual one hour. Apparently *A. subpictus* has a marked tendency to develop resistance.

It may be mentioned here that the authors also carried out similar experiments with wild caught zoophilic *A. fluviatilis* in Baramati, Poona District, and found that the M.L.Cs., as visually determined from charts, were 0·5 per cent in 1955 and 0·65 per cent in 1956 with p,p'-D.D.T. and 0·9 per cent with technical D.D.T. in 1956.

SUMMARY.

The measurements of susceptibility of wild caught females of *A. culicifacies* by the World Health Organization technique (Technical Report Series 80) were made in Bombay State in widely scattered localities, both unsprayed and sprayed with D.D.T., for one to eight years. The M.L.Cs. of *A. culicifacies* ranged between 0·24 and 0·54 per cent with p,p'-D.D.T. and between 0·50 to 0·60 per cent with Technical D.D.T. In these unspayed areas, the M.L.Cs. were between 0·24 and 0·38 per cent with p,p'-D.D.T. while in sprayed areas they were between 0·41 and 0·54 per cent. The M.L.Cs. were plotted in a chart against the number of years for which the area had been under D.D.T. spraying, and the regression co-efficient as well as the co-efficient of correlation were insignificant. It is concluded that no significant lowering of susceptibility has yet occurred even in places where D.D.T. has been sprayed for eight years. It is, however, stressed that a vigil has to be maintained because of the theoretical possibility, even though remote, of the sudden appearance of ineffectiveness of D.D.T. due to mutations.

ACKNOWLEDGEMENTS.

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relation to insecticides, recommended by the Indian Council of Medical Research. The authors express their thanks to Shri A. V. Halgeri, Shri V. G. Majumdar, Shri R. B. Deobhankar and Shri P. S. Jadhev of this Organization who have given technical assistance at some time or another during the course of these experiments. The statistical evaluation has been made by Shri H. N. Ranganathan, Statistician of the Public Health Department, and his contribution is gratefully acknowledged. The authors are also thankful to Dr. T. B. Patel, Director of Public Health for the Government of Bombay, for the numerous useful suggestions made from time to time.

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A PILOT STUDY ON THE SUSPENSION OF D.D.T. SPRAYING AND THE SETTING UP OF A SURVEILLANCE ORGANISATION IN KANARA DISTRICT COVERING THE WORK UP TO OCTOBER 31, 1956.

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[May 23, 1957.]

INTRODUCTION.

KANARA and Dharwar were the first two districts of Bombay State to be taken up in 1946 for extensive malaria control by the use of indoor residual spraying of D.D.T. While the whole of Kanara District, except a few villages in the coast, was hyperendemic for malaria and was taken up practically in its entirety for purposes of spraying, malaria prevalence in Dharwar District was patchy with areas of varying grades of endemicity interspersed with areas of hypoendemicity. Therefore, substantial portions of Dharwar District had remained hitherto unsprayed. The total number of villages and the population of the districts and those actually directly protected by D.D.T. spraying till the end of 1955, are given below:—

District	Area in square miles	Total number of villages and towns	Total number of villages and towns sprayed	Total population	Population directly protected
Kanara ...	3,977	1,235	1,163	517,780	477,702
Dharwar ...	5,285	1,362	720	1,575,386	1,080,885

The results achieved in the first three years of work, in these two districts, have been published (Viswanathan and Ramachandra Rao, 1947:1948:1949).

For the first seven years, D.D.T. in the form of an emulsion, prepared from aromex, soap and water, was sprayed in both the districts at a dosage of 56 mg./sq. ft. at intervals of eight weeks in Kanara District and six weeks in Dharwar District during the malaria transmission season. In Dharwar, three rounds of spraying were given between July and November. In Kanara, three rounds were given in the eastern rice zone with the transmission season extending from July to November, and four rounds were given in the remaining western garden zone between October and June. During the last three years, that is from 1953-1954, however, the emulsion was replaced by a 75 per cent water wettable powder of D.D.T. and Dharwar District received two rounds of spraying at 112 mg./sq. ft. and Kanara District continued to get the same number of rounds as before at 56 mg./sq. ft.

EPIDEMIOLOGY OF MALARIA IN KANARA DISTRICT.

The epidemiology of malaria in Kanara District was studied by Jaswant Singh and Jacob (1944) and subsequently amplified by the studies made by the Malaria Organization of Bombay State (Viswanathan, 1950).

The whole of the district, except a narrow belt along the coast, was highly malarious with spleen rates ranging in most cases between 50 and 80 per cent. All the three species of plasmodia were prevalent, *P. falciparum* and *P. vivax* being found in approximately equal numbers. In the first year of study, however, an unusually high prevalence of *P. malariæ* was noticed, but in subsequent years the incidence of *P. malariæ* did not exceed ten per cent of all the positive blood smears. The childhood parasite rate varied from zero to 40 per cent and in 1942-1943 the average for the district was 21·6 per cent. The infant parasite rates also showed considerable variations and were generally between 20 and 40 per cent in most hyperendemic villages. Though 23 species of anophelines were found in the district, only *Anopheles fluviatilis* was found to be the vector with an average sporozoite rate of about ten per cent. But in certain months and in certain localities, sporozoite rates as high as 33 per cent were met with. The bulk of *A. fluviatilis* collected was in human dwellings. But a small negligible fraction was found in cattlesheds. The anthropophilic index as determined by precipitin tests was nearly 90 per cent. Though *A. fluviatilis* was found in fair numbers in human dwellings during day time, investigations (Viswanathan, Ramachandra Rao and Rama Rao, 1944) revealed that a considerable part of the total adult population at any time rested outdoors during day time. The chief breeding places of *A. fluviatilis* were streams and channels with slow current of water. Ricefields, particularly those which were terraced and which had moving water in them and located in the proximity of human dwellings, were also found to breed the species in small numbers (Ramachandra Rao, 1945). No period of the year was climatologically unfavourable for malaria transmission, but the actual seasons of transmission were determined mainly by the facilities existing for the uninterrupted breeding of the vector species which in turn was determined by the topography and rainfall. Even within the confines of this district two

distinct zones, having quite different seasons of transmission, were noticed. The eastern rice zone having gently undulant terrain, a moderate rainfall and no perennial streams had a season of transmission from July to November, coincident with the breeding of *A. fluviatilis* occurring mainly in the channels and ricefields during monsoon months. The western garden or ghat zone which on the other hand is characterized by the presence of deep valleys, heavy rainfall, extending sometimes up to 150 inches per year, and a large number of perennial streams, had the season of transmission between November and June coincident with the progressively increasing breeding occurring in the streams and channels during the post-monsoon months. In the latter zone, no breeding of *A. fluviatilis* occurred during the monsoon months because of the heavy flushing in the streams and channels. There were a few intermediate zones having characteristics of both the zones.

RESULTS OF MALARIA CONTROL.

The highly satisfactory results of the control measures have been reported from time to time. The cumulative spleen, childhood parasite and infant parasite rates in Kanara District for each year from 1945 to 1955, as well as the number of malaria cases treated in selected public dispensaries, are summarized below:—

Year	Spleen rate per cent	Childhood parasite rate per cent	Infant parasite rate per cent	Number of malaria cases treated in 12 public dispensaries	Percentage of malaria cases to all cases
1945	75·0	12·5 to 40·0	32·3	43,813	Over 50·0
1946	72·2	...	...	32,095	
1947	14·4	3·8	0·8	28,252	
1948	11·6	2·7	0·4	17,455	
1949	7·1	2·1	0·8	15,353	18·2
1950	2·7	0·1	0·0	11,903	15·4
1951	0·9	0·0	...	8,019	10·1
1952	0·3	0·0	0·0	5,854	7·1
1953	0·2	...	...	6,582	7·2
1954	0·0	0·0	0·0	4,638	4·0
1955	0·0	0·0	0·0	5,562	4·2

In the year 1955, only one out of 5,639 children examined in the district showed an enlarged spleen. Not one of the 293 blood smears from children and 314 blood smears from infants, was found positive. The number of malaria cases treated (entirely on clinical diagnosis) in the selected dispensaries came down from

over 43,000 in 1945 to below 6,000 in 1955 and the percentage of malaria cases to all cases was reduced from above 50 per cent to hardly 4·2 per cent.

There have also been significant changes in the demographic indices. Given below are the birth rates, death rates, infant mortality rates and malaria death rates in Kanara District as a whole from 1944 to 1955:—

Year	Birth rate per mille	Death rate per mille	Infant mortality rate per mille	Malaria death rate per mille
1944	29·5	26·2	176	2·3
1945	34·7	27·6	153	1·9
1946	39·5	20·0	137	1·3
1947	23·8	23·1	151	1·3
1948	34·7	16·3	107	0·8
1949	38·4	16·3	111	0·6
1950	38·9	17·3	118	0·6
1951	36·2	13·8	105	0·9
1952	36·5	13·9	100	0·4
1953	36·6	13·7	98	0·4
1954	33·4	12·5	89	0·4
1955	37·3	14·2	99	0·4

The infant mortality rates have declined from 176 per 1,000 live births in 1944, to 99 in 1955. The excess of birth rates over death rates which was only 3·3 in 1944, was as high as 23·1 per mille in 1955. For the Bombay State as a whole, the same index has risen from about 18 in 1944 to about 21 in 1955. A number of blood smears examined from public dispensaries, as well as from other sources, have not yielded a single positive blood smear since 1953-1954.

The effect on the vector species of this extensive indoor residual spraying of D.D.T. has been equally spectacular. While prior to the institution of control measures, the average densities of *A. fluviatilis* adults in human dwellings during the whole period of the transmission season stayed well above the minimum of four per ten man-hours, a level which was postulated (Viswanathan, 1944) as the critical limit below which the transmission would be interrupted, not a single specimen of *A. fluviatilis* has been collected in the several thousand hours spent on routine mosquito collections carried out in the district both in human dwellings and cattlesheds from the year 1952. A special series of investigations carried out by the staff sent from the headquarters of the Malaria Organiaztion, however, revealed the presence of a few specimens of *A. fluviatilis* in isolated localities. The results of these special collections are embodied in Tables I and II. Even in April 1956, ten adults and four larvæ of *A. fluviatilis* were found in Kanara District but all the ten adults were found in only one village, Kadalgadde in Yellapur Taluka,

TABLE I.

Results of special collections of adult anophelines in Kanara District from 1950 to 1956.

Peirod of survey	1950 April				1951 February				1952 May and August				1953 October				1954 April		1954 December				1956 April			
Number of villages visited	32				8				24				3*				14		35				41			
Talukas	Sirsi, Siddapur, Yellapur.				Sirsi, Siddapur, Yellapur.				Sirsi, Siddapur, Yellapur, Mundgod, Haliyal.				Sirsi, Mundgod, Haliyal.				Sirsi, Siddapur, Yellapur.		Karwar, Yellapur, Ankola, Siddapur, Sirsi.				Sirsi, Kumta, Siddapur, Yellapur, Mundgod.			
Hours	62·0		65·0		9·7		19·5		Collected by pyrethrum spraying				30·3		6·3		73·0		49·8		50·2		50·3		53·3	
Number of searches	HD —		CS —†		HD 23		CS 33†		HD 248		CS 28		HD —		CS —		HD —	CS —	HD 175		CS 145		HD 214		CS 211	
	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
A. fluvilaitis ...	1	8‡	1	49	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	10
A. aconitus ...	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A. aitkeni ...	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A. annularis ...	0	3	1	37	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	3	0	0	1	8
A. barbirostris ...	0	0	0	0	0	0	0	1	0	0	0	8	0	31	0	27	0	0	0	0	0	0	0	0	0	0
A. culicifacies ...	1	0	0	10	0	0	0	28	9	70	0	1	0	0	0	0	0	0	0	0	2	40	0	0	0	1
A. hyrcanus ...	0	0	0	0	0	0	0	0	0	0	0	0	0	21	0	23	0	0	0	0	0	1	0	0	0	1
A. jamesi ...	1	16	1	243	0	0	0	47	0	4	1	6	2	9	0	17	0	5	0	0	0	12	0	0	1	14
A. jeyporiensis ...	1	29	7	248	0	1	1	135	1	11	1	19	0	0	0	2	0	1	0	2	3	184	0	1	0	12
A. karwari ...	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	3
A. pallidus ...	0	0	0	7	0	0	0	10	0	5	0	0	0	7	0	8	0	3	0	0	0	1	0	0	0	1
A. splendidus ...	0	0	0	2	0	0	0	4	0	0	0	0	0	2	0	3	0	0	0	0	0	2	0	0	0	0

TABLE I—(*Concld.*)

Period of survey	1950 April				1951 February				1952 May and August				1953 October				1954 April		1954 December				1956 April			
Number of villages visited	32				8				24				3*				14		35				41			
Talukas	Sirsi, Siddapur, Yellapur.				Sirsi, Siddapur, Yellapur.				Sirsi, Siddapur, Yellapur, Mundgod, Haliyal.				Sirsi, Mundgod Haliyal.				Sirsi, Siddapur, Yellapur.		Karwar, Yellapur, Ankola, Siddapur, Sirsi.				Sirsi, Kumta, Siddapur, Yellapur, Mundgod.			
Hours	62·0		65·0		9·7		19·5		Collected by prethrum spraying				20·3		6·3		73·0		49·8		50·2		50·3		53·3	
Number of searches	HD —		CS —†		HD 23		CS 33†		HD 248		CS 28		HD —		CS —		HD —	CS —	HD 175		CS 145		HD 214		CS 211	
	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
A. stephensi ...	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A. subpictus ...	3	2	2	6	0	0	0	46	1	24	4	13	0	0	0	0	0	3	0	2	0	41	0	1	1	0
A. tassellatus ...	0	0	0	0	0	0	0	3	1	0	0	1	8	18	6	34	0	0	0	2	0	29	0	0	0	0
A. theobaldi ...	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A. turkhudi ...	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
A. vagus ...	0	4	0	22	0	0	0	6	1	16	0	2	0	7	0	3	0	0	0	2	0	4	0	3	0	8
A. varuna ...	0	0	0	3	0	0	0	7	0	3	2	2	0	0	0	0	0	0	0	0	0	8	0	0	0	2

HD = Human dwellings. CS = Cattlesheds.
M = Males. F. = Females.
*Includes night catches also.
†Note that cattlesheds were not being sprayed in Kanara District in 1951.
‡All found in unsprayed villages

Places where *A. fluviatilis* were collected:—

M.	F.	
		1954
0	1	Kadalgadde (Taluka Yellapur).
0	1	Kansur (Taluka Siddapur).
0	1	Hostot (Taluka Siddapur).
0	1	Hunsekop (Taluka Siddapur).
0	1	Bhairumbe (Taluka Sirsi).
0	7	
		1956
0	10	Kodalgadde (Taluka Yellapur). All the ten were found in cattlesheds and eight were found in one unsprayed newly built cattlesbed.

TABLE II.

Results of special collections of Anopheles larvæ in Kanara District from 1950 to 1956 — All searches in streams and channels only.

Period of survey	1950 April	1951 February	1952 May	1952 August	1953 October	1954 April	1954 Dec.	1956 April
Number of villages visited	...	...	20	9	8	19	35	32
Taluks	Sirsi, Yellapur, Siddapur.	Sirsi, Yellapur, Siddapur.	Sirsi, Yellapur, Siddapur, Kumta, Ankola.	Mundgod, Haliyal.	Mundgod, Sirsi, Haliyal.	Siddpur, Sirsi, Yellapur.	Karwar, Yellapur, Ankola, Siddapur, Sirsi.	Sirsi, Kumta, Siddapur, Yellapur, Mundgod.
Time spent: Hours and minutes	H. M. 121 30	H. M. 47 10	H. M. 45 0	H. M. 135 0	H. M. 112 15	H. M. 113 0	H. M. 79 0	H. M. 57 0
A. fluviatilis ...	16	5	5	5	0	1	2	4
A. aconitus ...	0	0	0	0	0	0	0	1
A. aitkeni ...	23	95	24	0	9	112	73	88
A. annularis ...	0	0	0	2	13	52	0	17
A. barbirostris ...	48	0	315	492	402	118	276	233
A. culicifacies ...	5	0	58	54	2	64	3	0
A. hyrcanus ...	2	63	5	13	420	0	0	0
A. insulaeflorum ...	0	0	0	0	0	1	0	0
A. jamesi ...	90	17	501	409	42	305	92	569
A. jeyporiensis ...	44	80	108	41	10	14	65	55
A. karwari ...	5	13	109	3	0	93	0	91
A. maculatus ...	17	19	66	4	0	18	0	0
A. pallidus ...	24	0	0	129	8	39	0	0
A. philippinensis ...	1	0	0	0	0	0	0	0
A. splendidus ...	0	0	0	0	0	0	0	0
A. stephensi ...	9	5	0	0	0	0	0	0
A. subpictus ...	5	1	31	390	0	148	208	98
A. tessellatus ...	0	0	0	0	0	0	0	0
A. theobaldi ...	0	0	0	0	0	0	0	0
A. turkhudi ...	0	0	0	0	0	0	0	0
A. vagus ...	0	3	4	503	299	0	21	0
A. varuna ...	27	5	174	28	12	27	52	20

below Arbail Ghat. This village had been sprayed year after year from 1946. It is, however, significant that all the specimens were collected in cattlesheds only. It is worth mentioning that cattlesheds in Kanara District were excluded from spraying in the initial stages and it was only from 1952 that all of them are being regularly sprayed. The four larvæ were found in four widely scattered villages in Sirsi, Yellapur and Mundgod talukas. Therefore at the beginning of 1956, it was safe to say that *A. fluviatilis* had reached such low levels of prevalence that it could be regarded as having virtually disappeared from vast sections of the district. At present, one can only speculate on the nature of these lingering specimens. Firstly, it may be assumed that the D.D.T. spraying operations over a number of years have so greatly reduced the natural densities that the remnants are those which escaped destruction in some isolated parts of the district. Secondly, one can postulate that there were, to commence with, two races—a predominant anthropophilic and weaker zoophilic—in the district, and because of intense spraying of all the human dwellings the entire anthropophilic moiety of the population has disappeared and the few lingering specimens are the remnants of the zoophilic moiety only. This is an interesting possibility when it is realized that the few lingering specimens are collected only from cattlesheds. Thirdly, one could also consider the possibility that the anthropophilic habits of this species were unstable and the few species that have survived have reverted to the more stable zoophilic habits. If the first conjecture is correct, with the disappearance of the adverse pressure of D.D.T. and with the disappearance of the remaining particles of D.D.T. from the walls of houses in course of time, there should be a gradual increase of *A. fluviatilis* population. If in future studies, adequate numbers of adult *A. fluviatilis* are collected, it would be interesting to examine their blood contents by means of precipitin tests.

Therefore, at the end of the tenth successive year of D.D.T. spraying in Kanara District, the district as a whole was in a condition suitable for an experiment in the suspension of spraying. It had all the criteria necessary for such suspension. But such a course could not be undertaken without the establishment of an adequate surveillance organization. It was, therefore, decided that with effect from June 1956, D.D.T. spraying should be interrupted in the district as a whole and the surveillance procedures established.

SURVEILLANCE ORGANISATION.

From the first week of June 1956, a limited surveillance organization was established for work in the Kanara eastern rice zones in Sirsi, Mundgod, Haliyal and Yellapur talukas with a total population of about 82,000. The work in the remaining parts of the district (with a population of 4,20,000) was to commence from December because the normal season of malaria transmission would not commence in them till then. Moreover, the last D.D.T. spraying in the eastern zone, now taken up for surveillance, was in September-October, 1955, while the last spraying in the western zone was done only in May, 1956. The limited objective of this tentative organization was a scientific study of the after effects of the interruption of spraying, both on the mosquito populations and on the course of malaria prevalence if it did reappear, keeping in mind the deductions

made by Macdonald (1956) on the possible course of a malaria epidemic in the wake of suspension of spraying. In Kanara, however, the vector species was practically on the point of elimination while in his thesis Macdonald (1956) had assumed that the potential of increase in the vector population had not undergone any change though their numbers might have been greatly reduced. Therefore, it would have been interesting to watch the course of events after the interruption of spraying and the elimination of the adverse pressure of D.D.T. on the vector population. In view of this limited objective only a part, though a major part totalling more than half the population, of the eastern sections of the district, was selected for the fortnightly enquiry and no arrangements were made for the treatment by drugs of individual cases of fevers.

From the beginning of August, however, the surveillance organization was made fully comprehensive and arrangements were made for enquiry among the population living in all the villages of the tract and for treating every person having fever of any origin with antimalaria drugs. The functions of the surveillance organisation, the structure of the teams and the results so far obtained, that is till 31st October, 1956, are dealt with below.

The functions of the surveillance organization were:

(*i*) House to house visit in every village once a fortnight to discover cases of fever;

(*ii*) Taking blood smears from all fever cases, irrespective of the cause for the fever, and sending them to the headquarters for examination;

(*iii*) Administration of chloroquine in a suitable single dose to all persons having fever;

(*iv*) If any blood smear is found positive, to treat the case with a drug of the 8-aminoquinoline group, under the direct supervision of a qualified medical officer;

(*v*) Routine mosquito collections in selected villages; and

(*vi*) Compilation and submission of weekly reports.

The proformas used by the staff and instructions regarding them are reproduced here as appendices I to IV.

For the total population of 82,000 living in the eastern rice tracts of Kanara District in Haliyal, Sirsi, Mundgod and Yellapur Talukas, a staff consisting of six Investigators working full time, three Investigators working for three days in the week only and five full time Assistants were appointed. The part-time Investigators were not provided with Assistants. The Investigators were either Malaria Supervisors who are graduates in science or qualified Sanitary Inspectors who had had long experience in malaria work. The Assistants were Insect Collectors who had been in service for ten years. The work was locally supervised by the Medical Officer of Health, Kanara East Division, assisted by the Assistant Medical Officer. Each team, consisting of an Investigator and an Assistant, was allotted a certain group of villages for being visited once a fortnight on the appointed day of the week. The headquarters of the seven teams were two at Sirsi, three at Haliyal, one at Mundgod and one at Yellapur. The number of villages and total population allotted to each team are given below:—

Team	Number of persons in the team	Taluka	Number of villages allotted	Total population allotted	Average population per day per worker
I	2	Sirsi	98	14,500	725
II	2	Sirsi		13,500	675
III	3*	Mundgod	63	17,000	650
IV	2	Haliyal	87	13,000	650
V	2*	Haliyal		10,000	660
VI	2	Haliyal		13,000	650
VII	1*	Yellapur	2	1,000	500
Total	14		250	82,000	

*One of them on part-time.

The average population to be surveyed by each team per working day was about 1,350 or a total population of 13,500 for each fortnight. In addition, two Insect Collectors were set apart specially for routine fortnightly mosquito collections in a total of ten villages and the Assistants to the Investigators, who are also qualified Insect Collectors, were made to collect mosquitoes in the headquarters place on one day of the week. Standard forms devised for the submission of the weekly data and instructions for their use are appended as Appendices I to IV. The dose schedule recommended for the administration of 'Nivaquine' tablets of 200 mg. each, containing chloroquine base of 150 mg., was as follows:—

1–3 years	A single dose of 1 tablet.
3–9 years	A single dose of 2 tablets.
9–15 years	A single dose of 3 tablets.
15 years and above (adult dose)	A single dose of 4 tablets.

The drug had to be taken with a cupful of water on full stomach.

Actual experience showed that the three teams in Haliyal Taluka fully covered all the villages allotted to them. In Sirsi and Mundgod talukas, because of a mis-interpretation of the instructions, a number of scattered villages, comprising small portion (4,000 out of 25,000 in Sirsi and 2,000 out of 17,000 in Mundgod) of the population, had been omitted. This was discovered in September and rectified. The number of houses visited has varied somewhat from fortnight to fortnight (Table V). For a population of 82,000 approximately there were 16,000 houses. As already stated, a population of some 6,000 located in a number of scattered small villages had been omitted. Therefore, the teams should have visited about 14,500 houses each fortnight from August onwards. Their actual output of work has however been as follows:—

August	First half	13,003 houses.
	Second half	10,962 ,,
September	First half	11,664 ,,
	Second half	12,684 ,,

October	First half	11,348	houses.
	Second half	9,396	,,

The reduction in the second half of October is mainly because the teams were directed to concentrate on the scattered small villages which they had missed. On the whole, the teams have covered 70 to 80 per cent of the work allotted to them.

Transports were not provided to the teams except to a limited extent in Sirsi. Most of the visits to the villages were made on foot.

OBSERVATIONS ON SURVEILLANCE.

Prevalence of fevers. —In Table V are given the total number of houses visited and the number of fever cases actually reported in them, week by week, from the beginning of June till the end of October 1956. From August, practically all cases of fever except infants, pregnant women, and those who were obviously suffering from fevers other than malaria, were given adequate doses of cholorquine. The population surveyed in June and July was 46,000, in August and September 74,000 and in October 80,000. 1,564 blood smears were taken and sent to the headquarters of the Malaria Organisation. Examination of these blood smears had revealed only one positive, that is a *P. malariæ* infection in a person living in Khomadulli Village in Haliyal Taluka (Slide N-270). The smear was taken on August 14, 1956. The person was later treated from October 1, 1956 with a complete course of primaquine tablets for five days. The history of this patient, who is a man aged 22 years, was that he is a permanent resident of the village and had an attack of high fever accompanied by delirium about ten years ago. At that time he was treated by a doctor who gave him some injections. During the last two years, he was again getting attacks of fever at long intervals accompanied by rigors and the attacks would subside of their own accord without any special treatment. He had a slight fever on the day of the visit of the surveillance team and was given chloroquine. Subsequent blood smears taken from this patient have been negative. 437 blood smears taken from infants, with or without fever, have also been found negative for malaria parasites.

The average number of fever cases, seen in 100 houses for the entire period, is 1·5. Omitting the figures for the first week, which obviously are not complete, the weekly figure has ranged from 0·9 to 3·1. Though the highest figure 3·1 was found in the middle of August, there is no particular trend in these figures and incidence of fevers has fluctuated around the mean throughout the season. If the fevers had been due to malaria, there should have been a progressive increase in the number of fever cases seen.

In Table IV is given a summary of the entomological data collected during surveillance. While species like *A. jeyporiensis*, *A. jamesi*, *A. annularis*, *A. subpictus* and *A. vagus* are gradually reappearing in houses from which they had practically disappeared during the last five or six years, not a single specimen of *A. fluviatilis* had yet been taken till the end of October. Regular searches for larvæ in channels and streams have also been made routinely in the selected villages. No larvæ of *A. fluviatilis* have been collected during the period. A special search, made by an additional investigation party from the headquarters during the month of

September, has also not revealed presence of any *A. fluviatilis* either as larvæ or as adults. Special care was taken to preserve every doubtful specimen, particularly of *A. varuna*, for further study and confirmation of the identification by a senior worker.

TABLE III.

Collection of mosquitoes as a part of surveillance in human dwellings, Kanara District (eastern rice tract), in villages in which spraying was suspended: June to October 1956.

(Month of last spray: October, 1955).

Month	June		July		August		September		October		Total	
Time spent	Hrs. 157	mts. 50	Hrs. 337	mts. 20	Hrs. 172	mts. 20	Hrs. 241	mts. 40	Hrs. 149	mts. 30	Hrs. 1058	mts. 40
Species	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
A. annularis ...	0	2	0	2	0	3	0	13	...		0	20
A. barbirostris ...	0	1	0	1	0	8	0	24	0	5	0	39
A. culicifacies ...	...		0	2	...		...		...		0	2
A. culiciformis ...	...		0	2	...		...		...		0	2
A. fluviatilis ...	...		...		...		...		...		...	
A. hyrcanus ...	...		...		0	3	0	19	0	12	0	34
A. jeyporiensis ...	0	6	0	5	1	4	4	46	1	8	6	69
A. jamesi ...	0	3	0	11	3	9	2	10	0	4	5	37
A. karwari ...	...		0	8	0	1	0	2	...		0	11
A. leucosphyrus ...	...		1	2	...		...		...		1	2
A. maculatus ...	...		...		...		...		...		...	
A. pallidus ...	...		0	10	1	10	0	4	0	8	1	32
A. philippinensis ...	...		...		...		...		0	1	0	1
A. subpictus ...	8	29	9	55	6	38	5	17	2	8	30	147
A. splendidus ...	...		...		...		...		...		...	
A. sundaicus ...	...		...		...		...		...		...	
A. tessellatus ...	0	1	0	13	0	2	1	14	1	23	2	53
A. vagus ...	2	6	14	64	1	24	3	15	0	5	20	114
A. varuna ...	...		1	4	...		...		0	1	1	5
Indet. species ...	...		0	1	...		...		...		0	1
Total ...	10	48	25	180	12	102	15	164	4	73	66	569
Culicines ...	31	127	57	489	35	538	16	494	7	158	146	1806

M = Males. F = Females.

TABLE IV.

Collection of mosquitoes as a part of surveillance in cattlesheds, Kanara District (eastern rice zone), in villages in which spraying was suspended: June to October 1956.

(Month of last spray: October, 1955).

Month	June		July		August		September		October		Total	
Time spent	Hrs. 65	mts. 0	Hrs. 116	mts. 50	Hrs. 55	mts. 0	Hrs. 79	mts. 0	Hrs. 49	mts. 0	Hrs. 364	mts. 50
Species	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
A. annularis ...	0	10	0	20	1	28	2	66	0	26	3	150
A. barbirostris ...	...		1	10	0	37	4	249	0	164	5	460
A. culicifacies ...	0	2	0	2	...		...		0	1	0	5
A. culiciformis ...	...		...		...		...		...		...	
A. fluviatilis ..	...		...		...		...		...		...	
A. hyrcanus ...	0	1	0	7	0	10	2	142	0	205	2	365
A. jeyporiensis ...	0	11	1	97	3	49	9	409	7	610	20	1176
A. jamesi ...	1	36	4	127	1	134	2	158	1	169	9	624
A. karwari ...	0	2	1	80	0	38	1	71	0	14	2	205
A. leucosphyrus ...	0	1	0	5	0	1	...		...		0	7
A. maculatus ...	...		...		...		...		0	1	0	1
A. pallidus ...	0	9	0	39	0	42	1	104	0	119	1	313
A. philippinensis ...	...		...		...		...		...		...	
A. subpictus ...	9	83	28	363	10	132	6	51	3	56	56	685
A. splendidus ...	...		0	1	...		0	2	...		0	3
A. tessellatus ...	0	6	1	65	1	36	5	95	15	109	22	311
A. vagus ...	9	68	43	433	15	169	11	140	2	61	80	871
A. varuna ...	0	1	1	14	0	1	0	3	0	5	1	24
Indet. species ...	...		0	16	0	1	...		...		0	17
Total ...	19	230	80	1279	31	678	43	1490	28	1540	201	5217
Culicines ...	11	227	75	1128	21	770	11	976	2	681	110	3782

M = Males. F = Females.

Though the monsoon had ended and the normal season of prevalence of *A. fluviatilis* for the year in the rice tracts of Kanara District had terminated by the end of October, 1956, it was too early to attribute the complete absence of

this species during the four months, even in the absence of D.D.T. spraying, as due to an eradication of the species. It would be prudent to postpone any opinion on this matter till investigations are carried out for at least another season in the same region. Additional investigations proposed for the next season are (1) more thorough searches in the ecologically suitable breeding places, *viz.*, streams and channels and (2) capture of adult mosquitoes by use of pyrethrum knock-down sprays, supplementing the collections by hand.

RECOMMENDATIONS FOR FURTHER WORK.

Recommendations have been separately made for institution of an expanded surveillance organization in the Kanara western ghat sections commencing from December, 1956. While the population of the eastern zone is only 80,000 the western zone has a population of over 4,20,000. It has been proposed that the entire malaria staff of the neighbouring districts should be mobilized and posted to Kanara District for this work. Experience has shown that provision of a motor transport would greatly facilitate surveillance work and, therefore, it has been proposed that all the vehicles of the Malaria Control Schemes in the neighbouring districts, should also be drafted for the purpose.

PRECAUTIONARY D.D.T. SPRAYING.

One of the precautions to be taken in connection with surveillance is the prevention of entry of malaria carriers from other parts of the country into the district where interruption of spraying has been achieved. Fortunately there is little danger of such introduction of carriers from across the border from Belgaum and Dharwar or Shimoga districts where the villages are continued to be sprayed with D.D.T. as before. There is, however, an unknown risk of introduction of carriers from across the border in Goa as the exact status of malaria control in that region is not known. Therefore, as a precaution, it has been proposed that the villages situated within a belt of ten miles along the Goa border, should continue to receive the usual two rounds of spraying. Further, owing to the presence of several forest and mining industries in and around Dandeli in the northern portion of the district, there is some risk of labourers carrying malaria parasites coming into the district from other parts of South India. It has, therefore, been proposed that Dandeli and the other mining centres should also continue to be sprayed with D.D.T. and should receive special attention from the surveillance staff.

DISCUSSION AND CONCLUSIONS.

Considering the fact that both the terrain and the monsoon of Kanara District were most unfavourable for any kind of field activity from June to November, the successful execution of this experiment gives scope for optimism that similar work in the other and more easy districts could be carried through without much technical difficulty. The average population allotted to each Investigator was roughly 650 per working day or about 6,500 in a fortnight. They have actually covered about 70 to 80 per cent of the work allotted. Therefore, the coverage of an average population of 10,000 per Investigator in the districts in

TABLE V.

Summary of surveillance work in eastern part of Kanara District—Number of houses visited and fever cases seen, by weeks—June to October 1956.

Week beginning	Jun. 3	Jun. 10	Jun. 17	Jun. 24	Jul. 1	Jul. 8	Jul. 15	Jul. 22	Jul. 29	Aug. 5	Aug. 12	Aug. 19	Aug. 26	Sep. 2	Sep. 9	Sep. 16	Sep. 23	Sep. 30	Oct. 7	Oct. 14	Oct. 21	Oct. 28	Grand Total
Number of houses visited each week	54	1,822	2,038	2,225	2,120	17,34	1,542	1,434	1,529	9,810	3,193	4,737	6,225	5,624	6,045	7,158	5,427	4,977	6,371	4,196	5,200	5,331	88,823
Number of houses per fortnight										13,003		10,962		11,669		12,685		11,348		9,396			
Number of fever cases seen	7	42	30	28	29	33	26	39	29	155	97	81	119	75	74	48	59	42	65	73	90	32	1,275
Mean number fevers per 100 houses	15	2·3	1·5	1·2	1·4	1·9	1·6	2·7	1·9	1·6	3·1	1·7	2·5	1·4	1·2	0·7	1·1	0·9	1·0	1·8	1·8	0·6	1·5
Number of blood smears taken from fever cases	7	74	65	33	30	33	26	39	29	175	100	100	146	86	96	67	77	55	101	82	92	51	1,564
Infant fever cases seen		1	...	...	...	1	...	...	...	7	3	1	3	1	...	...	...	...	...	...	...	...	17
Infant blood smears taken both with and without fever	1	5	7	...	38	81	60	15	3	10	3	3	3	1	...	4	1	...	1	1	...	1	438

the plains seems to be entirely within the realms of practicability. If motor transports are made available, there is no doubt that the output can still further be improved.

The present work was organized merely by mobilizing only the available staff without extra grants and it was made possible because the area and the population surveyed were comparatively small, *i.e.*, about 1,000 square miles and 32,000 population. When a large district with a population of over a million is taken up for surveillance, a field staff of over 100 Investigators alone would be required in addition to the staff required for supervision, technicians for blood examination, transport drivers, clerical staff for compilation, etc. This study has shown that provided adequate funds are made available for the employment of such staff, surveillance on a big scale is practicable.

The economics of nation-wide surveillance programmes have to be considered very carefully. All efforts must be made to keep the budgets at the absolutely minimum levels consistent with technical requirements. In a district like Kanara where control measures are well established for a number of years and all indices have long reached and have been maintained at their end-points for a number of years, it is for consideration whether a house to house fortnightly surveillance should be done *throughout* the year or whether surveillance could not be restricted with perfect safety to the actual seasons of transmission and whether the house to house visits could not be carried out only once a month. Such measures may make it possible to keep the expenditure at a low level without sacrificing safety.

Among the technical results of this study is the observation that *A. fluviatilis* has been practically absent from large sections of Kanara District. It is significant that no larvæ of this species were also collected in spite of intense searches in the most suitable habitats, namely the channels and streams. Whether the same conclusion would hold good for the garden tracts of Kanara District, is difficult to say at present and can only be decided after completing work during the ensuing season. Though mosquitoes of other species were found returning to the human dwellings, their numbers had not yet reached those which were obtaining in the pre-D.D.T. days. It may be that D.D.T. particles still persisted on some of the sprayed surfaces in the houses and were still effective enough to prevent the build up of the original densities. Traces of deposits of D.D.T. powder were noticeable in several houses even in July. Scrapings from walls and other sprayed surfaces should now be taken and tested to see whether D.D.T. in an effective state still persists in them. *A. culicifacies* of which fair numbers of adults were collected in the pre-D.D.T. days has also undergone a very considerable reduction. Only two adults were collected in houses and five in cattlesheds. The experience in the neighbouring district of Dharwar also has been similar and *A. culicifacies* has become a rarity there also in villages which were being sprayed for the last ten years.

The complete absence of *P. vivax* and *P. falciparum* infections in the blood smears of over 1,500 persons suffering from fever, is noteworthy. The single case of quartan infection may well be a relapse, for it is well known that *P. malariæ* infections can persist in human beings for a number of years. In the face of these observations it is surprising that the 12 dispensaries of Kanara District are still showing over 5,000 cases of malaria annually. None of the slides, taken from patients diagnosed as suffering from malaria in the dispensaries located in the zone

where investigations were carried out, was positive for malaria parasites. Further studies are being undertaken to evaluate the validity of the diagnosis made in the out-patient departments of public dispensaries all over the State.

Kanara District has now become a part of the enlarged Mysore State as a result of the reorganization of States in India and from November 1, 1956, the malaria work in the district is no longer under the direct supervision of the authors but they have every reason to believe that the experiment, which has been started, will be carried through most vigorously by the new authorities.

ACKNOWLEDGEMENTS.

This work is the result of collaboration of several individuals over a period of several years. The incentive given to the general idea of interruption of spraying and surveillance by Dr. D. K. Viswanathan, the former Director of Public Health for the Government of Bombay, is gratefully remembered. Among those who have materially contributed to the technical aspects of the work in connection with surveillance are Shri S. C. Bhatia, Shri A. V. Halgeri, Shri V. S. Karandikar, Shri R. M. Naik and Shri P. S. Shirwadkar. The very loyal and painstaking work, done by them and the other field staff on surveillance duty, merits special commendation.

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APPENDIX I.

INSTRUCTIONS FOR USING FORMS S1, S2 and S3.

1. These are the proformas of the pages of the three registers which the Investigators are to maintain.

2. Copies of the pages of each register should be made out in the same proformas once a week and sent to the Medical Officer of Health of the Division with a copy to the Assistant Director of Public Health (Malaria), Poona, *on every Saturday*. The week beginning always refers to the date of a Sunday.

3. *Form S1.*

This should be the first page of the weekly report.

In column 5, the number of fever cases should mean not only cases of fever actually seen at the time of the visit but also cases of fever which occurred during the last fortnight.

Form S2.

Serial number.—Each Investigator has his own series of numbers commencing with 1. But to distinguish the serial numbers of different Investigators, the number will be preceded by an approved letter of the alphabet. The approved letters at present are:—

G—Shri Gokarn.
H—Shri Hegde.
J—Shri Ijari.
N—Shri Nair.
A—Shri Shirwadkar, Assistant Medical Officer.

The numbers should be written as G1, H-7, J-4 and so on in the form and the same number also written with *hard pencil* boldly on the thin smear of the slides. When more Investigators are appointed, they should be assigned a letter.

Taluka and village.—First write name of taluka and then names of all villages visited in it.

Door number or address.—This is to ensure retracing the case for treatment if his blood smear is positive. Therefore, no doubt should be left regarding the exact address.

Name of fever case.—First personal name, next father's name and third surname.

Age.—Write number of years as 4, 8, 9, 70 and so on. If the case is an infant, write boldly INF followed by the number of months, like INF three months, INF six months, etc. If INF is not written, it will be taken that the slide is that of a person above the age of one year. No smears from infants, other than those which show fevers, should be taken.

Remarks.—There is no need to elaborately describe the case. In cases, not having fever at the time of visit, state how many days ago the fever occurred and for how long.

Taken by.—Write initials of the person who takes the smear.

Columns "Examined by", "Date of examination", "Result" and "Remarks" will be entered in at the headquarters.

Examined by.—The initials of the person who actually examines the slide.

Results.—Write *Neg.* if negative. If positive, write species as V, F or M and stages as tr. (=trophozoites) and g. (=gametocytes) as V.tr or F.g. and so on.

Remarks.—Write only when necessary, as for instance in case of heavy infections, or mixed infections, etc. Also if the smear is of poor quality, or broken in transit, say 'Rejected'.

(The slides should be sent securely packed every Monday to the Assistant Director of Public Health (Malaria), Poona, by parcel post).

Form S3.

For each type of shelter, record number of males and females collected.

If any species not listed is found, write its name and enter the figures.

APPENDIX. II.

Form S-1.

Kanara District. Malaria Surveillance Team,..... Name of Investigator.....

Investigator's Weekly Report Week beginning.....

Note: *This should include data for villages visited by the Assistants also.*

Taluka	Village	Date of visit	Number of houses visited	Number of fever cases seen	Number of persons to whom drug administered	Number of smears taken	Initials of persons who did the surveillance	Remarks (only when absolutely necessary)
1.	2.	3.	4.	5.	6.	7.	8.	9.

Public Health Department. No. Date.....Camp.....

Submitted to the Medical Officer of Health, Sirsi, with a copy to the Assistant Director of Public Health (Malaria), Poona, together with.....sheets of mosquito collection report and..... sheets of blood smear reports.

Name

Investigator:——

Designation.

APPENDIX III.

Form S-2

Kanara District. Malaria Surveillance Team.....; Name of Investigator.....Week beginning.....

Serial number 1.	Taluka and village 2.	Date 3.	Door number or address 4.	Name of fever case 5.	Age 6.	Sex 7.	Remarks 8.	Taken by 9.	Examined by 10.	Date of examination 11.	Result 12.	Remarks 13.

APPENDIX IV.

Form S-3.

Kanara District. Malaria Surveillance Team .....; Mosquito collections Report for week commencing.....

Name of village																							
Date																							
Adults																							
Number of houses/cattlesheds			...																				
Time spent: Hrs. Mts.	...	...																					
Routine or general	...	...		M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
A. fluviatilis	...	...	...																				
A. culicifacies	...	...	...																				
A. annularis	...	...	...																				
A. hyrcanus	...	...	...																				
A. jamesi	...	...	...																				
A. jeyporiensis	...	...	...																				
A. pallidus	...	...	...																				
A. subpictus	...	...	...																				
A. tessellatus	...	...	...																				
A. vagus	...	...	...																				
A. varuna	...	...	...																				
Other species	...	...	...																				
Total Anopheles	...	...	...																				
Culicines	...	...	...																				

APPENDIX IV (*Concld.*)

Name of village	
Date	
Number of houses/cattlesheds	
Number of structures yielding Anopheles adults	
Number of structures yielding *A. fluviatilis*	
Larvæ	
Number of streams and channels examined	
Time spent: Hrs. Mts.	
A. fluviatilis larvæ	
A. varuna larvæ	
Other Anopheles larvæ	
Total	

Remarks, if any:

Insect Collector:

Investigator:

Indian Journal of Malariology, **11**, 3, *September 1957*.

FILARIASIS IN ASSAM STATE*.

BY

P. C. BASU.

(*Malaria Institute of India, Delhi.*)

[June 29, 1957.]

THIS report sets out the results of a filariasis survey carried out in some tea-garden areas of Assam State during February-April, 1957.

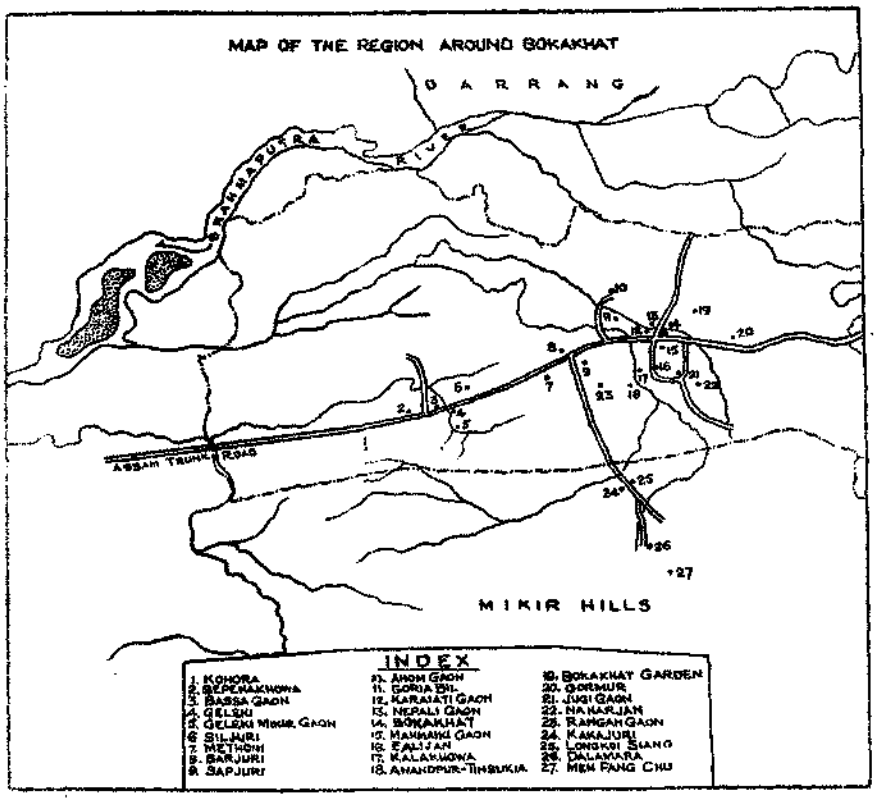
DESCRIPTION OF AREA.

The survey was conducted on both sides of the Assam Trunk Road between mile-stones 40 and 55, in Bokakhat area of Bokakhat Sub-division. On the north, the area is limited by the River Brahmaputra and Kaziranga reserve forest and on the south by the Mikir Hills. Villages and tea-gardens are situated in the forest clearings. A number of hill streams, draining into the Brahmaputra, cross from south to north. The northern part of the area is subject to flooding during the monsoon and large perennial swamps exist (Map 1). Communications are good during the dry season. Tea and wet crops form the main cultivation. The main sources of water supply are the swamps, streams, wells and tube-wells.

In Chabua area, the survey was conducted in Chabua Thana of Lakhimpur District, extending from the Dibru River in the north to the Dibrugarh-Tinsukia track of N. E. Railway in the south (Map 2). On the east, the area surveyed extended to the Dinjan Tea Estate and in the west up to Rohmoria Gaon. In the northern part of the area, many of the villages close to Dibru River, are subject to annual flooding when the inhabitants are evacuated to a settlement in Dinjan Tea Estate. The area is evenly flat with two big tea plantations in the Centre known as Balijan North and Balijan South Tea Estates. Villages with surrounding

* The survey was carried out to obtain an idea of the degree of filariasis in the indigenous population due to the introduction of symptomless but infected immigrant labour, and consequent endemicity of the areas. The surveys currently reported were confined to two areas, one around Bokakhat in Sibsagar District and the other around Chabua in Lakhimpur District.

MAP 1.

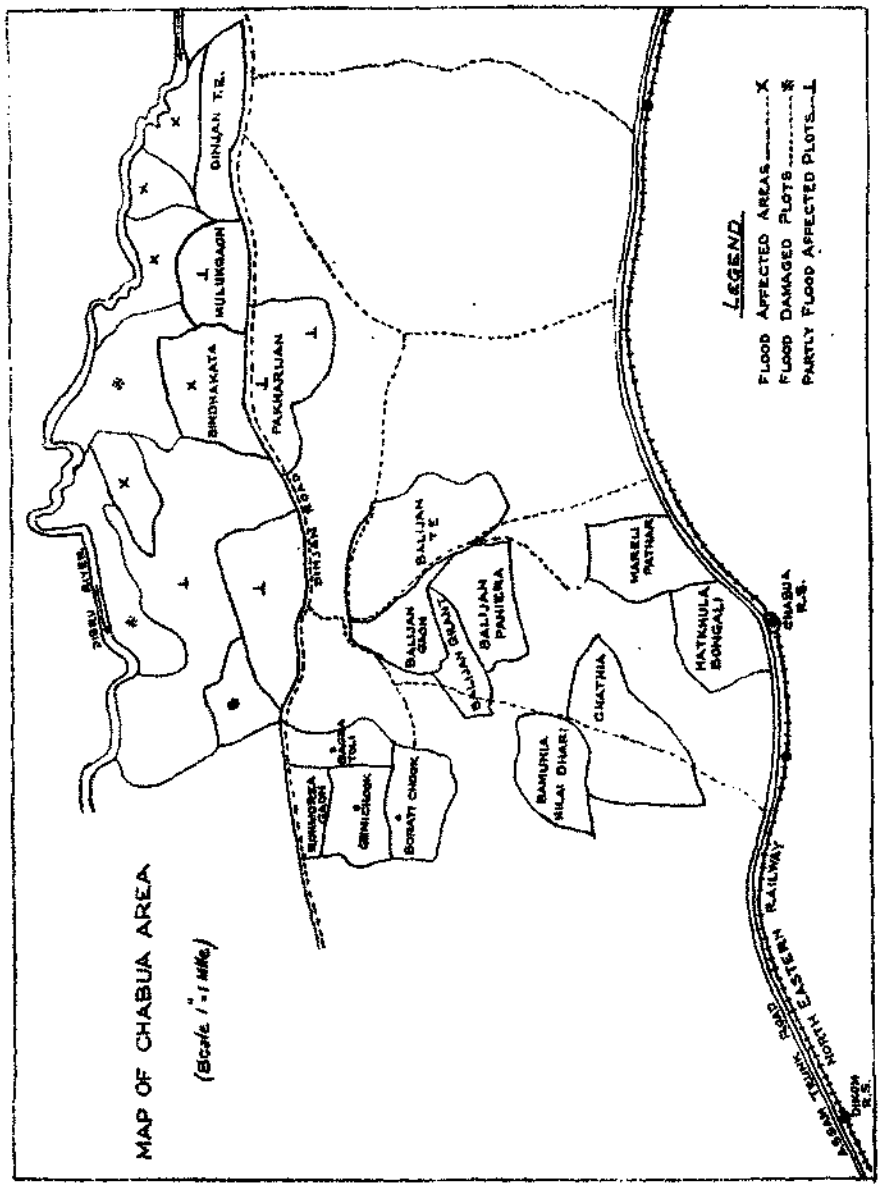


PEOPLE AND THEIR ECONOMIC CONDITION.

In Bokakhat area, indigeonous people, chiefly agriculturists from the plains and hills (mainly Mikirs), live in organized villages. There are other small villages founded and populated by ex-tea garden labourers mainly from Bihar, Orissa, Madhya Pradesh and Madras who have permanently settled since at least two generations. The third category of persons are the tea-garden labourers living in organized labour lines. While a small part of the garden population are recent arrivals, the majority are residents of the area for over 20 years. The labour population in the gardens are economically better off and have better environmental sanitation than the village population. Almost every house in both the plantations and in the villages has several heads of cattle and other domestic animals.

In Chabua area, the composition of the labour population in the gardens is about the same as in Bokakhat area. The village population belong mostly to the plains of Assam. In addition, a large number of immigrants, from the area now forming part of East Pakistan, have also settled since generations in some of the villages in this area. The economic condition of the people in this area is about the same as in Bokakhat area.

MAP 2.



METEREOLOGICAL CONDITIONS.

Temperature, rainfall and relative humidity values for five years (January, 1952 to December, 1956) for Bokakhat area, are given in Table I, and temperature and rainfall values for Chabua area in Table II.

TABLE I.

Representative temperature and rainfall values for Bokakhat Area.

(From Methoni Tea Estate Records: January 1950 to December 1956.)

Month	Temperature (°F.)			Rainfall (inches)			Relative humidity (per cent). Mean 1951–56
	Mean daily maximum	Mean daily minimum	Mean	Mean 1950–56	Maximum 1950–56	Minimum 1950–56	
January ...	69	53	61	0·97	2·03	0·08	...
February ...	74	69	71	0·80	1·45	0·16	...
March ...	78	64	71	4·49	11·69	0·34	...
April ...	81	70	75	6·86	11·61	3·77	83
May ...	84	74	79	11·48	13·93	7·79	82
June ...	86	78	82	13·93	22·44	9·16	85
July ...	87	79	83	14·68	16·53	13·49	87
August ..	87	79	83	16·14	21·79	11 08	86
September...	86	77	81	9·17	17·07	2·93	84
October ...	81	72	76	7·16	12·75	2·70	82
November...	75	63	69	1·33	2·97	0·42	91
December...	69	55	62	0 72	2·77	0·02	79

TABLE II.

Representative temperature and rainfall values for Chabua Area.

(From Balijan North Tea Estate Records: January 1952 to December 1956.)

Month	Temperature (°F.)			Rainfall (inches)		
	Mean daily maximum	Mean daily minimum	Mean	Mean 1952–56	Maximum 1952–56	Minimum 1952–56
January ...	66	58·9	62·4	1·06	1·39	0·74
February ...	72	64	68	2·11	6·29	0·36
March ...	72·5	67	69·7	6·03	9·55	2·03
April ...	79·5	72·5	76	8·86	13·20	4·00
May ...	84	77·6	80·8	12·37	16·77	6·63
June ...	84·8	80	82	15·72	20·94	11·52
July ...	84	79	82	20·55	23·13	7·61
August ...	84·9	79·9	82·4	15·47	18·49	12·55
September ...	83·1	77·9	80	14·75	21·79	7·06
October ...	76·8	71·8	74·3	9·17	14·82	5·81
November ...	70·6	63·8	67·2	0·87	1·83	0·09
December ...	65·3	58·3	61·8	1·02	2·57	0·09

paddy fields are situated in the region south of the plantations. Big sullage and storm water drains pass through the plantations.

In both the areas, it would be observed that the mean of the daily maximum and minimum temperature is about 75° F. (23·9° C.) during April through September. The relative himidity from April through December, for which data are available, is above 75 per cent. The temperature and humidity conditions would thus appear to be favourble for transmission of filariasis during the greater part of the year.

PRESENT SURVEY.

Bokakhat area.—Representative parts of six gardens with a population of 5,850 and of 19 villages with a population of 8,695 were examined at night between 7.30 p.m. and midnight. The total number examined was 2,213 or 15 per cent. The data are presented in Table III.

Disease manifestations, mainly of the lower limbs and few genital cases (Table IV), were observed in all the gardens and in 14 of the 19 villages; the overall disease rate for the area being 3·3 per cent. The age distribution of disease manifestations are given in Table V. It would be observed that disease manifestations are present in all age-groups, starting even from three to five years. The sex distribution of disease manifestations, was found to be 58 per cent for males and 42 per cent for females. Disease was noted in a child as early as three years of age with *elephantiasis* of the lower extremities.

Infection was observed amongst the labour population in all the gardens and amongst 15 villages out of the 19 surveyed; the overall infection rate being 4·5 per cent. Both *W. malayi* and *W. bancrofti* infections were noted, the former in 74 per cent and the latter in 26 per cent of the persons showing microfilaria in their blood. The incidence of filarial infection covered all the age-groups from one to five to above 50 years. The sex distribution of the infected persons was found to be almost equal (54 per cent males and 46 per cent females). The earliest age at which infection was observed was in an infant 12 months old, showing *W. bancrofti*. *W. malayi* infection was noted in a child as young as 18 months. The average infestation was 9 per 20 c.mm. of blood. The average infestation for *W. malayi* was 7·8 per 20 c.mm. and 26·7 per 20 c.mm. of blood for *W. bancrofti*. The overall endemicity rate was 7·7 per cent. In four of the five villages free from disease manifestations, the infection rates varied from 0·7 to 1·4 per cent. Three of the fourteen villages, with persons showing disease manifestations, were negative for filarial infection. Thus of the nineteen villages surveyed, eighteen were noted to be endemic for filaria.

Chabua area:—Two gardens with a population of 7,000 and 12 villages with a population of 5,938 were surveyed, the number of persons examined being 1,739 or 13 per cent of the population. The findings are presented in Table VI.

Disease manifestations were observed amongst the labour population in both the gardens. Amongst the village population, however, disease manifestations were noted in four of the twelve villages. The overall disease rate for the area was found to be 1·8 per cent and consisted chiefly of *elephantiasis* of the lower limbs,

TABLE III.

Disease rates etc. in Bokakhat Area.

Area	Population	Number examined	Number of persons with disease manifestation	Disease rate per cent	Number positive for infection	Infection rate (per cent)	Endemicity rate (per cent)	Average Infestation (per 20 c. mm.
Dalamara (Garden) ...	350	82	4	4·9	3	3·6	8·5	26·3
Basangaon (Village) ...	1,200	119	2	1·7	1	0·8	2·5	...
Sapanakhwa (Village) ...	200	39	2	5·0	...	...	5·0	...
Methoni (Garden) ...	900	156	6	3·8	23	14·7	18·5	8·1
Borjuri (Garden) ...	500	121	8	6·6	10	8·2	15·7	8·0
Kakojuri and Longkolsiang (Villages) ...	200	41	3	7·3	3	7·3	14·6	11·0
Sujuri (Garden) ...	500	77	18	25·0	5	7·04	32·3	5·8
Geleki - Mikir Gaon (Village) ...	200	41	4	9·7	8	19·5	26·8	7·0
Geleki (Village) ...	175	29	1	3·4	...	...	3·4	...
Borjuri - Mikir - Rangang Gaon and Kania Tarang (Village) ...	200	40	2	5·0	5	12·4	17·5	28·6
Sapujuri (Village) ...	400	86	5	5·8	11	12·7	18·6	7·5
Ahom Gaon (Village) ...	300	32	1	3·1	4	12·5	15·6	15·5
Moumaki Gaon (Village)	1,150	88	...	...	...	...	...	...
Gariabill (Village) ...	300	28	2	7·1	4	14·2	21·4	3·7
Karoiati Gaon (Village)	600	121	2	1·6	2	0·8	2·4	12·0
Nepali Gaon (Village) ...	500	85	1	1·1	2	2·3	3·5	3·0
Kalakhwa (Village) ...	400	60	2	3·3	...	...	3·3	...
Nuandapur - Tinsukia (Village) ...	350	77	...	...	1	1·2	1·2	...
Bokakhat (Garden) ...	1,350	150	3	2·0	2	1·3	3·3	4·5
Gormur (Village) ...	700	139	...	...	1	0·7	0·7	...
Nahorjan (Garden) ...	2,250	293	4	1.4	4	1·4	2·8	3·0
Balijan (Village) ...	350	70	...	...	1	1·4	1·4	...
Jugigaon (Village) ...	500	101	...	...	1	1·0	1·0	...
Men Fang Cho (Village)	370	73	2	2·7	8	10·8	13·5	13·3
Kobura (Village) ...	600	66	1	1·5	2	3·0	4·5	4·0
Total ...	14,545	2,213	73	3·3	100	4·5	7·7	9·0

Average infestation for *W. malayi* 7.7 and for *W. bancrofti* 14 per 20 c.mm.

TABLE IV.

Analysis of disease manifestations in Bokakhat and Chabua Areas.

Area	Number of persons with disease	Type of manifestations			
		Lower extremity (one or both)	Upper extremity (one or both)	Both upper and lower extremities	Genital
Bokakhat ...	73	65	3	...	5
Chabua ...	32	28	...	2	2

genital affections being observed only in a very few persons (Table IV). The age distribution of disease manifestations is given in Table VII from which it would be noted that disease manifestations occurred in persons over 21 years, the sex distribution being almost equal. Infection amongst the labour population was observed in both the gardens and amongst the villages in nine out of twelve villages. The overall infection rate was 7·9 per cent. Both *W. bancrofti* and *W. malayi* infections were noted, the former in 81·3 per cent and the latter in 18·7 per cent of the persons showing microfilaria in blood. The incidence of microfilarial infection covered all age-groups above three years (Table VII). The sex distribution of infected persons was almost equal. The earliest age at which infection was noted was in a child aged 3½ years, the species being *W. bancrofti*; *W. malayi* infection was noted in a child as young as six years. The overall endemicity rate was 9.7 per cent. It would be noticed that in five villages, free from persons with disease manifestations, infection rate varied from 1·5 to 4 per cent. Thus of the twelve villages surveyed, nine were noted to be endemic for filaria.

ENTOMOLOGICAL INVESTIGATION.

The plantation lines in both the areas receive residual B.H.C. spraying at the rate of 22 mg. per square foot every month from March through November as an antimalarial measure.

It was the second year of B.H.C. spraying when this survey was conducted and during the preceeding three years, D.D.T. has been used as a residual insecticide.

The villages in Bokakhat area, being included in the National Malaria Control Programme, received residual spraying with D.D.T. since July, 1955, whereas the villages in Chabua area had no such spraying.

ADULT MOSQUITOES.

Only in-door mosquito collections were made from three types of shelters, *viz.*, human dwellings, mixed dwellings and cattlesheds. Mosquito catches were made in the mornings between 7.00 and 11.00 a.m., and in the evenings between 7.30 and 11.00 p.m.

TABLE V.

Age distribution of disease and infection of persons examined in Bokakhat Area.

Details of examination	Age groups — in years															
	0—5		6—10		11—20		21—30		31—40		41—50		Above 50		Total	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Number of persons examined	230	242	152	176	177	221	253	229	157	126	94	64	59	33	1122	1091
Number positive for microfilariæ ...	1	3	2	3	8	10	16	11	12	10	11	5	4	4	54	46
Percentage	0·4	1·2	1·3	1·7	4·5	4·5	6·3	4·8	7·6	7·9	11·7	7·8	6·7	12·1	4·8	4·2
Number positive for disease	3	1	1	2	2	4	13	6	8	9	9	5	7	2	44	28
Percentage	1·3	0·4	0·6	1·1	1·1	1·8	5·1	2·6	5·0	7·1	9·5	7·8	1·18	6·0	3·9	2·5
Number positive for both	...	...	...	...	...	...	...	...	1	...	...	...	...	...	1	...
Percentage	...	...	...	...	...	...	...	...	0.6	...	...	...	...	...	0.08	...
Average infestation per 20 c.mm. of blood ...	1	5	2	9·5	11·4	9·5	8·5	7·1	9·5	21·3	4·3	11·2	5·0	11·0	...	...

M=male. F=female.

TABLE VI.

Results of filaria survey in Chabua Area.

Serial number	Area	Population	Number of persons examined	Number showing disease manifestation	Disease rate per cent	Number showing microfilariæ in blood	Infection rate (per cent)	Endemicity rate (per cent)	Average infestation (per 20 c.mm.
1.	Bindhakata Gaon and Pakharijan ...	403	66	4	6·0	5	7·5	12·1	4·4
2.	Muluk Gaon ...	235	31	...	...	1	3·2	3·2	2
3.	Dinjan Tea Estate (Villagers from flooded area rehabiliated in this area) ...	550	75	...	...	2	2·7	2·7	4
4.	Bammia Hilly Darny...	700	76	...	...	...	...	...	...
5.	Chathia	300	28	...	...	1	3·2	3·2	3
6.	Marily Pathar ...	500	75	...	...	3	4·0	4·0	16·3
7.	Hathola Bangali ...	450	66	...	...	...	...	...	...
8.	South Balijan Tea Estate	3,000	495	3	0·6	70	14·02	14·6	9·4
9.	North Balijan Tea Estate	4,000	534	22	4·1	53	10·1	14·06	6·1
10.	Begotolia ...	500	32	...	...	...	...	...	...
11.	Rohmoria ...	500	75	2	2·6	1	1·3	4·0	1
12.	Genichak... ...	800	82	...	...	1	1·2	1·2	2
13.	Baratichak ...	1,000	106	1	0·9	1	0·9	0·94	2
	Total	12,938	1,739	32	1·87	138	7·9	9·7	7·7

Average infestation for *W. malayi* 4 and for *W. bancrofti* 10 per 20 c.mm.

In Bokakhat area 115·5 man-hours in morning and 102·4 man-hours in the evening were spent for mosquito collection. The following species were noted:—

C. fatigans
C. vishnui
C. sitiens
C. tritæniorhyncus
M. uniformis
A. albopictus
A. aconitus
A. barbirostris
A. hyrcanus
A. turkhudi
A. kochi
A. minimus

TABLE VII.

Age distribution of disease, and infection of persons, examined in Chabua Area.

Details of examination	Age groups — in years															
	0—5		6—10		11—20		21—30		31—40		41—50		Above 50		Total	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Number of persons examined	199	212	142	126	72	138	139	186	129	120	88	73	55	60	824	915
Number positive for microfilariæ ...	1	3	7	8	3	13	11	13	26	12	14	7	11	9	74	64
Percentage	0·5	1·4	4·9	6·3	4·1	9·4	7·9	6·9	20·1	10·0	15·9	9·5	20·0	15·0	8·9	6·9
Number positive for disease	...	...	...	...	...	...	4	3	6	3	5	3	2	6	17	15
Percentage	...	...	...	...	...	...	2·8	1·6	4·6	2·5	5·6	4·1	3·6	10·0	2·0	1·6
Number positive for both	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Average infestation per 20 c.mm. of blood ...	3·0	2·0	10·4	3·1	13·6	8·0	6·2	7·0	6·4	10·8	9·6	6·7	3·4	11·2	...	...

M = males. F = females.

The above collections were made in 13 villages and plantations from 1,254 shelters and the consolidated data are presented in Table VIII and the density figures in Table IX.

TABLE VIII.

Morning and evening collections of C. fatigans *and* Mansonioides uniformis *from Bokakhat Area.*

Man-hours morning collections Hrs. Mts.	Number of *C. fatigans* females	Number of *Mansonioides* females	Man-hours evening collections Hrs. Mts.	Number of *C. fatigans* females	Number of *Mansonioides* females
115–30	923	28	102–45	1,021	174

TABLE IX.

Mosquito density data in Bokakhat Area.

Type of shelter	SPRAYED AREAS							
	Human dwellings		Mixed dwellings		Cattle-sheds		Total	
	Hrs.	Mts.	Hrs.	Mts.	Hrs.	Mts.	Hrs.	Mts.
Time spent ...	158	45	9	55	49	55	218	15
	M	F	M	F	M	F	M	F
Culicines ...	186	1,526	11	100	44	688	241	2,314

M=male. F=female.

Density per ten man-hours.

SPRAYED

C. fatigans ... 99·31

Mansonioides ... 9·5

Out of these collections, 195 female *M. uniformis* and 1,908 female *C. fatigans* were dissected. No developmental stages of the *wuchereria* larvæ were detected except the freshly ingested microfilaria in the stomachs of 4 *C. fatigans* mosquitoes. These microfilariæ, when stained, resembled those of *W. malayi*. All other species of mosquitoes dissected did not show any infection. The disssection results are presented in Table X. It would be noticed that *M. uniformis* were collected in comparatively large number during the evening hours between 7.30 and 11.00 p.m. The majority of the *M. uniformis* caught were from cattlesheds (Table XI). Blood meals from 39 *M. uniformis*, tested for precipitin reaction, showed that five were positive against human antiserum and 13 were positive against bovine and goat antisera. The remaining 11 samples were found negative amongst human bovine,

goat, dog and pig antisera. Thus, *M. uniformis* showed a zoophilic index of 33·33 per cent while the anthropophilic index was only 12·8 per cent.

TABLE X.

Dissection results in Bokakhat Area.

Species dissected	Number dissected	Number positive	Remarks
C. fatigans ...	1,908	4	Positive for microfilaria in abdomen. Species *W. malayi*.
M. uniformis ...	195	Nil	...
Other Culicines	161	Nil	...
Anophelines ...	15	Nil	...

TABLE XI.

Shelter preferences for C. fatigans *and* M. uniformis *in Bokakhat Area caught in 217 man-hours.*

	Human dwellings	Mixed dwellings	Cattle-sheds
C. fatigans ...	1,398	74	472
M. uniformis ...	43	12	147

In Chabua area, 50·7 man-hours in the morning and 11 man-hours in the evening were utilized for mosquito collection.

The mosquitoes were *C. fatigans*, *C. vishnui*, *C. sitiens*, *M. uniformis* besides *A. hyrcanus*, *A. barbirostris* and *A. annularis*. The collection was made from eight villages and two plantations. The collection figures and mosquito densities are given in Tables XII to XIV. It would be observed that infective larvæ have been detected in two, third-stage larvæ in five, second-stage of development in the thorax in seven mosquitoes. First-stage developing forms have been noted in the abdomen and thorax of 42 mosquitoes. Other species of mosquitoes did not show any infection. A comparison of the density for ten man-hours would show that intensive residual spraying of insecticides was having apparently no effect on *C. fatigans* density in the garden lines. The successful completion of the developmental cycle of *wuchererian* larva showed that the longevity of *C. fatigans* was not being affected either by the insecticide. This provided circumstantial evidence that the susceptibility of local *C. fatigans* to B.H.C. was probably low.

TABLE XII.

Morning and evening collections of C. fatigans *and* Mansonioides sp. *in Chabua Area.*

Man-hours morning collections		Number of *C. fatigans*: Females	Number of *Mansonioides*: Females	Man-hours evening collections		Number of *C. fatigans*: Females	Number of *Mansonioides*: Females
Hrs.	Mts.			Hrs.	Mts.		
60—40		1,576	...	11—10		152	1

TABLE XIII.

Mosquito density data in Chabua area.

Type of shelter	SPRAYED								UNSPRAYED							
	Human dwellings		Mixed dwellings		Cattle-sheds		Total		Human dwellings		Mixed dwellings		Cattle-sheds		Total	
	Hrs.	Mts.	Hrs.	Mts.	Hrs.	Mts.	Hrs.	Mts.	Hrs.	Mts.	Hrs.	Mts.	Hrs.	Mts.	Hrs.	Mts.
Time Spent	25	50	2	10	2	00	30	00	22	30	2	10	7	10	31	50
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Culicines	307	399	21	60	1	16	329	975	114	695	4	54	1	55	119	804

M=males. F=females.

Density of female C. fatigans *for ten man-hours.*

SPRAYED		UNSPRAYED	
Human dwellings	... 346	Human dwellings	... 303·5
Mixed dwellings	... 277	Mixed dwellings	... 207·7
Cattlesheds	... 80	Cattlesheds	... 35·0

TABLE XIV.

Dissection results of mosquitoes collected from Chabua area.

Species dissected	Number dissected	Number positive	NUMBER OF MOSQUITOES SHOWING DIFFERENT STAGES OF INFECTION														Remarks
			Head		Thorax				Abdomen				Malpighian tube				
			3	4	1	2	3	4	1	2	3	4	1	2	3	4	
C. fatigans	1,725	45	...	...	6	7	5	2	36	...	...	...	...	...	...	...	Species *W. bancrofti*
Other Culicines	23	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...

BREEDING PLACES.

In Bokakhat area, extensive breeding of *C. fatigans* was noted in perennial swamps with putrefying vegetations such as water-hyacinth. *Mansonioides* breeding could not be detected in spite of careful and prolonged search.

In Chabua area, the blocked sullage and rain-water drains in the plantations provided excellent sanctuaries for *C. fatigans*. In some of the villages also, sullage water drains provided similar breeding places.

FILARIAL ENDEMICITY AMONGST PURELY INDIGENOUS, LOCAL-BORN AND IMMIGRANT PEOPLE.

A classification of the persons examined, according to their origin in relation to the area surveyed, showed that the endemicity is highest amongst the immigrants, next amongst the local-borns and lowest amongst the indigenous people (Table XV). The term indigenous has been applied to denote the persons such as Mikir, Ahom and other tribes indigenous in Assam and who are known to move little outside the district. The term local-born has been applied to persons born in the area whose parents or grand parents were immigrants from other States; these people also do not move out of the district. The term immigrant is applied to persons who themselves have migrated from other States either recently or since a number of years; some persons amongst the last category periodically visit their parent States.

TABLE XV.

Final endemicity in different categories of persons.

	Number of persons examined	Number of persons showing disease	Disease rate (per cent)	Number of persons showing micro-filaria	Infection rate (per cent)	Endemicity rate (per cent)	Percentage of infection *W. bancrofti*/*W. Malayi*
Immigrants...	308	24	7·8	43	13·9	21·4	76/24
Local-born ...	2,296	54	2·3	157	6·8	9	54/46
Indigenous ...	1,348	18	1·3	38	2	4	50/50

PERIODICITY.

Nocturnal periodicity was observed in both types of *Wuchererian* infections.

DISCUSSION.

Previous filariasis survey of the nine villages in tea-gardens of Cachar District in Assam had revealed the prevalence of the disease due to *W. malayi* infection in seven and due to *W. bancrofti* infection in one village (Rao, 1942).

It was also noted in these areas that the infection rate had remained steady compared with figures for previous years and that it did not appear to have spread to the neighbouring villages.

In the present investigation, two widely separated areas in the districts of Sibsagar and Lakhimpur showed that filarial disease manifestations and infection are prevalent in all the eight gardens surveyed, the disease rates varying from 0·6 to 25·3 per cent, the infection rate from 1·3 to 14·7 per cent and endemicity rate from 2·8 to 32·3 per cent amongst the garden population. Disease manifestations have been observed in 18 out of 31 villages surveyed, the disease rates varying from 0·9 to 9·7 per cent whereas infection has been observed in 24 villages, the rates varying from 0·7 to 19·5 per cent. The endemicity rates for these villages varied from 0·7 to 28·6 per cent. So it is noted that 27 villages of the total of 31, *i.e.*, 87 per cent of the villages surveyed, showed filarial endemicity. Some percentage of these villages are inhabited only by the indigenous people such as Mikirs and Ahoms, who usually do not go out of the districts and as such have acquired the infection locally. A classification of the persons examined, according to their origin in relation to the area surveyed, shows that the endemicity is highest amongst the immigrant labourers, the next in order of decreasing endemicity being in those born locally whose parents or grand-parents were immigrants, and lowest amongst the indigenous people. Thus the existence of foci of both types of *Wuchererian* infections and their spread to the neighbouring villages affecting the indigenous population, is quite apparent. Further, it may be stated that the villages close to the gardens are found to be more endemic than the villages away from the gardens. Both *W. malayi* and *W. bancrofti* infections have been noted in the areas surveyed, the former infection being predominant in Bokakhat area and the latter in Chabua area. The average infestation for *W. malayi* in the Bokakhat area is much lower than the average infestation of *W. bancrofti*. Probably, the low density of *Mansonioides*, coupled with their high zoophilisms as noted at the time of the survey, is responsible for not showing the infective larvæ in *M. uniformis* in this area. But in Chabua area, the moderately high infection rate of *W. bancrofti* associated with high density of *C. fatigans*, readily showed the infection in the latter.

Humidity and temperature conditions are found suitable for transmission of *W. bancrofti* for the greater part of the year as experimentally both infection and infestation rates in *C. fatigans* have been shown to be high when relative humidity is above 60 per cent and the temperature is between 80° and 90° F. (Acton and Rao, 1930; Rao and Iyengar, 1930).

Thus, it can be concluded that the plantations provide filarial foci from which active transmission of the infection (both types of *Wuchererian* infections), affecting the local-borns in the immediate neighbourhood and the indigenous people in the contiguous villages, is taking place.

This fact should provide logical incentive for similar investigation in all the districts of Assam where tea plantations exist and in order to prevent the further spread of filariasis immediate control measures are indicated.

The four major tea districts in the plains of Assam, *viz.*, Lakhimpur, Sibsagar, Cachar and Darrang have a total of 4,86,134 adult persons engaged in plantation (Census of India, 1951). The other three minor tea districts, *viz.*, Goalpara,

Kamrup and Nowgong have a total of 19,057 persons engaged likewise. Thus, roughly about half a million persons and their children, distributed in seven districts in the plains, may be residing in endemic areas with a possible spill of the infection in the contiguous areas involving the local population. Although a precise determination of the population at risk can only be based on actual delimitation of the problem a rough estimation would put the figure around a million persons.

In tea plantation, a liberal application of insecticides is the usual practice. In spite of repeated applications, the density of *C. fatigans* is recorded to be comparable to that in unsprayed areas. This, associated with the observation that complete development of filarial larvæ to the infective stage occurs in *C. fatigans* within the plantation lines, provides circumstantial evidence of the lowered susceptibility of this species of mosquito locally. Studies on the median lethal concentration of the synthetic insecticides to the local *C. fatigans*, will throw useful light on this subject and help planning of the control programme.

SUMMARY.

Filariasis survey of two areas, one in the Sibsagar and another in the Lakhimpur district of Assam, was carried out. Six tea gardens and nineteen villages were included in the former and two gardens and twelve villages in the latter area. The total population in the areas was about 27,843 of whom 3,952 were examined.

Both the areas were found endemic for filariasis (*W. bancrofti* and *W. malayi*) and involved the indigenous population residing in the neighbouring villages.

Infection, involving both the *Wuchereria* species, affected almost all the age-groups in local-borns and indigenous people, indicating active transmission.

Both *C. fatigans* and *M. uniformis* were found and infective larvæ were noted in the former.

The climatic conditions appeared to be suitable for transmission during the greater part of the year.

The possible public health hazard from filariasis in different districts of Assam, where tea plantations exist, is discussed and the immediate need for further investigations and control measures suggested.

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STUDIES ON THE RAPID LOSS OF BIOLOGICAL EFFICACY OF D.D.T. AND DIELDRIN SUSPENSIONS ON MUD SURFACES AGAINST CULICINE MOSQUITOES*.

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CONTROL of culicine mosquitoes, with residual insecticides, is a difficult problem because physiologically culicine mosquitoes have been found to be comparatively less susceptible to synthetic insecticides and require heavier dosages than those needed for the control of anopheline mosquitoes (Giglioli, 1946; Wharton and Reid, 1950; Davidson, 1952; Pal and Sharma, 1954; Busvine, 1957). In the case of mud surfaces, the insecticidal deposits loose their toxicity quickly and make their control still more difficult in areas with houses having mud walls.

Use of solutions or emulsions of insecticides for the control of mosquitoes was replaced with aqueous suspensions prepared from water dispersible concentrates, particularly in the case of absorbent surfaces like mud walls. On such surfaces, insecticides in solution or emulsion are mostly sorbed into the inner layers and thus are not available to be biologically effective against insects. For some years, it has been believed that suspensions leave most of the insecticide particles on the surface and thus give better results. Several reports, however, are now available on the loss of activity of insecticide residues in case of suspensions sprayed on mud surfaces (Hadaway and Barlow, 1951: 1952; Downs *et al.*, 1951; Jaswant Singh *et al.*, 1951; Pal and Sharma, 1952; Viswanathan, 1952; Bordas *et al.*, 1953).

For the prevention of sorption of insecticide into the inner layers of mud surfaces, several methods have been tried with varying degree of success. Recently

* These studies were carried out under the auspices of Insecticides and Mosquito Repellent Enquiry financed by Indian Council of Medical Research.

50 per cent dieldrin water dispersible powder, containing resins, was prepared by Shell workers at their Woodstock Research Centre in U.K. Comparative studies with this product in the laboratory revealed that it was sorbed to a lesser degree than the ordinary 50 per cent dieldrin water dispersible powder. It was observed that to produce appreciable 24 hours mortality in the test insects, *C. fatigans*, an exposure of six hours to the insecticidal deposits on mud panels was necessary; indicating that even with such a formulation, most of the insecticide was ineffective due to sorption (Sharma and Bami, 1957).

To investigate thoroughly the cause or causes for rapid loss of effectiveness of insecticides on mud surfaces *vis-a-vis* control of culicine mosquitoes, detailed field and laboratory investigations were undertaken with 75 per cent D.D.T. water dispersible powder, 50 per cent dieldrin water dispersible powder, and 50 per cent dieldrin water dispersible powder containing resins.

FIELD STUDIES.

Experimental area.—Field investigations on the efficacy of 50 per cent dieldrin water dispersible powders with and without resins, and 75 per cent D.D.T. water dispersible powder, against culicine mosquitoes, were undertaken in four villages of Meerut District, Uttar Pradesh during March-April, 1957. These villages were completely isolated from each other and consisted of houses with mud walls and roofs made of thatch or wooden planks. Each one of the three villages was sprayed with a particular insecticidal formulation and the fourth village was kept unsprayed for purposes of comparison. All structures, whether human dwellings or cattlesheds, were sprayed. Inner surfaces of walls and ceilings were treated. The number of houses and total population in each village and other partiulcars are shown in Table I.

TABLE I.

Particulars of experimental villages, formulations and dosages of insecticides employed.

Serial number	Name of village	Number of houses	Population	Insecticide and formulation used	Dosage (mg. per sq. ft.)
1	Mirzapur ...	80	400	75 per cent D.D.T. water dispersible powder	200
2	Bhandapur ...	30	175	50 per cent dieldrin-resin water dispersible powder	50
3	Asratnagar ...	35	200	50 per cent dieldrin water dispersible powder	50
4	Karkar Manda	125	550	Unsprayed (Comparison)	...

Insecticidal formulations and dosages applied.—All the insecticidal formulations were completely analysed for their physical and chemical characteristics according to World Health Organization Specifications (1956) and were found to conform

to the specifications (Table II). Dosages of insecticides applied were dieldrin 50 mg., dieldrin-resin 50 mg., and D.D.T. 200 mg. per sq. ft.

TABLE II.

Particulars regarding the physical and chemical properties of the insecticidal formulations used.

Serial number	Insecticide and formulation	Insecticide content per cent	Clay content per cent	Dispersing agent content per cent	Acidity or alkalinity	Dispersibility per cent	Dispersibility after tropical storage (per cent)	Particle size as per cent below microns			
								10	20	40	74*
1.	75 per cent D.D.T. water dispersible powder ...	77·5	18·6	3·9	Neutral to litmus	59·5	52·1	73	95	98	100
2.	50 per cent dieldrin-resin water dispersible powder ...	52·1	38·0†	9·9	,,	83·5	78·0	78	91	94	100
3.	50 per cent dieldrin water dispersible powder ...	48·5	36·8	14·7	,,	84·5	85·5	80	93	96	99·0

*Values obtained through sieve analysis.
†Resin only.

Spraying was carried out by experienced workers under the direct supervision of one of the authors. The accuracy of spraying was further checked by removing wall scrapings and estimating the quantity of insecticides in them. For instance, analysis of nine scrapings from D.D.T. sprayed village, according to Alessandrini's method (1950), gave an average of 240 mg. D.D.T. per sq. ft. Similarly six scrapings (6 × 6 inches area) were removed from each of the dieldrin and dieldrin-resin sprayed villages. The scraped material was extracted with benzene and the dieldrin content estimated by total chlorine method (World Health Organization, 1956; Bami, 1957). Average dosages applied, of dieldrin and dieldrin-resin, were found to be 56·4 and 61·0 mg. per sq. ft., respectively.

Entomological data.—In each of the experimental villages, six suitable catching stations were established; in each treated village three of these catching stations were not sprayed. Mosquito catches were made from all catching stations once weekly for half an hour by means of a mosquito sucking tube. The catch consisted almost exclusively of *C. fatigans*. The mosquitoes so caught were kept in cages in the villages for recording 24 hours survival rates. It would be evident from the perusal of the entomological data (Table III) that in all the treated villages there was only slight decline in mosquito population for one week after spray. Majority of the mosquitoes, caught from experimental villages, also survived for 24 hours. The survival rates (female mosquitoes only), one day after spray, were 91·2, 96·0 and 70·8 per cent in D.D.T., dieldrin-resin and dieldrin treated villages, respectively, which indicated that the insects did not pick up the insecticides in lethal dosages.

TABLE III.

Density of culicine mosquitoes caught from the sprayed and the unsprayed catching stations (total of three catching stations) and their 24 hours' survival rates.

Number of days after spray	D.D.T. 200 MG./SQ. FT.						DIELDRIN-RESIN 50 MG./SQ. FT.						DIELDRIN 50 MG./SQ. FT.						COMPARISON		
	Sprayed catching stations			Unsprayed catching stations			Sprayed catching stations			Unsprayed catching stations			Sprayed catching stations			Unsprayed catching stations			Total of six catching stations		
	Total number of mosquitoes	Survival rate		Total number of mosquitoes	Survival rate		Total number of mosquitoes	Survival rate		Total number of mosquitoes	Survival rate		Total number of mosquitoes	Survival rate		Total number of mosquitoes	Survival rate		Total number of mosquitoes	Survival rate	
		M	F		M	F		M	F		M	F		M	F		M	F		M	F
0	250	...	...	211	...	...	157	...	...	66	...	...	42	...	...	49	...	...	329	...	...
1	34	0·0	91·2	140	100	82.3	76	95·8	96·0	22	100	100	29	40	70.8	43	61·5	76·5	222	50	92·6
7	29	100	96·4	284	100	100	58	88·9	98 0	23	100	100	28	33·3	100	32	100	100	175	100	100
14	148	100	99·3	285	100	100	201	96·5	99·5	207	85·7	95·9	41	88·9	90·6	212	100	94·6	525	95·3	94·9
21	140	80·0	98·4	332	96·3	95·1	218	100	100	144	68·9	93·9	52	83·7	79·9	182	64·3	85·8	325	94·7	95·8

M = males. F = females.

These findings were rather unusual because both D.D.T. and dieldrin formulations, when applied at these dosages on non-absorbent surfaces, have been found to be quite effective against culicine mosquitoes in South India (Jaswant Singh *et al.*, 1956). The possible reasons for the lack of biological efficacy were considered to be comparatively low susceptibility of *C. fatigans* to insecticides in this area and non-availability of the insecticide on the surface due to sorption phenomenon. To elucidate the factors involved, detailed laboratory investigations were undertaken.

LABORATORY INVESTIGATIONS.

DURATION OF EFFECTIVENESS OF INSECTICIDAL FORMULATIONS WHEN SPRAYED ON THE MUD PANELS.

Mud panels (6 ×6 inches) were prepared from clay used in the construction of houses in the experimental villages. The panels, when completely dry, were sprayed with dieldrin (both formulations at 50 mg. per sq. ft.) and D.D.T. (200 mg. per sq. ft.) suspensions prepared from their water dispersible concentrates. Three to four days old laboratory-bred mosquitoes (*C. fatigans*♀♀, normal strain) were exposed to the panels for three and six hours in accordance with the technique described by Pal (1951), using 20 insects in each test. For each insecticidal formulation, three replicates were done. An average percentage mortality after 24 hours was recorded. Same panels were used for subsequent exposures at weekly intervals for four weeks. The results are presented in Table IV. It would be seen that even with long exposures of three and six hours, mortality in insects was not heavy except in the case of D.D.T. where 24 hours percentage mortality figures, one day after spray, with three and six hours exposures, were 76·6 and 88·3 per cent, respectively. This is in conformity with the observations in the field where insecticidal sprays showed only slight biological efficacy during the first week after spray.

To assess the efficacy of insecticides when almost whole of the dosage applied would be available to the insects on the surface, above mentioned laboratory tests were repeated by spraying these insecticides at the same dosages on glass and wood panels (non-absorbent surfaces). Three to four days old *C. fatigans*♀♀, raised in the laboratory from eggs of the mosquitoes collected from the comparison village, were used in these tests. The insects were exposed to the sprayed surfaces for half an hour, one hour and three hours; and 24 hours' mortality recorded. Insecticidal deposits on both wood and glass surfaces produced 90 to 100 per cent mortality with one hour and three hours exposures but with half an hour exposure, the mortality figures were 50, 45 and 40 per cent for glass panels and 70, 32 and 60 per cent for wood panels in case of D.D.T., dieldirn and dieldrin-resin, respectively.

The above mentioned data indicated that the insecticides at the dosages applied were effective against *C. fatigans* on non-absorbent surfaces but substantial survival in insects after half an hour exposure to the insecticidal deposit, would mean that bulk of the normal population of *C. fatigans* might be having natural low suscpetibility to D.D.T. and dieldrin.

TABLE IV.

Details regarding the degree of sorption of insecticides into mud surfaces and their duration of effectiveness against C.fatigans.*

Insecticidal formulation	Number of days after insecticidal treatment	Chemical estimation (insecticide mg./sq. ft.)			Biological effectiveness	
		Total quantity on panels *A*	Quantity sorbed into panels *B*	Quantity at surface (*A* minus *B*)	24 hours percentage after 3 hours' exposure	Mortality after 6 hours' exposure
75 per cent D.D.T. water dispersible powder	1	234·0	210·0	24·0	76·6	88·3
	7	218·0	210·0	8·0	53·3	60·0
	14	210·0	†	...	33·3	46·6
	21	144·0	112·8	31·2	28·3	21·6
50 per cent dieldrin-resin water dispersible powder	1	55·2	43·0	12·2	36·6	51·0
	7	42·8	38·6	4·2	16·5	25·0
	14	36·2	24·2	12·0	5·0	6·6
	21	42·4	37·0	5·4	6·6	4·3
50 per cent dieldrin water dispersible powder	1	45·6	41·4	4·2	23·3	45·0
	7	32·8	25·2	7·6	8·3	8·3
	14	26·8	21·5	5·3	10·0	10·0
	21	31·6	20·8	10·8	12·0	8·0

*Biological experiments were conducted on the same set of panels during the entire period, whereas chemical estimations of insecticides were made on different sets of panels each week.

†Not estimated.

CHEMICAL ESTIMATION OF DEGREE OF SORPTION OF INSECTICIDES INTO THE MUD SURFACES.

Several sets of dried mud panels (same as employed in the above mentioned studies) were sprayed with D.D.T. and the two dieldrin formulations as described before. Twenty-four hours after spraying, four panels (one sq. ft.) from each of the three sprayed sets, were taken for estimation of total and sorbed insecticide. One half of each of the four panels in a set, was covered with 'Sello-tape' and the remaining uncovered surface (½ sq. ft.) was thoroughly scraped to a depth of 3 to 4 mm. This scraped material represented the total insecticide available on and under the surface. 'Sello-tape' was well pressed on the remaining half of the panel surface and later removed and discarded. The 'Sello-tape' treated surfaces (½ sq. ft.), were scraped similarly and this represented the amount of insecticide sorbed into the mud. The two scraped samples, representing total and sorbed insecticide only, were thus removed in each of the three cases. The scraped samples were extracted with benzene for ten hours using soxhlet extractors. The solvent was removed and the residues were used for estimating their insecticide content. Estimation of dieldrin, in case of both the formulations, was done by total chlorine method as already discussed (Sharma and Bami, 1957). D.D.T. was estimated by slightly modifying the hydrolysable chlorine method as described by Bami (1957). Similar estimations were repeated during the subsequent three weeks using new sets of panels (taken from the originally sprayed stock) every time

and the results of analysis are given in Table IV. From the data, it would be evident that nearly 90 per cent of D.D.T. and about 30 per cent of dieldrin and dieldrin-resin were sorbed into the mud surface immediately on spraying and that position was maintained during the subsequent period. There also appeared to be slight chemical deterioration of the insecticides with time as judged from the figures of total insecticide on the panels (Table IV). It is also well-known that fine insecticide particles between 10 and 20 micron size, are readily sorbed into the active mud surfaces and that the penetration is far more rapid and complete when the particle size is below ten microns (Hadaway and Barlow, 1952). Under the circumstances, causes of extensive sorption in relation to the type of mud surfaces involved and particle size of insecticidal formulations sprayed, were also investigated.

COMPOSITION, POROSITY AND SORPTIVE POWER OF MUD.

The sample of mud used in the above studies was analysed for its straw content and amount of colloidal clay contained in it (Table V). The mud was made into small dry panels, whose apparent density as well as actual specific gravity were estimated. Percentage porosity of the mud was calculated from these figures using the formula:

$$\text{Porosity in per cent.} = \left(1 - \frac{d}{p}\right) \times 100$$

Where d = apparent density of mud.
p = specific gravity of mud.

TABLE V.

Composition of mud and its physical characteristics.

Serial number	Type of analysis	Result
1.	Straw content ...	0·49 per cent
2.	Colloidal clay content. ...	19·3 per cent
3.	Free iron (Fe_2O_3) ...	1·28 per cent
4.	Free alumina (Al_2O_3) ...	0·44 per cent
5.	Apparent density ...	1·86 to 1·97
6.	Specific gravity ...	2·67
7.	Porosity ...	26·3 to 30·4 per cent
8.	Sorption (Carbon tetrachloride method) ...	5·5 to 6·5 per cent

The porosity values varied from 26·3 to 30·4 per cent while the theoretical limits for porosity for any mud sample should range between 25·94 and 47·64 per cent,

depending upon degree of compactness of the mud and arrangement of particles in it. Alessandrini and Placucci (1956) have stated that sorption occurs by capillary diffusion through the pores and hence the sorption rate would be greater in case of smaller pores *i.e.*, low porosity percentage. The figures obtained in the present studies, which are closer to the lower limit, support the above hypothesis in view of the fact that this mud had shown very high sorption power for insecticidal particles.

Another reliable method for assessing the sorption power of a mud sample is to determine the extent of sorption of a non-polar solvent like carbon tetrachloride by the mud sample. The original method of Barlow and Hadaway (1955), as modified by Paulini (1956), was used for this estimation with the exception that dry mud blocks were kept at 25°C in an atmosphere saturated with carbon tetrachloride vapours for 48 hours instead of 24 hours. The sorption values (Table V) varied from 5·5 to 6·5 per cent. According to Paulini (1956), these values should correspond to moderately inactivating muds (medium sorption). Similarly 19·3 per cent colloidal clay content of the mud sample also indicated the material to be fairly sorptive and inactivating in character. Correlating carbon tetrachloride sorption and percentage mortality of *Aedes ægypti* in case of various mud discs sprayed with D.D.T. and dieldrin, Bruce-Chwatt (1955) had observed that a sorptive power of about 6 per cent led to maximum inactivation of the materials due to machanical sorption. The sorption values of 5·5 to 6·5, determined in the present studies, and the relevant chemical and entomological data, support his results. It may be fair to conclude that whatever are the factors (colloidal clay, iron content, percentage porosity etc.) responsible for the sorption of carbon tetrachloride by the mud, degree of such sorption could be correlated with the extent of inactivation of non-volatile insecticides like D.D.T. and dieldrin. However, it will be more correct to state that this correlation holds good specially in case of sorptive muds (Barlow and Hadaway, 1955).

PARTICLE SIZE DETERMINATION OF 75 PER CENT D.D.T., 50 PER CENT DIELDRIN AND 50 PER CENT DIELDRIN-RESIN DISPERSIBLE POWDERS.

Highly sorptive nature of the mud under test and relevant chemical and entomological data, made it evident that inactivation of the insecticides is due to sorption. The size of the particles, sprayed on a sorptive surface would, therefore, be a major factor governing the extent to which insecticide particles can penetrate into the surface. Insecticidal particles, below 20 micron size, are readily sorbed into the active mud surfaces with subsequent rapid loss of biological efficacy (Hadaway and Barlow, 1952). The formulations under test had offered dispersibility values which were much above the minimum requirement of 50 per cent as per World Health Organization Specification (1956), particularly in the case of dieldrin and dieldrin-resin formulations (Table II). Previous studies on particle size distribution of 50 per cent D.D.T. water dispersible powders, meeting the World Health Organization Specifications on dispersibility, had shown that at least 50 per cent of the D.D.T. particles were below 10 microns size (Bami and Cheema, 1957). Particle size of insecticide constituents of the three formulations was determined by the sedimentation method described by Bami and Cheema (1957) and the results are given briefly in Table II. 75 to 80 per cent

of the insecticidal particles, in all the three cases, were found to be below 10 microns size while over 90 per cent were below 20 microns size. Considering the fact that particles below 20 microns are rapidly and readily sorbed into mud surfaces with almost complete loss of biological efficacy (Hadaway and Barlow, 1952), the behaviour of these three formulations in the present investigations was understandable.

DISCUSSION.

The degree of mosquito control, resulting from any insecticidal treatment, is primarily dependant on the amount of insecticide available on the surface to be effective against the insects and the inherent susceptibility of the species to be controlled. During these investigations, sorptive power of the mud, as determined by carbon tetrachloride (Barlow and Hadaway, 1955) and percentage porosity (Alessendrini and Placucci, 1956) methods, has been found to be fairly high. This has naturally led to insecticide being easily lost due to sorption. Particle size determinations of the insecticidal formulations have also confirmed the findings that very fine particles are easily sorbed into the mud surface. High sorptive power of the mud, coupled with very fine nature of the insecticidal particles involved, led to 80 to 90 per cent of the insecticide sprayed being readily sorbed into the inner layers, leaving only a fraction of the dosage applied on the surface. Had sufficient quantity of insecticide been available on the surface to the insects, the biological efficacy on mud surfaces in the laboratory and field would have been adequate as is supported by the mortality produced in test insects by exposing them to the insecticidal deposits on glass and wood panels representing non-absorptive surfaces.

Analysis of data, concerning the resting habits of *C. fatigans*, showed that 6·6 per cent of the individuals rest on ceilings, 83·9 per cent on the walls and 9·5 per cent on other articles lying in the rooms. Therefore, for the greater fraction of the population resting on walls, the amount of insecticide available on the surface was very inadequate. The slight initial decline in mosquito population in the treated villages might be due to the so called 'particulate effect' (Davidson and Burnett, 1952; Giglioli 1954) where airborne insecticide particles from sprayed surfaces remain biologically effective for sometime. It is also reasonable to presume that bulk of the *C. fatigans* population in nature, consists of individuals having low susceptibility to D.D.T. and dieldrin, and that for their effective control even alternative insecticides, to which the whole population is highly susceptible, may have to be employed. The initial low susceptibility seems to result in quick selection of resistant strains of insects after residual spraying of houses. This is supported by the fact that insecticide resistance in *C. fatigans* has been recorded from a number of States in India* after implementation of D.D.T. spraying programmes (Pal *et al.*, 1952; Krishnan, 1956).

The use of insecticide suspensions, instead of aqueous emulsions or oily solutions, was substituted with the hope that on absorbent mud surfaces, insecticide particles would be left on the surface and insect control would be more effective.

* *Technical Report of the Scientific Advisory Board of the Indian Council of Medical Research* for the year 1956.

But it appears that in order to have stable suspensions meeting the World Health Organization Specifications (1956), the present day insecticidal formulations are being ground to such a fine particle size, that their sorption into mud surfaces becomes exceedingly great. The advantages, obtained by using stabler suspensions, are being far out-weighed by the loss of material on active mud surfaces in rural areas. Sorptive nature of mud could be corrected by some type of pretreatment of the surface (Pal and Sharma, 1952; Clapp *et al.*, 1947; Maier *et al.*, 1948; Hadjinikolau and Busvine, 1948; Maes, 1952; Bordas *et al.*, 1953; Barlow and Hadaway, 1955), but so far none of the methods advocated have been found to be both practicable and economical. The incorporation of resins in dieldrin formulation has also offered little advantage in this respect. The other possibility worth exploring is to limit the size of particles in water dispersible powders, if possible. This objective might be achieved by fixing both the upper and lower limits of size of particles in water dispersible powders. According to the current World Health Organization specifications (1956), only the upper limit is fixed which stipulates that at least 98 per cent of the particles should be below 74 microns size. However, the lower limit is not indicated which largely governs the degree of sorption on a given surface. Attempts have to be made to improve the suspensibility of a water dispersible powder by using better quality dispersing agents in larger amounts rather than grinding the material to such fineness where it is physically perfect but biologically unsatisfactory. The technical difficulties involved are no doubt there but an effective method to check sorption will have to be found out in order to have successful mosquito control operations in rural areas.

SUMMARY.

To investigate thoroughly the causes for rapid loss of effectiveness of insecticides on mud surfaces *vis-a-vis* control of culicine mosquitoes, field and laboratory investigations were undertaken with 75 per cent D.D.T., 50 per cent dieldrin and 50 per cent dieldrin-resin water dispersible powders.

Field investigations were carried out in four villages of Meerut District, Uttar Pradesh, during March-April, 1957. Three of the villages were sprayed with D.D.T. (200 mg./sq.ft.), dieldrin (50 mg./sq.ft.) and dieldrin-resin (50 mg./sq.ft.) suspensions and the fourth village was kept unsprayed for purposes of comparison. Mosquito collections from these villages consisted almost exclusively of *C. fatigans*. The entomological data collected from experimental villages showed that the insecticidal treatments produced slight decline in mosquito density for only one week after spray. Similar results were obtained in the laboratory with *C. fatigans*, when these insecticides were applied on mud panels.

Studies on the composition, porosity and sorption capacity of the mud, normally used in the construction of houses in the experimental villages, indicated that this mud was of sorptive nature causing inactivation of the insecticides applied. Particle size determination of the insecticide constituents of the formulations showed that over 90 per cent of the particles were below 20 microns size and about 75 to 80 per cent below 10 microns. The extreme fineness of the particles also led to their rapid sorption into the inner layers of mud surface.

It is concluded that the lack of efficacy of the insecticidal treatments against *C. fatigans* in the field and laboratory, was due to the loss of 80 to 90 per cent of the insecticidal dosage applied on mud surfaces by sorption into the inner layers, and possibly also due to the bulk of *C. fatigans* population in nature consisting of individuals having low susceptibility to D.D.T. and dieldrin.

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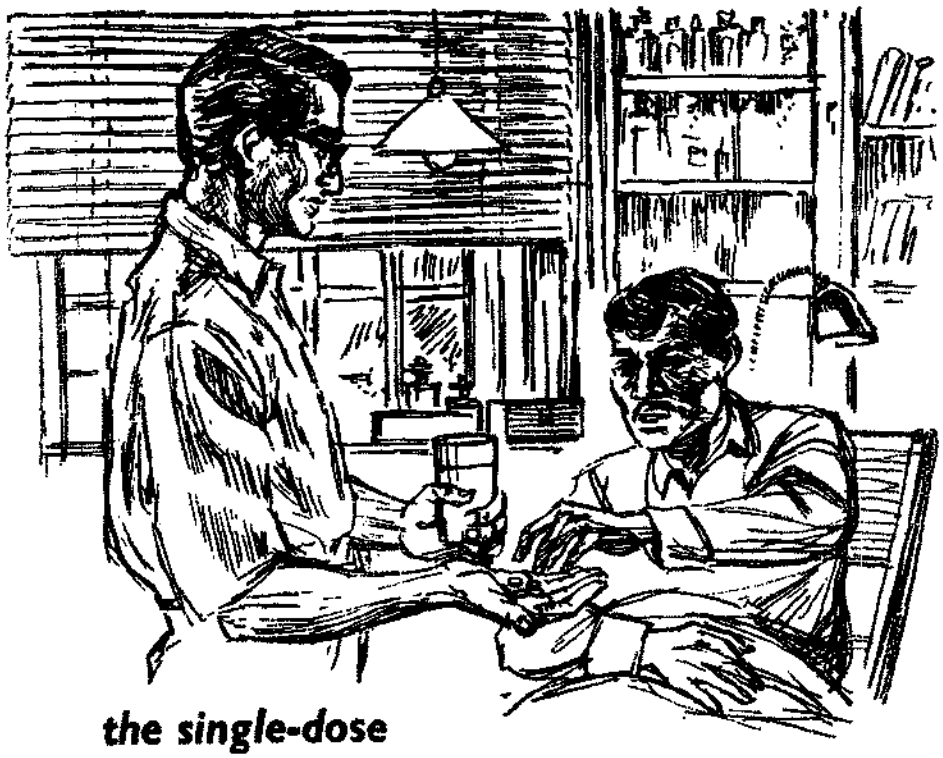

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NOTICE.

INSTRUCTIONS TO AUTHORS.

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MSS. should be sent in **duplicate** only after careful revision and correction, otherwise they are liable to be refused. Authors are asked especially (*a*) to verify the proper logical arrangement and sequence of their headings and sub-headings, and (*b*) to maintain uniformity in the use of contractions, capitals, italics, etc. A short summary of the salient points of the paper should be placed at the end.

Tables, charts, etc., should be numbered, and alluded to in the text as 'Table I', etc., and not as 'the following table', etc.

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The following contractions are in use in the *Indian Journal of Malariology*, whether the number to be expressed is one or more :—

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N.B.—In order to avoid ambiguity the word 'grain' should be written in full.

Where degrees of temperature are given, the scale used should always be specified, e.g. 37°C., 98°F.

To indicate a percentage, 'per cent' should be used, and not the symbol %.

The decimal point should never be placed at the beginning of a number ; e.g. the form 0·5 should be used, not ·5.

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When there are references to two or more papers by the same author published in the same year the letter *a* should be placed immediately after the year in the first case, the letter *b* in the second case, and so on ; e.g. Blank, J. (1936*a*).

The following are some of the abbreviations in use :—

Amer. J. Hyg.
Ann. Trop. Med. Parasit.
Brit. Med. J.
Bull. Ent. Res.
Bull. Soc. Path. Exot.
C. R. Soc. Biol.
Geneesk. Tijd. Ned.-Ind.
Ind. J. Mal.
Ind. J. Med. Res.
J. Mal. Inst. Ind.
Jl. R. A. M. C.
J. Trop. Med. Hyg.
Meded. Volk. Ned.-Ind.
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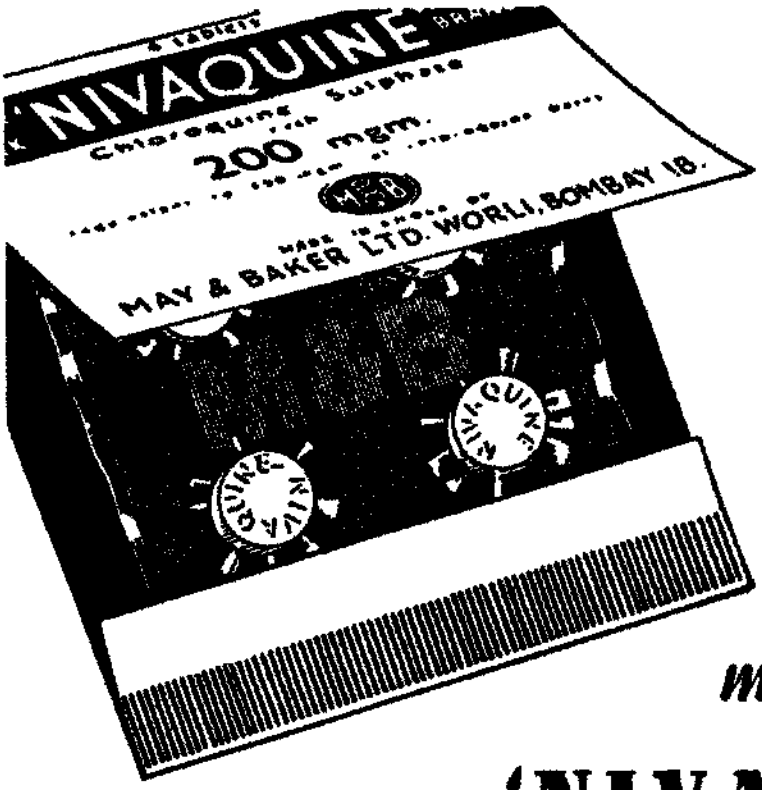

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VECTORS OF MALARIA IN MID-JAVA.

BY

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[August 5, 1957.]

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INTRODUCTION.

THE Province of mid-Java has been the site of activities of a malaria control demonstration and training project jointly sponsored by the Government of the Republic of Indonesia and the World Health Organization since September, 1951. Until July, 1956 the activities of the team were confined to the south coast area with headquarters at Tjilatjap. With the launching of the nationwide campaign against malaria by the Ministry of Health, the rôle of the team was extended to cover the whole province of mid-Java with headquarters at the provincial capital Semarang since 1956. The observations described in this paper were made during these assignments.

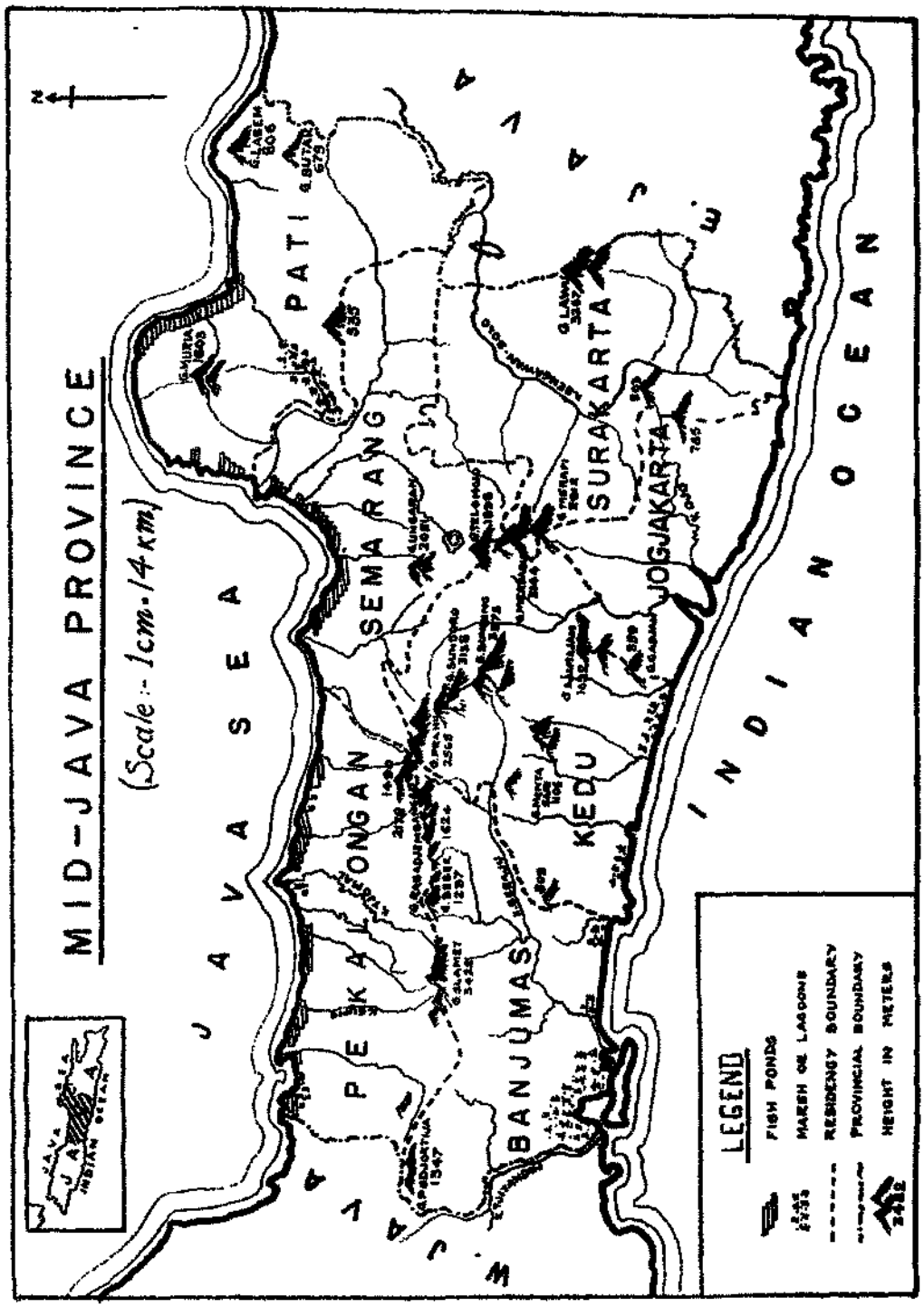
THE PROVINCE OF MID-JAVA.

Topography.—The province of mid-Java, which is situated between east longitude 109° and 111° and south latitude 7° and 8·5°, has an area of approximately 40,000 square kilometers and is about a third in size of the entire island of Java. It faces the Java sea and the Indian ocean on the north and the south, respectively, and is bounded on the east and the west by the East and the West Java provinces. A greater part of the central region from east to west is hilly in terrain, studded with a number of dead volcanos and an active one Merapi and consists of extensive plateaux and valleys with an average altitude of about 800 meters above seal level, sloping steeply to the north and the south.

The coastal region is mostly flat with a gentle slope up to a distance varying from 15 to 30 kilometers inland from the coast. The soil, which is of volcanic origin, is mostly alluvial and extremely rich. A large number of rivers and streams of varying sizes originate from the central elevated regions and flow towards the seas on the north and the south for most of the year. The brackish water lagoons, which run along the coastline and parallel to its close to the estuaries of the rivers, are the exclusive features of the south coast just as the extensive, man-made fish ponds (*tambaks*) are to be found only in the regions of the north coast (Map 1).

Divisions.—Administratively, the Province consists of six large divisions known as Residencies which are as follows:—(*i*) Banjumas, (*ii*) Kedu, (*iii*) Semarang, (*iv*) Pati, (*v*) Pekalongan, and (*vi*) Surakarta. Besides, there is also the territory of Jogjakarta which has a special autonomous administration of its own. Each Residency is subdivided into a number of Regencies or *Kabupatens* and there are 28 such sub-divisions in the Province excluding the territory of Jogjakarta. The regencies are further subdivided into districts and sub-districts and finally to villages or *desas*.

Population.—According to 1955 census figures, the total population in mid-Java, inclusive of Jogjakarta territory, is 17·9 millions of which 15·6 millions are in the Province and 2·3 millions are in Jogjakarta territory. The average density of population is about 450 inhabitants per square kilometer. In some parts (as for instance in the Residency of Pekalongan) along the north coast of the Province, the density of population exceeds 1,200 per square kilometer whilst in the hilly areas the population is sparse with the density around 250 per square kilometer. The population is chiefly rural in character living in over 8,500 villages or *desas*,


MID-JAVA PROVINCE
(Scale:- 1cm=14km)
JAVA SEA
INDIAN OCEAN
PATI
SEMARANG
SURAKARTA
JOGJAKARTA
PEKALONGAN
KEDU
BANJUMAS
LEGEND
FISH PONDS
MARSH OR LAGOONS
RESIDENCY BOUNDARY
PROVINCIAL BOUNDARY
HEIGHT IN METERS

Agriculture is the chief occupation of a large proportion of the people inland. Cultivation of rice is the most important branch of agriculture. Those on the coasts live on fishing in the sea, fishponds, rivers and canals. The houses are situated largely in the midst of ricefields or coconut groves. The building material, chiefly used for constructing the houses, consists of a temporary or semi-permanent character such as wood or bamboo for the structural frame work and dried leaves of the nipa palm tree or bundles of alang grass for the roof. More permanent materials, such as bricks and tiles, are used by the well-to-do. The houses are generally large in size and have a number of compartments used as living or bedrooms. Kitchens form separate annexes of considerable size. Mostly the walls of the houses are lime-washed. Cattle are usually tied in the open or in sheds with all the sides open. In the hilly regions, the animals are sheltered in one of the compartments of the house itself.

Climate.—The annual rainfall is plentiful and from the data of over sixty years (published in "*Rainfall in Indonesia*" by Prof. H. P. Berlage), it may be concluded that the average annual rainfall amounts to approximately 3,500 mm. in about 180 rainy days in the north coast and to approximately 4,000 mm. in about 200 days in the south coast. The yearly average minimum temperature in the coastal areas was 23°C. (74°F.) and the average maximum temperature 36°C. (96·0°F.). The highest monthly temperature is usually recorded during April and the lowest during August. Seldom has a temperature of 37·8°C. (100°F.) been recorded anywhere in Java according to the observations during the five years. The relative humidity varies from 70 to 90 per cent in the coastal areas and 50 to 60 per cent in the inland areas.

ANOPHELINE FAUNA.

The following 18 species have so far been recorded in the Province:—*A. aconitus*, *A. annularis*, *A. bæzai*, *A. barbirostris*, *A. hyrcanus* var. X (*venhuisi*), *A. hyrcanus sinensis*, *A. kochi*, *A. leucosphyrus*, *A. maculatus*, *A. minimus flavirostris*, *A. philippinensis*, *A. ramsayi*, *A. schuffneri*, *A. subpictus subpictus*, *A. subpictus malayensis*, *A. sundaicus*, *A. tessellatus* and *A. vagus*.

Of these, the most commonly found species in the coastal areas are *A. sundaicus*, *A. subpictus subpictus* and *A. subpictus malayensis*. Their monthly distribution in the house collections in the south coast area, recorded during the period 1952-1955, are summarised in Table I.

In the inland irrigated areas, with extensive rice cultivation, the species commonly met with are *A. aconitus*, *A. subpictus malayensis*, *A. barbirostris* and *A. vagus*. In Table II are shown their monthly distribution observed in the Regency of Purworedjo during the period 1955-1956.

In the hilly terrain too, at altitudes above 500 meters from the sea level, the species *A. aconitus* is the most common one making up nearly 60·0 per cent of all anopheline collections. The other species were *A. subpictus malayensis* 15·0 per cent and *A. minimus flavirostris* and *A. hyrcanus* 15·0 per cent. The species *A. maculatus* was extremely scarce in daytime house collections even in those localities (as Kalikadjar in the Regency of Wonosobo) where typical breeding places for *A. maculatus* exist and an infected one was found recently. During the eight

months of observation since July, 1956, in Kalikadjar area *A. maculatus* constituted less than 5·0 per cent of all anophelines collected in that area.

TABLE I.

Monthly distribution of female anophelines in daytime house collections in the south coast area during 1952-1955.

Month	Man-hours spent	Total anophelines found	Per cent distribution of the different species			
			A. sundaicus	*A. subpictus*	*A. subpictus malayensis*	Other species
January ...	391	9,330	25·1	17·8	55·5	1·6
February ...	399	5,027	18·0	38·0	30·3	14·0
March	502	9,695	17·9	30·7	42·7	8·7
April	686	13,996	17·8	18·0	60·4	3·8
May	657	10,014	18.3	14.0	60·1	7·6
June	634	13,948	10·0	16·6	63·3	10·1
July	732	19,037	12·0	22·3	60·2	5·5
August	702	12,637	22·8	26·4	47·2	3·6
September ...	616	9,604	29·9	15·0	51·0	4·1
October ..	634	15,102	37·6	23·3	36·9	2·2
November ..	500	10,619	30·2	37·8	20·5	11·5
December ...	543	14,198	40·8	29·6	27·5	2·1
Total	6,996	1,43,207	23·5	23·4	47·1	6·0

VECTORS AND THE TRANSMISSION SEASON.

Of the above-mentioned species of anophelines, the two species *A. sundaicus* and *A. aconitus* have been definitely incriminated as the main malaria vectors in their respective areas of prevalence, namely, the coastal areas and the inland rice-field areas. Two other species of secondary but not of inconspicuous importance are *A. subpictus subpictus* and *A. maculatus*, the former in the coastal areas and the latter in the central hilly areas.

A. sundaicus.— On 38,164 dissections of the species *A. sundaicus* in the south coast area during the five years 1952 to 1956 by the World Health Organization Team, the species had a gross sporozoite rate of 0·26 per cent and the yearly infectivity rates during that period are shown in Table III.

TABLE II.

Monthly distribution of the female anophelines in daytime house collections in the localities of Purworedjo Regency during 1955-1956.

Month	Man-hours spent	Total anophelines found	Per cent distribution of the different species		
			A. aconitus	*A. subpictus malayensis*	*A. barbirostris* and *A. vagus*
January ...	135	2,352	68·0	27·8	4·2
February ...	119	1,858	79·1	19·5	1·6
March	76	1,092	79·1	8·9	12·0
April	143	2,874	51·4	40·5	8·1
May	174	2,163	50·5	30·7	18·8
June	205	3,143	69·5	27·2	3·3
July	221	4,319	73·4	24·5	2·1
August	230	4,256	91·5	4·9	3·6
September ...	223	3,551	76·3	1·6	22·1
October ...	167	3,761	61·0	30·9	8·1
November ..	121	1,618	62·4	26·5	11·1
December ...	81	1,069	94·3	4·6	1·1
Total	1,895	32,056	70·0	23·4	6·6

TABLE III.

Infectivity of A. sundaicus *in south coast area during 1952 to 1956.*

Year	Number dissected	Number with sporozoites	Sporozoite rate per cent
1952	2,592	1	0·04
1953	2,270	3	0·13
1954	24,093	73	0·30
1955	4,850	11	0·23
1956	4,359	12	0·28
1952–1956	38,164	100	0·26

Earlier records show that sometimes very high infectivity rates have been observed in East and West Java. Walch and Walch (1921) found 17·5 per cent infected out of 58 and Stoker is said to have reported a rate of 39 per cent in a locality in East Java (Overbeek and Stoker, 1937). Although highly susceptible to infection, *sundaicus* has also been found in large numbers in certain regions where the incidence of malaria is indeed very low. (Walch and Soesilo, 1929).

Based on five years detailed observations in the south coast, there is clear evidence that *A. sundaicus* is capable of being a vector all the year round. Frequency of the infections, especially during the period 1954-1956, was such that infective *sundaicus* were found in all the months of the calendar during one year or the other, indicating perennial transmission. The peak of transmission occurred during the second half of each year.

A. aconitus.—The sporozoite rate of this species in Bruno area, at an altitude 200 meters above sea level in Purworedjo Regency, was found to be 0·09 per cent during the years 1954-1956.

TABLE IV.

Infectivity of A. aconitus *in Bruno area during 1954-1956.*

Year	Number dissected	Number with sporozoites	Sporozoite rate per cent
1954	2,440	1	0·04
1955	3,142	4	0.12
1956	4,362	4	0·09
1954–1956	9,944	9	0·09

Mangkoewinoto (1918), Swellengrebel *et al.* (1919) and Overbeek and Stoker (1937) have recorded infection rates of 7·3, 2·3 and 18·8 per cent, respectively, in Java. In the Solo Valley, an infection rate of 43·0 per cent is said to have been reported but details as to numbers dissected are not available. According to Covell (1944) " it appears probable that these variations in the effectiveness of *aconitus* as a malaria carrier in the East Indies are related to its zoophilic tendencies, for it appears to feed on man only where cattle are scarce. If it is present continuously and in great numbers in such areas, hyper-endemic conditions may result ".

A. aconitus is a vector for most of the year in its area of prevalence. During the two years 1955 and 1956, sporozoite infections were found in this species in the months of January, February, March, June, September and December.

A. maculatus.—As far as this species is concerned, the evidence available so far is limited. The sporozoite rate for this species was found to be 0·7 per cent of 130 dissected during September, 1956, in Kalikadjar area of Wonosobo Regency. *A. aconitus* was also found infected at the same time in this area. Over 300

specimens of *A. maculatus* dissected subsequently during the last quarter of that year, proved negative. In Java, this species is considered as a vector of some importance in hilly areas according to several previous records. Venhuis (1941) during an epidemic at Kediri in East Java, is reported to have found 1 gland infection among 393 specimens caught in houses and 2 with gland infections out of 136 caught along stream banks.

In mid-Java the infection detected in *A. maculatus* was in the month of September, 1956. Examining the conditions prevalent in the areas where this species is suspected as vector, the period April to October is believed to be the season of this species and the months November to March would appear to be very unfavourable on account of heavy rainfall and consequent flushing of the breeding places of this species in the hilly region. This needs confirmation by further observations.

A. subpictus subpictus.—This species has also been found to play a secondary but not an inconspicuous rôle as vector during some seasons, as happened during the years 1953 and 1954 in the south coast area when the species had a sporozoite rate of 0·055 per cent on a total of 22,896 dissections made between the years 1952 and 1956.

TABLE V.

Infectivity of A. subpictus subpictus *in south coast area during 1952 to 1956.*

Year	Number dissected	Number with sporozoites	Sporozoite rate per cent
1952	2,473	0	0·0
1953	2,889	5	0·17
1954	15,326	8	0·05
1955	2,034	0	0·0
1956	175	0	0·0
1952–1956	22,896	13	0·06

The infections occurred in the month of October in 1953 and in February, March, May and June in 1954.

Several infections in the species *A. sundaicus* were also found simultaneously in the same area and this period coincided with extremely favourable conditions for *A. sundaicus* and *A. subpictus* larvæ in the lagoons and high densities of adults of these species in houses. Previous records indicate that only occasionally this species has been found naturally infected. Brug and Walch (1927) found a single specimen of this species (then known as *M. rossi*) infected out of 221 dissected, and suspected that " there was an indication that *M. rossi* played a part as carrier during the epidemic at Solo in 1926 ". The number of infections found in *A. subpictus subpictus* in the south coast of Java in 1953-1954 by the World Health Organization Team appears to be the highest ever recorded in that species in Java.

Other species.—The other species that were dissected during the years 1952-1956 without finding any plasmodium infection, are as follows:—

A. subpictus malayensis	41,280
A. hyrcanus var. *X* (*venhuisi*)	1,342
A. hyrcanus sinensis	640
A. minimus flavirostris	913
A. kochi	172
A. tessellatus	125
Total ...	44,473

BIONOMICS OF THE VECTORS.

A. ACONITUS.

Breeding places.—Ricefields constitute the most important breeding places of *A. aconitus*. Owing to the richness and fertility of the soil and the even distribution of the rainfall for most of the year, the rice cultivation is a continuous process in most parts of the country and it is not uncommon to notice as much as five crops being raised in two years. But generally speaking, in most of the areas with well developed irrigation system, the planting is done between the months October and January and the harvesting takes place between the months April and July. In the hill slopes, plateaux and valleys the characteristic pattern is terrace cultivation and the ricefields there depend to a large extent on irrigation from seepage and on rainfall. In such areas, there is no definite season for rice cultivation and one can see throughout the year paddy plants at all stages from seedlings to fully mature ones. There is undoubted evidence that in such places the breeding of *A. aconitus* goes on without interruption.

Intensive observations on the extent of larval breeding in ricefields, with different stages of plant growth, showed that paddy nurseries with young growing seedlings are free from *A. aconitus* larvæ.

The breeding starts in the main ricefields approximately two to three weeks after the transplantation of the seedlings from the nurseries to the fields. For nearly three full months, the breeding goes on without interruption and when the paddy plants are well grown and heavy with drooping ear-heads, the breeding of larvæ ceases in these ricefields. With the increase of organic pollution following the harvest of the ear-head and ploughing in of the stalks, conditions for *aconitus* breeding in such ricefields appear to be extremely unfavourable and consequently no larvæ are found there. They reappear if the land lies fallow for some time or after the next transplantation.

Besides ricefields, the irrigation channels and margins of clear streams are the other important breeding places of this species.

A. aconitus may be considered as the most widely spread vector in the Province with its distribution from the coastal plains at sea level to the central plateaux up to an altitude of about 1,000 meters above sea level. There is no

Regency in this Province wherein this species does not play a minor or major rôle in the transmission of malaria. It is estimated that not less than 4,500 out of the total 8,500 villages in the Province are situated within the zone of influence of this species.

Bionomics of adult aconitus.—

(*i*) The adults are found in houses as well as in the animal sheds close by. The density is greater in cattlesheds than in houses. While the daytime density in houses was on the average about 25·0 per man-hour, the night-time density was about 100 per man-hour in some localities.

(*ii*) The adults are found to rest on the walls as well as under the ceilings.

(*iii*) The species is considered to be very exophilic and numbers of adults of the species are found in the various types of outdoor shelter, especially, plantbushes or moist and damp hollows in stream banks (Venhuis, 1942).

(*iv*) The species has strong zoophilic tendencies for according to precipitin tests carried out at Lister Institute, England, on 944 blood samples sent between July, 1955 and March, 1956 from here, 831 were found positive for ox or buffalo blood. The anthropophilic index was found to be 7·1 per cent and the variation in the anthropophilic indices ranged anywhere from 0·0 per cent to 60·0 per cent among the seven batches analysed so far, as shown in Table VI.

TABLE VI.

Results of precipitin tests carried out at Lister Institute, England, on blood samples from A. aconitus *caught in houses in Purworedjo Regency (Mid-Java).*

Period	Locality	Number tested	Reaction				
			Man	Ox/B.	M/ox	Horse	Negative
July, 1955	Bruno	96	25	62	1	0	8
September, 1955	Brunoredjo	96	6	87	1	1	1
October, 1955	Kaliwungu	16	10	5	0	0	1
November, 1955	,,	160	11	140	0	0	9
December, 1955	,,	64	7	51	0	0	6
February, 1956	Tegalsari	224	0	224	0	0	0
March, 1956	Brunoredjo	288	7	262	0	4	15
Total ...	...	944	66	831	2	5	40

B=Buffalo. M=man.

Similar variations have been recorded by Walch (1932) in other parts of Java, in some cases 12 per cent with human blood (out of 229) and in other places 61 per cent with human blood (out of 137).

A. SUNDAICUS.

Breeding places.—*A. sundaicus* is highly selective in regard to its breeding places. It needs stagnant water of a definite range of salinity, plenty of sunlight, plaques of long fibred floating algæ such as enteromorpha, chætomorpha or spirogyra and a certain amount of organic contamination also. The optimum salinity was found to be 15 to 20 parts per thousand although larvæ of *A. sundaicus* were found in situations with salinity range as low as 4 and as high as 30 parts per thousand. In the salt water fish ponds (*tambaks*) which are exclusively confined to the northern coastal districts of Java and in the closed-off lagoons along the south coast of Java, such a combination of these conditions in several places make them ideal breeding places for this species. In the coastal swamps with extensive mangrove growth, the species has not been found to breed so far. But when the mangroves are cleared and an attempt is made to reclaim the land for fish-culture or agriculture by constructing bunds and arresting tidal action, then the species tries to invade. This is in accordance with the finding of Strickland (1936) in Malaya.

In contrast to the fish ponds which are confined to the north coast with its shore line exposed to the comparatively calm Java sea, the lagoons of the south coast of Java, facing the rough Indian Ocean, are subjected to a greater degree of wave action and tidal influence. And even in the lagoon areas, all the breeding grounds are not subjected to the same degree of tidal movements. One lagoon may suddenly get exposed to the sea because of breaches on sand banks at either end and may get salinified to the extent to make it unfavourable for *A. sundaicus* for several weeks. Another lagoon may be left untouched for several months to cause uninterrupted and prolific breeding. As long as the fish ponds or the lagoons satisfy the optimum conditions for the species, it continues to thrive in large numbers and get extremely scarce to the extent of creating the impression of virtual disappearance of the species, with the elimination of such breeding places.

The Province of mid-Java has a total shore line of 830 kilometers on the north and south coasts put together. According to the monograph of fish culture in the coastwaters of Java by W. H. Schuster (1949), out of the total of 81,618 hectares of fish-ponds in Java and Madura, 18,361 hectares or 22·4 per cent of the total, are to be found in mid-Java alone and *all of them in the north coast only*, the south coast being unsuitable for such fish-ponds on account of the fierce surfs. The lagoons are the exclusive features of the south coast and the area occupied by the lagoons in the south coast is very much less in comparison with the extent of fish ponds in the entire Province.

Sweet water *sundaicus* has so far not been found in Java. Nor does *sundaicus* breed in water collections which are subjected to the daily tidal influence.

Bionomics of adult A. sundaicus.—

During the period 1952-1956, this species has been studied in great detail by the World Health Organization Team in the south coast area. The results of these studies are being reported separately. The salient findings may be summarized as follows:—

(*i*) Adult *sundaicus* prefer houses to cattlesheds for daytime resting. During a series of intensive test collections made in different kinds of stables

during 1952-1954, only 13 *sundaicus* were found among 1,833 anopheline mosquitoes. Though in fluctuating density inside houses throughout the year, the main seasonal peak of its prevalence was found to be between September and December with a secondary peak between March and May. If local breeding conditions were extremely unfavourable, the species may get so scarce, as to give the impression of virtual disappearance in that area. During the most favourable conditions and especially in situations very close to the breeding places, the species may constitute nearly 80·0 of all anopheline collections.

Adults of *A. sundaicus* are extremely scarce in areas beyond a range of approximately two kilometers from the nearest fish pond, lagoon or brackish water marsh and are absent in the inland areas beyond a maximum distance of three kilometers from the coast. The flight range of *A. sundaicus* is well within three kilometers.

(*ii*) Indoors, mostly they may be found resting on the walls as well as under the ceilings to the extent of 55·0 per cent and 35·0 per cent, respectively, of all *sundaicus* that could be collected. Other resting places indoors were the hanging clothes, fishing nets, paddy bags and beneath furniture. Out of over 11,000 *A. sundaicus* collected in a series of test collections indoors, the distribution in the different types of resting places were as follows:

On walls	55·0 per cent
Ceilings	35·0 ,, ,,
Beneath cots, benches etc.	3·0 ,, ,,
Hanging clothes	5·0 ,, ,,
Paddy bags, fishing nets etc. ...	2·0 ,, ,,

(*iii*) The species has marked exophilic tendencies as shown by the finding of approximately 40·0 per cent of those caught in window traps in a freshly fed state. Freshly fed females have also been found in out-door resting places to the extent of over 26·0 per cent (Table VII). Precipitin tests on 492 blood samples from out-door collections of *A. sundaicus* showed an anthropophilic index of 21·9 per cent.

TABLE VII.

Stomach and ovary conditions among A. sundaicus *caught inside huts, window traps and outdoor shelters.*

Source of collections	Number examined	Stomach and ovary conditions							
		Freshly fed	Per cent	Half gravid	Per cent	Gravid	Per cent	Unfed	Per cent
Morning collections.									
1. Inside huts ...	32,43	1,641	50·6	540	16·6	696	21·4	366	11·4
2. Window traps ...	664	261	39·3	118	17·8	170	25·6	115	17·3
3. Outdoor shelters	1,013	270	26·6	117	17·0	119	19·6	4·2	42·1

(*iv*) The most favoured resting places outdoors were found to be the moist and dark crevices in the rocky promontories close to the breeding places. Hollows in sand banks in the vicinity of lagoons were also found to be favourite outdoor resting places of *A. sundaicus*. The average density per man-hour of collection in these outdoor shelters was found to be 4·0 in rock crevices and less than 2·0 in the hollows in sand banks. The distribution of *A. sundaicus* in the different types of out-door shelter searched in the south coast area during the period 1952-1955, is given in Table VIII.

TABLE VIII.

Distribution of A. sundaicus *in the different types of outdoor shelters searched in the south coast during 1952-1955.*

Type of resting place out-door	Time spent in man-hours	Number collected (*A. sundaicus*)	Percentage of the total in all types
Rock crevices	290	805	54·4
Hollows in sand banks ...	190	331	22·4
Plant bushes	240	48	2·7
Firewood or bamboo heaps, miscellaneous types	430	298	20·5

(*v*) On 3,436 blood samples from house collections and 492 from outdoor collections, the anthropophilic indices were found to be 51·0 per cent and 21·9 per cent, respectively.

(*vi*) The duration of the gonotrophic cycle is 48 hours.

(*vii*) Of the *A. sundaicus* that enter human habitations at night, approximately 60·0 per cent are found to do so during the second and third quarters of the night.

A summary of 20 observations in the south coast area in 1954 on the time of entry of *A. sundaicus* at night, is given in Table IX.

TABLE IX.

Time of entry of A. sundaicus *at night.*

Time	Number of *A. sundaicus* caught	Percentage out of of the total
19·00	78	3·34
20·00	135	5·80
21·00	187	8·09
22·00	230	9·80
23·00	238	10·60
24·00	198	8·50
1·00	193	8·20
2·00	270	11·50
3·00	324	13·80
4·00	235	10·50
5·00	210	9·00
6·00	44	1·90
Total ...	2,342	100·00

(*viii*) Observations on the time of feeding indicated that a great majority of them get their blood meal during the third and fourth quarters of the night.

TABLE X.

Time of feeding of A. sundaicus *at night.*

Time	Total examined	Number found unfed	Percentage out of total unfed	Number found fed	Percentage out of total fed
19·00	42	23	8·3	19	4·1
20·00	49	27	9·8	22	4·7
21·00	46	23	8·3	23	5·0
22·00	60	35	12·7	25	5·4
23·00	68	27	9·8	41	8·8
24·00	71	40	14·0	31	6·7
1·00	46	25	9·0	21	4·5
2·00	72	10	3·6	62	13·4
3·00	94	22	8·0	72	15·5
4·00	92	11	4·0	81	17·4
5·00	70	16	5·8	54	11·6
6·00	30	17	6·2	13	2·8
Total ...	740	276	100·0	464	100·0

(*ix*) Based on over three hundred night collections in the coastal areas during the period 1952-1956, the nocturnal density of *A. sundaicus* is estimated to average 50 per night, person and room.

A. MACULATUS.

Breeding places and habits of A. maculatus.—

The distribution of this species in the Province is very patchy and the only locality (Kalikadjar, in the Residency of Kedu) where appreciable numbers of larvæ and adults of *A. maculatus* have been found so far, is in the central hilly region at an altitude of 800 meters above sea level. Though *A. maculatus* had been found infected in this area (in September, 1956) the species *A. aconitus* which is present in larger number in the same area, had also been found infected during the same month.

Cool springs, seepages and streams well exposed to sunlight have been found to be the chief breeding places of *A. maculatus*. Larvæ of this species have

also been found along the still margins of clear streams in the midst of terrace cultivation of rice in the slopes of hills.

The adults of this species are scarce within houses during day time but many enter at nights and bite actively. Day time density in houses was as low as 2·0 per man-hour and night time density 40·0 per man-hour. This suggests that this species uses habitations as day-time resting places to a very limited extent only. Some of the outdoor resting places, where they were found so far, are the shady moist banks of small streams.

More investigations are required locally to determine the extent to which it is exophilic and other details relating to its nocturnal habits and feeding preferences.

A. SUBPICTUS SUBPICTUS

Breeding places and habits of A. subpictus subpictus.—

A. subpictus subpictus is a more ubiquitous breeder as the larvæ of this species could be found in brackish as well as fresh water and in clear as well as turbid water. Often times it has been found to breed side by side with *A. sundaicus* in the lagoons and with *A. aconitus* in the ricefields.

During daytime, the adults are found in houses as well as animal shelters. They rest on walls and in the ceilings. They are also found in various types of outdoor shelters. On 500 blood samples tested from house collections, the anthropophylic index was found to be 20·0 per cent. In night time collections in houses, the species is extremely scarce. It has been found to be a malaria carrier of some importance only when the desnity in houses was extremely high.

CONTROL BY INDOOR RESIDUAL SPRAY.

A. sundaicus *and D.D.T.*—Based on trials on an experimental scale in some villages on the north coast of west-Java, the first encouraging results with D.D.T. against *A. sundaicus* were reported by Bonne-Wepster and Swellengrebel (1950) and Van Thiel and Winoto (1951). This was followed by a number of trials in other areas in Java as well as in Sumatra and Celebes, a summary of which has been made by Soeparmo and Stoker (1952). These led to the general adoption of a dose of 2·0 gm. per square meter in suspension applied once a year in many areas of north coast of Java and elsewhere in Indonesia with fairly successful results (Soeparmo and Laird, 1954).

Resistance.—In 1954, in the Djakarta coastal area, about three years after the first house spraying with D.D.T. and after two annual house sprayings had been completed, *A. sundaicus* was first shown to be resistant to D.D.T. by Crandell (1954). Later, resistance was detected in other places also such as Tjirebon, Semarang and Surabaja, all on the north coast of Java, during 1954 and 1955. As all or most of these areas showed a history (during the war) of larvicidal control with oil containing D.D.T., either from the air or from the ground, it was believed

that such a larviciding was probably a contributory factor in the development of resistance. High densities of *A. sundaicus* were reported to rest, even on freshly sprayed surfaces, without being affected adversely. The degree of resistance of *A. sundaicus* at Semarang was estimated in 1955, after three rounds of spray, to be of the order of nearly 18 times by Davidson (1955)*.

Behaviouristic change.—In the south coast of the Province where the World Health Organization Team was functioning since 1952 onwards, detailed observations showed that no such physiological resistance, as was found in the north coast, resulted in the south coast areas even after four rounds of D.D.T. spray.

Issaris and Sundararaman (1954) noticed that *A. sundaicus*, which normally rested in significant proportions on walls, behaved differently following the spray and avoided contact with a lethal dose of D.D.T. and in some cases any contact whatsoever but continued simply to enter, feed and leave, and such a behaviouristic change interfered with the efficacy of residual insecticide application. The median lethal concentration of D.D.T. for *A. sundaicus* from some villages in the south coast, after four rounds of D.D.T. spray, was found to be 0·6 per cent in April, 1956, as compared with the median lethal concentration of 0·5 per cent among *A. sundaicus* from another area in the south coast which had ever been sprayed with insecticides, whereas the median lethal concentration of D.D.T. for *A. sundaicus* at Semarang in the north coast after three rounds of spray, was found by Davidson to be in the region of 9·0 per cent in 1955, showing clearly that while the north coast strain of *A. sundaicus* was found to have developed physiological resistance to D.D.T. in 1955, the south coast strain had not done so up to 1956.

A. sundaicus *and dieldrin.*—In the north as well as in the south coast, when it became evident that malaria transmission was not interrupted when D.D.T. was used, a change of insecticide to dieldrin was adopted and its use continues in those areas since 1956. At the dosage of 0·5 gm. per square meter, dieldrin produced high mosquito mortalities (100 per cent) for six months after which a decline became apparent. In the south coast where D.D.T. had previously been sprayed and the behaviouristic change of *A. sundaicus* avoiding any contact with sprayed surface earlier referred to had been induced, the same changed behaviour seemed to continue even with the application of dieldrin. But this is under further study. More investigations under local conditions are necessary to establish the optimum dosage and the number of rounds per year to achieve the full degree of interruption of transmission.

A. aconitus *and D.D.T.*—In mid-Java Province the use of D.D.T. residual spray in *aconitus* areas is of recent origin (from August, 1956) and available evidence is limited. By field experiments with D.D.T. solution in kerosene in Sundanese houses in West Java, Swellengrebel and Lodens (1949) found no repellency in *A. aconitus* to D.D.T. Van Goor and Lodens (1952) observed in Tjiramaewah in West Java that " although the experiments showed that *A. aconitus* malaria can probably be suppressed by house spraying with D.D.T., the authors

*Official report on his visit to Java, Indonesia, regarding insecticide resistance of *Anopheles Sundaicus* (World Health Organization document).

could not prove it" (authors summary). According to observations in mid-Java, there has been complete disappearance of *A. aconitus* from treated houses for full six months following the spray up to the most recent observations in 1957.

A. aconitus *and dieldrin.*—Observations in the limited areas where dieldrin at 0·5 gm. per square meter had been used in September, 1956, show that similar complete absence of *A. aconitus* from treated houses has been maintained for full six months up to date (March, 1957).

There is no report of any resistance of *A. aconitus* to D.D.T. from anywhere so far. As recommended by the experts at the W.H.O. sponsored Inter Regional Conference on Malaria, held in Athens in 1956, " it is probably best to use D.D.T. in the first instance and to change to another insecticide only when D.D.T. fails ".

A. maculatus *and residual insecticides.*—There is no evidence yet in this Province on the nature of reaction of this species to D.D.T. or dieldrin indoor residual spray since such spraying operations have not been extended to the typically *maculatus* areas in this Proivnce so far. In view of the fact that this species does not normally rest in houses after feeding, careful investigations are necessary to obtain a knowledge about the behaviour of the species in relation to treatment with residual insecticides.

SUMMARY.

As the entomological observations recorded in this note relate to the province of mid-Java, some of the essential features of the area are briefly described.

A list of all the anopheline species so far encountered in the Province and the species most commonly found in the coastal strip, the inland plains and the hilly terrain, is furnished.

A. sundaicus breeding in the brackish waters of the fish ponds and lagoons in the coastal areas, and *A. aconitus* breeding in the ricefields, are the principal malaria vectors. The secondary vectors of some importance are *A. maculatus* breeding in the clear jungle streams in the hills and *A. subpictus subpictus* which is ubiquitous in its prevalence in this province.

A brief account is given of the transmission season and infectivity of the vectors, their distribution and bionomics.

The present status of the reaction of these vectors to control by indoor residual spray with D.D.T. and dieldrin, is summarized.

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ON THE IMPORTANCE OF LIMNOLOGICAL STUDIES IN SOME MALARIA ERADICATION SCHEMES.

By

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[July 19, 1957.]

THE new concept of malaria eradication is based on two important observations. Firstly, malaria infections are self-curing and do not normally last for more than three years. Though, in a very few cases, *P. falciparum* and *P. vivax* infections have been shown to last longer, they do not invalidate the general statement that, if no new cases of malaria have occurred in a given locality for three years, its population no longer represents a source of infection for anophelines (Pampana, 1955). Secondly, anopheline mosquitoes usually take time to develop resistance against residual insecticides, possibly five or six years or more. Therefore, where malaria transmission can be completely interrupted for a period of at least three years and the vector anophelines do not show evidence of resistance meanwhile, malaria eradication will not remain a distant or impracticable ideal.

There are, however, many regions where vector anophelines have not developed resistance in spite of the continuous use of D.D.T. over a period of about ten years. There is also evidence that, in a few such regions, the local vectors, far from exhibiting any tendency to resistance, are observed to disappear altogether. In Thailand, where a pilot-project started in 1949 has since been expanded to cover all the malaria-infected areas of the country, *A. minimus*, the local vector, has disappeared from many of the sprayed areas (Dy, 1954). Gabaldon (1949) and Giglioli (1951) have reported the eradication of *A. darlingi* from Venezuela and British Guiana whereas Dowling (1951) and Hamon and Dufour (quoted by Gabaldon, 1953) have reported similar disappearance of *A. funestus* from Mauritius and Reunion, respectively. The vector of Peninsular India, *A. fluviatilis*, has become a rarity in most of the sprayed regions of that region.

On the other hand, there are certain other instances where definite resistance to insecticides has been observed. The first authentic report of resistance to D.D.T. was received from Greece in respect of *A. sacharovi* (Livadas and Georgopoulus, 1953). Later, in the same country, *A. superpictus* was also reported to have become resistant to D.D.T. (Belios, 1954). When this phenomenon was observed in Greece, malaria had already practically ceased to be an important public health problem it once had been and the hope was entertained that the residual malaria might be dealt with by the proper administration of antimalaria drugs. The situation in other regions is not, however, so encouraging. Garret Jones and Grammiccia (1954) reported resistance to D.D.T. in *A. sacharovi* at Arida

in the Lebanon. Crandell (1954) has shown that, in the Djakarta area of Indonesia, *A. sundaicus* became resistant to D.D.T., though not yet to dieldrin. In another area of the same country, the same Anopheles has reacted in a different manner; it continues to feed in sprayed houses but avoids contact with D.D.T. and rests in outdoor shelters (Issaris and Sundararaman, 1956). Elliot and Ramakrishna (1956) observed that *A. gambiæ* in North Nigeria had developed resistance against dieldrin within about a year of the first application of the insecticide, though its sensitivity to D.D.T. had not diminished to any appreciable extent. It is, however, the experience of many workers that when resistance to one insecticide is developed, cross-resistance to other chemically related substances would soon become evident. In all these cases, resistance was observed when malaria had just been brought under control but by no means eradicated.

If malaria eradication on a global scale is contemplated, the problems mentioned above have to be solved and can probably be ultimately solved only by recourse to antilarval measures, applied either independently or in conjunction with other methods. When some vector anophelines have developed resistance to one or more of the residual insecticides, it is logical to assume that other vector species would also undergo a similar change and to prepare for that eventuality. Even if insects were not developing resistance, it is reasonable to continue to apply sanitary measures to deposits of waste water as these measures will not only inhibit the development of mosquito larvæ but will mitigate other water-borne diseases and eliminate useless and unsightly waters (Knipe, 1953). The concept of prevention is based on the approach that vectors are symptoms of environmental delinquencies and that the real place of vector control lies more in altering the causes than in dealing primarily with the effects (Henderson *et al.*, 1955). Antilarval measures have thus not been entirely out-dated and appear still to be an essential feature in some eradication schemes, specially in the case of vectors like *A. sergenti* and *A. minimus flavirostris*.

As might be expected, the first antilarval measures to be adopted consisted mainly of engineering works such as elimination of breeding places, reduction of water surface to the smallest possible area and fluctuation of water surface at the periphery etc. Chemical larvicides were employed in respect of those waters which could not otherwise be disposed of. However, owing to their high initial cost and their general inapplicability to rural areas, these measures were later sought to be substituted by naturalistic measures, which had the desirable effect of shifting the emphasis from engineering to biological studies. The result is very gratifying in that a large amount of literature on the biology of anopheline larvæ has been produced. However, the limnological aspect of the breeding places has perhaps not received the attention that it deserves and this may operate as a gap in our knowledge of the subject. Some valuable limnological observations were no doubt made in India and elsewhere by certain authors several years ago. Waddel (1903)* observed that 1 part in 4,000 of strong liquor ammonia in the water was fatal to mature larvæ and computed that a dilution of 1 part in 30,000 would inhibit newly hatched larvæ. Hodgson and King (1914)* and Hodgson (1920)* studied the temperature of water in the various breeding places and concluded that

*Quoted by Senior White (1928*a*)

the optimum temperature for Anopheles was 60° to 78° F. (20°-26°C.) while temperatures of an above 95° F. (35°C.) were rapidly fatal. Hodgson (1920)* also concluded that eggs were rarely laid in water above 70° F. (21°C.) and that, though larvæ managed to exist when the temperature is raised to 80°F. or more, the greater number would fail to turn into adults, or if they did, these were weaklings of short life. Certain Russian authors (Adowa and Selenzov, quoted by Senior White, 1928*a*) made similar studies in respect of *A. maculipennis* and concluded *inter alia* that it is conductivity which indicates the threshold of anopheline breeding and that high values are favourable. Senior White (1926:1928*a*) studied the physical factors in the ecology of some mosquitoes of Ceylon and India and concluded as follows:—

(*a*) study of *pH*, conductivity, carbonates and albuminoid ammonia in connection with mosquito breeding is "useless";

(*b*) saline ammonia exceeding one in a million parts is inhibitory to anopheline breeding except in the case of *A. subpictus* (*sundaicus* could not be included in the study); and

(*c*) it was impossible to confirm Hodgosn and King (1914)'s values for optimum and limiting temperatures.

Studies on the temperature of the water in the breeding places were subsequently continued by Muirhead Thomson (1951) with particular reference to *A. minimus* in Assam and by Pal (1945) in respect of *A. culicifacies* in the Punjab. The results are not conclusive enough, though it has been shown that *culicifacies*, a pool breeder, can withstand a higher temperature than the stream-breeding *minimus* and that the ovipositing female anophelines of these two species are also sensitive to water temperatures though to a lesser extent. The studies so far made have not, of course, led to any definite conclusions but they have clearly indicated the great scope for and importance of further observations, without which knowledge of anopheline ecology remains incomplete.

All inland water can be divided into two series, *viz.*, the running water series (lotic environment) and the standing water series (lentic environment).†

The essential difference between the two series is as follows: In the lotic series, a tiny rivulet gradually deepens, widens its bed and cuts back at its head, thus in time extending its length and increasing its cross-section to the size which justifies the designation of brook. This process continues by the same general type of action, ultimately producing a creek and then finally a river, with all intergrading conditions incidental to such a gradual transformation. In the lentic series, on the other hand, natural processes work towards extinction, mainly by the gradual filling of basins. Filling is due primarily to the accumulation of wind-blown materials, sediments brought in by in-flowing streams, aquatic plants producing deposits of organic matter and wave action cutting exposed shores and depositing the eroded material in the basin. Thus, whereas the lotic series have a tendency to increase and expand, lentic series are constantly exposed to the risk of extinction.

*Quoted by Senior White (1928*a*).

†Much of the limnological material presented in this note is quoted from Welch (1952).

LENTIC ENVIRONMENT.

Ponds and lakes, which serve as breeding places for some vector anophelines, constitute the main source of interest in the lentic series to the malariologist. Ponds are generally of four classes, *viz.*, (*i*) those which represent the pond stage of dying lakes, (*ii*) those formed by cut-off or dead streams and beach ponds, (*iii*) those whose basins are shallow and the extent is limited and, therefore, cannot be included in lakes and (*iv*) those whose basins are the result of man's activities, e.g., "borrow-pits", quarries, impoundments etc. All ponds are either permanent (aestival ponds) or temporary (vernal ponds), containing water at certain times of the year and becoming dry at others. The physical, chemical and biological factors mentioned below are usually characteristic of a large variety of ponds.

At the surface film, a phenomenon called "surface tension" occurs, due to unbalanced attractions between molecules at the surface, since at the surface the liquid molecules are pulled downward and laterally by the neighbouring molecules and upward attraction is lacking because there are no liquid molecules above them. The surface film serves as a mechanical support for organisms and miscellaneous particulate materials, but, it is the lower surface of the film—the infraneuston—that supports temporarily the larvæ of certain insects including mosquitoes.

Owing to limited areas involved, water movements in ponds are minimal. Growth of aquatic plants and the proximity to hills and jungles help the ponds to maintain a calm surface even in times of high winds. The temperature of the water, specially at the surface, tends to follow the atmospheric temperature, which varies greatly at different parts of the day and night. The turbidity of ponds varies widely according to season, productivity, nature of the basin etc. The shallow nature of ponds usually makes possible an illumination of the bottom by effective light so that plants may occupy the entire basin, specially in unshaded ponds.

There are dissolved solids in all pond waters but they differ both in quality and quantity and also show conspicuous seasonal variations. They depend upon various factors such as the source and nature of bottom deposits, presence or absence of inlets and outlets, area of drainage, wind-blown material and the character of the biota.

The suspended matters in ponds are of two groups, *viz.*, settling suspended matters and non-settling suspended matters. The latter consist mainly of (*i*) plankton organisms and those coarsely divided solids whose specific gravity is less than that of water and (*ii*) very finely divided non-living materials and organisms of exceedingly small size.

The dissolved gases in ponds vary mainly according to the nature of the bottom and it has been shown that, (*a*) over sandy bottoms of younger ponds, abundant dissolved oxygen prevails, (*b*) water over vegetation has a moderate dissolved oxygen supply at least during the day; (*c*) in the older ponds, the bottom water is supplied with oxygen only during certain periods of the year and (*d*) the free carbon dioxide content is greater in the deeper water of older ponds. In plant-filled ponds, almost complete exhaustion of dissolved oxygen supply may occur at night, though it is recouped after dawn through photochemical processes.

The biological productivity of ponds differs from that of lakes in that pond organisms are essentially of the littoral and benthic type, though there are all possible gradations manifesting, in many instances, mixtures of both lake and pond features.

The faunal characteristics of ponds are as follows:—

(*a*) Among the vertebrates, amphibians constitute the most important group. Fishes are few or may be entirely absent.

(*b*) Of the invertebrates, the protozoa, rotifers, insects and snails are the most important.

(*c*) Pond faunas, particularly those of the temporary variety, are largely composed of species which pass a part of their life cycle out of water.

(*d*) Surface film animals and those which must come to the surface for breathing, are often abundant.

As the ponds grow older, bacteria, plankton, vegetation and the larger animals increase quantitatively. There are, however, great variations in the faunal composition of ponds from year to year. A species may be abundant in a pond one year, disappear for three or four years and then suddenly re-appear. A good example of this phenomenon is afforded by *A. sundaicus* in areas south of Calcutta, where for no apparent reason, it disappears for years and, when coming back, colonises even a wider area than it did previously (Senior White and Venkat Rao, 1946).

For the purpose of plant growth, ponds may be divided into two general classes, (*i*) those with bare bottoms, and (*ii*) those with bottoms composed of soft, accumulated deposits. In general, the former are devoid of larger vegetation while abundant growth of higher plants is present in the latter. The older and deeper waters usually exhibit three different zones of vegetation, *viz.*, (*i*) the marginal zone of emergent vegetation, (*ii*) the middle zone of floating and rooted plants and (*iii*) the inner zone of submerged vegetation. Algal growth is also often conspicuous. Emergent vegetation is rooted at the bottom and projects out of the water surface for part of its length like *Scirpus* species. Floating vegetation is either wholly or partly floating on the surface (example *Pistia* species). Submerged vegetation is continuously under the surface except for infloroscences like *Ottelia* spp. The nature and extent of these plants may vary according to environmental factors such as light, temperature, wave action and character of the bottom. Too little depth eliminates certain groups whereas too great depth excludes the larger plants completely. When decreasing light, due to increasing depth, falls below the effective limit, the plants disappear. Changes of temperature at different levels are, however, seldom great enough to limit plant growth.

The amount of aquatic plants produced by ponds in certain regions like West Bengal and Orissa is often surprisingly high, though quantitative studies have not been made to substantiate the statement. These ponds, however, make large demands upon the supply of essential materials in the water and bottom soils. A certain return of essential substances results from the decay of plants but the degree of completeness of the return varies with circumstances and the substances involved.

Though it is held that the chemistry of water is the most important influence in determining the volume and distribution of plant growth, certain physical factors such as temperature and turbidity also play an important rôle. Particles, specially silt, floating in water absorb heat more rapidly than does the water itself; then these particles transfer their heat to the surrounding water, thereby raising its temperature. The unfavourable effect of this is the restriction of photosynthetic processes in plants, which, therefore, tend to disappear.

Besides plants, pond waters support green and blue-green algæ, which may exist independently or find mechanical support in the plants. Except in very small and highly transient pools, there is also the plankton composed of heterogeneous assemblage of minute organisms which, along with the associated finely divided non-living matter, floats on the water at the will of waves and other water movements. It has been observed that the amount of plankton produced by fresh water bodies is, other things being equal, in some inverse ratio to the amount of its gross aquatic vegetation, specially of the submerged kind. This does not mean that all vegetation is inimical to the development of plankton but only such vegetation is inimical which successfully competes with the phytoplankton for the available plant food and thus brings by its decay no additional source of plant nutrition into the water.

Let us now briefly examine the relationship between the various limnological factors described above and the growth and development of anopheline larvæ.

Surface tension.—If the surface tension is lowered, larvæ and pupæ cannot exist at the surface and are drowned and destroyed. However, the surface tension of pond waters varies somewhat widely according to different situations. There is reduced surface tension in absolutely still waters. Though a rise of temperature has the effect of further lowering the tension, the variation is usually slight and probably not significant. A steady wind may blow about the surface film and cause local variations in surface tension. There is reduced surface tension in regions of foam formation. Substances which have the ability to produce an appreciable reduction in surface tension of a liquid on being dissolved in it, are called surface active agents. In this category are included soaps, certain sulphonic acids and various types of organic compounds. The most important single factor, responsible for reduced tension, is the presence of substances of organic origin, which exert a greater influence than other chemical factors.

Water movements.—It is well known that mosquito larvæ are absent from ponds where there is perceptible wave action. Movement of water in pond situations is often restricted by (*a*) protection from closely surrounding hills, forests and similar features and (*b*) luxuriant plant growths. While the former is unalterable, the latter is subject to man's interference and can be removed or altered by mechanical or naturalistic means.

Temperature.—There appear to be optimum and upper tolerence limits of temperature for most anophelines. That the pool-breeding *A. culicifacies* can withstand a higher temperature than the stream-breeding *A. minimus*, has already been mentioned. Muirhead Thomson (1951) showed that the temperature of standing waters like ponds and ricefields in Assam was about 41°C. (106°F.) at a time when, in the vicinity, the temperature of water in grass-edged streams seldom exceeded 34°C. (90°F.). But in the deeper ponds, the temperature at the

surface tends to follow the atmospheric temperature and thus varies to a somewhat wide extent, whereas the temperature below the surface is not so much influenced by atmospheric variations owing mainly to the plant growth reducing mechanical mixing of water by wind action. Therefore, the anopheline species breeding at the surface must be capable of adjusting themselves to varying temperatures and specially so in respect of maximum limits. The interesting suggestion made by Hodgson (1920) (quoted by Senior White, 1928*a*) that a large majority of anopheline larvæ fail to emerge when the water temperature is raised to 27°C. (80°F.) or more or, if they do so, the emerging adults are weaklings of short life, remains still to be verified and confirmed.

Dissolved solids and gases.—The relationship between dissolved solids and anopheline breeding has not been studied but some investigations have been made of the influence of certain dissolved gases. The oxygen content of pond waters is determined by the nature of the basin and by the presence of or absence of aquatic plants. It is interesting to observe that certain younger ponds are richer in dissolved oxygen than the older ones. The amount of dissolved oxygen, present in the water, is important for several anophelines, particularly the vectors. Senior White (1926) showed a significant relationship between the high oxygen content of water in the ricefields of Ceylon and the breeding of "*A. funestus* (*listoni**)". He also showed that, in foul waters in which anopheline larvæ are rarely found, free oxygen is not present; the small amounts, if any, found being probably in all cases nitrites.

It is recognized that, where saline ammonia is present in a higher proportion than one part in a million, it inhibits the development of several vector species. Senior White (1928*a*) found 100 per cent of *A. annularis*, 98·6 per cent of *A. culicifacies* and 95·8 per cent of *A. stephensi* larvæ at values of less than one part in a million though 53 per cent larvæ of *A. subpictus* were found beyond that figure.

Turbidity.—Generally speaking, in turbid waters, vector larvæ are not found in any numbers. Muirhead Thomson (1951) is of the view that, at least in the case of *A. minimus*, this is due to the increased water movement occasioned by the presence of silt but not to the silt itself. But, in still waters, turbidity exercises a profound influence on the growth of aquatic plants, which are essential for anopheline breeding, as already mentioned.

Aquatic plants etc.—The main function of aquatic plants with which we are presently concerned, is to afford food, shelter and protection to anopheline larvæ. Though this is generally true of a majority of plants, there are certain species of plants which favour certain larvæ and appear to inhibit others. Larvæ of *A. ramsayi* and *M. annulifera* are often associated with *Pistia stratiotes*. Iyengar (1946) has shown that a thick growth of *Eichhornia speciosa* inhibits the breeding of *A. philippinensis* and *A. sundaicus* in Bengal† but it has been observed that *A. aconitus* is frequently met with in association with this plant. Lemna and Wolffia have been cited as anti-anopheline plants, though they sometimes harbour larvæ of a few species like *A. nigerrimus* and *A. barbirostris*. On the other hand, the same plant may favour more than one species of Anopheles or the same Anopheles may

*The reference probably applies to *A. Varuna*.

†Venkat Rao and Ramakrishna (1947*b*), however, observed no incompatibility between *Eichhornia* and *A. sundaicus* in Orissa.

have equal preference for more than one species of aquatic vegetation. There are also certain species like *A. culicifacies* and *A. varuna* whose larvæ are found mostly in waters without the higher plants. In fact, there are instances where, with the mechanical removal of higher plants, *A. annularis* was quickly substituted by *A. varuna* in Orissa (Venkat Rao and Ramakrishna, 1940).

In the larger ponds without vegetation, anopheline larvæ are found only at the shallow edges but, if the ponds are full of floating vegetation, larvæ are liable to be found all over the water surface. One function of vegetation is, therefore, to vastly increase the extent of effective breeding area. Eggs are laid on the leaves, while eggs, larvæ, and pupæ find mechanical support among the plants. But the fundamental dependence of animals upon green plants is as important in water as it is on land. There is evidence that the larger aquatic plants are directly consumed by insect larvæ. In his studies made on seventeen species of *Potamogeton*, Berg (1949:1950) (quoted by Welch, 1952) found more than two dozen species of insects feeding regularly and extensively on these plants. The third function of aquatic plants in relation to anopheline larvæ is the protection they afford to the latter. The protection may be dispensed in diverse ways. The larvæ are sheltered from excessive light and unfavourable wave action; the water temperature is maintained at optimum, or at least within bearable limits and a safe refuge is afforded from predators.

It has already been stated that introduction of silt into plant-filled ponds has a very unfavourable effect on plant growth. It has, however, been observed that, when the plants are removed by mechanical or other means, the hitherto clear water becomes turbid and polluted. This change has been attributed to rotting pieces of vegetation left in the ponds and to the rapid growth of the green alga, *Microcystis auriginosa*, which quickly occupies the entire water surface (Venkat Rao and Ramakrishna, 1947*a*). Muirhead Thomson (1951) thinks, however, that the increase in organic matter of these de-weeded ponds may probably have been brought about by the bottom mud being stirred up by the labourers engaged in de-weeding operations. The presence of *Microcystis auriginosa* is observed to indicate the absence of at least the vector larvæ.

Non-plankton and plankton algæ are an important source of food for anopheline larvæ. Senior White (1928*b*) carried out a study of algæ as food for anopheline larvæ and observed that *A. maculatus* feeds on whatever organisms occur in abundance in their larval habitats but diatoms and fungal spores are its preferred foods. Sen (1954) noted that algæ form the main source of nutrition for anopheline larvæ and discussed the association of *A. sundaicus* with some of the algæ. Covell and Singh (1942) observed the association of the same species with eight types of algæ *Lyngbya*, *Anabena* and *Spirogyra* being the most important. Venkat Rao and Ramakrishna (1947*a*) noted the close association of fresh-water breeding *sundaicus* with *Spirogyra*. In a recent study on the nutrition of certain anopheline larvæ, Neogy and Kachroo (1956) concluded that larvæ depend mainly on algal food, of which 95 per cent is constituted by green algæ.

Non-plankton algæ may exist independently in water but they are usually found along with the larger plants, living or dead, in which they find mechanical support. Senior White and Adhikari (1939) observed that, in the Chika Lake, *Potamegaton pectinatus* and *Halophila ovata*, uprooted by wave action, were bound

together into large plaques by the alga, *Lyngbya aestuarii*, in which larvæ of *A. sundaicus* could be seen in large numbers.

Plankton, including plankton algæ, also constitutes a source of food supply for several species of anopheline larvæ. It has already been stated that the amount of plankton in any body of water is in some inverse ratio to the amount of its gross aquatic vegetation, specially of the submerged sort. Where the vegetation is abundant and the plankton is minimal, species like *A. annularis*, *A. philippinensis* and *A. sundaicus* are found in large numbers, whereas in waters which have very little gross vegetation and which, therefore, presumably have greater amounts of plankton growth, larvæ of *A. varuna*, *A. culicifacies* etc., are found. Probably, the former are capable of thriving on gross plants and algæ whereas the latter depend on plankton (chiefly plankton algæ) for their nutrition.

The case of the small and purely temporary rain water collections may require some passing discussion, as larvæ of *A. culicifacies*, besides other non-vector anophelines, are often found in significant numbers in such situations. These small and highly transient pools do not maintain any plankton or aquatic plants. The water is clear and does not also appear to support suspended organic material. Where then is the food supply for these larvæ? Neogy and Kachroo (1956) have shown that anopheline larvæ are primarily herbivorous and cannibalism was never noticed. They, however, observe that, where vegetation is scarce, the fleshy epidermis of leaves of plants such as *Aponogeton crispus* and *Vallisneria spiralis* provide good substitutes but the incidence of such foods is very meagre. It, therefore, appears that, in such conditions, organic materials present at the bottom (which is usually very near the surface) and wind-blown materials constitute the only source of food for the larvæ. Thus, larvæ of *culicifacies* may be capable of sustaining themselves on plankton (mainly phytoplankton) in permanent and semi-permanent habitats and on bottom and wind-blown materials in purely temporary habitats.

Among lakes, only the Chilka Lake in Orissa will be mentioned here as the present writer can claim personal knowledge of no other lake in India. The Chilka, which is about 500 square miles in extent, was originally a baylet in the Bay of Bengal and served as a natural harbour for sea-going vessels. It became a lake when the access to the sea was completely cut off by littoral sand drift. With large quantities of flood water and silt flowing into it during each rainy season, the water has become increasingly fresh (the salinity at present rarely exceeds 1 per cent) and the level of the basin has been greatly raised, the maximum summer depth being now only about ten feet. The main interest of this lake to the malariologist is in its being an excellent breeding place for *A. sundaicus*, the larvæ of which are found in large numbers among the aquatic plants and algæ which it supports. But, the *sundaicus* breeding in the lake varies very greatly from year to year. During certain years, the larvæ are virtually absent in the area, whereas in other years they are present in fairly large numbers. During certain periods, which recur at long and uncertain intervals, there is a phenomenal increase in the amount of *sundaicus* breeding as during the early part of 1942 (Venkat Rao *et al.*, 1942). These variations in density have been attributed to the idiosyncrasies of this " elusive " insect but there may be certain limnological factors also involved in them. For instance, during 1942, the whole lake was choked with a dense growth of *Potamegaton pectinatus* to an extent unknown within living memory

and it may be reasonable to ask whether this can be associated with the equally heavy breeding of *A. sundaicus*. The factors responsible for the abundance or absence of certain plants during certain years, therefore, require detailed study.

It has already been stated that the basin of the lake is being rapidly filled by silt from inflowing streams and other flood waters on the one hand and by deposits of organic matter produced by aquatic plants on the other. Very soon, the lake may be converted during the process of extinction into a series of ponds containing water of low salinity, which, in this area, has been observed to provide optimum breeding conditions for a very virulent vector form of *A. sundaicus* (Senior White *et al.*, 1947). Thus, in this instance at least, a dying lake is liable to constitute a greater source of danger to public health than the live lake itself.

LOTIC ENVIRONMENT.

Running waters are familiar features of most land areas and vary in size from tiny rivulets to mighty rivers. They differ from the lentic series in the following fundamental respects:—

(*a*) *Depth.*—As a rule, the depth of running waters, specially the smaller ones, is small compared to ponds and lakes.

(*b*) *Width of basin.*—The water is often confined to relatively narrow channels.

(*c*) *Current.*—The whole volume of water flows in one direction.

(*d*) *Gradient.*—All conditions, physical, chemical and biological, gradually change from source to mouth in a definite direction.

(*e*) *Absence of prolonged stagnation.*—Constant flowage eliminates stagnation except in intermittent streams where a significant fall in water level converts the river into a series of isolated pools.

(*f*) *Basic food materials.*—Most streams depend much upon the contribution of the surrounding land for food materials.

Water movements.—The velocity of the water varies with many circumstances and is not uniform in all parts of the transverse section of a stream. The velocity is reduced at the surface because of surface tension and diminishes at the sides owing to frictional effects. Muirhead Thomson (1951) has observed that (*a*) in a stream with bare edges, the velocity of the current falls off rapidly towards the margins, (*b*) where there is a fringe of vegetation or a grassy edge, the velocity is immediately reduced and a zone of still, or very slowly flowing water is produced and (*c*) if the vegetation is sufficiently dense, a zone of perfectly still water may be produced even in a rapidly flowing river. Of course, the situations are radically changed in times of flood.

Temperature.—It has already been shown that, in any particular locality, the temperature of running waters at the surface is lower than that of still waters, often surprisingly so. However, there is a greater tendency in running waters at all depths to follow air temperatures more closely than in the standing waters.

Turbidity.—Running waters are generally more turbid than still waters. In some streams, high turbidity is more or less a permanent feature but in the

majority of cases, it occurs only during the season of heavy rains. Sometimes, ingress of domestic sewage and other forms of stream pollution increase turbidity. However, in the rivulets which form the earliest stages of rivers, the water is much less turbid except in times of flood.

Dissolved solids and gases.—In respect of dissolved solids, lotic and lentic environments have few general differences but, in the former series, there is the influence of various kinds of contamination at various points in their course, some of which produce an increase in the quantity of dissolved solids.

Owing to mechanical conditions involved in current, the dissolved oxygen supply of uncontaminated waters is high at all levels. Decomposition gases tend to be minimal.

Aquatic plants.—The larger aquatic plants do not usually occur in streams except where the current is greatly reduced and lagoons are formed. Grass and other minor terrestrial vegetation is, however, found at the edges.

Algæ.—Certain types of algæ, like *Cladophora*, are characteristic of slow running streams but they are usually attached to stones and other supports. When pulled out of these supports, they may float freely on the water, sometimes in large plaques.

Plankton.—It has been stated that plankton is a feature of the lower reaches of streams and that the upper reaches are practically devoid of it. Many streams are observed to maintain a plankton often in considerable quantity. The plankton of streams is designated as potamoplankton, though there is no basic distinction between the plankters of running and standing waters. In certain streams, zooplankton predominates, while in others phytoplankton is dominant.

As Muirhead Thomson (1951) has stated in reviewing the range of breeding places used by Anopheles, it soon becomes evident that certain species are found invariably in standing waters while others thrive at the edges of streams and rivers and that, among the stream breeders themselves, some seem to prefer very slowly flowing streams while others are found at the edges of fairly rapid streams. The stream breeding species are there probably because they possess an "inherent demand for current" and their almost universal absence from standing waters may be due directly to the absence of the necessary current and not to the high dissolved oxygen demand postulated by certain workers (Wu, quoted by Welch, 1952). The maintenance of position in the face of water current may be successfully met by a body form which offers least resistance such as the "stream-lined" form, unusually well-developed clinging habit and special forms of attachment to supporting objects. Muirhead Thomson (1951), on the other hand, observed that, at least in the case of *A. minimus*, this is a mistaken idea and that other and much more effective means of protection to larvæ are afforded by their reactions to light and shade, which are intimately bound up with water movements. He goes on to show that it is not primarily the stillness of the water but the shade afforded by the vegetation that attracts the larvæ to the edges of streams. In a series of experiments, he removed the shade away from the edge into the mid-channel and found that the larvæ left the edges and followed the shade. The larvæ maintained their position in the current by active swimming movements but, when the critical velocity of 0·3 feet per second was reached, they were washed away. The sharp

reaction of larvæ to shade will, therefore, almost inevitably keep them in the vegetation as well as out of the danger zone of rapidly flowing water. However, in the experience of many workers, a slight movement of water appears necessary for larvæ of the *minimus* group. Except where the vegetation is very dense, there is some movement of water at all spots favoured by *minimus*, e.g., in pot-holes in ricefields and in side-pools in streams under the roots of overhanging trees and even in what are described as "seepage ricefields".

A slight water movement is not unfavourable for certain species like *A. culicifacies* and *A. stephensi* which are normally pool breeders but which are found along the edges of streams during the dry season. Large numbers of larvæ of these two species were found by Sinton and Majid (1935) drifting downstream when the water velocity was not more than 0·25 feet per second. Such drift may be occasioned by the larvæ being displaced from their resting places due to mechanical disturbances or to a slight rise in the water level of the stream. The larvæ are not destroyed by the current at this velocity and, in the course of drifting, many of them find anchorage at safe places downstream and are enabled to develop there normally. It may, however, be remembered that larval drift occurs only in one direction downstream, and areas in that direction are thus liable for invasion by (possibly) new species of Anopheles if environmental conditions are otherwise suitable there.

Mondal (1940) observed that larvæ of *A. minimus* and the closely related *A. fluviatilis* and *A. varuna* of the Jeypore Hill Tracts, are found along the earth banks of streams but not along sandy edges, where only larvæ of *A. culicifacies* are encountered. In fact, control of the *minimus* group in that area was obtained by filling up side pools abutting earth banks and diverting the flow of water along a central channel made entirely in the sandy bed. It thus appears that shade, inherent demand for some current and contact with earthy edges, are all necessary elements in the ecology of *A. minimus* and the related species.

If a certain amount of water movement is necessary for stream breeders, the reverse appears to apply in the case of confirmed pool breeders like *A. sundaicus* and possibly also *A. annularis* and *A. philippinensis*. Venkat Rao (1951) succeeded in eliminating the breeding of *sundaicus* from ponds and borrowpits in Orissa by converting them into running waters. This incompatibility of certain species to standing or running waters, as the case may be, is difficult to explain except on the hypothesis of body-form adjustments and, in spite of what Muirhead Thomson (1951) said to the contrary, a fresh approach in this direction is clearly indicated.

The food supply of stream breeders is evidently different from that of pool breeders. In the absence of major aquatic plants and algæ, the larvæ may have to depend to a great extent on adventitious food materials such as those which the streams obtain from the surrounding land. Stream breeders appear to be definitely at a disadvantage in this respect.

GENERAL DISCUSSION.

It is evident that a number of factors of limnological significance are involved in the ecology of anopheline larvæ and that many of them are interdependent.

For instance, introduction of silt into a clear water pond results in raising the temperature which greatly limits the growth of the larger plants, whereby the larvæ are deprived of food, shelter and protection and therefore disappear. Removal of vegetation gives rise, in many instances, to the growth of algæ like *Microcystis* and render the water turbid and thus unfavourable for Anopheles. Where algal growth does not result from the elimination of the larger plants, water movement at the surface is facilitated and unfavourable wave action is produced. Vegetation may also be eliminated by increasing or decreasing the depth of ponds according to the type of vegetation to be dealt with. In areas where *A. culicifacies* is the vector, the problem may probably be solved by encouraging the growth of aquatic plants.

There is the general observation that certain species appear to thrive in waters exposed to sunlight while others prefer overhanging shade or even complete cover from sunlight. Among the Malayan vectors, *A. umbrosus* favours shaded waters which are avoided by *A. maculatus* and the problem becomes complicated when, with the provision or removal of shade, one species is replaced by the other. The importance of overhanging shade for *A. minimus* has already been mentioned but Muirhead Thomson (1951) has also shown that a complete cover, which eliminates sunlight altogether, is inimical to the same species. The preference of *A. leucosphyrus* for shade is well known, though Colless (1956) has recently observed larvæ even in puddles fully exposed to sunlight. Among the sun-loving vectors, perhaps *A. culicifacies* and *A. sundaicus* are prominent examples. Whether this attraction to light or shade reflects the need for a lower or higher temperature or can be attributed to other peculiarities of the species concerned, is not clearly understood. Muirhead Thomson's (1951) experiments quoted above indicate that the preference for shade is inherent in *A. minimus* as the temperature does not rise immediately when the shade is removed but still the larvæ are observed to follow the movement of shade even into the mid-stream. Stream-breeding anophelines thus appear to possess an inherent demand, not only for current, but also for shade. Again, while most anophelines breed in natural waters, at least one vector species (*A. stephensi* type form) always prefers confined waters like masonry wells and other equally confined artificial water collections like household cisterns. These waters are usually cut off from sunlight and do not also support any vegetation or algal growth but larvæ may be found in fairly large numbers. It will be interesting to ascertain the source of food supply for these larvæ.

The population densities of many vector species vary from year to year, an extreme instance, as already stated, being *A. sundaicus*. The heavy prevalence of *A. culicifacies* during epidemic years has also been noted in several regions. Among other species too, variations do occur, though within narrower limits. Working in the Eastern Satpura Hill Tracts, Senior White and Adhikari (1940) observed that, even in those hyperendemic areas, the house densities of *A. fluviatilis* and *A. varuna* showed fluctuations which were more conformable to epidemic than to endemic conditions. One of the causative factors for these fluctuations may be the preponderance or otherwise of predators. If limnological (and other) factors are favourable for the multiplication of predators, the victims are necessarily reduced to small or negligible proportions. If, on the other hand, conditions are unfavourable for the predators, the victims are enabled to multiply to a great exetent. Quoting the case of *A. sundaicus* again, its presence in a particular year in exceptional

numbers may be due to the complete, or almost complete, absence of its predators, which, in this particular instance, may result from the abundant growth of *Potamegeton pectinatus*, greatly limiting their movements and thus cutting off their food supply. It is admitted that a heavy increase in the population of *A. culicifacies* is usually due to a rapid augmentation of its breeding places but, in this case also, the absence of predators in sufficient numbers is understood. In this view, a broader study of the various predators of mosquito larvæ seems to be necessary. The various insect populations in any breeding place are in dynamic balance with one another and any limitation placed on the propagation of one insect, is likely to set in motion a chain of circumstances which may effect, adversely or otherwise, many other insects in the same habitat.

It is well known that most, if not all, anopheline species breed in clean waters and that, if such waters are converted into foul waters, the breeding usually stops. This was supposed to be due to Anopheles, specially species like *A. maculatus* and *A. minimus*, not being able to develop their aquatic stages in foul waters but Muirhead Thomson (1951) showed that it was not so. He introduced eggs of *A. minimus* into foul water, where they developed in the normal way and ultimately became adult mosquitoes. He then observed that absence of *minimus* in herbage-packed streams was due to the adult females avoiding foul waters and preferring clean waters for oviposition. However, the question was not pursued by him with a view to ascertain whether the adult *minimus* females, emerging from foul waters, were susceptible to plasmodial infection. The critical experiments carried out by Russell and Mohan (1939; 1940) have, however, shown that vector adults, emerging from grossly polluted waters, were still capable of developing malaria parasites but Muirhead Thomson (1951) observed that, in these experiments, the pollution was of animal origin and that it still remained to be seen if pollution with vegetable organic matter—a much more widespread phenomenon in nature—would have any effect on the susceptibility of the adults. It is recognized that *C. fatigans*, which breeds in sullage waters, carries the infection of one form of filariasis, *viz.*, *Wuchereria bancrofti* but it has been observed by Senior White (1936) that the same species breeding in herbage-packed streams did not bite man and, therefore, could not be a vector of that disease. Venkat Rao (1955) has suggested that the inability of the salt water form of *A. sundaicus*, to be an effective malaria vector in Orissa, was probably due to the organic pollution of salt waters in that area. Commenting on the observation of Huxley (1942) that many adult characters (of insects) may well be non-selective *qua* adult characters, being consequential results of juvenile adaptations, Senior White (1948) stated as follows:—

> "This opens up the possibility that vectorial capacity, which cannot be selective as an adult character,.....may be brought about by larval adaptations that lead to the development in adult tissue of suitability (or otherwise) to plasmodial development. The possibility of biological control of malaria by sub-specific and not specific substitution has already been envisaged. If vectorial capacity in the adult is a resultant of larval adaptation to some particular habitat or pabulum, it is a step further towards such an achievement ....."...

This statement supports the view that, even if certain species continue to breed in an environment which is radically altered by pollution etc., malaria control

can still be achieved as the resultant adults may have lost their vectorial capacity.

This note, obviously, is not intended to be a treatise on applied limnology but has been prepared with a view to re-emphasise the importance of a study of the limnological factors involved in anopheline ecology and to suggest that, in those malaria eradication projects where "antilarval works" have to be carried out, the malaria teams be strengthened by the addition of limnologists, whose services may prove to be as necessary as those of engineers and entomologists.

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HÆMATOLOGICAL STUDIES IN SIMIAN MALARIA.

Part I. Blood picture in normal *M. mulatta mulatta* and those infected with *P. knowlesi* infection.

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In India, anæmia of varying degrees and types is wide-spread, particularly amongst the poorer section of the population. Many workers had observed that malnutrition, coupled with tropical diseases of bacterial and parasitic infections, was primarily responsible for the condition (Napier and Das Gupta, 1937; Macdonald, 1939; Thomson, 1944; Ramalingaswami and Patwardhan, 1949). But Hare (1940) was of the opinion that in Assam anæmia was mainly due to long continued dietary conditions. During their studies on a large number of cases with hepatomegaly, anæmia and symptoms of malnutrition, Girdwood (1948) and Passmore (1949) observed that majority of their patients had had attacks of malaria.

However, ordinarily malaria or malnutrition, each by itself, can and does produce different types and degrees of anæmia. Destruction of a large number of erythrocytes on account of invasion by plasmodia is common knowledge, but, by some process not fully understood, quite a large number of unparasitized cells are also destroyed (Maegraith, 1948). The degree of anæmia, due to malaria, depends on a variety of factors like age of the host, species of parasite, duration and severity of infection. For example, anæmia is often severe in children. Again according to some observers, it is very pronounced in *P. falciparum* in man and *P. knowlesi* in monkeys. Ordinarily in *P. vivax* infection, the degree of anæmia is usually less than that seen in *P. falciparum*, but in chronic infection due to repeated relapses of *P. vivax*, severe anæmia is not of infrequent occurrence. But, since vast tracts of the country are malarious and malnutrition is rampant, it is likely that anæmia, observed in a large section of the population, could be due to a number of factors, particularly malaria and malnutrition.

It is, therefore, only natural that hæmatological findings during clinical studies should quite often be influenced by the above factors, especially when dealing with the poorer section of the population residing in malarious districts. Further, it may be difficult to determine as to what extent and in what manner

one or the other factor is responsible for alteration in the blood picture in such cases. In view of these the need for detailed investigations may well be realised. Since such studies in human subjects present quite a number of problems, observations on experimental animals would appear to be the obvious course.

The present paper records the findings on the hæmatological studies undertaken in *M. mulatta mulatta* (rhesus monkeys) kept under various dietary regimes and infected with simian malaria (acute and chronic). Parasite counts were made for proper appraisal of the results. Since these observations cover a wide field, investigations undertaken have been recorded in three parts. Part I refers to the studies on the blood picture in normal *M. mulatta mulatta* (kept on standard diet) and those infected with *P. knowlesi* (which runs a fatal course in these animals). Part II deals with similar investigations during acute and chronic stages of *P. cynomolgi* infection in monkeys kept under standard and low protein dietary conditions. Although, urobilinogen estimations in urine and faeces were carried out simultaneously along with the investigations indicated in Parts I and II above, for convenience the findings have been recorded separately in Part III.

MATERIALS.

Host.—Wild-caught rhesus monkeys, obtained from the neighbourhood of Delhi, were utilised for these investigations. On their arrival in the laboratory, they were weighed, numbered and kept in cages. As some animals suffer from tuberculosis in nature, all the animals were subjected to tuberculin test as a routine procedure (Jaswant Singh *et al.*, 1951; Nair and Ray, 1954). Only those monkeys showing negative reactions were accepted for experiments. The monkeys were also subjected to repeated blood examination for evidence of any protozoal infection.

In the present series (Part I), the animals were fed on a standard diet provided to the monkeys at the Malaria Insititute of India. The diet consisted of wheat, gram, vegetables or fruit, and the caloric value ranged between 800 and 900.

Parasite.—A strain (Nuri) of *P. knowlesi*, recently isolated in *M. mulatta* from Kra monkeys (*M. irus*) found in Nuri Valley in Malaya, was used (Jaswant Singh, Ray and Nair 1953; Edeson and Davey, 1953). The strain is maintained in the laboratories of this Institute by blood passage. It has a periodicity of 24 hours sporulation occurring at about 4 p.m. daily. It is extremely virulent to the *simian* hosts which succumb within a few days, once patent infection is established. An Ehrlich's eye piece, adjusted to count 100 erythrocyte per field of thin film under the oil immersion objective, was used for parasite count. Before recording a negative report, at least 50 fields of the thick film were examined. All smears were stained with J.S.B. stain (Jaswant Singh and Bhattacharji, 1944).

METHODS.

The hæmatological studies included enumeration of total erythrocytes, estimation of hæmoglobin, determination of volume of packed cells, mean corpuscular volume, mean corpuscular hæmoglobin concentration, reticulocyte count, and the estimation of urobilinogen in faeces and urine (described in Part III).

Enumeration of erythrocytes.—Total erythrocyte count was done with a Thoma-Zeiss haemocytometer in the usual manner.

Estimation of hæmoglobin.—Fishers' electro-haemometer (Fishers Scientific Co., Pennsylvania, U.S.A.) was used for estimation of hæmoglobin. Blood was collected from the ear vein in the haemometer pipette up to the mark below the bulb, then N/10 hydrochloric acid was drawn into it (the dilution being 1:251). The diluted sample was left either in the pipette or transferred to Fisher-photometric cell and kept for at least 30 minutes. After necessary adjustments, the standardising coil containing distilled water was inserted into the instrument. Then bringing in the pointer to B, distilled water tube was replaced by photo electric cell containing the sample. The reading was made direct from the scale in gm. per 100 c.c. of blood.

Determination of volume of packed cells.—Wintrobe's haematocrit tubes were used for the purpose. Five c.c. of blood, obtained from the dorsal leg vein of the monkey, was placed in the tube containing 4 mg. solid potasium oxalate and 6 mg. solid ammonium oxalate. Blood was then transferred to haematocrit tube with the help of a long drawn-out pipette which was lowered to touch the bottom of the tube. Care was taken to avoid introduction of air bubbles. The tube was filled up to the mark (mark 10 to the right side), allowed to stand vertically for one hour and then centrifuged at 2500-3000 r.p.m. for 30 minutes (Wintrobe, 1951). The volume of packed cells (V.P.C.) was read directly (in percentage).

MEAN CORPUSCULAR VOLUME (M.C.V.) AND MEAN CORPUSCULAR HÆMOGLOBIN CONCENTRATION (M.C.H.C.).

From all the three data mentioned above, i.e., total erythrocytes, V.P.C. and hæmoglobin estimation, calculations (as shown below) were made to determine M.C.V. and M.C.H.C. (Napier and Das Gupta, 1942).

$$(a) \quad \text{M.C.V.} = \frac{\text{V.P.C.} \times 10}{\text{R.B.C. (in millions)}}$$

$$(b) \quad \text{M.C.H.C.} = \frac{\text{Hb.} \times 100}{\text{V.P.C.}}$$

RETICULOCYTE COUNT.

Clean slides were first dipped in one per cent brilliant crystal blue solution (in absolute alcohol) for a few minutes and allowed to stand on racks. When dry, a drop of blood, from the ear vein of a monkey, was spread uniformly over the slide. The slides were then stained with J.S.B. stain (Jaswant Singh and Bhattacharji, 1944). For counting, an Ehrlich's eyepiece adjusted to count 100 erythrocytes per field, was used. One hundred such fields were examined and the number of reticulocytes per 10,000 erythrocytes thus obtained was recorded.

EXPERIMENTAL RESULTS.

(*A*) STUDIES ON UNINFECTED *M. MULATTA MULATTA*.

During preliminary studies, the hæmoglobin level in *M. mulatta mulatta* showed that as a rule the wild-caught animals had lower hæmoglobin content than those kept under standard diet for few weeks. A brief review of the findings is indicated below.

In 37 wild-caught monkeys, the hæmoglobin level, carried out soon after they were received and subsequently for three consecutive weeks, indicated a mean average rise by 1·8 mg. per 100 c.c., which remained fairly constant for weeks under the normal dietary conditions. In view of this, studies were undertaken in another lot of 100 monkeys of all age groups at the end of four weeks after they were received at the laboratories. The range of hæmoglobin content was found to vary between 7 to 13·5 gm. per 100 c.c. blood, though in 77 per cent of them it ranged between 8 and 11 gm. The mean hæmoglobin reading in respect of all animals was 9·7 gm. per 100 c.c.

Out of the 137 monkeys indicated above, 36 were between 11 and 36 months old and were selected for detailed studies. The particular range was selected because most types of investigations are carried out in monkeys of this age group. Age was determined by computation of sitting height and body weight indicated earlier by Ray (1955).

The hæmoglobin level of these 36 monkeys varied between 8 and 10·8 gm. (mean 9·27 gm.). Out of these, 16 were males and the mean hæmoglobin level read was 9·15 gm., while in the 20 females it was 9·4 gm. The volume of packed cells (V.P.C.) was estimated to be 39 per cent (mean) with range between 35 to 46 per cent. The total erythrocyte count showed a range between 4·2 and 5·4 millions per c.mm. with 4·88 million as the mean count.

From the above data, the M.C.V. and M.C.H.C. were computed as 80 c.μ and 24 per cent respectively.

The reticulocyte count in 32, out of the 36 monkeys, showed a range from 0 to 43 per 10,000 (0 to 0·43 per cent).

Since the investigations were carried out in detail and on a fairly large number of monkeys within limited age groups, the present findings may be taken as standard for normal monkeys obtained from the neighbourhood of Delhi.

(*B*) STUDIES ON MONKEYS INFECTED WITH *P. KNOWLESI*

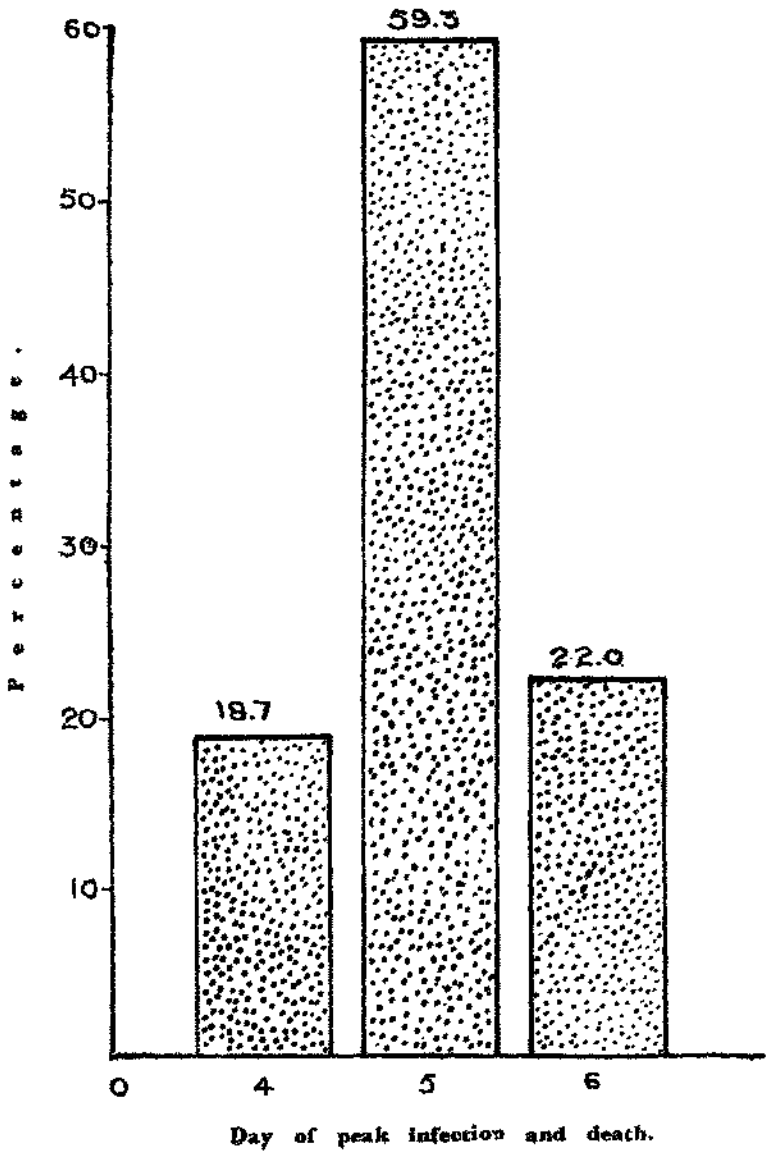
As indicated earlier, parasitological studies were also undertaken side by side, for proper appraisal of the hæmatological findings. For convenience, the former data is recorded first.

(*I*) *Parasitological findings.*—A group of 32 monkeys (age group 12 to 34 months) were inoculated intravenously each with 5×10^6 parasitized cells. Patent infection was established within a few hours to Day-1 (Day of inoculation being 0). In seven animals peak parasitæmia reached on Day-4, in 19 (about 60 per cent) it was on Day-5, and in the rest 6 it was Day-6 (Graph 1). The

course of parasitæmia in 10, out of the 32 selected at random, has been shown in Graph 2. The intensity of infection at peak varied from 48 to 96 per cent cell infection and the proportion of rise during the last 2 to 3 days was found to be 12, 50 and 100-fold as compared to the density during the first day or two (Table I).

GRAPH I.

Percentage of M.mulatta *which showed peak infection and died on different days of infection* (P. knowlesi).



GRAPH 2.

Course of infection in P. knowlesi *in ten* M. mulatta mulatta.

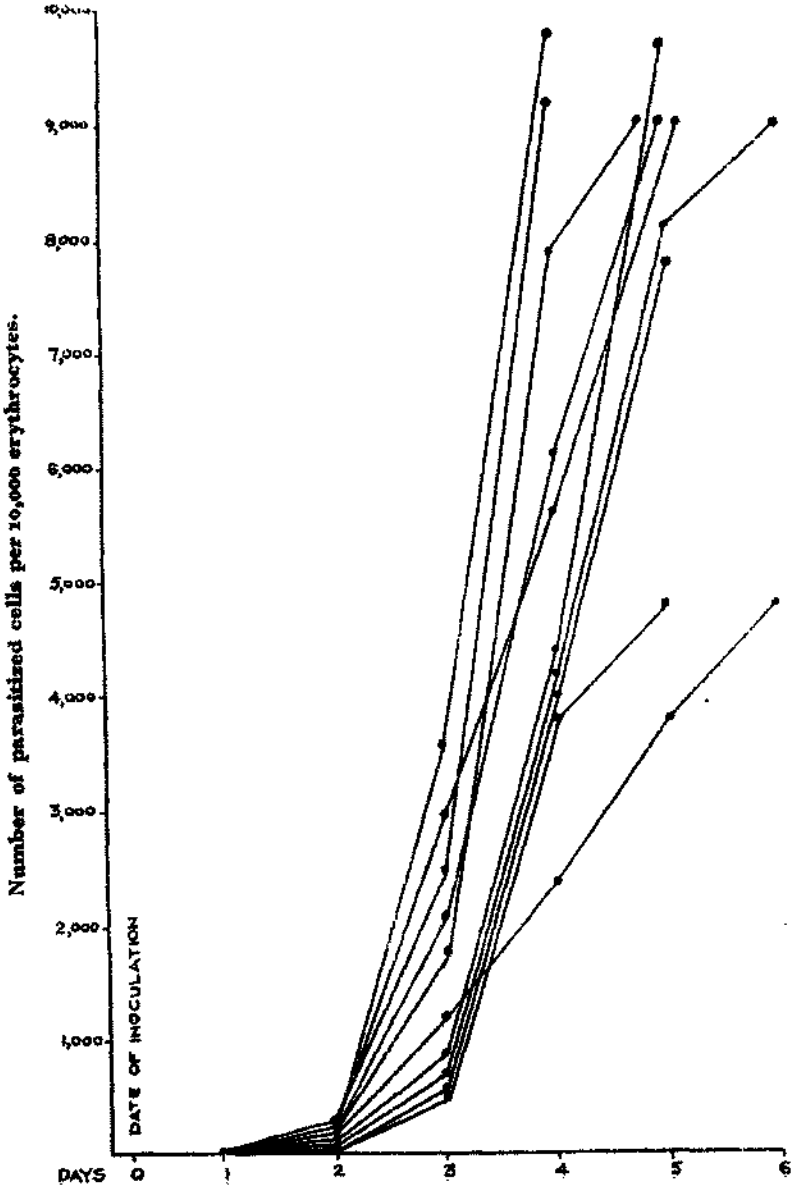


TABLE I.

Number of parasites per 10,000 erythrocytes on days after infection.

Day of inoculation is Day-0.

Monkey number	Days after inoculation					
	1	2	3	4	5	6
4152	0	200	2,100	7,900	9,000	
4394	4	40	900	5,200	8,700	
4416	0	150	2,500	6,600	9,200	
4502	0	18	230	2,700	9,000	
4613	5	45	800	4,500	8,000	
4647	0	100	900	4,400	9,800	
4705	0	30	500	2,400	9,800	
4711	0	300	3,000	5,600	9,000	
4759	0	20	200	2,300	8,900	All died by Day-6
4771	0	15	720	3,900	6,400	
4790	5	250	1,800	6,100	9,000	
4794	0	35	500	4,000	7,800	
4857	0	17	690	4,700	9,300	
4865	0	10	600	1,900	7,200	
4864	0	40	385	3,720	7,800	
4868	0	120	1,680	3,000	6,800	
4877	2	5	600	3,800	4,800	
4893	2	125	750	2,800	8,800	
4902	1	60	650	5,600	9,000	
Total	19	1,580	19,505	80,220	1,58,300	
Mean	1·0	83·1	1,026·5	4,222·2	8,331·5	
Per cent of erythrocytic infection ...	0·01	0·83	10·26	42·22	83·31	
Degree (fold) of increase compared to Day-2 ...	...	1	12·3	50·8	100·0	

(2) *Hæmatological findings.*—Estimation of hæmoglobin, total erythrocyte count etc. were undertaken in 12 out of the 32 monkeys on which parasitological

TABLE II.

Hæmatological findings in 12 M. mulatta mulatta *infected with* P. knowlesi.

Monkey number	Hæmoglobin in gm. per 100 c.c. blood						Erythrocytes (millions)						V.P.C. (per cent)					
	Initial	Days after infection					Initial	Days after infection					Initial	Days after infection				
		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5
4857	9·2	8·5	7·8	8·0	7·6	6·2	4·21	4·07	3·86	3·29	2·17	1·38	35	35	34	33	25	23
4864	8·8	8·5	8·0	7·8	7·4	5·5	5·96	6·22	6·10	4·46	3·15	2·09	32	33	31	28	28	20
4868	9·6	8·5	8·5	9·5	7·6	6·1	4·12	4·57	4·09	3·48	2·35	1·37	42	41	35	30	29	26
4877	8·4	8·2	8·0	7·4	7·9	6·2	5·6	5·91	4·10	3·67	3·21	2·55	31	32	26	26	22	24
4790	8·8	8·0	7·0	7·0	6·0	4·5	5·23	4·79	4·21	3·85	3·56	2·30	36	35	29	32	26	14
4794	9·6	8·8	8·0	6·7	5·4	4·2	4·71	4·80	4·70	4·30	3·71	2·60	40	37	35	32	27	15
4863	10·5	9·5	8·5	6·8	5·9	5·0	4·92	4·59	4·12	3·81	3·32	2·30	44	41	35	28	24	14
4902	10·4	10·2	9·2	9·6	8·2	6·11	5·10	4·97	4·65	3·60	2·57	1·8	42	42	38	31	24	16
4893	9·6	9·0	9·5	8·5	6·4	4·7	4·47	5·01	4·15	2·93	3·13	2·75	37	36	35	31	23	17
4771	10·4	10·0	8·5	7·4	5·8	4·0	5·22	4·97	4·12	2·79	3·01	1·92	43	42	34	27	20	16
4705	9·5	8·8	7·8	7·3	6·5	4·0	4·85	4·73	3·75	3·21	2·8	2·62	40	41	35	30	22	18
4759	9·8	9·5	8·6	7·1	6·9	5·8	5·06	4·65	4·38	3·95	3·02	3·03	42	40	32	24	18	14
Total ...	114·6	107·5	99·4	93·1	81·6	62·4	59·45	59·28	52·33	44·34	36·00	26·71	464	455	399	352	288	218
Average ...	9·55	8·9	8·2	7·7	6·8	5·2	4·95	4·94	4·35	3·69	3·00	2·22	38·6	37·9	33·2	29·3	24·0	18·2
Percentage*	100	92·18	85·85	80·61	71·19	54·4	100	99·78	87·87	74·53	60·6	44·84	100	98·16	85·98	75·88	62·16	47·13

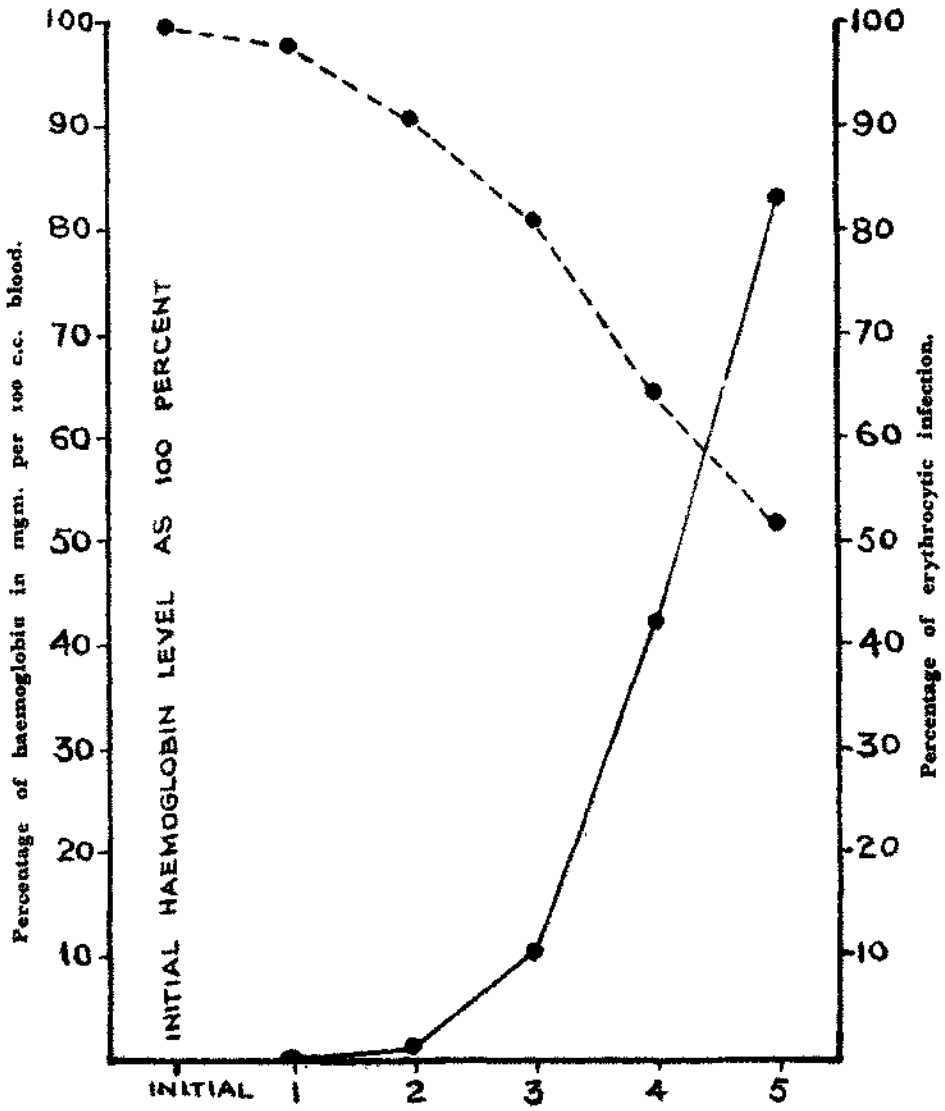
*Initial reading has been taken as 100 per cent.

studies were recorded earlier. In all cases, peak density was attained on Day-5 and they died either the same day or, as in two instances, on the following day but further sporulation had occurred. Details are indicated below.

(*a*) *Estimation of hæmoglobin.*—The initial mean hæmoglobin level, as observed on the day of inoculation (just prior to), was observed to be 9·55 gm. per 100 c.c. (Table II). During the subsequent days, the level was found to fall slowly at first till it reached 5·2 gm. (mean) at the terminal stage. The hæmoglobin level had, therefore, gone down to 54 per cent of the initial; that is, during the course of five

GRAPH 3.

Rise in infection and concomitant fall in hæmoglobin.



days there was a reduction by 46 per cent. The meteoric rise in parasitæmia, as mentioned earlier, and a similar rate of fall in hæmoglobin have been depicted in Graph 3.

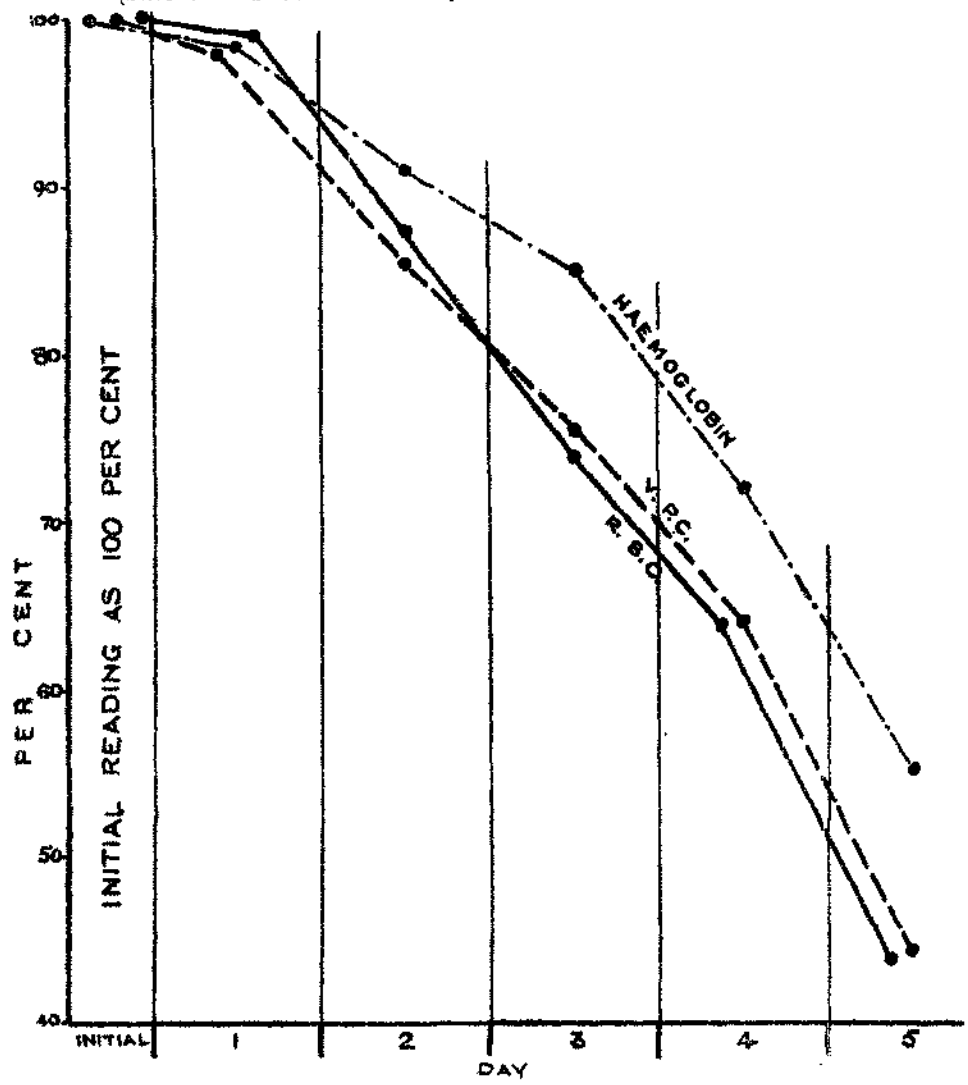
(*b*) *Volume of packed cells.*—From Table II, it would be evident that the mean initial V.P.C. was 38·6 per cent and by Day-6 it was lowered by 18·2 per cent, that is by about 50 per cent of the initial level.

GRAPH 4.

P. KNOWLESI INFECTION.

Percentage of fall in hæmoglobin, V.P.C., and R.B.C. (mean of 12 monkeys).
Dose of inoculum 5×10^6 *parasitized cells.*

(Selection made from those monkeys which died on Day 5 after inoculation).



(*c*) *Total erythrocyte count.*—The mean total erythrocyte count on Day-0 was recorded to be 4·95 million per c.mm. Along with rise in parasitæmia, there was also a rapid fall in the erythrocyte count so much so that on Day-5 it was reduced to 44·84 per cent of the original level (Table II), i.e., there was a reduction by about 55 per cent. From Graph 4, it would be noted that the rates of fall of erythrocytes and V.P.C. seemed to run parallel.

(*d*) *M.C.V. and M.C.H.C.*—From the data presented above, the initial M.C.V. was recorded to be 78 c.μ (mean) and the M.C.H.C. as 24 per cent (mean). During the last three days, there appeared to be slight rise in both the findings as indicated in Table III.

TABLE III.

Mean data on hæmatological studies on 12 M. mulatta mulatta *infected with* P. knowlesi.

Day of infection	Mean haemoglobin (gm.)	Erythrocytes (millions.)	V.P.C. (per cent.)	M.C.V. (c.μ.)	M.C.H.C. (per cent.)
Initial	9·55	4·95	38·6	78	24
1	8·2	4·94	37·9	76	23
2	8·2	4·35	33·2	76	24
3	7·7	3·49	29·3	84	26
4	6·8	3·00	24·0	80	28
5	5·2	2·22	18·2	82	28

(*e*) *Reticulocyte count.*—The number of reticulocytes counted once a day in ten of the monkeys showed up to Day-3 a variation from 0 to 85 per 10,000 erythrocytes. Subsequently, the range varied from 0 to 37. However, it was observed that the mean reticulocyte count was always somewhat higher than observed on Day-0. The highest count of 25 (0·25 per cent) was recorded on Day-3.

From blood smears obtained every hour for five hours from 5 out of the 10 monkeys, it was observed that comparatively fewer parasites invaded the reticulocytes whereas the majority of them were found to be inside mature erythrocytes. For every 100 infected cells counted, the number of reticulocytes was found to be between 3 to 16. Thus the ratio of reticulocytes to mature erythrocytes was found to be about 1:32 to 1:5.

DISCUSSION.

During the current investigations, observations have been made in a fairly large series of infected *M. mulatta mulatta*, out of which detailed parasitological studies have been recorded in 32 animals. The infection has been found to be so severe that in all cases death has been the inevitable result (between the 4th and 6th day after infective inoculation). The day to day parasitic rise can be considered as

phenomenal. Erythrocytic invasion at about the time of death has been recorded to be as high as 96 per cent and from this the extent of loss of erythrocytes, and of hæmoglobin, would be clearly evident. The course and intensity of infection have great degree of similarity to that seen in *P. gallinaceum* infection in chicks (Jaswant Singh, Basu and Ray, 1952) or *P. berghei* in mice (Ramakrishnan and Satya Prakash, 1950) and are not altogether dissimilar to those seen in some fatal infections due to *P. falciparum* in man.

During the present series of studies, it has been observed that the level of hæmoglobin rises quickly in freshly caught monkeys when they are fed on standard diet. During the present investigations, the increase has been found to be by about 1·8 gm. (mean) per 100 c.c. of blood within 2 to 3 weeks. Thereafter, the level seems to remain static. It is, therefore, obvious that in nature the local monkeys live on a comparatively poorer diet than those maintained in these laboratories. The nutritional status of monkeys has been dealt separately in an earlier communication by Jaswant Singh and Ray (1957). In view of these observations, all the animals have been conditioned prior to undertaking any investigation so that correct appraisal could be made of any subsequent changes due either to parasitic infection, nutritional status or both.

The overall second week (in captivity) hæmoglobin level of 137 animals (of all age-groups) has been found to be 9·7 gm. per 100 c.c. of blood with a range between 7 to 13·6 gm. However, in a group of 36 animals of younger age-group (ranging from 11 to 36 months), the hæmoglobin has been shown to be 9·4 gm. of females and 9·15 gm. for males, the mean of the two groups, male and female, being 9·27 gm. The volume of packed cells in the same group of monkeys has been observed to vary between 35 and 46 per cent with the mean value of 39, whereas the mean erythrocyte count (in 30 out of the 36 animals) has been recorded as 4·88 millions. Therefore, the M.C.V. and M.C.H.C. may be calculated as 80 c.μ. and about 24 (23.7) per cent respectively.

Shukers *et al.* (1938) and Mazumdar and Das Gupta (1944) have also undertaken similar investigations on the same species of monkeys, and Rao and Rao (1940) on *M. sinicus* (a long tailed South Indian species of monkey). For ready appraisal, the results have been summarised as shown below:—

	Present author	Shukers *et al.* (1938)	Mazumdar and Das Gupta (1944)	Rao and Rao (1940)	
	M. mulatta mulatta			*M. sinicus*	
				Group A	Group B
Haemoglobin ...	9·27	12·2	13·59	14·1	14·1
Erythrocytes ...	4·88	5·2	5·76	6·3	5·6
V.P.C. ...	39	40	44·98	43·7	49·7
M.C.V. ...	80	77	78·5	70·5	87·6
M.C.H.C. ...	23·7	30·5	30·4	32·5	27·5

The striking difference appears to be in the hæmoglobin level, as in the present series it is somewhat less than seen in the same species as reported by earlier workers. But, it should be borne in mind that in their series, the monkeys have been taken from all age-groups as against only the younger ones in the present series. Taking into account all age-groups in the present series, the range has been observed to vary between 7 and 13 gm. Further, in the present series a fairly large number of animals have been studied and also photoelectric cell (electro-haemometer) has been used instead of naked eye observations (Sahli's). It should, therefore, be assumed that the present readings are generally accurate.

As regards the findings on the total erythrocyte count and V.P.C., there do not appear to be much difference in the present series and those presented by Shuker *et al.* (1938), although those obtained by Mazumdar and Das Gupta (1944) appear to be slightly different.

Inspite of these differences, the M.C.V. readings in all three groups appear to be more or less similar. But findings on the M.C.H.C. have been observed to vary widely. This is attributed to the differences in the hæmoglobin readings in the different groups. The differences observed in the findings by Rao and Rao (1940) are obviously due to different species of animals used.

Hynes (1944), Hutchison and Hunter (1949) and others have emphasized the importance of the three main measurements in anæmia, namely, the hæmoglobin, M.C.V. and M.C.H.C. The hæmoglobin readings indicate the degree of anæmia, the M.C.V. shows whether the anæmia is macrocytic, normocytic or microcytic, and the M.C.H.C. indicates whether it is orthochronic, hypochronic or hyperchronic. In view of these, special attention has been paid to these findings during the current investigations.

In *P. knowlesi* infection in *M. mulatta mulatta* kept under standard diet, the fall in hæmoglobin has been noted to be acutely steep as within five days the mean hæmolgobin level has been found to drop from 9·55 to 5·2 gm. per 100 c.c., i.e., a reduction by 46 per cent. Taking all factors into account, the M.C.V. readings indicate that the anæmia is of normocytic type, though there is a slight tendency towards macrocytosis during the final phase of the infection. This has also been confirmed recently in *P. berghei* infection in white mice (Ray, 1954). According to Wintrobe (1951), anæmia is usually normocytic in malaria but when blood destruction is very rapid it may become slightly macrocytic. Whitby and Britton (1953) are of the opinion that in malaria, anæmia may be of any hæmatological type, including macrocytic. According to many, the tendency towards macrocytosis is purely physiological phenomenon and is caused by anoxaemia on account of rapid destruction of blood.

It would then be observed that in acute infection of *P. knowlesi* the anæmia is progressively rapid, and during the terminal stage it is of severe degree. The type of anæmia is normocytic though there is a slight tendency towards macrocytosis.

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HÆMATOLOGICAL STUDIES IN SIMIAN MALARIA.

Part II. Blood picture in monkeys during acute and chronic stages of *P. knowlesi* infection.

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In Part I of this communication (Ray, 1957*a*), the normal values on the blood picture of *M. mulatta mulatta* were indicated. It was also reported that when such animals were infected with virulent strain of *P. knowlesi*, the course of infection was extremely rapid irrespective of the dietary condition of the host and there was severe degree of anæmia. All such monkeys died within a few hours after the last sporulation.

During the present investigations, the effect of a benign type of plasmodium, like *P. cynomolgi*, in the same species of monkeys was studied during both the acute and chronic stages of infection and also under various dietary conditions. For proper appraisal of the findings, parasitological studies were also taken up simultaneously.

The experimental studies were grouped under several series. Series I records observations on the parasitological and hæmatological findings in *P. cynomolgi* infection in *M. mulatta mulatta* during acute and chronic stages of infection at a time when the monkeys were under standard dietary conditions. Under Series II, observations were recorded on the blood picture of normal monkeys kept under various dietary conditions while Series III records the findings on the blood picture in monkeys kept under various dietary conditions and at the same time infected with *P. cynomolgi*.

MATERIALS AND METHODS.

The materials and methods used were similar to those indicated in Part I of this communication, except for the following:—

Diet of the host.—During these experiments, some monkeys were fed on a standard diet while others received low protein diet consisting of sago, jaggery, sweet potatoes and bananas. The caloric values of such a diet were maintained at the same level as the standard diet (isocaloric low protein diet) in some series

while in others they were intentionally lowered (low caloric low protein diet) by reducing the quantity of food proportionally. The sago diet was introduced through a stomach tube.

Parasite.—A strain of *P. cynomolgi*, originally isolated by Sinton and Mulligan (1932), was used for these investigations. As a rule, the infection rises progressively after inoculation but the peak period and density vary to a great extent according to the intensity of the challanging doses. The peak is usually followed by crisis and then the infection runs a chronic course. This stage is characterized by latent periods punctuated at intervals of recrudescences. At the time of crisis, the monkeys appear extremely pale and weak. Occasionally a few of them succumb at this stage even though no demonstrable parasitic infection could be found.

EXPERIMENTAL RESULTS.

SERIES I (UNDER STANDARD DIETARY CONDITION).

(A) PARASITOLOGICAL STUDIES.

1. Acute stage.

Various grades of challanging doses ranging from 0·1 to 100 million parasitized cells per kg. body weight were injected intravenously to 43 *M. mulatta mulatta* (Table IV)*. The groupings have been shown as per Table IV from which it would be noted that under the lowest dosage schedule, the prepatent period was the longest (four to five days) whereas under the highest, patent infection was established within a few hours. The results were somewhat intermediate when the infective doses were 5×10^6 or 50×10^6.

TABLE IV.

Dose of inoculum, prepatent period, intensity of infection and survival rate in monkeys.

Serial number.	Dose of parasitized cells per kg. body weight	Number of monkeys	Prepatent period (days)	Peak parasitæmia		Survival rate, per cent
				Per cent cell infection (mean)	Days after inoculation (mean)	
1.	$0·1 \times 10^6$	5	4 – 5	7·80	9 – 11	80
2.	$0·5 \times 10^6$	5	2 – 3	10·34	9 – 10	80
3.	$1·0 \times 10^6$	5	2 – 3	10·37	8 – 10	80·0
4.	$2·5 \times 10^6$	7	1 – 3	10·58	8 – 9	71·4
5.	$5·0 \times 10^6$	10	0 – 2	13·5	7 – 8	70
6.	50×10^6	5	0 – 1	18·2	6 – 8	60
7.	100×10^6	6	0	23·00	5 – 8	50

* Tables I to III are included in Part I of this communication (Ray, 1957*a*).

As to the course and intensity of infection, the rise in parasitæmia was slow under the lowest infective dose and the mean peak density of 7·8 per cent was attained in 9 to 11 days. On the other hand, with 50 or 100×10^6 the rise in parasitæmia was progressively rapid and the peak density of 18·2 to 23 per cent was attained within a period of five to eight days. With an infective dose of 5×10^6, the results were somewhat intermediate, the peak being attained in seven or eight days and the density reached up to 13·5 (mean) per cent. In all cases, the stage of crisis was marked by sudden fall in parasitæmia and subsequently complete parasitic clearance from the peripheral circulation for varying periods.

The maximum mortality of 50 per cent was observed under the highest dosage schedule, and it was the lowest under the first three schedules varying from 0·1 to 1×10^6. Here again, the rate was intermediate under 2·5 and 5×10^6 dosage schedules.

2. Chronic stage.

Follow-up for studies on the recrudescence pattern was made on 15 of the 43 monkeys, selected at random. It was observed that after the initial clearance of parasitæmia, recrudescences occurred in all monkeys at intervals of one to three weeks up to a period of two to three months. The frequency and the intensity of infection were not to be found dependent on the initial infective dose. In all cases, the parasitic density was usually of a low order and remained patent for three to five days. Subsequent recrudescences up to an observation period of 30 weeks, in 12 out of the 15 monkeys, were found to be few and far between occurring at intervals of four to eight weeks and lasting for two to three days only. Splenectomy resulted in heavy parasitæmia within a week and death usually occurred soon thereafter. All animals died after a week to ten days.

It would be observed that while the prepatent period varied inversely to the dose of inoculum, there was a direct correlation between dose of inoculum, the course and the intensity of acute infection and also the mortality rate. A dose of 5×10^6 seemed to show an intermediate reaction between the various dosage schedules and, therefore, this particular infective dose was chosen for hæmatological investigations. Recrudescences occurred in all cases and the pattern appeared to be more or less similar in all groups irrespective of the infective dosage schedules.

(B) HÆMATOLOGICAL STUDIES.

Hæmatological studies were undertaken in six *M. mulatta mulatta* infected with a challanging dose of 5×10^6 parasitized cells per kg. body weight. The monkeys weighed between 7 and 9 lbs. and the peak parasitæmia attained was on Day 8 (mean).

1. Acute stage.

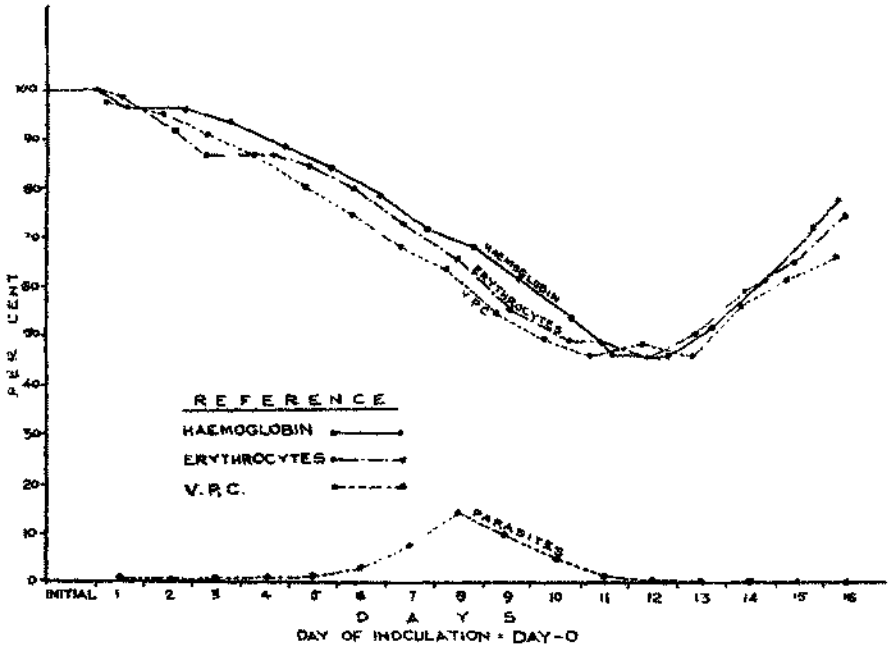
(*a*) *Hæmoglobin, total erythrocyte count and volume of packed cells.*—The initial mean hæmoglobin level was estimated to be 9·4 gm. per 100 c.c. of blood on the day of inoculation. For the subsequent four days there was a very gradual fall in

the hæmoglobin level but subsequently it was rapid and the lowest level of 4·5 gm. was attained on Day 11 (day of inoculation being Day 0). Thus there was a reduction by 53 per cent. More or less the same low level was maintained for the following two days, thus indicating a trough like depression as shown in Graph 5. Thereafter the rise in hæmoglobin level was rapid and by Day 16 it had reached 66 per cent of the initial level.

Against an initial V.P.C. (Volume of Packed Cells) reading of 30 per cent, the lowest reading of 19 per cent (reduction by 51 per cent) was observed on Days 11 and 12. Thereafter there was rapid rise till the reading had reached 69·5 per cent of the former level on Day 16 (Graph 5)*.

GRAPH 5.

The course of infection due to P. cynomolgi *in* M. mulatta mulatta *and readings in haemoglobin, total erythrocytes and V.P.C. during acute phase of infection.*



The trend of fall in the total erythrocyte count reading was somewhat similar. The lowest level of 2·35 million per c.mm. was reached on Day 12. The initial mean reading being 4·94, the reduction was calculated to be to the extent of 52·6 per cent.

Thus the maximum reductions, in respect of all the three readings, were over 50 per cent of the initial level reached in four to six days after peak parasitæmia, and anæmia was severe.

* Graphs 1 to 4 are included in Part I of this communication (Ray, 1957*a*).

(*b*) *M.C.V.* (*Mean Corpuscular Volume*) *and M.C.H.C.* (*Mean Corpuscular Hæmaglobin Concentration*).—From the above data the initial M.C.V. and M.C.H.C. were calculated to be 79 c.μ. and 23 per cent respectively. The overall (mean) M.C.V. from Day 1 to Day 16 was observed to be 81 c.μ. and the M.C.H.C. was 23 per cent. However, it was observed that there was some degree of rise in M.C.V. shortly before and after peak parasitæmia, reaching a maximum level of 87 c.μ. on Day 9. The M.C.H.C. during this period varied from 20 to 24 per cent.

Thus during the acute phase of *P. cynomolgi* infection there was severe degree of anæmia and since there was no significant overall variations in the M.C.V. and M.C.H.C. readings, the anæmia appeared to be of normocytic orthochronic type.

(*c*) *Reticulocyte count.*—Observations on reticulocyte count showed certain interesting features. The mean initial reading was 5·8 per 10,000 erythrocytes. Later, there appeared to be a slight degree of rise varying from 6·2 to 11·4 up to Day 9. But from Day 10, there was an abrupt rise in reticulocytes and the peak was reached on Day 14. The mean readings on these days were 86·4, 157·4, 303·4, 548 and 860·6 per 10,000 erythrocytes. This period coincided with the stage of crisis. Thereafter there was a marked fall and final reading on Day 23 was 6·4.

The number of reticulocytes increased suddenly after the peak parasitæmia had reached, and the high readings were maintained for about 10 to 11 days.

Examination of blood smears, taken at interval of two hours for a period of eight hours before and after sporulation in three monkeys, showed that the proportion of parasitized to unparasitized reticulocytes varied between 1 : 8 and 1 : 5. Majority of the parasites seemed to have an affinity for mature erythrocytes.

2. Chronic stage.

During the chronic phase, hæmoglobin readings were taken weekly in respect of five monkeys up to a period varying from six to ten weeks. The readings fluctuated between 7·1 and 8·3 gm. per 100 c.c. against the normal initial reading of 9·7 gm., thus indicating that complete recovery was not attained at least up to the tenth week, possibly on account of periodic recrudescences during the latent phase.

During this period, no significant rise in reticulocytes could be observed except of a transient and mild nature immediately after a recrudescence of moderate severity.

SERIES II (UNDER DIFFERENT DIETARY CONDITIONS).

Hæmatological studies were undertaken in 34 normal monkeys kept under various dietary conditions as indicated below.

(*i*) *Isocaloric standard diet.*—Observations were made on five monkeys for eight weeks and these formed the control group. It was observed that the level of hæmoglobin fluctuated between 9·0 and 9·7 gm. per 100 c.c. as compared to the initial level of 9·2 gm. The variation in V.P.C. was between 35 and 38 per cent

against the initial reading of 36 per cent. During the same period, the total erythrocyte count varied from 4·1 to 4·65 millions per c.mm. against the initial level of 4·38 millions.

(*ii*) *Isocaloric high protein diet.*—Five monkeys were subjected to such investigations for a period of six weeks and two out of the five up to a period of 14 weeks. Against an initial level of 8·7 gm., the hæmoglobin level was found to be 10·2 gm. at the end of six weeks. The V.P.C. also increased to 44 per cent from the initial reading of 37 per cent. The increase in total erythrocyte count was found to be 5·11 millions against 4·28 millions initial level.

Two monkeys, which were followed up to the end of 14 weeks, showed a hæmoglobin level of 12·6 gm. (mean). The erythrocyte count and V.P.C. were recorded as 5·65 millions and 46 per cent respectively. Thus the animals under high protein diet showed better results than those under standard diet.

(*iii*) *Isocaloric low protein diet.*—Observations on 16 monkeys were made up to ten weeks. Thereafter these were followed up in ten monkeys up to fourteenth week, and in eight up to the end of eighteenth week. The data have been presented in Table V. For brevity, only the findings of alternate weeks have been incorporated in the table.

TABLE V.

Hæmatological data on monkeys kept on isocaloric low protein diet.

Week	Mean haemoglobin in gm. per 100 c.c. blood	Mean total erythrocytes in millions	Mean volume of packed cells (Per cent)	Mean M.C.V. (c. μ.)	Mean M.C.H.C. (Per cent)
Initial ...	8·8	4·9	37	76	23
2nd ...	8·2	4·5	35	77	23
4th ...	7·1	4·03	28	71	24
6th ...	7·0	3·8	27	71	25
8th ...	7·0	3·09	26	83	27
10th ...	7·4	3·24	28	87	26
12th ...	7·2	3·6	29	80	24
14th ...	6·6	3·1	28	90	23
16th ...	6·9	3·2	27	84	25
18th ...	6·5	2·73	26	95	25

M.C.V.=Mean corpuscular volume.
M.C.H.C.=Mean corpuscular hæmoglobin concentration.

From the hæmoglobin readings (mean), it may be observed that during the first four weeks there was a fairly progressive reduction. However, for the following eight weeks the hæmoglobin level was maintained between 7·0 and 7·4 gm. per

100 c.c., but during the last five weeks further reduction (though slight) was evidenced. The reduction in the total erythrocyte count and the V.P.C. were equally marked up to the end of the eighth week. Thereafter, while the V.P.C. was more or less steady between 26 and 29 per cent, the erythrocyte count fluctuated between 3·1 and 3·6 millions up to the end of 16th week. During the last two weeks, further fall in the total erythrocyte count was noted.

During the first six weeks, the M.C.V. was recorded to be between 71 and 77. Subsequently, there was a slight rise during the following two weeks. The level fluctuated between 80 and 90 c.μ. during the following eight weeks. The final reading at the end of eighteenth week was 95 against the initial of 76 c.μ. The fluctuations in the M.C.H.C. were hardly significant.

It would appear that under the low protein dietary regime, anæmia developed, and became more evident from about the fourteenth week onwards. At this stage, there was definite tendency towards macrocytosis as observed from the M.C.V. readings. However, it has to be borne in mind that from about the sixteenth week onwards, diarrhœa was more frequent than noticed earlier.

(*iv*) *Low caloric low protein diet.*—Investigations were carried out for four weeks in eight monkeys, four of which were fed on half and the other four on one-third isocaloric diet. The results showed that in both series there was a progressive reduction in hæmoglobin, total erythrocytes and the V.P.C. However, the reductions appeared to be more rapid in the series kept on one-third isocaloric diet. Further, there was a marked tendency towards macrocytosis in both the series, particularly noticeable from the third week. The M.C.H.C. variation was little, if any.

In these series also, the picture was complicated by diarrhœa, particularly in the second group where it became more or less intractable by the fourth week.

(*B*) RETICULOCYTE COUNT.

Reticulocyte count was carried out once in every week in ten monkeys kept on isocaloric low protein diet. The mean count varied from 4 to 56 per 10,000 erythrocytes (0·04 to 0·56 per cent) during the first eight weeks. Five monkeys, which were followed up to the eleventh week, showed somewhat higher count from after the ninth week. The findings ranged between 36 and 117 per 10,000 erythrocytes (0·36 to 1·17 per cent). From this, it would appear that at least up to the eighth week there was no siginficant rise in reticulocyte count, as compared to those seen in normal monkeys. However, in those in which observations were continued beyond that period, higher counts were often observed, though not to the same extent as seen in monkeys with plasmodial infections.

SERIES III (UNDER VARIOUS DIETARY CONDITIONS AND ALSO INFECTED WITH *P. CYNOMOLGI*).

(*A*) PARASITOLOGICAL STUDIES.

1. Acute stage.

During the current studies, observations on the host parasite relationship were made in the same species of monkeys kept under various dietary regimes.

Five out of 35 monkeys were fed on high protein diet and infected with a dose of 5×10^6 parasitized cells. The pattern of infection was almost similar to that observed in monkeys kept under standard diet. As such, presentation of separate data would not appear to be necessary.

The doses of infective inoculation, the number of monkeys, the intensity of infection and the survival rates in respect of 30 monkeys kept under low protein diet, have been presented in Table VI. It was observed that the intensity of infection was independent of the dose of inoculum as the variation in the parasitic density

TABLE VI.

Doses of infective inoculation, intensity of infection and survival rate in monkeys.

Serial number	Dose of inoculum (Millions)	Number of monkeys	PEAK			Survival rate (per cent)
			Range (Days)	Mean (Days)	Parasitæmia (per cent)	
1.	0·1	3	10 – 11	10·6	10·0	66
2.	0·5	3	9 – 10	9·6	12·31	66
3.	1·0	3	9	9·0	12·03	66
4.	2·5	6	9	9·0	10·75	66
5.	5·0	10	7 – 8	8·0	10·2	60
6.	100	5	6	6·0	12·2	0

during peak infection was slight. Even with the highest dose of inoculum, the cell infection during peak was 12·2 per cent as against 23 per cent in animals under standard (Graph 6) diet. It would also be observed that under standard dietary conditions, there was usually complete clearance of parasitæmia after the crisis, whereas under low protein diet patent parasitæmia was observed for a much longer period as indicated in Graph 7. Further it was observed that irrespective of the dose of inoculum the mortality rate, amongst monkeys kept under low protein diet, varied between 34 and 40 per cent in all the series except the one under which the dose of inoculum was the highest. In the last group, the mortality rate was 100 per cent as against 50 per cent under standard diet.

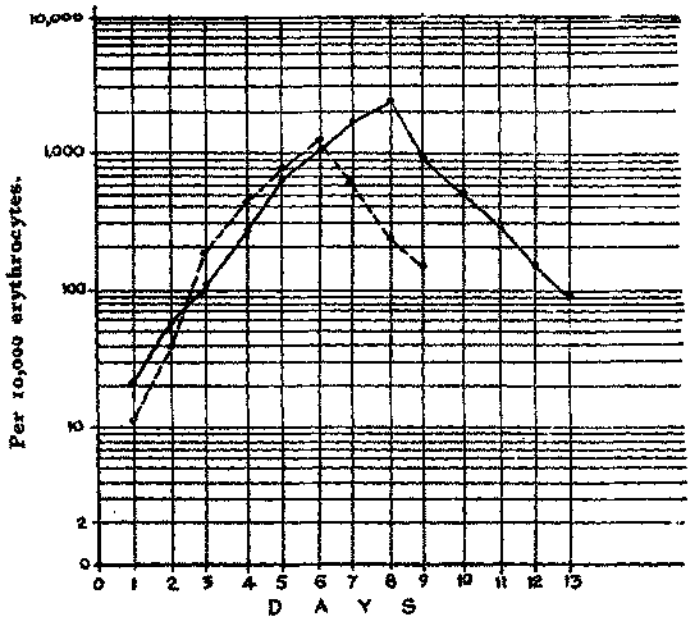
2. Chronic stage.

Eight of the 30 monkeys were followed up during the chronic phase for a period of eight to twelve weeks. Although the frequency of recrudescences was not much altered, the density was much lower than seen in the acute condition but patent parasitæmia persisted for much longer period, sometimes up to 10 to 12 days seen under standard dietary regimes.

Since severe degree of anæmia developed quickly in monkeys kept under low caloric low protein diet, even without any parasite infection, it was not considered necessary to include this group under the present series.

GRAPH 6.

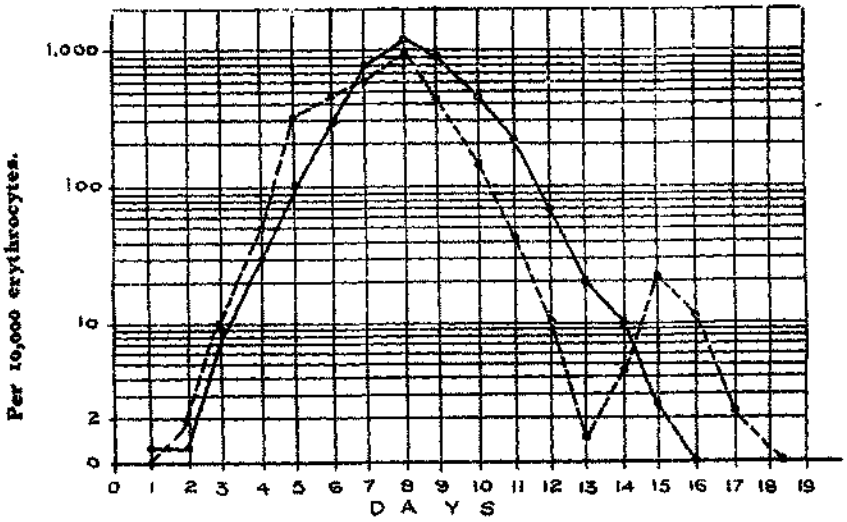
P. cynomolgi *infection in monkeys under standard and low protein dietary conditions.*
(Dose of inoculum 100×10^6).



Standard diet.
Low protein diet.

GRAPH 7.

P. cynomolgi *infection in monkeys under standard and low protein dietary conditions.*
(Dose of inoculum 5×10^6).



Under standard dietary conditions.
Under low protein dietary conditions.

HÆMATOLOGICAL STUDIES.

1. General.

The hæmatological investigations were undertaken during the acute and the chronic phases of *P. cynomolgi* infection in *M. mulatta mulatta* kept under isocaloric high and low protein diet. Blood picture in the series, kept under high protein diet, appeared to be identical to those observed under standard dietary condition.

During the acute phase of the infection, six out of the ten monkeys kept under low protein diet and which had received a challanging dose of 5×10^6 parasitized cells and on which detailed parasitological investiations were undertaken, were at the same time subjected to detailed hæmatological studies. Observations during the chronic phase were taken up in eight monkeys, which included four on which detailed studies were undertaken during the acute stage as mentioned above.

2. Blood picture during the acute phase.

(*i*) *Hæmoglobin, total erythrocyte count and V.P.C.*—Since complete parasite clearance, after the acute infection, was not attained until the eighteenth day subsequent to the infection inoculation, the estimates were continued up to Day 20. The mean initial hæmoglobin reading was 8·8 gm. After patent parasitæmia was established, there was a progressive reduction but the maximum hæmoglobin loss was not registered until Day 14 (six days after peak parasitæmia) when the level had reached as low as 3·2 gm. (mean) per 100 c.c. of blood or 36 per cent of the original level. As such, the anæmia developed appeared to be of very severe degree. Thereafter, there was a steady recovery up to the period of observations.

The reduction in the total erythrocyte count was equally marked and the maximum loss registered was by 69 per cent of the original level of 5·1 millions per c.mm. Thus the loss in erythrocytes was not only severe but even appeared to be more than the loss in hæmoglobin. However, from Day 15 onwards, there was a very rapid rise in the count and the initial rate of recovery appeared to be much faster than that of hæmoglobin.

Compared to these findings, the reduction in V.P.C. was not up to the same extent though the lowest reading was observed to be 17·2 as against the initial mean of 40 per cent. Therefore, the maximum reduction in V.P.C. was by 57 per cent in the initial level. The phase of recovery was fairly rapid and by Day 20 the level had reached up to 80 per cent of the original reading.

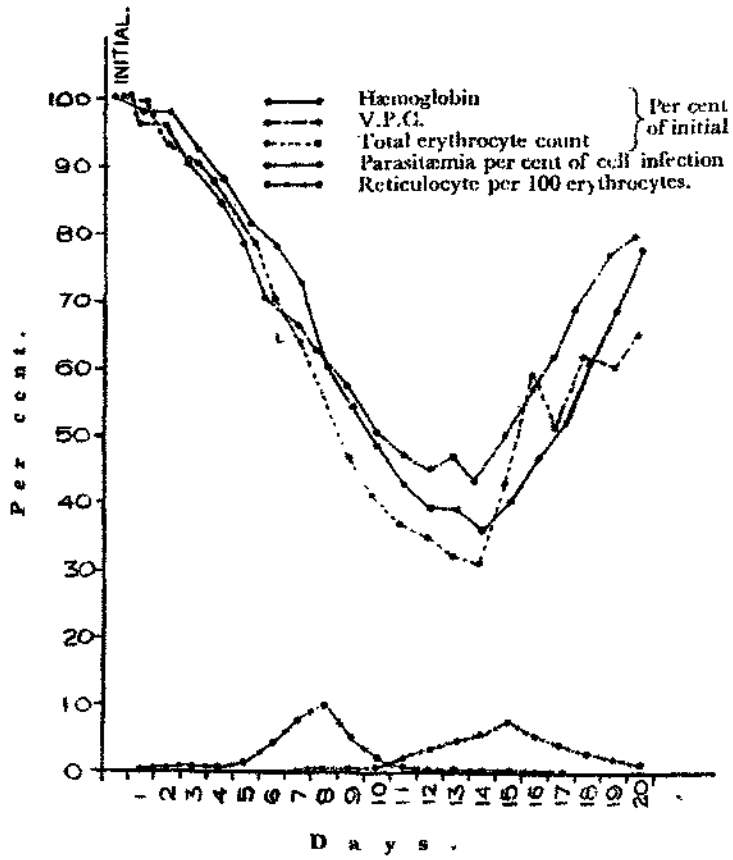
A comparative data in respect of all these three readings, along with the course of parasitæmia and reticulocyte count, have been shown in Graph 8.

(*ii*) *M.C.V. and M.C.H.C.*—The initial M.C.V. reading was observed to be 78 c.μ. (mean). During the early phase of infection, the readings varied only slightly but from Day 7, there was a general tendency towards rise and the maximum reading registered was 114 on Day 13 as shown in Graph 9. Subsequently there was a sudden fall followed thereafter by moderate rise. However, from Day 4 to 20 the overall mean was observed to be 91 c.μ. which was somewhat higher than the initial reading. Against the initial M.C.H.C. reading of 22 per

cent, the findings up to Day 7 were between 23 and 25 per cent. From Day 8, the M.C.H.C. registered a persistently low level varying between 17 and 21 as shown in Table VII.

GRAPH 8.

Course of P. cynomolgi *infection and the hæmatological picture during the acute phase of infection in* M. mulatta mulatta *kept under isocaloric low protein diet.*



There was a general tendency towards macrocytosis during the acute phase, particularly from about the peak infection to the stage of crisis. Taking the overall mean M.C.H.C. reading as 20 against the initial of 23, it may be said that the reduction was not very significant, though occasionally the level had gone down to 17-18 per cent.

The hæmatological studies, therefore, indicated that under low protein dietary conditions, *P. cynomolgi* infection caused anæmia of such a severe degree as had not been encountered previously in *P. cynomolgi* or *P. knowlesi* infection in monkeys kept under standard diet nor in uninfected monkeys fed on isocaloric low protein diet. Further the anæmia could be considered to be of macrocytic type.

GRAPH 9.

M.C.V. readings during the course of acute P. cynomolgi *infection in* M. mulatta mulatta *fed on isocaloric low protein diet.* (Dose of inoculum 5×10^6)

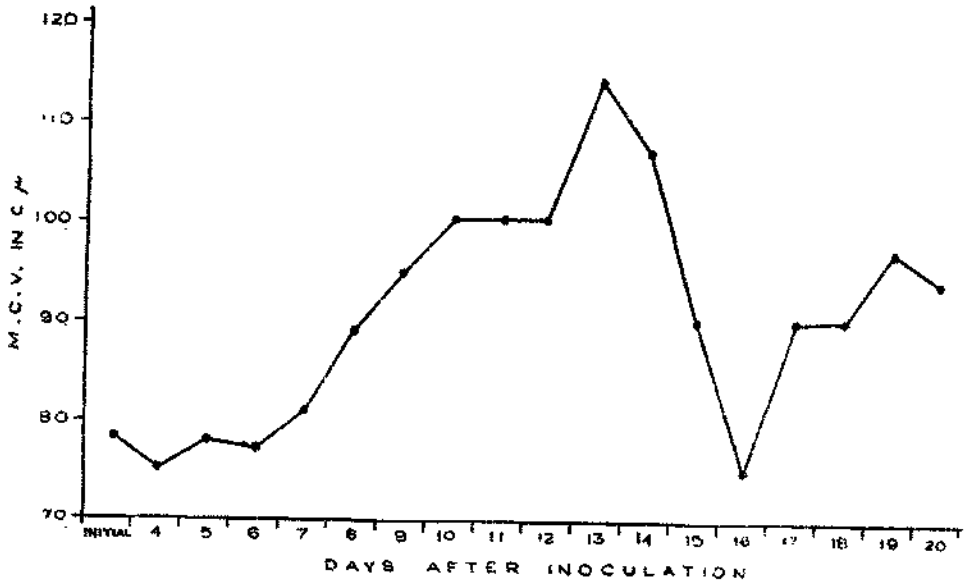


TABLE VII.

Hæmatological data relating to hæmoglobin, erythrocyte count, V.P.C., M.C.V. and M.C.H.C.

Weeks after peak parasitæmia	Mean hæmoglobin gm./100 c.c.	Mean total R.B.C. in millions	Mean V.P.C. per cent	Mean M.C.V. c.μ.	Mean M.C.H.C.	Remarks
3	7.3	3.25	35	107	20	
4	7.2	3.50	38	108	18	
5	7.6	3.78	42	111	18	Macrocytic hypochronic type of anæmia.
6	7.8	3.66	47	128	16	
7	8.1	3.31	44	132	18	
8	7.7	3.85	44	114	17	
9	7.5	3.94	41	104	17	
10	7.2	3.50	46	131	15	
11	7.5	3.00	40	133	17	
12	7.4	2.78	39	140	18	

V.P.C. = Volume of packed cells.
M.C.V. = Mean corpuscular volume.
M.C.H.C. = Mean corpuscular haemoglobin concentration.

(*iii*) *Reticulocyte count.*—During the acute phase of the infection, reticulocytes were counted in five monkeys, on which detailed hæmatological studies were undertaken as indicated above. The trend of events is well depicted as per Graph 8. During the stage of early infection up to peak parasitæmia, reticulocyte response was definitely slow as compared to the period of crisis. After the peak parasitæmia, there was a sudden rise which reached a maximum level of seven per cent (mean). Subsequently there was a gradual reduction in the number though it still remained much higher than the initial level, even long after the clearance of parasites from peripheral circulation. The remarkable features observed during these studies were the low counts prior to and during peak parasitæmia followed by sudden rise at a time when parasitæmia was on the decline.

(3) Hæmatological picture during the chronic phase.

The course of study included latency and recrudescences, and covered a period of 4 to 12 weeks after the peak parasitæmia. A total of eight monkeys were closely followed up and these included four of the monkeys on which detailed studies were carried out during the acute phase of the infection. All the observations were carried out once a week.

The mean weekly hæmoglobin level varied within a close range from 7·2 to 8·1 gm. per 100 c.c. of blood which, according to the previous experience, was definitely lower than that observed in uninfected monkeys on standard diet. In the same way, the total erythrocyte counts ranged between 2·78 and 3·94 millions. On the contrary, the variations in the V.P.C. were fairly wide, and ranged between 35 and 47 per cent.

Throughout the period, the M.C.V. readings were much higher than those observed in the series of 30 uninfected normal monkeys kept on standard diet. Compared to the mean initial level of 79 c.μ. in normal monkeys [Part I of this communication (Ray, 1957*a*)], the range between 104 and 140 c.μ. should be considered as fairly high (Table VII).

During the third week (after peak infection), the M.C.H.C. was recorded to be 20 per cent. But, thereafter the variation was between 15 and 18 per cent. When compared with the observations made previously on normal animals [Part I of this communication (Ray, 1957*a*)] and also those in respect of infected ones kept on standard diet, it would be evident that there had been a considerable lowering of the mean M.C.H.C. readings.

DISCUSSION.

From the results of observations, it was noted that *P. cynomolgi* in *M. mulatta mulatta* runs a benign course and resembles somewhat the pattern of infection due to *P. vivax* but fundamentally different to that seen in *P. knowlesi* infection (Ray, 1957*b*). After infective inoculation by the intravenous route, the prepatent period and the time interval for attaining the peak density, seem to vary inversely according to the dose of infection irrespective of the dietary condition of the host. The higher the dose, the shorter the prepatent period as well as the interval between infection and peak parasitæmia. To be more precise, it may be said that with doses varying

from 0·1 to 100 millions the peak period fluctuated between 11 and 5 days respectively. But under standard diet, the intensity of infection during peak and the mortality rate are directly proportional to the infective doses. With the heaviest dose, the infection reached the highest level of 23 per cent with a mortality rate of 50 per cent as against 7·8 per cent cell infection and 20 to 30 per cent mortality rate respectively when infected with the lowest challanging dose of $0{\cdot}1 \times 10^6$ parasitized cells per kg. of body weight.

On the other hand, the course and intensity of infection, as well as the mortality rate seem to differ somewhat in monkeys kept under low protein dietary conditions. Firstly with the highest dose of inoculum, the mortality rate is 100 per cent and in the rest of the series it varies from 34 to 40 per cent. Though the period, till peak infection has been attained, is found to be between 6 and 10·6 (mean) days, depending on the dose of infection, the peak density is not proportional to the dose, as the range is found to vary from 10 to 12 per cent cell infection. Even with the infective inoculum of 100 million parasite per kg., the highest intensity attained is 12·2 per cent as against 23 per cent under standard diet. In other words, the intensity of infection under low protein diet would appear to be reduced by about 50 per cent though the mortality rate is higher than observed under standard diet.

Comparing the results of the two series, one under the standard and the other under low protein diet, both having received the same infective dose of five millions, it may be observed that the peak density attained is somewhat higher in the former.

Another interesting feature is that comparatively speaking the reduction in parasitæmia, during and after the crisis, is somewhat slower in animals kept under standard diet as against the other group in which it is of abrupt nature. Further, while complete clearance of parasitæmia is attained within a few days after the peak in the former group, patent parasitæmia is detectable (though in small number) for a much longer period in the latter. This phenomenon and the lower density at peak infection, particularly when compared to the series infected with 100 million parasites, may have perhaps some bearing with the nutritional status of the host and also the essential needs of the parasites. It may be argued that having reached the peak infection, the host is no longer able to provide sufficient nutrient to the parasites on account of their own nutritional deficiency, and as such the fall in parasitæmia has been found to be abrupt in the series of animals kept under low protein diet. From these observations, one may assume that a sub-standard diet may prove somewhat detrimental to parasitic growth. These findings may seem to differ somewhat from those observed by Passmore and Sommerville (1940) who have not found any significant difference in the intensity of infection due to *P. cynomolgi* in *M. mulatta mulatta* kept under the two types of diet, one containing liberal amount of milk, vegetables and fruits in addition to wheat; and the other consisting of milled rice and certain supplementary items. The supplementary food provided, consisted of fat and pulses also. But in the present series, the low protein diet is made up of sago, jaggery, and sweet potatoes and, therefore, the protein content is almost negligible. The deficiency in the protein content of the diet may perhaps explain for the differences observed. Working with avian malaria, Seeler and Ott (1945) have recorded that *P. gallinaceum*

infection in chicks runs a more severe course in protein deficient diet as compared to that seen in chicks kept under high protein diet. But again, the diet is made up of pure chemical food, as against natural food. On the other hand, Ramakrishnan (1954*a*) has observed that in *P. berghei* infection in white mice, if meat is excessive in the diet the course of infection is severe, whereas under-nourishment of the host affects the nutritional requirements of the parasite and, therefore, its multiplication and still further its capacity for antigenic stimulation (Ramakrishnan, 1954*b*). If this were so, then it should explain for the low parasitic density in the present series kept under low protein diet. It will also explain for the delay in the complete clearance of parasites from the peripheral circulation. Lack of sufficient density, during early infection, may have perhaps delayed the immune mechanism and hence the parasitic clearance. Similar views are also held by Cannon (as quoted by Seeler and Ott, 1945) who records that, in hypoproteinæmia animals are unable to produce normal antigenic stimulus and fail to produce sufficient antibody. This would perhaps account for the delay in the clearance of parasitæmia in chicks kept on low protein diet. Further it may also explain for the reasons for prolonged parasitæmia during recrudescences under low protein dietary condition in contrast to standard diet under which parasitæmia has been usually observed to be purely of transient nature.

The overall picture, therefore, appears to be that under low protein dietary conditions the intensity of infection may not be the same as in cases under standard diet. Moreover there is likely to be some delay in the initial parasite clearance after the acute infection, perhaps on account of defective immune mechanism. The same factor may also be responsible for prolonged parasitæmia during recrudescences. In human malaria, according to Williams (1940), resistance to infection depends on nutritional status. It is believed to be decreased in children who are breast fed by mothers (who themselves are suffering from malnutrition), by late weaning, a poor weaning diet, presence of helminths and gastroenteritis.

As to the blood picture in monkeys kept under standard diet and infected with *P. cynomolgi*, the degree of anæmia during the course of acute infection is quite severe, even much more than observed under *P. knowlesi* infection, even though cell infestation is much less than seen in the later infection. Similar observations have been made in respect of *P. vivax* infection in which the resulting anæmia could be disproportionately severe (Maegraith, 1948). The variation in M.C.V. has been found to be 76 to 87 c.μ. against the initial level of 79. But the overall mean for the 16 days may be noted as 81 c.μ., which is very close to the mean initial of 79 c.μ. Thus, although shortly before and after the peak infection the M.C.V. has been a little over the initial, the anæmia could judiciously be called normocytic. The M.C.H.C. has been very close to the initial of 23 per cent and as such it may be called orthochronic type of anæmia. In the final analysis, it may be said that *P. cynomolgi* produces normocytic orthochronic type of anæmia under standard diet similar to that seen in monkeys which have been made anæmic by repeated bleeding (Ray, 1955). The blood picture has been found to be in no way different in this infection in monkeys kept under high protein diet. Under isocaloric low protein dietary conditions in uninfected monkeys, the hæmoglobin level has been found to be somewhat lower, particularly during the fourth to the sixth week. The fluctuation of the level is between 85 and 93 per cent of the original.

From the M.C.V. readings it is quite obvious that there is a general tendency towards macrocytosis from about the seventh week. The M.C.H.C. is found to be more or less near the initial level though occasionally it has been higher. Thus the anæmia developed under low protein diet, during a period of about two months, is slight and it is usually of a macrocytic orthochronic type.

In contrast, the anæmia has been observed to be of severe degree in the animals kept on half or one third isocaloric low protein diet. The M.C.V. in both the series is found to be on the rise. This is more conspicuous in the latter group. The variation in the M.C.H.C., if at all, is considered to be negligible. In these two series, therefore, the anæmia has been severe and it is definitely of macrocytic orthochronic type. This is understandable as according to Orten and Orten (1946), Joliffe *et al.* (1950) and others, deprivation of protein in the diet leads to anæmia, and this can be corrected by protein as has been shown in laboratory experiments with rats. Defective dietary conditions in human host, leading to macrocytic anæmia, are also a well established fact (Castle, 1933; Wills, 1934). Fairley *et al.* (1938) have also noticed macrocytic anæmia in Macedonians living on diet without any protein of animal origin. In India, Napier (1934: 1939), Taylor and Chuttani (1945), Patwardhan (1952) and many others have made similar observations in cases of deficiency anæmias amongst Indian adults living purely on vegetarian diet.

In view of the above, the hæmatological picture in the series of infected animals (*P. cynomolgi*) under low protein dietary conditions (with or without additional factors), would appear to be quit understandable. The fall in the hæmoglobin level is noted to be of acute nature and as compared to the initial level the loss is to the extent of 64 per cent. Thus the anæmia produced is the severest seen in the course of the present studies. The M.C.V. is within normal limits prior to peak parasitæmia but subsequently there has been a gradual rise up to 114 c.μ. *Pari pasu* a fall in the M.C.H.C. has also been registered. The anæmia may thus be called macrocytic hypochromic type, similar to that reported by Rodriguez-Melina (as quoted by Wintrobe, 1951). According to Hynes (1944), deficiency of Will's factor causes macrocytic anæmia whereas iron deficiency leads to normocytic or microcytic anæmia. Diet of Indians, consisting of little food of animal origin, is deficient of Will's factor and the most important cause of increased Will's factor utilization is chronic malaria. The same view is held by Thomson (1944) who is of the opinion that " In India, anæmia as a simple dietary deficiency state is extremely rare. It is the product of parasitic infestation and bare subsistance level of nutrition ". Sharma (1944) also believes that "the incidence of malaria and ankylostomiasis, in victims of dietary deficiencies, exaggerate the anæmic condition ". From the present series of investigations, it would appear to be quite evident that malarial infection severely accelerates the degree of anæmia already present due to deficient dietary condition. The type of such anæmia would appear to be of macrocytic nature.

Another interesting feature observed during these studies is the reticulocyte response in *P. cynomolgi* infection irrespective of the dietary condition of the host. During early infection, the reticulocytes have been found to be within normal limits or somewhat lower, but subsequently up to the day following peak parasitæmia the count has been observed to be much less. Therefore, the sudden rise, reaching

its peak density of 8·6 per cent on Day 14 against 0·06 per cent on Day 9 (95-fold increase) would, no doubt, appear to be remarkable. In this connection, it may be noted that along with the rise in reticulocyte count, there is an increase in the erythrocyte count and V.P.C. It would also be observed that at this period the anæmia has a tendency towards macrocytosis. These three phenomena would appear to have a bearing on the increased reticulocyte count. Whitby and Britton (1953) consider that if anæmia is severe and regeneration is rapid, macrocytosis is common. Since rise in hæmoglobin level has been found to be as sharp as the other findings, the explanation may lie in the low hæmoglobin content of reticulocytes.

The reason for the very low count during the acute phase and the sudden rise after peak infection may perhaps need more explanation. Fairley and Bromfield (1933) and Fairley (1934) have reported that in natural or induced infections due to *P. falciparum* or *P. vivax*, the reticulocyte count ranges from 0·6 to 7·8 per cent, but after treatment it usually rises considerably. The same has been observed by Thonnard-Neumann (1945). In view of this, the latter has suggested that there is an inverse relationship between the presence of parasites in the peripheral blood and the height of the reticulocyte count. Repeated examinations of bone marrow, during the acute phase, have not revealed any restriction in the production of reticulocytes. On the contrary, there has been an increase. But after treatment, when there is reduction in parasitæmia, " the reticulocytes formed in the bone marrow were apparently flushed out into the circulation, giving rise to reticulocytosis in the peripheral blood ". In view of these, he has further suggested that the peripheral parasitæmia may have perhaps interfered with the discharge of reticulocytes, and thus causing a " damming up " in the bone marrow. This is perhaps what has happened in the present investigations, when soon after peak the reticulocytes have been on the increase quite abruptly. Gramiccia (1945) has also discussed this point and he considers that there appears to be a barrier in the bone marrow during the acute stage of infections and this obstructs the release of reticulocytes.

After the peak reticulocyte density (six days subsequent to peak parasitæmia), a rapid fall has been observed, and during the latent period it has been fluctuating between 0 and 0·13 per cent. Similar observations have also been recorded by Ramakrishnan *et al.* (1953) in respect of *P. berghei* in white rats. But after recrudescences in *cynomolgi* infection, bouts of reticulocytosis of transient nature have been observed. Thus if any significant rise in reticulocyte count is any indication of large scale erythrocytic destruction, it may be conjected that during latent stage of infection there is no abnormal destruction of the red blood cells.

As regards differential invasion of erythrocytes, studies at the time of schizogony, as recommended by Macgraith (1948), have shown that the ratio of reticulocytes to erythrocytes invaded, varies between 1:8 and 1:5. In other words, the proportion of reticulocytes found invaded is less than mature erythrocytes. According to many authors *P. vivax* preferentially invades reticulocytes as compared to *P. falciparum* and *P. malariæ* (Hegner, 1938; Kitchen, 1938; Ferrebee *et al.*, 1946). Thus *P. cynomolgi* would appear to behave differently to *P. vivax* as has been observed by Malamos (1937) and during the present studies.

SUMMARY.

In summing up the observations recorded in Parts I and II of these series of communication, it would be evident that:—

(*1*) The hæmoglobin level in wild caught *M. mulatta mulatta* was somewhat less than those in captivity and fed on standard diet. This could very well be due to the lower nutritional state in nature. The range of hæmoglobin level in monkeys of all age-groups, kept in captivity, had been found to vary between 7 and 13·5 gm. per 100 c.c. of blood, and between 8 and 10·8 gm. (mean 9·27 gm.) for monkeys between 11 and 36 months age-group. The V.P.C. range was found to be between 35 and 46 per cent (mean 39) and the same for total erythrocyte count was 4·88 million per c.mm. (mean). The normal M.C.V. and M.C.H.C. were calculated to be 80 c.μ. and 24 per cent, and the fluctuations in the reticulocytes were observed to be from 0·0 to 0·43 per cent (mean).

(*2*) When these animals were infected with *P. knowlesi* (Nuri strain), the course of infection was rapid and the peak density (sometimes reaching 90 per cent infection or over) was extremely high.

The fall in hæmoglobin level was also phenomenal as within a short period of four to five days, it came down to almost 50 per cent of the original level. Equally rapid was the fall in the total erythrocytic count and V.P.C. The degree of anæmia was, therefore, found to be quite severe, and it was, as a rule, of normocytic type. There was hardly any significant increase in the reticulocyte count and the parasites seemed to prefer the mature erythrocytes than the reticulocytes.

Death invariably followed a few hours after peak infection, but this could be prevented by transfusion of whole blood or even washed cells alongwith anti-malarials which by themselves failed to prevent the course of events.

(*3*) Compared to the above, the picture in *P. cynomolgi* was somewhat different as the intensity and mortality rates seemed to vary according to the dose of infection. As a rule, with a moderate dose of 5×10^6 parasitized cells the peak density of about 13 per cent was attained in eight days. This was followed by a period of crisis when there was a fall in parasitæmia. At this period, about 30 per cent of the animals died. In 70 per cent of animals, which survived, there was complete clearance of parasitæmia for varying periods. The latent period was punctuated by recrudescences at periodic intervals.

Although the parasitic infection did not appear to be severe, the anæmia developed was somewhat of a higher order than observed in *P. knowlesi* infection. Soon after the peak infection, the hæmoglobin level was lowered by 50 per cent. The same was observed to be the case in respect of total erythrocyte count and V.P.C.; the lowest level being attained about six days after peak infection. Thereafter there was a rapid recovery but the original level was not reached at least up to a fortnight up to peak infection.

The overall picture appeared to be severe degree of anæmia of normocytic orthochronic type even though there was a slight tendency towards macrocytosis shortly after peak infection.

During the chronic stage, the hæmoglobin level was maintained at a somewhat lower level than the original and this was attributed to periodic recrudescences.

The reticulocyte count presented an interesting feature. During the rising infection and peak, the counts were low but soon after there was an abrupt increase and the highest count was recorded five to six days after peak. As compared to the initial reading of 0·058 per cent, the rise up to 8·6 per cent (about 15,000 fold) seemed to be most striking.

(*4*) From a study of the blood picture it was evident that uninfected monkeys, kept under high protein diet, improved significantly. But during plasmodial infection (*P. cynomolgi*), the fall in hæmoglobin level and other counts were more or less similar to that seen in monkeys kept under standard diet.

(*5*) However, when uninfected monkeys were kept under low protein diet, and if this diet was of isocaloric value, there was a slight degree of anæmia with a tendency towards macrocytosis. If the caloric value of the diet was lowered by 50 or 30 per cent, the anæmia of macrocytic type developed quickly.

(*6*) When such animals were kept under isocaloric low protein diet and infected with *P. cynomolgi*, the picture appeared to be somewhat different to that seen in animals kept under standard diet. Firstly, the density at peak infection was comparatively low and not much influenced by the dose of infective inoculum. During crisis, the fall in parasitic density appeared to be sudden, but all the same there was some delay in complete clearance of parasites from the peripheral blood. During recrudescence, patent parasitæmia lasted for a longer period than observed under standard diet.

As to the blood picture the fall in hæmoglobin, during acute infection, was quite severe as the reduction was by over 65 per cent. There was a corresponding fall in other counts as well. From the M.C.V. readings, it was evident that the anæmia was of macrocytic type. Although M.C.H.C. was recorded to register low level during and soon after peak infection, it was generally orthochronic in nature. However, during chronic infection under low protein dietary conditions, the M.C.V. was persistently high and M.C.H.C., as a rule, was low. Thus in chronic malaria, under low protein diet, the anæmia appeared to be macrocytic hypochromic type.

There was no appreciable degree of change in reticulocyte response in this series as compared to that in infected animals kept under standard diet.

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HÆMATOLOGICAL STUDIES IN SIMIAN MALARIA.

Part III. Estimation of fæcal and urinary urobilinogen.

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EARLIER investigations (Parts I and II) (Ray, 1957*b*:1957*c*) were made on the blood picture in simian malaria, particularly in respect of the changes in the hæmoglobin level, the degree and type of anæmia, lreticulocyte response, etc., in *P. knowlesi* and *P. cynomolgi* infections. As to the fate of the excess of hæmoglobin due to breakdown of erythrocytes (normal and parasitized) in such cases a number of observations had been carried out in the past, especially in respect of the iron containing moiety including hæmozoin (malarial pigment) as well as the iron-free element like bilirubin. With regard to the urobilinogen output, there appears to be some degree of controversy. According to many, urobilinogen output in urine is raised during malarial infection (Gordon, 1924; Kingsbury, 1926). However, some workers are of the opinion that excess of urinary urobilinogen may appear in malaria but it is not always present during an acute attack. Further, since urobilinogen output is considerably increased during or immediately after passing of hæmoglobin in blackwater fever, its presence can be associated with hæmolysis but the same relationship would not appear to be obvious in respect of malaria (Maegraith, 1948).

In view of the divergent views mentioned above, the present investigations were focussed on the studies relating to urobilinogen output in malarial infection and also under different conditions indicated in earlier papers (Ray, 1957*b*:1957*c*). Estimation of urobilinogen in fæces was also carried out simultaneously along with the estimation in urine. This was in view of the fact that increased output of urinary urobilinogen occurs in other conditions as well, and is not necessarily the sole indication of excessive erythrocytic breakdown.

MATERIALS AND METHODS.

The details regarding the host, parasites, dietary conditions, etc., have already been indicated in Parts I and II (Ray, 1957*b*:1957*c*). In the present paper, the technique adopted for quantitative estimation of urobilinogen has been described.

Estimation of urobilinogen.—The technique* adopted was that of King (1951) details whereof are indicated below. A Klett-summerson photo-electric colorimeter was used for quantitative evaluation of urobilinogen in fæces and urine.

TECHNIQUE.

(*a*) *Estimation in fæces.*—1·5 gm. of fæces was transferred into a test tube and emulsified thoroughly with 9 c.c. water. To this, was added 10 c.c. of ferrous sulphate solution and then 10 c.c. of 2·5 N. sodium hydroxide. After allowing the mixture to stand for two hours, it was filtered. To 2 c.c. of the filtrate, 2 c.c. of Ehrlich's reagent was added. This was mixed in a 100 c.c. conical flask and allowed to stand for ten minutes and then 5 c.c. of sodium acetate solution was added.

For blank, 2 c.c. of 6-N-hydrochloric acid was added to 2 c.c. of filtrate and then 5 c.c. of sodium acetate.

The standard used was phenolphthalein.

After reading the samples, calculations were made as follows (King, 1951):—

$$\left.\begin{array}{l}\text{Urobilinogen in mg.}\\ \text{per 100 gm. of fæces}\end{array}\right\} = \frac{\text{Reading of test-reading of blank}}{\text{Reading of standard}} \times 0{\cdot}00387 \times V \times \frac{100}{0{\cdot}1}$$

V = Final volume of the coloured solution.

(*b*) *Estimation of urobilinogen in urine.*—The samples of urine were collected from metabolic cages and rarely there was any contamination with fæces. The material was always rejected if found contaminated.

To 2 c.c. of urine, equal volume of Ehrlich's reagent was added and the procedure adopted thereafter was the same as for fæces.

Calculations.—Calculations were made in the same way as for fæces. The figures thus obtained were divided by 20 to obtain the final result. This is because the amount of fæces taken was 1·5 gm. which was emulsified and finally made up to 30 c.c. and thus 2 c.c. of that (filtrate) represented an equivalent to one-twentieth of 1·5 gm. of fæces. As no such dilutions were made in respect of urine for calculations on the same basis as for fæces, the data should naturally be divided by 20.

Investigations were taken up in normal monkeys as well as those infected with *P. knowlesi* or *P. cynomolgi*.

**Reagents.*—Phenolphthalein Standard was prepared by dissolving 50 mg. phenolphthalein in 100 c.c. of alcohol. This was diluted with alkaline water before use by adding 1 c.c. of phenolphthalein standard to 5 c.c. of 15 per cent sodium carbonate solution in a 100 c.c. volumetric flask and diluted to the mark with water.

Ehrlich's reagent was prepared by dissolving 0·7 gm. of P. dimethyl-aminobenzeldehyde in a mixture of 150 c.c. of concentrated hydrochloric acid and 100 c.c. water.

The other agents were ferrous sulphate solution, 2·5 N.sodium hydroxide and sodium acetate solution.

OBSERVATIONS.

A. Under standard diet*.

SERIES I. STUDIES IN NORMAL MONKEYS.

Estimation of urobilinogen in fæces was made in 35 normal monkeys, and in 27 monkeys in respect of urinary urobilinogen.

(*i*) *Fæces* (*wet*).—Urobilinogen was estimated in 35 normal animals and the range varied between 6·27 and 54·18 mg. (mean 24·6) per 100 mg. of the fæces of the usual consistency. In two animals which developed diarrhoea for two days, the variations were considerable and the urobilinogen estimated was observed to be lowered by 35 to 50 per cent (on account of dilution).

(*ii*) *Urine.*—The urine of 27 normal monkeys, was tested for determination of urobilinogen. The range of urobilinogen varied between 0·25 and 1·8 mg. per 100 c.c. of urine, the mean being 0·85 mg.

SERIES II. STUDIES OF MONKEYS WITH *P. KNOWLESI* INFECTION.

Fæcal and urinary urobilinogen was determined from the day prior to inoculation to Day 4 (day of inoculation being 0) in ten monkeys infected with *P. knowlesi.* Since the infection always ends fatally during the acute stage, studies were carried out only during acute infection. It was not possible to carry out the investigation on Day 5 or Day 6 as rarely any fæces was available then. Further, invariably there was hæmoglobinura at that period. During the latter part of the infection, some of the monkeys infected with *P. knowlesi* usually developed diarrhoea, making it difficult to assess the actual amount of urobilinogen on account of dilution. Therefore samples were obtained only from those animals which did not develop diarrhoea.

Fæces.—Although there were considerable individual variations, by and large there was a progressive rise in urobilinogen as the infection developed rapidly.

*STANDARD DIET FOR MONKEYS AT THE MALARIA INSTITUTE OF INDIA.

Ingredients	Quantity in ounces	Fat	Protein (*g*)	Carbohydrate	Caloric value
Gram	4	6·0	19·6	69·6	360
Wheat flour	4	2·0	13·6	82·0	400
Vegetables or fruits† ...	4				
	(*a*) Carrots or	0·4	1·2	12·0	52
	(*b*) Guava or	0·4	1·6	16·4	76
	Sweet potato	0·4	1·2	25·2	148
Total		8·4	34·4 to 34·8	163·0 to 186·8	812 to 908

† The items vary according to the season of the year.

On Day 4, the final reading of estimated urobilinogen in fæces was 19·7 mg. (Table VIII) * as compared to the initial level of 13·0 mg. per 100 gm. (Graph 10) *.

Urine.—The mean initial urobilinogen reading in the ten monkeys was 0·85 mg. per 100 c.c of urine. Subsequently there was a steady rise and by Day 3 the amount was almost double of the initial. The final mean reading was higher still and it was recorded as 2·17 mg. (Table VIII).

TABLE VIII.

Urobilinogen in fæces and urine of monkeys under standard diet with P. knowlesi *infection.*

Serial number	Monkey number	Urobilinogen in fæces (mg. per 100 gm. of fæces)						Urobilinogen in urine (mg. per 100 c.c. of urine)					
		Initial	Day of infection					Initial	Day of infection				
			1	2	3	4	5		1	2	3	4	5
1	5518	20·3	21·3	16·0	19·0	23·6	...	0·76	0·8	S.B.Z.	1·67	1·9	...
2	5543	19·0	19·0	17·3	19·0	31·2	...	0·65	0·62	0·85	1·41	1·72	...
3	5561	18·6	S.N.A.	14·1	16·0	11·4	...	0·92	S.N.A.	1·45	1·68	2·32	...
4	5562	8·5	10·6	12·0	15·5	21·3	...	1·2	0·86	S.N.A.	1·98	2·2	...
5	5586	17·0	14·3	5·9	6·6	14·3	...	0·58	0·88	0·95	1·56	1·82	...
6	5587	8·2	17·7	16·2	24·4	21·8	26·9	0·62	0·94	1·6	2·2	2·86	...
7	5663	11·1	13·8	14·3	20·4	S.N.A.	...	0·74	0·83	0·65	S.N.A.	1·65	...
8	5667	9·5	11·9	10·1	11·7	17·2	...	1·31	1·52	1·2	1·86	2·61	...
9	5672	9·8	10·6	8·7	17·4	21·2	...	0·96	1·41	1·5	1·66	2·4	...
10	5696	8·0	10·2	14·0	S.N.A.	15·9	...	0·83	0·88	0·54	0·95	S.N.A.	...
Mean		13·0	14·3	12·8	16·6	19·7	...	0·85	0·97	1·09	1·66	2·17	...

S.N.A. = Samples not available.

From these data, it would be evident that there was a progressive rise in both fæcal and urinary urobilinogen as the infection developed (Graph 10) *.

SERIES III. STUDIES IN *P. CYNOMOLGI* INFECTION.

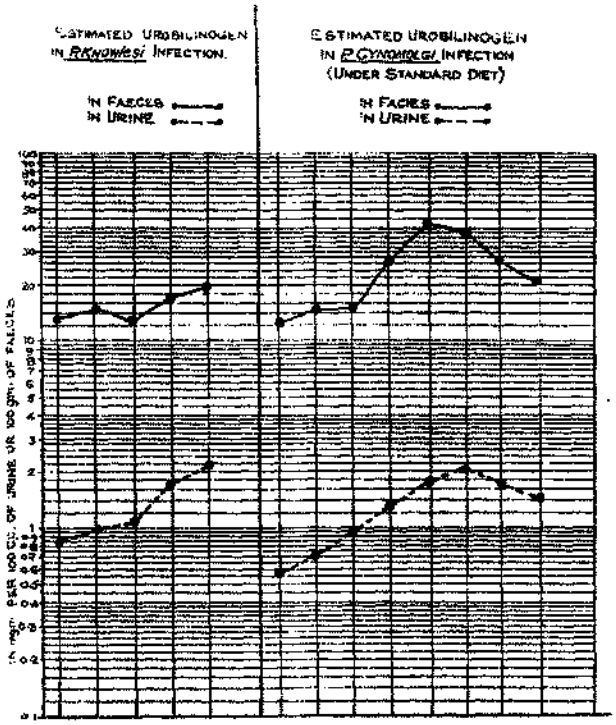
(*A*) DURING ACUTE INFECTION.

Urobilinogen estimation of fæces and urine was undertaken in six monkeys infected with *P. cynomolgi* during the phase of acute infection and the readings were taken on every alternative day up to a period of 14 days. The rise or fall,

* Tables I to VII and graphs 1 to 9 have appeared in parts I and II of this paper (Ray, 1957*b*:1957*c*).

if any, was determined by comparison with the initial mean reading obtained prior to inoculation.

GRAPH 10.



Fæces.—The highest reading on fæcal urobilinogen was recorded as 42·1 (mean) on Day 8 as against 13·4 mg. per 100 mg. fæces prior to inoculation (the peak parasitic infection in this series was observed to be between Day 7 and 9). Subsequently there was a rapid fall but the final reading on Day 14 was still higher than the initial level.

Urine.—The maximum urinary urobilinogen was recorded to be 2·13 on Day 10 as against the initial level of 0·59. The final reading on Day 14 was about three times as high as the mean initial reading.

The results have been presented in Tables IX and X from which it may be observed that along with the rise in infection there was progressive increase in fæcal and urinary urobilinogen (see also Graph 10).

(*B*) IN CHRONIC INFECTION.

In this series altogether nine monkeys were utilized for the studies at a time when the infection had become chronic and after four to six weeks after the acute phase. In six animals, no parasitæmia was detectable at the time of the

investigation while in three others recrudescence was observed, though the parasite density appeared to be of a very low grade ranging from 3 to 14 per 10,000 erythrocytes.

TABLE IX.

Estimation of urobilinogen in fæces in P. cynomolgi *infection (in mg. per 100 gm).*

Monkey number	Initial	Day 2	4	6	8	10	12	14
4840	14·9	16·0	18·3	20·5	38·9	41·2	32·5	27·9
4856	16·5	14·5	20·4	30·0	54·0	33·5	40·5	18·4
4927	20·2	22·0	15·8	26·3	39·5	30·9	21·0	22·8
4932	9·6	13·5	19·8	25·0	29·4	27·1	18·5	13·5
5039	11·9	12·8	15·4	38·8	53·5	55·3	26·4	22·0
5041	7·7	11·5	9·9	22·4	37·5	32·4	18·9	18·2
	80·9	90·3	89·6	163·0	253·0	220·4	157·8	122·8
Mean	13·4	15·06	14·9	27·1	42·1	36·7	26·3	20·4

TABLE X.

Estimation of urobilinogen in urine in P. cynomolgi *infection (in mg. per 100 c.c.).*

Monkey number	Initial	Day 2	4	6	8	10	12	14
4840	0·51	0·84	1·54	1·72	2·06	2·32	2·01	1·43
4856	0·63	0·77	0·96	1·08	1·46	1·87	1·50	1·71
4927	0·52	0·46	0·78	1·39	1·83	2·43	2·13	1·60
4932	0·40	0·61	0·80	0·67	1·20	1·66	1·41	0·96
5039	0·83	0·90	1·05	1·25	2·02	2·37	1·67	1·03
5041	0·65	0·68	0·85	1·44	1·86	2·18	2·62	1·82
	3·54	4·26	5·98	7·55	10·42	12·83	10·74	8·55
Mean	0·59	0·71	0·99	1·25	1·73	2·13	1·79	1·42

In view of this, the findings had been recorded in two groups. Group I represents those without parasitæmia while Group II showing recrudescence. Estimation of urobilinogen in fæces and urine was carried out on three consecutive days in each animal. The data are presented in Table XI.

TABLE XI.

Estimation of urobilinogen in fæces and urine.

Group	Monkey number	Urobilinogen in Urine (Average of 3 readings)	Urobilinogen in Fæces (Average of 3 readings)
I	8048	0·45	10·8
	8123	0·41	20·7
	3069	0·28	12·4
	8093	0·32	14·8
	8096	0·48	18·0
	8203	0·20	19·6
Mean		0·35	16·05
II	8168	0·39	25·7
	8176	0·65	24·0
	8192	0·88	72·2
Mean		0·64	40·6

Although the individual day-to-day variations in the readings appeared to be from 0·12 to 0·91 mg. per 100 c.c. of urine under Group I and 0·19 to 0·96 under Group II, the overall average (mean of 18 readings from six animals) reading under the former group was 0·35 mg. as against 0·64 under the latter (mean of 9 readings). Similar data of fæcal urobilinogen was observed to be 16·05 mg. per 100 gm. fæces under Group I and 40·6 mg. under Group II. The daily range of variation of individual readings was 4·1 to 29·3 mg. under Group I and 16·8 mg. to 125·0 mg. per 100 gm. of fæces under Group II. These observations would, therefore, indicate that the excretion rate of both urinary and fæcal urobilinogen was of a higher order in those animals where recrudescence had occurred. The urinary urobilinogen was almost doubled while fæcal urobilinogen was increased by more than double of that seen in chronic cases but without parasitæmia.

B. Under low protein dietary condition.

SERIES I. UNINFECTED MONKEYS.

Estimation of urobilinogen in fæces and urine was undertaken for a period of ten weeks in ten monkeys and up to 16 weeks in six. These were kept under isocaloric low protein diet.

(*i*) *Fæcal urobilinogen.*—The initial mean reading on ten monkeys was 21·5 mg. per 100 c.c. of fæces. Subsequent studies showed that although there was considerable individual variations, the overall mean data did not vary significantly from

the initial at least up to ten weeks. The subsequent readings during the next six weeks were 19·5, 26·0, 23·0, 27·2, 18·4 and 20·5 mg. per 100 gm. of the fæces. During this period, particularly from about the fourteenth week, occasional diarrhœa was noted and as such the specimen had to be rejected.

(*ii*) *Urinary urobilinogen.*—The initial reading was observed to be 0·85 mg. per 100 c.c. The subsequent readings during the first eight weeks showed fluctuations between 0·7 and 1·5 mg. During the subsequent eight weeks, the readings varied from 1·25 to 1·6 mg. It would, therefore, seem that there was slight rise in urinary urobilinogen during the latter half of the observation period.

SERIES II. MONKEYS INFECTED WITH *P. CYNOMOLGI.*

ACUTE INFECTION

Urobilinogen in fæces and urine.

The estimation were undertaken in eight monkeys on every alternative day during the phase of acute infection.

The mean initial urobilinogen in fæces was recorded to be 9·8 mg. per 100 gm. of fæces. It was observed that from Day 4, there was a tendency towards rise and the level had reached 21·5 mg. (mean) by Day 8. The maximum recorded was 26·0 on Day 12. After that, there was a gradual decrease but the amount of urobilinogen recorded on Day 18 (the last day of observation) was still much higher than the initial level.

From the data obtained, it was observed that up to Day 4 the urobilinogen in urine was nearabout the initial level of 0·42 mg. per 100 c.c. of urine. Then there was a sudden rise and this was maintained up to Day 10. The maximum amount of urobilinogen recorded was 1·97 mg. on Day 8. From Day 12, there was a gradual fall and the final amount recorded on Day 18 was 0·36 mg.

From the results of the series of investigations thus carried out, it was observed that the urinary urobilinogen put out varied from 0·25 to 1·8 mg. per 100 c.c. of urine, the mean normal value being 0·85 mg. The fæcal urobilinogen in the same animals recorded at the same time ranged between 6·27 and 54·18 mg. (mean 24·6 mg.) per 100 gm. of the fæces of the usual consistency. In this connection, it may be mentioned that during diarrhœa the normal fæcal urobilinogen reading seemed to be very considerably lowered on account of dilution.

In *P. knowlesi* infection, the mean reading of urinary urobilinogen in the group of 10 *M. mulatta mulatta* was found to be 2·17 mg. on Day 4 as compared to the mean initial (pre-inoculation) level of 0·85 mg. per 100 c.c. of urine. The urobilinogen in fæces was recorded to be 19·7 mg. against the mean initial level of 13·0 mg. per 100 gm. of fæces. It would thus be evident that in acute infection like *P. knowlesi* in which the infection develops rapidly and the erythrocytic invasion is of extremely high order, there was a corresponding rise in both fæcal and urinary urobilinogen output.

More or less similar observations were made in the acute phase of *P. cynomolgi* infection. Against the pre-inoculation mean reading of 0·59 mg., the highest

urinary urobilinogen output was observed to be 2·13 mg. per 100 c.c. of urine on Day 10. Just prior to and during the peak parasitic infection (between Day 6 and 8), the readings varied between 1·25 and 1·73; at that time the fæcal urobilinogen output being 27·1 and 42·1 mg. respectively (on the different days) against the mean initial level of 13·4 mg. per 100 gm. of fæces. Even the final readings on Day 14 were higher than the initial.

During chronic *P. cynomolgi* infection, the mean readings for urinary and fæcal urobilinogen were 0·35 and 25·7 mg. respectively during the latent phase as compared to 0·64 and 40·6 mg. recorded in the series showing recrudescence, thereby indicating that the urobilinogen ouptut increased considerably during recrudescence as compared to the latent phase.

Investigations carried out in normal monkeys, kept under low protein diet, showed hardly any significant rise in urobilinogen either in urine or fæces during an observation period up to 16 weeks. However, it was recorded that under similar conditions when monkeys were infected with *P. cynomolgi* (between the fourth and sixth week after being placed on low protein diet), the urinary urobilinogen output was rapidly increased to 1·97 mg. on Day 8 during the acute phase of the infection, and also at the time when the peak parasitaemia was recorded. The mean pre-inoculation reading was 0·42 mg. per 100 c.c. of urine. During the same period, the mean fæcal urobilinogen reading was found to be 21·5 mg. against the initial reading of 9·8 mg. per 100 gm. of the fæces.

DISCUSSION.

Most authors believe that both bilirubin and urobilinogen output is increased in urine during an attack of malaria, and this is particularly noticeable during the acute phase (Gordon, 1924; Kingsbury, 1926; Owen, 1928; Fredricks and Hoffbauer, 1945). Some workers have also reported that this is more noticeable in *P. falciparum* than in others (Kingsbury, 1926; Owen, 1928) though Gordon (1924) and Hebir (1927) were unable to establish any variation on account of difference in the species of plasmodia. Similar controversial issues exist on the question of the time when such increased output occurs, that is, whether they occur, before, during or after the onset of the malarial attack.

In this connection, it may be indicated that most of the above mentioned workers had studied the urobilinogen output in urine only and not in fæces. Thus from their finding, it is difficult to establish if the rise in the pigment could really be due to hæmolysis alone as increase in urinary urobilirubin and urobilinogen could also be associated with hepatic insufficiency (Mac Cormac and Dodds, 1923; Fredrick and Hoffbauer, 1945; Wintrobe, 1951). It should also be mentioned here that signs of liver damage, as determined by liver function tests, have been established in all forms and all stages of human malaria (Sinton and Hughes, 1924; So, 1941; Kopp and Solomon, 1943; Kern and Norris, 1944; Fredricks and Hoffbauer, 1945; Wahi and Arora, 1951). Further, it had been demonstrated that mild to severe degree of parenchyma damages do occur in simian malaria like *P. knowlesi* infection, irrespective of the dietary conditions of the host (Taliaferro and Mulligan, 1937; Rigdon and Stratman Thomas, 1942; Macgraith *et al.*, 1951; Ray, 1954*a*: 1955: 1957). Parenchyma lesions have also been observed in

P. cynomolgi infection when the hosts are kept under low protein diet even for as short a period as five to six weeks (at a time when normally there should not be any liver damage under the dietary conditions alone (Ray, 1953; 1954*b*: 1955).

According to Whitby and Britton (1953), bilirubinæmia and increased urobilinogen in urine may be due to hepatic inefficiency and not necessarily to increased hæmolysis. In elaborating this point further, they have indicated that the amount of urinary urobilinogen excreted depends not only upon the amount derived from flood destruction but also on the efficiency of the liver. It has been further indicated that quantitative determination of fæcal urobilinogen is of great value, as high output is usually due to excessive hæmolysis. The value of estimation of fæcal urobilinogen in increased hæmolysis has also been stressed by Sherlock Sheila (1956).

In view of these observations, equal importance was placed on the estimation of fæcal urobilinogen as well, througout the course of present studies and the finding would appear to be highly interesting. It has been shown that during acute infection due to either *P. knowlesi* or *P. cynomolgi*, there is significant increase in the output of urobilinogen both in fæces and urine, irrespective of the dietary conditions of the hosts (in respect of the series infected with the latter plasmodium). Further, the increased output of the pigment would appear to follow the course of infection reaching its highest level during or about peak parasitæmia. In the series infected with *P. cynomolgi* in which studies could be continued after the peak, urobilinogen output has been found to be gradually lowered along with crisis. These findings, would, therefore, clearly indicate a direct correlationship between erythrocytic invasion, by the parasites and thus its destruction, and the output of urinary and fæcal urobilinogen.

Further, it may also be borne in mind that the output of urobilinogen has been recorded to be higher during recrudescence than during latent period.

Another interesting feature may be noteworthy. It has been observed that in uninfected monkeys kept on low protein diet there has been no significant rise in fæcal urobilinogen output for as long a period as 16 weeks. But during the latter part of this period, slight increase in urinary urobilinogen output has been recorded. In this connection, it may be mentioned that under such conditions frank liver injury has been observed from about the eighteenth week onwards (Ray, 1955). It could, therefore, be due to hepatic inefficiency on account of the low protein dietary condition as the question of excessive hæmoglobin does not arise under such circumstances.

In view of these observations, one would be tempted to draw the inevitable conclusion that the increased output of the pigment during infection is mainly due to excessive hæmolysis caused by destruction of large number of erythrocytes on account of parasitic invasion. Besides, there is also some breakdown of normal erythrocytes as well in malarial infections for reasons not clearly known (Maegraith, 1948). Further it is considered that the need for estimation of fæcal urobilinogen during such studies is of paramount importance, as increased output of the pigment in urine, in the absence of similar data in fæces, could not be correlated to hæmolysis alone since such increase does occur in hepatic dysfunction as well. This has been observed during the present investigations as well. It is also felt that periodic studies on fæcal urobilinogen may be helpful in cases of long continued

anæmia and when chronic malaria is suspected. It is common knowledge that quite often presence of plasmodial infection may be missed during routine blood examination, particularly at a time when the parasitæmia is at a sub-patent level.

In this connection, it may be worthwhile to point out that the technique followed for determination of fæcal urobilinogen involves accurate weighing of wet specimen for subsequent treatment as recommended by Maclagan (1946) or King (1951). But certain limitations of this technique have been observed during the conduct of the present investigations, as for example, the estimated amount of urobilinogen would vary widely in fæces of normal consistency as against the samples obtained from cases suffering from diarrhoea.

In view of this, it may be considered whether it would not be better to follow the techniques which involve in using samples of over-dried fæces as have been recommended by some workers.

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BIOMETRIC STUDIES ON THE SUSCEPTIBILITY OF SOME SPECIES OF ANOPHELINE MOSQUITOES TO TECHNICAL D.D.T., GAMMA B.H.C. AND TECHNICAL DIELDRIN*.

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INTRODUCTION.

In India, with the extensive use of insecticides for malaria control, continuous vigilance is necessary for the detection of variation in the susceptibility to insecticides in mosquitoes immediately on its appearance anywhere in the country. This can be done by determining the initial susceptibility of mosquitoes in unsprayed areas and then periodically assessing their susceptibility in sprayed areas. With this object in view, specific studies by way of determination of median lethal concentrations (M.L.Cs.) of different insecticides against *A. culicifacies*, *A. fluviatilis*, *A. annularis* and *A. subpictus* have been carried out since 1956 in some States of India and a summary of the results has been published by Sharma *et al.* (1957) to serve as base line data to be used for purposes of comparison by other workers engaged in similar studies. Detailed analysis of the data and full particulars of the investigations are presented in this publication.

EXPERIMENTAL TECHNIQUE.

Susceptibility tests were carried out with anopheline mosquitoes against technical D.D.T., gamma B.H.C., and technical dieldrin in the villages of Uttar Pradesh, Rajasthan and Delhi. Particulars of the villages are given in Table I.

*These studies were carried out under the auspices of "*General behaviour of vector species of anophelines, their resistance to insecticides etc.*." an enquiry sponsored by Indian Council of Medical Research.

TABLE I.

Particulars of experimental villages.

Village	District and State	Sprayed/Unsprayed. If sprayed, history of spraying	If unsprayed, distance from sprayed areas	Topographical features of the area	Approximate number of houses in the village
Arthala	Meerut, U. P.	Unsprayed	4½ miles	Plain, canal and well irrigated area.	35
Kailashpuri	Udaipur, Rajasthan	,,	12 ,,	Foot-hill area with perennial streams.	160
Jwalaheri	Delhi	Sprayed with D.D.T. as malaria control measure since 1947.	...	Plain, well irrigated area.	100
Nangli Sahidan	Delhi	,,	...	Plain, canal irrigated area.	65

Technique recommended by the World Health Organization (W.H.O. Technical Report Series 80, 1954) for assessing the susceptibility of mosquitoes to residual insecticides was followed. The W.H.O. technique was slightly modified in respect of time of exposure of test insects to insecticidal deposits in case of *A. subpictus* collected from D.D.T. sprayed villages to obtain mortalities within the critical range. There was no mortality in the test insects after 24 hours with exposures of one and two hours to the insecticidal deposits. The period of exposure was, therefore, increased from one to 24 hours*.

Wild-caught gravid mosquitoes were used for the tests. During the observation period, the test insects were provided with glucose solution soaked in cotton pads. Mosquitoes which were found " dead beyond doubt " were recorded as dead. Total number of insects in all cases, for each concentration, varied from 72 to 324 and mortality in control was never more than six per cent†. Complete details of the susceptibility tests for each species, with different insecticides, are given in Table II. Mean temperature, relative humidity figures and period of tests are given in Table III.

BIOMETRIC TECHNIQUE.

In order to obtain maximum information from the results of the susceptibility tests, the experimental data were subjected to precise and critical statistical examination. For this purpose, the probit analysis method was followed. The mean tolerance distribution, i.e., median lethal concentration (M.L.C.) and the

*Mortality in insects was recorded after 24 hours' exposure of the insects to insecticidal deposits. In such cases, D.D.T.-impregnated filter papers were snipped in a way to cover the full length of the tubes, and corks were replaced with mosquito nettings.

†The tests were carried out at the village sites to avoid injury and consequent mortality in insects during transportation. Low mortality in controls indicated careful and satisfactory handling of test insects.

TABLE II.

Basic data of susceptibility tests.

Locality of capture of mosquitoes. Sprayed (S.)/Unsprayed (U/S.)	Species of mosquitoes	Insecticide, and concentration per cent		Number of mosquitoes: Exposed n	Dead r	Corrected mortality rate (Per cent) p
Kailashpuri Village Udaipur District, Rajasthan (U/S.)	*A. annularis*	D.D.T.	1·0	144	97	67·4
			0·5	144	57	39·6
			0·25	144	31	21·5
			0·125	144	10	6·9
			Control	144	...	C = 0
		Dieldrin	0·5	108	65	60·2
			0·25	108	30	27·8
			0·125	108	13	12·0
			0·0625	108	4	3·7
			Control	108	...	C = 0
		B.H.C.	0·05	108	71	65·7
			0·025	108	49	45·4
			0·0125	108	10	9·6
			0·00625	108	2	1·8
			Control	108	...	C = 0
	A. culicifacies	D.D.T.	0·5	138	103	73·9
			0·25	138	83	58·9
			0·125	138	29	18·6
			0·0625	102	27	24·5
			Control	138	4	C = 2·9
		Dieldrin	0·125	144	134	92·9
			0·0625	144	95	64·8
			0·03125	144	47	31·7
			0·015625	144	32	21·1
			Control	144	2	C = 1·39

TABLE II. (*Contd.*)

Locality of capture of mosquitoes. Sprayed (S.)/Unsprayed (U/S.)	Species of mosquitoes	Insecticide, and concentration per cent		Number of mosquitoes: Exposed n	Number of mosquitoes: Dead r	Corrected mortality rate (Per cent) p
		B.H.C.	0·05	108	90	83·3
			0·025	108	85	78·7
			0·0125	108	77	71·3
			0·00625	108	44	40·7
			Control	108	...	C = 0
	A. fluviatilis	D.D.T.	1·0	108	90	83·3
			0·5	108	57	52·8
			0·25	108	47	43·5
			0·125	108	29	28·8
			Control	108	...	C = 0
		Dieldrin	0·5	96	63	65·6
			0·25	96	54	56·2
			0·125	96	29	30·2
			0·0625	96	22	22·9
			Control	72	...	C = 0
		B.H.C.	0·05	96	71	73·7
			0·025	96	63	65·3
			0·0125	96	25	25·2
			0·00625	96	9	8·5
			Control	96	1	C = 1·04
Arthala village, Meerut District, Uttar Pradesh (U/S.)	*A. culicifacies*	D.D.T.	2·0	90		90·8
			1·0	90		78·2
			0·5	90		43·7
			0·25	88		27·1
			Control	90		C = 3·3
			0·125	313		20·3
			Control	53		...

TABLE II. (*Contd.*)

Locality of capture of mosquitoes. Sprayed (S.)/Unsprayed (U/S.)	Species of mosquitoes	Insecticide, and concentration per cent		Number of mosquitoes		Corrected mortality rate (Per cent)
				Exposed n	Dead r	p
		Dieldrin	0·05	72	45	62·5
			0·025	72	30	41·7
			0·0125	72	19	26·4
			0·00625	72	12	16·7
			0·003125	72	6	8·3
			Control	72	...	C = 0
		B.H.C.	0·0078125	81	61	74·0
			0·00390625	81	49	58·4
			0·001953125	81	18	18·2
			Control	81	4	C = 4·94
	A. subpictus	D.D.T.	2·0	216	204	94·3
			1·0	216	182	84·3
			0·5	216	154	70·8
			0·25	216	126	57·5
			0·125	216	56	24·5
			Control	216	4	C = 1·85
		Dieldrin	0·5	252	169	66·8
			0·25	252	138	54·4
			0·125	252	74	28·8
			0·0625	216	27	11·8
			Control	252	2	C = 0·79
		B.H.C.	0·05	324	235	72·3
			0·025	324	204	62·6
			0·0125	324	101	30·5
			0·00625	324	48	14·0
			Control	324	3	C = 0·93

TABLE II. (*Concld.*)

Locality of capture of mosqutioes, Sprayed (S.)/Unsprayed (U/S.)	Species of mosquitoes	Insecticide, and concentration per cent		Number of mosquitoes		Corrected mortality rate (Per cent)
				Exposed n	Dead r	p
Jwalaheri, Delhi (S.)	*A. subpictus*	D.D.T.	5·0	212	169	78·4
			4·0	216	122	53·7
			3·0	216	104	44·8
			2·0	216	84	35·0
			1·0	216	45	15·8
			Control	216	13	C = 6·02
		Dieldrin	0·5	216	203	93·9
			0·25	216	139	64·2
			0·125	216	107	49·3
			0·0625	216	35	15·8
			Control	216	1	C = 0·46
		B.H.C.	0·05	216	202	93·4
			0·025	216	194	89·6
			0·0125	216	77	34·1
			0·00625	216	21	7·59
			Control	216	5	C = 2·31
Nangli Sahidan, Delhi (S.)	*A. subpictus*	D.D.T.	5·0	288	211	72·2
			4·0	288	212	72·6
			3·0	288	154	51·6
			2·0	288	151	50·5
			1·0	288	74	22·7
			Control	288	11	C = 3·82

N.B.—Mortality rates in the concentrations were corrected by Abbott's formula when some mortality in control was found.

Abbot's formula, $P = \frac{(p^1 - C)}{100 - C} \times 100$

Where P = Mortality rate due to insecticide alone, i.e., corrected mortaility rate.
C = Mortality rate in control.
p^1 = Mortality rate observed in the concentration.

TABLE III.

Mean temperature and relative humidity figures during test.

Species of anopheline	Place of collection of mosquitoes	Insecticide	Period of experiment	Mean temperature °F.	Mean relative humidity (Per cent.)
A. annularis	Kailashpuri, Rajasthan	D.D.T.	Nov. 12-16, 1956	70·2±2·9	72·0±8·9
	,,	Dieldrin	Nov. 19-24, 1956	70·0±2·6	79·7±7·0
	,,	gamma B.H.C.	Nov. 19-25, 1956	69·7±2·8	74·0±0·0
A. culicifacies	,,	D.D.T.	Nov. 14-19, 1956	69·8±2·7	85·7±3·3
A. culicifacies	,,	Dieldrin	May 4-5, 1957	89·0±3·2	...
	,,	gamma B.H.C.	Nov. 20-23, 1956	68·3±2·3	76·6±3·2
A. culicifacies	Arthala, Uttar Pradesh	D.D.T.	Sep. 17-20, 1956	82·1±1·4	92·0±0·0
	,,	Dieldrin	May 25-28, 1957	87·2±3·7	48·7±5·7
	,,	gamma B.H.C.	Jun. 6-9, 1957	83·3±2·8	85·0±4·1
A. fluviatilis	Kailashpuri, Rajasthan	D.D.T.	Nov. 21-23, 1956	67·3±1·3	82·3±4·9
	,,	Dieldrin	Nov. 24-25, 1956	67·3±1·2	79·9±5·7
	,,	gamma B.H.C.	Nov. 24,25, 1956	67·5±1·5	82·5±8·6
A. subpictus	Arthala, Uttar Pradesh	D.D.T.	Sep. 17-20, 1956	82·1±1·4	92·0±0·0
	,,	Dieldrin	Oct. 11-28, 1956	75·0±1·1	87·0±1·4
	,,	gamma B.H.C.	Oct. 11-28, 1956	75·0±1·1	87·0±1·4
A. subpictus	Jwalaheri, Delhi	D.D.T.	Sep. 29-Oct. 4, 1956	78·1±1·1	81·0±5·9
	,,	Dieldrin	Oct. 10-17, 1956	72·9±0·9	89·8±1·7
	,,	gamma B.H.C.	Oct. 10-17, 1956	72·9±0·9	89·8±1·7
A. subpictus	Nangli-Sahidan, Delhi	D.D.T.	Oct 21-Nov. 6, 1956	70·6±1·8	87·4±1·8

regression coefficient,* which is a measure of diversity of response (Hoskin and Gordon, 1956) were estimated following the maximum likelihood estimation method outlined by Finney (1952). For precision of the estimates, standard errors (S.Es.) of M.L.C.s and regression coefficients were worked out.

None of the log concentration probit graphs showed any systematic departure of the points from the regression line and heterogeneity in the test mosquitoes with respect to their response to the insecticides as revealed by χ^2 tests with K-2 degree of freedom, where K=total number of batches of insects for all concentrations

*The regression coefficient may be defined as the change in the dependant variate (probit of mortality rate in insects) per unit change in the independant variate (log concentration of insecticide).

in the experiment*. The fiducial limits† of each M.L.C. were calculated by both the approximate and precise methods. Full details of analysis of data for one experiment are given in Table IV. A summary of the results of similar analysis for all tests are given in Table V.

RESULTS AND DISCUSSION.

Out of the four species of anopheline mosquitoes reported in this communication, only *A. culicifacies* of two widely separate regions, with different topography, was tested against all the three insecticides. M.L.Cs. of technical D.D.T., technical dieldrin and gamma B.H.C. for *A. culcifacies* of Uttar Pradesh were $0{\cdot}43 \pm 0{\cdot}01$, $0{\cdot}032 \pm 0{\cdot}005$ and $0{\cdot}0039 \pm 0{\cdot}0003$, respectively, and of Rajasthan were $0{\cdot}22 \pm 0{\cdot}018$, $0{\cdot}0436 \pm 0{\cdot}0025$ and $0{\cdot}0071 \pm 0{\cdot}0010$, respectively. From these M.L.C. values, it may be seen that *A. culicifacies* of Uttar Pradesh and Rajasthan differ significantly in respect of their mean tolerance distribution to D.D.T. and B.H.C. Variations in the M.L.Cs. of D.D.T. have also been reported in *A. culicifacies* of Bombay State (Ramachandra Rao and Bhatia, 1957). There are a number of factors such as vigour, age, etc., of the test insects and prevalent temperature and humidity which influence the susceptibility of insects to insecticides. In the present case, it may be observed from Table III that the temperature was higher during the susceptibility tests with D.D.T. in Uttar Pradesh than in Rajasthan. It is, therefore, considered that the higher M.L.C. of D.D.T. to *A. culicifacies* of Uttar Pradesh may probably be due to the effect of higher temperature. A significant increase (as high as two times) in M.L.C. of D.D.T., without any associated change in the slope of *ld-pr* line, with the increase of temperature in the case of house-flies has also been reported by Menn *et al.* (1957). In the case of M.L.C. of B.H.C., it was observed that the values are low for *A. culicifacies* of Uttar Pradesh than those of Rajasthan. However, there is no significant difference in the slope of the *ld-pr* line. It may be seen from Table III that temperature during the experiments was higher in Uttar Pradesh than in Rajasthan. This shows that probably the relationship of temperature and mortality in the case of B.H.C. may not be the same as that of D.D.T.

The M.L.Cs. of technical D.D.T., technical dieldrin, gamma B.H.C. for *A. fluviatilis* and *A. annularis* of Rajasthan were $0{\cdot}35 \pm 0{\cdot}034$, $0{\cdot}238 \pm 0{\cdot}037$, $0{\cdot}022 \pm 0{\cdot}002$ and $0{\cdot}629 \pm 0{\cdot}067$, $0{\cdot}414 \pm 0{\cdot}058$, $0{\cdot}033 \pm 0{\cdot}002$, respectively. It may be seen from Table V that these two species show significant difference in their M.L.Cs. of D.D.T., B.H.C. and dieldrin. The mean tolerance distributions for these insecticides were found to be higher with *A. annularis* than with *A. fluviatilis* though mean temperatures during the tests were almost the same. Further, it may be seen that *A. culcifacies* of Rajasthan is most susceptible to all the three insecticides than the other species of the same area.

The upper fiducial limit of M.L.C. of B.H.C. for *A. culicifacies* (Rajasthan) is about 1·8 times as high as the lower limit (Table V) though both the values are

*Degree of freedom thus calculated is the sum of the degrees of freedom for the test of linearity and variation in mortality between the batches within a concentration.

†The fiducial limits may be defined as the two limiting values within which the parameter lies at a desired level of probability.

TABLE IV.

Details of analysis of data in respect of A. subpictus *of the sprayed village Nangli Sahidan.* (Exposed to D.D.T. for 24 hours).

							1st cycle of computation						2nd cycle of computation				
Concentration (z)	log 10 z (x)	Number exposed (n)	Number dead (r)	Corrected mortality rate (p) C=3·82	Empirical probit.	Y Expected probit.	nw	y	nwx	nwy	nwxy	Y(a)	nw	y	nwx	nwy	nwxy
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
5·0	1·70	288	211	72·20	5·59	5·8	137·5	5·57	233·750	765·875	1301·9875	5·6	151·9	5·58	258·230	847·602	1440·9234
4·0	1·60	288	212	72·56	5·60	5·6	151·9	5·61	243·040	852·159	1363·4544	5·4	162·6	5·60	260·160	910·560	1456·8960
3·0	1·48	288	154	51·62	5·04	5·3	166·2	5·04	245·976	837·648	1239·7190	5·2	168·6	5·05	249·528	851·430	1260·1164
2·0	1·30	288	151	50·54	5·01	4·9	167·5	5·02	217·750	840·850	1093·1050	4·9	167·5	5·02	217·750	840·850	1093·1050
1·0	1·00	288	74	22·74	4·25	4·2	121·0	4·26	121·000	515·460	515·4600	4·3	130·6	4·26	130·600	556·356	556·3560
Control		288	11	...	...	...	...	...	...	...	...	...	...	...	...	...	...
							744·1		1061·516	3811·992	5513·7529		781·2		116·268	4006·798	5807·3968

1st Cycle.—

$$\frac{1}{Snw} = 0{\cdot}001343905; \quad \bar{x} = 1{\cdot}4266; \quad \bar{y} = 5{\cdot}1229.$$

$$\begin{array}{rl} & 1554{\cdot}3585 \\ & -1514{\cdot}3339 \\ \hline Sxx^{*} = & 40{\cdot}0246 \end{array} \qquad \begin{array}{rl} & 5513{\cdot}7259 \\ & -5438{\cdot}0988 \\ \hline Sxy^{\dagger} = & 75{\cdot}6271 \end{array} \qquad \begin{array}{rl} & 19685{\cdot}2083 \\ & -19528{\cdot}6639 \\ \hline Syy^{\ddagger} = & 156{\cdot}5444 \\ & -142{\cdot}8986 \\ \hline \chi^2(38) = & 13{\cdot}6458 \end{array} \qquad \left\{ \chi^2 = Syy - \frac{(Sxy)^2}{Sxx} \right\}$$

$$b = \frac{Sxy}{Sxx} = \frac{75{\cdot}6271}{40{\cdot}0246} = 1{\cdot}8895$$

$Y_1 = 5{\cdot}1229 - 1{\cdot}8895\,(x - 1{\cdot}4266) = 1{\cdot}8895x + 2{\cdot}4275$ ... Equation by 1st cycle of computation.

$Y_p = 5 + 2{\cdot}3\,(x - 1{\cdot}35) = 2{\cdot}3x + 1{\cdot}895$ ... Equation of the provisional line.

Since mathematically worked out b, 1·8895 differes from the value of b, 2·3 estimated graphically, 2nd cycle of computation was taken up by computing the value of Y(a) from the regression equation of 1st cycle.

$^{*}Sxx = Sxwx^2 - \frac{(Sxwx)^2}{Sxw}$. $\quad ^{\dagger}Sxy = Sxwxy - \frac{(Sxwx)\,(Sxwy)}{Sxw}$. $\quad ^{\ddagger}Syy = Sxwy^2 - \frac{(Sxwy)^2}{Sxw}$.

Note.—S stands for summation.

TABLE IV. (*Contd.*)

2nd Cycle.—

$$\frac{1}{Snw} = 0{\cdot}001280082, \quad \bar{x} = 1{\cdot}4289, \quad \bar{y} = 5{\cdot}1290$$

	1638·2234		5807·3968		20719·6202
	—1595.0515		—5725·3721		—20550·9871
Sxx =	43·1719	Sxy =	82·0247	Syy =	168·6331
					—155.8433
				$x^2(38)$ =	12·7898

x^2 value is not significant at P. 0·05.

$$b = \frac{Sxy}{Sxx} = 1{\cdot}8999.$$

$$Vb = \frac{1}{Sxx} = 0{\cdot}0232 \left(\text{In case where } x^2 \text{ is significant, Vb is multiplied by the H.F.,} \frac{x^2}{df}\right)$$

S.E. of b = 0·15.

Since the difference between values of b, calculated by 1st and 2nd cycles of computation are not much, further computations are not undertaken.

The regression equation is therefore, Y = 5·1290 + 1·8999 (x—1·4289)
= 1·8999x + 2·4142 ... by 2nd cycle of computation.

From the equation of the 2nd cycle,
m = 1·361 in log.
= 22·961 (Antilog. value of *m*).

Since z was multiplied by 10 in the initial stage of computation, the antilog value of m, 22·961 is now divided by 10 to get MLC. in the concentration scale, which is 2·296 × 1.

$$Vm = \frac{1}{b^2}\left\{\frac{1}{Snw} + \frac{(m-\bar{x})^2}{Sxx}\right\} = 0{\cdot}000384 \left(\text{(In case } x^2 \text{ is significant, Vm is multiplied by H.F.,} \frac{x^2}{df}\right)$$

SE. = 0·019 in log.

In con. scale 22·961 × 2·3 × 0·019 = 1·003.

Dividing this value by 10, the true S.E. of M.L.C. is 0·1003.

Fiducial limit.—

A. *By approximate method*

m ± t × S.E. (The value of *t* is read from the *t* table, against P. 0·05 with K-2 degree of freedom, when x^2 is significant. When it is not significant the normal deviate, 1·96, is taken for *t*).

1·361 ± 1·96 × 0·019 = 1·361 ± 0·037

Upper limit = 1·398 } in log scale.
Lower limit = 1·324 }

The antilog values are 25·00 and 21·086.

In true con. scale values are 2·5 per cent and 2·109 per cent.

TABLE IV. (*Concld.*)

(*B.*) *By precise method*

$$m + \frac{g}{1-g}(m-\bar{x}) \pm \frac{t}{b(1-g)}\sqrt{\frac{(1-g)}{snw} + \frac{(m-\bar{x})^2}{Sxx}}$$

Where $g = t^2/b\,Sxx$.

In the present case

$$g = \frac{(1\cdot96)^2}{155.8341} = 0\cdot02465$$

and the fiducial limits are:

$$1\cdot361 + \frac{0\cdot02465}{0\cdot97535}(1\cdot361 - 1\cdot4289) \pm \frac{1\cdot96}{1\cdot8999 \times 0\cdot97535}\sqrt{\frac{0\cdot97535}{781\cdot2} + \frac{(1\cdot361 - 1\cdot4289)^2}{43\cdot1719}} = 1\cdot3593 \pm 0\cdot0391.$$

Upper limit: = 1·398 in log. and in true concentration scale is 2·5 per cent × 24.

Lower limit: = 1·320 in log. and in true concentration scale is 2·1 per cent × 24.

TABLE V

Summary of the analysis of susceptibility tests data in respect of different anopheline species.

Species of mosquitoes	Place of collection of mosquitoes Sprayed(S.) Unsprayed (U/S.)	Insecticide	Equation of the fitted lc–pr. line Y=	χ^2 (Degree of freedom)	M.L.C. Concentration Insecticide Per cent x 1hr.	Fiducial limits at P. 0·05 level: Upper limit (Con. %) xl hr. (By approximate method)	Lower limit (Con. %) xl hr. (By approximate method)	Upper limit (Con. %) xl hr. (By precise method)	Lower limit (Con. %) xl hr. (By precise method)	Regression co-efficient	Remarks x=10z.
A. annularis	Kailashpuri Village, Udaipur District, Rajasthan State (U/S.)	D.D.T.	2·103 x+3·3178	0·21(14)	0·6295 ±0·067	0·889	0·709	0·7396	0·5508	2·1±0·2	10z
		Dieldrin	2·2721x+1·3339	0·90(10)	0·414 ±0·058	0·55	0·31	0·5754	0·3243	2·3 ±0·3	100z
		B.H.C.	2·7714x+0·8079	4·18(10)	0·033 ±0·002	0·037	0·029	0·0371	0·0291	2·8 ±0·3	1000z
A. culicifacies	,,	D.D.T.	1·721 x+2·618	19·49(13)	0·222 ±0·018	0·261	0·190	0·260	0·193	1·7 ±0·2	100z
		Dieldrin	2·4549x+3·4276	8·34(14)	0·04365±0·00251	0·04886	0·03899	0·04898	0·03899	2·5 ±0·2	100z
		B.H.C.	1·3186x+3·8756	6·43(10)	0·0071 ±0·0010	0·0095	0·0054	0·0089	0·0049	1·3 ±0·2	1000z
A. culicifacies	Arthala Village, Meerut District, Uttar Pradesh (U/S.)	D.D.T.	1·7387x+3·9015	8·84(18)	0·4285 ±0·0108	0·4508	0·3981	0·3781	0·3221	1·7 ±0·14	100z
		Dieldrin	1·4105x+2·8742	0·53(8)	0·03214±0·0045	0·0433	0·0239	0·0461	0·0248	1·4 ±0·2	1000z
		B.H.C.	2·5285+3·5038	3·28(7)	0·0039 ±0·0003	0·0046	0·0033	0·0046	0·0033	2·5 ±0·4	1000z
A. fluviatilis	Kailashpuri Village, Udaipur District, Rajasthan (U/S.)	D.D.T.	1·5104x+4·1693	9·13(10)	0·35 ±0·034	0·427	0·295	0·431	0·292	1·5 ±0·2	100z
		Dieldrin	1·3879x+3·0887	2·19(10)	0·23823±0·037	0·32	0·17	0·31	0·19	1·4 ±0·2	100z
		B.H.C.	2·3685x+1·799	6·56(10)	0·0224 ±0·0016	0·0258	0·0194	0·0271	0·0204	2·4 ±0·24	1000z
A. subpictus	Arthala Village, Meerut District, Uttar Pradesh(U/S.)	D.D.T.	1·744 x+2·318	7·81(28)	0·264 ±0·018	0·2805	0·2157	0·2818	0·2158	1·7 ±0·1	10z
		Dieldrin	1·7822x+2·4886	3·73(26)	0·25645±0·0153	0·2884	0·22803	0·2904	0·2291	1·8 ±0·15	100z
		B.H.C.	1·9345x+1·4184	12·59(34)	0·0216 ±0·00099	0·0231	0·01928	0·0239	0·0194	1·9 ±0·12	1000z
A. subpictus	Jwalaheri Village, Delhi (S.)	D.D.T.	2·2893x+1·6109	13·39(28)	3·02 ±0·1320 x24	3·3113 x24	2·7542 x24	3·516 x24	2·698 x24	2·3 ±0·2	10z
		Dieldrin	2·5426x+1·9324	13·47(22)	0·16069±0·00702	0·17498	0·14757	0·1762	0·1432	2·54±0·27	100z
		B.H.C.	3·6439x+0·6313	31·8974(22)	0·0158 ±0·0054	0·0169	0·0148	0·01694	0·01476	3·6 ±0·208	1000z
A. subpictus	Nangli Sahidan Village, Delhi (S.)	D.D.T.	1·8999x+2·4142	12·7898(38)	2·2961 ±0·1003 x24	2·5x24	2·109x24	2·5x24	2·1x24	1·9 ±0·2	100z

N.B.—For positive value of log concentrations, the concentrations were multiplied by 10 or any power of 10 before taking the log values of concentration, x. In 'Remarks' Column, the factors by which the concentration, z, were multiplied are shown.

of the same population. In the case of experiments with significant value of χ^2, this ratio may show many-fold increase. It is, therefore, erroneous to estimate only times of tolerance to insecticide for establishing resistance in a particular case by merely comparing its M.L.C. with that of the susceptible strain of the same species. In deciding a case of resistance, a many-fold higher M.L.C., than the susceptible insect of the same species, is to be taken into account along with other physical and biological factors such as prevalent temperature during tests and vigour, age, nutritional conditions, etc. of test insects when the difference in susceptibility is statistically significant. This may be seen from the susceptibility data for *A. subpictus* which has been reported to be D.D.T. resistant in sprayed areas of Delhi State (Sharma and Krishnamurthy, 1957*a*:1957*b*). The M.L.Cs. of D.D.T. for the resistant and normal strains of the species were found to be $3{\cdot}02 \pm 0{\cdot}13 \times 24$* and $0{\cdot}264 \pm 0{\cdot}02 \times 1$† respectively. Even when the increased period of exposure of the resistant strain to the insecticidal deposits is not considered, its M.L.C. value of 3·02 is about ten times higher than that of the normal strain and the difference is statistically significant. In well-established cases of insecticide resistance in *A. sacharovi*, *A. sundaicus*, *A. stephensi* and *A. gambiae*, the degrees of tolerance, compared to normal strains, are stated to vary from six to fifteen times (Busvine, 1956). In view of this, ten times tolerance exhibited by *A. subpictus* to D.D.T. is fairly high. D.D.T. resistant *A. subpictus*, however, was as susceptible to gamma B.H.C. and dieldrin as the normal strain (Table V).

Regression coefficient which is reciprocal of standard deviation is an important parameter in toxicological studies of insects. M.L.C. alone does not give full information on the toxicity of an insecticide. Two insecticides may have the same M.L.Cs., but if the distribution of tolerances has lesser 'spread' for one than for the other, any increase or decrease from M.L.C. will produce a greater change in mortality in insects in the former case than in the latter. For this purpose, regression coefficient values are made use of. This statistic also provides information about the ratio of individuals with varying degree of susceptibility to insecticides under test in the population. It may be seen from the data presented in Table V that the mean values of regression coefficients for all the species were $1{\cdot}7 \pm 0{\cdot}1$ for technical D.D.T., $2{\cdot}2 \pm 0{\cdot}3$ for gamma B.H.C. and $2{\cdot}0 \pm 0{\cdot}2$ for technical dieldrin, which showed that the three insecticides, though differed significantly in their M.L.Cs. for different species of anophelines, did not show any difference in respect of the 'spread' of their toxicity to insects, i.e., the change in the probit of mortality rate in insects per unit change of log concentration of insecticide were almost identical for all the three insecticides.

In conclusion, it may be stated that the data presented in this paper are based on tests performed in a few States of India and on only four species of anophelines. It is hoped that it would stimulate interest in other workers to undertake similar studies on various species of anophelines in several representative areas of the country. The results of such studies will be very useful in computing precise parameters on the susceptibility of different species of anophelines to insecticides.

*Mortality in insects was recorded after 24 hours' exposure to the insecticidal deposits.
†Mortality in insects was recorded after 24 hours with one hour exposure to insecticidal deposits.

SUMMARY.

Details of susceptibility tests on *A. culicifacies*, *A. fluviatilis*, *A. annularis* and *A. subpictus* to technical D.D.T., technical dieldrin and gamma B.H.C. are given. The results of the tests have been analysed using the probit analysis method. Importance of regression coefficient is discussed and values of different parameters are given for use as base line data for comparison by other workers engaged in similar studies.

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PRELIMINARY REPORT OF THE FURTHER STUDIES ON THE 'SO-CALLED' NATURAL CRYPTIC (OCCULT) TRYPANOSOME OF THE HOUSE-SPARROW, *PASSER DOMESTICUS* LINNAEUS.

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INTRODUCTION.

A TRYPANOSOME of the house-sparrow, *Passer domesticus* Linnaeus, which was reported in the salivary glands of a mosquito, fed on a house-sparrow in April 1950, by Jaswant Singh *et al.* (1950) has been acclaimed as a cryptic trypansome by David and Nair (1955), Nair and David (1956), Ramakrishnan *et al.* (1956) and David and Satya Prakash (1957).

Further work on this trypansome, original strain of which was lost in 1950 and was again established in 1954 and since then maintained in the laboratory, was commenced when the senior author got the opportunity to work at the Malaria Institute of India in 1957. This short note is put forward to show that it is possible to see the trypanosome in the body of the vertebrate hosts (birds and mammals) if a sufficient amount of inoculum, containing meta-trypanosomes (metacyclic trypanosomes) is given. This was also shown by Grewal (1956) while dealing with some occult trypanosomes of mammals. This paper deals only with the experiments performed on two different birds, from the tissues (bone-narrow) and blood of which the adult trypanosome of the house-sparrow has been observed either in fresh or stained (thin smears) preparations.

MATERIAL AND METHODS.

To study the morphology of the flagellates, thin films of the bone-marrow obtained from the tibio-fibula and the blood from the pectoral vein of birds, were

dried in air, fixed in methyl alcohol and stained with J.S.B. stain according to the method of Jaswant Singh (1956) with satisfactory results.

Some other slides were dried in air, fixed in methyl alcohol for two to three minutes and stained for 20-45 minutes with Giemsa stain 1 in 10 dilution in buffered distilled water at *p*H 7·2-7·4. A few slides were exposed to osmic acid vapour for 10-20 seconds according to the technique of Taliaferro (1923) and stained with Giemsa stain. The results with Giemsa stain were equally satisfactory.

EXPERIMENT I.

One nightingale, *Molpastes cafer* (Bulbul 4), was inoculated intraperitoneally on September 5, 1957, with six and on September 6, 1957, with seven positive guts of mosquitoes, *C. fatigans*, fed on an infected bird (Bulbul 2TC) on August 13, 1957. During the nights between September 11 and 12, and 12 and 13, 1957, a lot of 385 laboratory-bred clean mosquitoes, *C. fatigans*, were fed on this infected bird. On the morning of September 13, 1957, this bird was found dead. Post-mortem examinations of the tissues—brain, liver, lung, kidney, spleen and bone-marrow—and blood were made immediately by fresh and stained preparations. Trypanosomes were demonstrated from the bone-marrow, both in fresh and stained preparations. The result of this observation is summarised in Table I. Examination of the mosquitoes, which had been fed on this bird (Bulbul 4) during the nights between September 11 and 12, and 12 and 13, 1957, was started from Septmeber 19, 1957. Infection in the mosquitoes was almost 100 per cent and the flagellates were invarialy found in the anterior portion of the mid-gut and sometimes throughout the mid-gut in various stages of development. Clusters of crithidia forms were quite common. Interspersed between the other flagellate forms, were the infective meta-trypanosomes.

EXPERIMENT II.

One parrot, *Palaeornis torquatus*, (Number 4), bought on August 30, 1957, has been under careful observation since then. Its blood was examined, under fresh preparations, twice, on September 1 and 2, 1957, which did not reveal any flagellates. On September 4, 1957, xenodiagnosis was performed on this bird which also confirmed the negative infection of this parrot with trypanosomes. It was inoculated on September 28, 1957, with the positive gut contents of 21 mosquitoes which had been fed on September 13, 1957, on an infected bird (Bulbul 4 of Experiment I). Examination made on October 2, 4, 6, 7 and 8, 1957, under fresh preparation, revealed the presence of trypanosome in its blood. The result of this experiment is shown in Table II. This parrot is still alive for further observations.

Trypanosome, in fixed and stained preparations, resembles *T. lewisi* of the rat with pointed posterior end, posteriorly placed kinetoplast, almost central nucleus and a free flagellum.

It is felt that if the doze of the inoculum is increased, trypanosomes may be observed in numbers, both from the bone-marrow, as well as from the blood; although it is known that for bird trypanosome the bone-marrow is the site of preference (Wenyon, 1926; Diamond and Herman, 1954).

TABLE I.

Results of the experimental inoculation of nightingale, M. cafer *(Bulbul 4) with the positive gut contents of 13 infected mosquitoes,* C. fatigans.

Number of mosquitoes used on each day	Date on which mosquitoes were fed on an infected bird (Bulbul 2TC)	Date of inoculation	Date of postmortem of the bird	Results of examination of tissues						Dates and results of blood examination		
				Brain	Liver	Lung	Kidney	Spleen	Bone-marrow	September 7, 1957	September 8, 1957	September 13, 1957
6	August 13, 1957	September 3, 1957	September 13, 1957	N	N	N	N	N	P	N	N	N
7		September 6, 1957										

N=Negative. P=Positive.

TABLE II.

Results of the experimental infection in parrot, Palaeornis torquatus *(Number 4).*

Dates and result of blood examination before inoculation		Dates and result of xenodiagnosis		Number of mosquitoes used	Date on which mosquitoes were fed on an infected bird (Bulbul 4)	Date of inoculation	Dates and result of blood examination after the inoculation			
September 1, 1957	September 2, 1957	Date on which mosquitoes were fed	Date of examination of mosquitoes				October 1, 1957	October 2, 1957	October 4, 1957	October 6, 1957
Negative	Negative	September 4, 1957	October 2, 1957	21	Nights between September 11 and 12 and 12 and 13, 1957	September 28, 1957	Negative	Positive	Positive	Positive

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NATIONAL MALARIA CONTROL PROGRAMME IN INDIA, 1953-1956.

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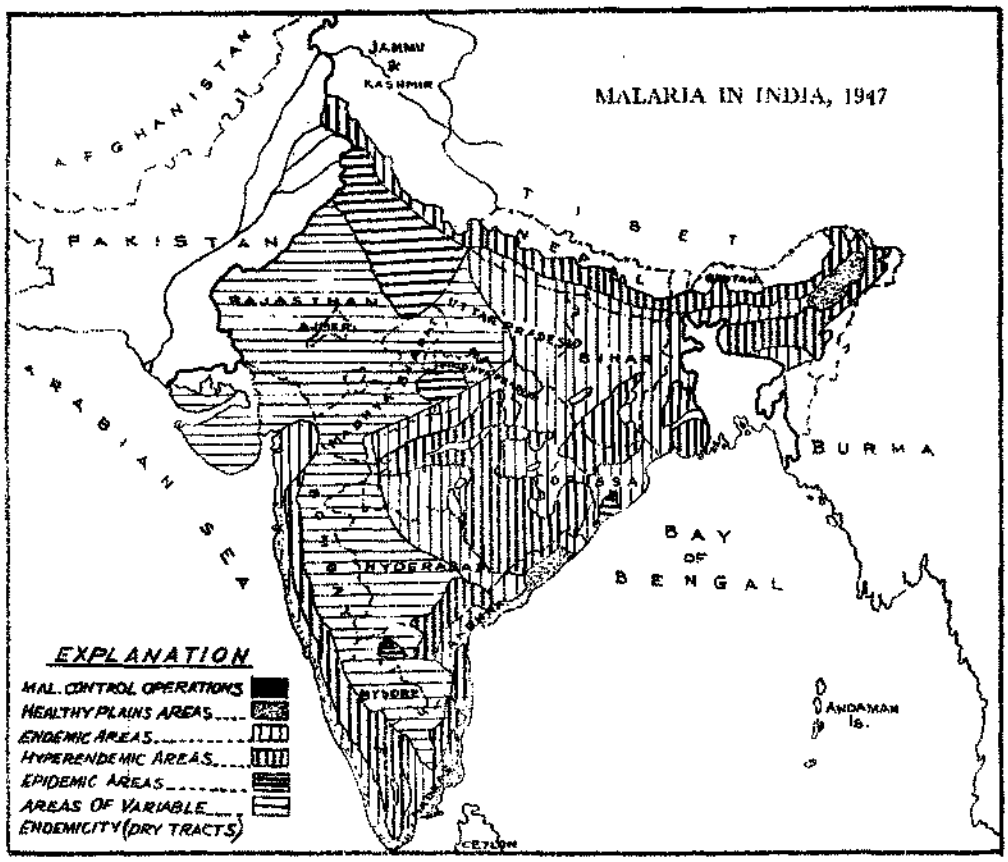
I. THE PLAN AND OBJECTIVES.

MALARIA has for centuries been one of the most formidable and serious health problems of India and a great obstacle to the development of the natural resources of the country. It debilitates its victims rendering them susceptible to other diseases and claims a greater number of deaths every year than any other single disease. Not only the individual but the entire nation suffers from its economic set-backs. The late Brigadier J. A. Sinton, the first Director of the Malaria Institute of India, estimated in 1935 that at least 100 million people suffered from malaria every year in India and one million deaths occurred annually in endemic areas and an equal number of deaths occurred due to the indirect effects of this disease. The intensity and distribution of malaria in India as it was in 1947 are shown in Map 1.

Studies on the different aspects of epidemiology of malaria have been in progress for many decades in India. Notable contributions in the field of malariology have been made from time to time by a number of workers in the country, most outstanding being that of Ronald Ross in 1897 on the rôle of mosquitoes in malaria transmission. Ever since the general acceptance of this epoch making discovery, efforts have been directed towards development of economical methods for the control of malaria. There have been several early examples of successful malaria control but only in limited areas. Larval control was the main technique employed in those sporadic attempts. One uniform conclusion therefrom was that such measures were too costly for general adoption in rural areas, where bulk of the problem exists. About the year 1936, trials made by workers in Africa held out the promise that effective control of malaria transmission could possibly be achieved more economically by killing adult mosquitoes

MAP I.



in houses by using pyrethrum contact insecticides (flit type). These trials were repeated in India in the same year with effective results. Experience, however, showed that the cost of such control was not uniform in all areas as it varied considerably with the feeding and resting habits of different species of anopheline mosquitoes transmitting malaria, as for example, malaria transmitted by *culicifacies* in the plains and by *fluviatilis* in foot-hill areas.

When towards the later stages of the Second World War, the main sources of pyrethrum, the principal contact insecticide, were cut off, the need for a substitute product became urgently manifest. As an almost immediate answer D.D.T., a very potent insecticide which possesses prolonged residual effect, was successfully used to fight all insect-borne diseases in the forward areas. Trials for civilian use in India were made in 1945 in small localized areas and by 1948 several States had demonstrated the almost spectacular effect on malaria produced by this insecticide, mainly in rural areas. Bombay State had been able to provide protection by this measure to a population of 6·8 million in known hyper-endemic rural areas in the pre-N.M.C.P. era. In Delhi with an urban population of

1·3 million, malaria incidence was brought down to a negligible proportion by the use of D.D.T. as a barrier spray in the peripheral dwellings reinforced by anti-larval measures in and around the city. Similar results from Mysore, Madras and Uttar Pradesh and implementation of malaria control programmes by residual spraying in a number of other countries generated such confidence both amongst the malariologists and the administrators that the time appeared ripe for thinking in terms of a country-wide control programme.

At the close of the year 1952-1953, it was roughly estimated that in India about 75 million persons suffered from malaria every year. Out of about 5·5 million annual deaths from all causes, just over 50 per cent were due to fevers out of which 0·8 million were directly attributed to malaria. The annual cost of malaria control programme in different parts of the country in 1952-1953 was estimated to be roughly Rs. 15 million, affording protection to about 30 million people in all. It was roughly estimated that a population of 200 million was living in areas where malaria was a serious problem (Table I) and just over 15 per cent thereof received protection in 1952-1953 (Map 2).

MAP 2.

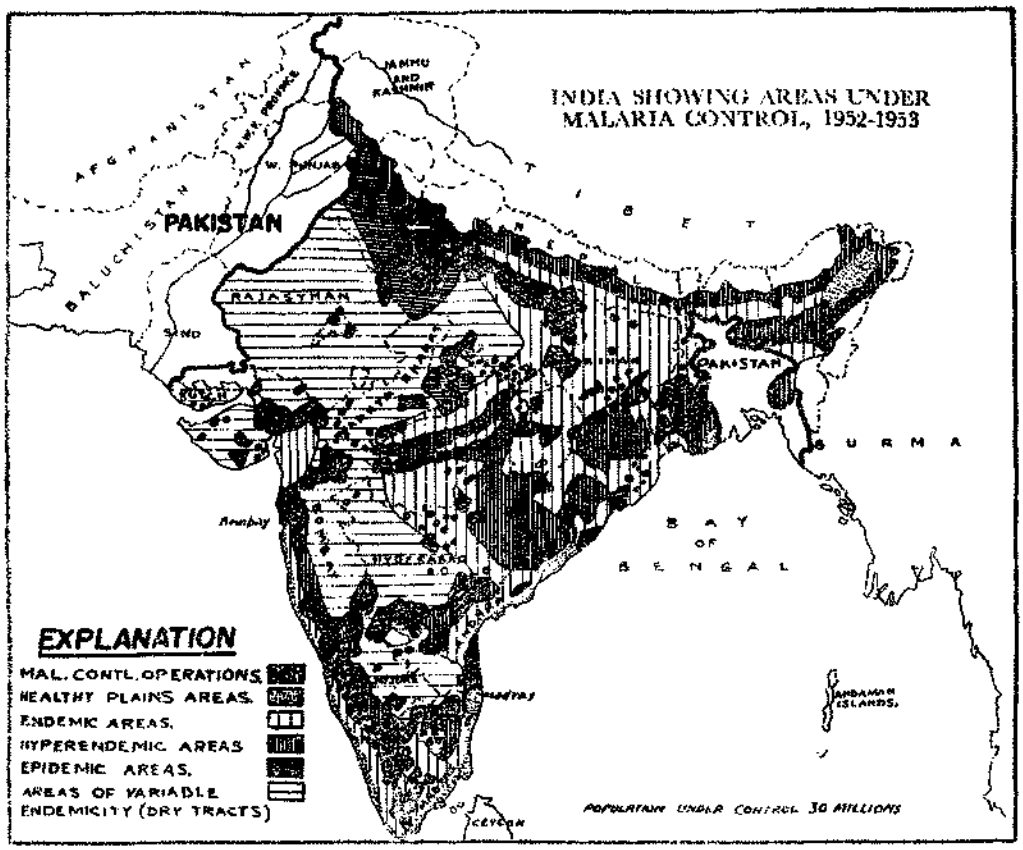


TABLE I.

Total population and that exposed to malaria risk, as estimated in 1952.

(Population in millions.)

States	Total population	Population exposed to malaria risk
Ajmer	0·69	0·69
Andhra	*	4·0
Assam	9·13	9·0
Bhopal	0·84	0·84
Bihar	40·02	21·0
Bombay	35·9	25·00
Coorg	0·23	0·23
Delhi	1·74	1·74
Himachal Pradesh	1·12	1·12
Hyderabad	18·65	6·0
Jammu and Kashmir	...	1·0
Kutch	0·57	0·57
Madhya Bharat	7·94	4·0
Madhya Pradesh	21·33	20·4
Madras	56·95*	4·0
Manipur	0·59	0·59
Mysore	9·07	6·0
Orissa	14·64	10·5
Patiala and East Punjab States Union	3·47	2·0
Punjab	12·64	7·0
Rajasthan	15·3	5·0
Saurashtra	4·14	4·0
Travancore-Cochin	9·27	2·0
Tripura	0·80	0·80
Uttar Pradesh	65·25	40·0
Vindhya Pradesh	3·58	3·3
West Bengal	24·79	22·0
Total ...	356·65	202·78

*Figures for Andhra are included in those for Madras.

The Health Survey and Development Committee of the Government of India, in view of the magnitude of the malaria problem in the country, recommended, in 1946, the establishment of anti-malaria organizations in all the States linked with a central organization of the Government of India. The problem was again taken up by the Planning Commission in 1951, which emphasized that malaria control should receive top priority and that the programme should be carried out on an all-India basis.

A comprehensive programme was drafted by the Director, Malaria Institute of India, in collaboration with malariologists in the States who had ample experience of rural malaria control using residual insecticides, and such international agencies as the Technical Co-operation Mission, U.S.A., World Health Organization, United Nations International Children's Fund and The Rockefeller Foundation. The Co-ordination Committee of the Ministry of Health recommended the Programme for consideration by the Planning Commission in reference to Point Four Indo-American Aid Programme.

The Programme as originally projected envisaged:

(*1*) In the remaining three years of the First Five Year Plan (1953-1954 to 1955-1956), 125 units, each unit affording protection to about a million people, should be established to protect nearly 125 million out of the estimated 200 million people living in more malarious areas.

(*2*) Each unit was to have an operation phase of three years, every house in endemic areas included in the programme receiving two rounds of spraying at a dosage of 100 mg. of D.D.T. per sq. ft. to cover the transmission period of about six months every year.

(*3*) The programme was to be phased so that 75 units would be established in the first year and the remaining 50, in the second and third years of the programme; to bring the total to 125 units.

(*4*) Maintenance programme for future years, continuing indefinitely, requiring alert watchfulness and continued control operations on a reduced scale, if necessary.

The cost of the plan, during the first three years of the operational phase, was estimated at Rs. 150 million including States' share. The major portion of this was received as American Aid in the form of material and equipment (D.D.T., transport, microscopes, spraying equipment, etc.). The Government of India bear the expenditure on the expansion of the Malaria Institute of India to intensify training of anti-malaria staff, and to co-ordinate the operations, and on the provision of anti-malaria drugs and locally manufactured insecticides. The State Governments meet the operational costs including the salaries of the staff. During the First Five Year Plan period, the actual expenditure incurred amounted to Rs. 113·67 million excluding Rs. 1·5 million on account of cost of D.D.T. donated by the U.N.I.C.E.F. during the year 1953-1954. The details of expenditure yearwise are shown in Table II.

Perhaps the most important characteristic of the plan was that while a general broad-based blue-print and a greater proportion of the finances required were made available by the Government of India with foreign aid, the responsibility and actual operation of the programme rested with the States.

TABLE II.

Expenditure on National Malaria Control Programme (1953-54 to 1955-56).

Year	Estimated expenditure during the first five year plan period. (Rupees, in lakhs)				Estimated actual expenditure during the first five year plan period. (Rupees, in lakhs)				
	Technical Cooperation Mission, U.S.A.	Govt. of India	States	Total	Technical Cooperation Mission, U.S.A.	United Nations International Children's Fund.	Govt. of India	States	Total
1952-53	...	...	...	...	26·76	...	...	...	26·76
1953-54	247·62	4·66	174·42	426·70	197·85	15·00	4·66	113·48	330·99
1954-55	221·91	71·39	186·35	479·65	259·82		26·65	125·00	411·47
1955-56	223·81	40·56	165·00	429·37	224·22	...	16·40	141·64	382·46
Unforeseen	...	190·05	...	190·05*	...	...	...	...	...
Total	693·34	306·66	525·77	1525·77	708·65	15·00	47·71	380·32	1151·68

*For D.D.T. plant	...	Rs. 75,00,000
Unforeseen expenditure	...	Rs. 115,05,000
		Rs. 190,05,000

2. ORGANIZATION AND ADMINISTRATION.

Saturday, December 13, 1952, will be remembered as a landmark in the history of malaria control in India when the operational agreement was signed in New Delhi between the Governments of India and the United States of America. The programme was actually launched in April, 1953, by the Hon'ble Rajkumari Amrit Kaur, Minister for Health, Government of India. Ninety units were sanctioned and allotted in the first year against 75 as originally proposed. In the second year, the number rose to 136 against the originally proposed 125. During the third year, the number of units allotted rose to 162. However, due to the late receipt of materials, transport and equipment, actually 84 units came into operation in 1953-54, 110¾ in 1954-55 and 132¼ in 1955-56 (Table III).

While formulating the National Programme for a vast country like India, it was inescapable to base the estimates on certain assumptions. The most important of these were:—

1. Average surface area to be treated per house was 1,000 sq. ft.;
2. Number of persons per house was 5;
3. A six-month transmission season on an average every year;
4. With the pattern of staff suggested, each unit could protect about one million people. It was, however, left to the discretion of the State authorities to make necessary adaptations of staff requirement to suit local conditions.

TABLE III.

Number of units allotted and the number of units formed in States during the years 1953-54, 1954-55 and 1955-56.

States	1953-54		1954-55		1955-56	
	Units allotted	Units functioning	Units allotted	Units functioning	Units allotted	Units functioning
1. Ajmer	⅔	⅔	⅔	⅔	⅔	⅔
2. Andhra ...	2	2	2	2	4	2
3. Assam	5	Not started	5	Not started	7	5
4. Bhopal ...	½	½	1	1	1	1
5. Bihar	7	7	14	14	17	17
6. Bombay ...	15	15	19	19	22	19
7. Coalfields ...	Nil	Not started	1	Not started	1	1
8. Coorg	½	½	½	½	½	½
9. Delhi	2	2	2	2	2	2
10. Himachal Pradesh ...	¾	¾	1¼	1¼	1¼	1¼
11. Hyderabad ...	4	4	6	5	6	6
12. Jammu and Kashmir	1	Not started	1	Not started	1	1
13. Kutch	½	½	½	½	½	½
14. Madhya Bharat ...	2	2	4	4	6	4
15. Madhya Pradesh ...	8	8	12½	10	17½	12½
16. Madras ...	2	2	3	2	3½	3
17. Manipur ...	½	½	1	1	2	1
18. Mysore ...	5	5	6	5	6	6
19. Orissa	2	2	5	5	6½	5
20. Patiala and East Punjab States Union	2	2	2	2	2	2
21. Punjab ...	4	4	7	7	7	7
22. Rajasthan ...	Nil	Not started	2	Not started	5	2
23. Saurashtra ...	1	1	3	3	4	3
24. Travancore-Cochin	1	1	2	2	2	2
25. Tripura ...	½	½	1	1	1	1
26. Uttar Pradesh ...	5	5	8	5	10	8
27. Vindhya Pradesh ...	2	2	3	2	3	3
28. North East Frontier Agency ...	Nil	Not started	¼	Not started	¼	Not started
29. West Bengal ...	16	16	22	16	22	16
30. Sikkim	Nil	Not started	¼	Not started	¼	Not started
Total ...	90	84	136	110¾	162	132¾

The allotment of units to the States was governed by two factors, namely, (1) the population exposed to the malaria risk and (2) the demand for units made by the States, which in turn depended upon (*a*) their appreciation of the situation, (*b*) the tempo and the expenditure on malaria control activities in the State previously, and (*c*) the financial appropriations considered possible by the Governments.

When inviting the State governments to participate in the programme, the Government of India stipulated the following conditions:—

1. That the State governments provide operational costs including the cost of personnel and that at the end of the active operational phase, suitable arrangements be made by the State Governments concerned to continue the programme at such level as may be found necessary to maintain and improve the results obtained.

2. That the Central assistance will be subject to the condition that the total expenditure on malaria control of the State government, receiving the assistance, will not thereby be reduced below the level of expenditure in 1952-53.

PATTERN OF ORGANIZATION FOR URBAN AREAS.

The technique used in the National Malaria Control Programme is one that suits rural malaria control eminently. In a sub-continent like India where the rural population predominates, planning had naturally to concern itself with control of malaria in the vast rural areas. Once this was regulated, attention could then be diverted to the control of urban malaria.

For the purpose of economy, ease and practicability of control measures, a place with a population of 40,000 or less has been considered as rural and others as urban (Table IV). Seventy-four cities, with a population of 1,00,000 or over, account for a total population of about 24 million. The exact population in 139 other towns, with population between 40,000 and 1,00,000, is not available. Allowing approximately 6 million people in such areas, it would seem that about 30 million come under the category of "urban malaria". In so far as the National Malaria Control Programme is concerned, it is known that malaria does not constitute an important health problem in all the cities. The question of the pattern of malaria control, best suited to such areas, has also been considered. The policy decided upon by the Government of India is that, under the National Malaria Control Programme, the insecticide requirement, on the basis of population, should be given free to the local bodies, provided, during the operational period, those bodies undertake to meet from their own resources, the requirements of staff, transport and equipment, and also to purchase insecticides during the maintenance phase. Thereafter the States or the municipal bodies could assume entire responsibility for the subsequent conduct of the programme. Advantage of such offer has been taken by some local bodies, perhaps not directly because of their malaria problem but because of the collateral benefits which accrue from insecticidal spraying.

In course of time, representations came in from certain Cantonment Boards that the population living in their areas should also be included in the scope of the National Malaria Control Programme. In the pre-D.D.T. era, the entire population, made up of civil and military components, in the Cantonment Board areas was treated as one group and the defence authorities were responsible for the control of malaria within the boundaries of the Boards; the measures were extended further to a belt of half a mile beyond the periphery of the Board limits for the protection of the defence personnel. Since D.D.T. came to be widely used, the defence authorities are restricting their activities of malaria control to premises sanitation of buildings occupied by the service personnel only, and have withdrawn the benefits of that measure from the civilian population in the Board areas. It is this change in practice that has led the Cantonment Board authorities to press for their inclusion in the National Malaria Control Programme in order to have the benefits of insecticidal spray.

The same policy as adopted in respect of urban areas should also be applicable to contonments with a population of 40,000 or more.

TABLE IV.

Number of towns and their population.

State	Number of towns with population			
	Upto 10,000	From 10,000 to 40,000	Above 40,000	Above 1,00,000
Ajmer	3	...	2	1
Andhra	See against Madras State			
Assam	7	9	...	...
Bhopal	...	1	1	1
Bihar	13	32	10	5
Bombay	94	107	29	8
Coalfields	...	...	...	...
Coorg	1	1	...	...
Delhi	3	1	3	1
Himachal Pradesh	9	2	...	...
Hyderabad	97	48	9	2
Jammu and Kashmir ...	21	1	2	...
Kutch	5	3	...	...
Madhya Bharat	44	18	5	3
Madhya Pradesh	34	65	9	2
Madras	...	52	47	13
Manipur	1	...	...	...
Mysore	79	25	6	3
Orissa	12	9	3	1
Patialaand East Punjab States Union	44	18	1	...
Punjab	72	38	13	3
Rajasthan	Information not available			3
Saurashtra	56	20	8	3
Travancore-Cochin	...	13	7	2
Tripura	...	...	1	...
Uttar Pradesh	6	81	32	16
Vindhya Pradesh	6	6	...	...
West Bengal	9	47	25	7
Total ...	616	597	213	74

According to the 1951 Census, population in the 74 cities, with over 1,00,000 population each, is 24·126 million.

Minimum population in 139 towns, with population over 40,000 or less than 1,00,000 each, is 5·6 million.

Estimated minimum population in "Urban" areas, for purposes of malaria control, is 29·7 million.

Anti-larval operations in urban areas and cantonments do not form at present a part of the National Malaria Control Programme. The general experience is that in such areas the emphasis is on mosquito control as distinct from malaria control. A permanent remedy for controlling mosquitoes is the provision of engineering works, which would, in all probability, be linked up with other national programmes like those of filaria control, water supply and drainage in pursuance of the recommendations of the Environmental Sanitation Committee set up by the Government of India in 1948 (*vide* its report published in 1956).

MAINTENANCE PHASE.

The Administrative Medical Officers of the various States in their meeting held in February 1955, proposed that:

1. The National Malaria Control Programme should be intensified and continued in the Second Five Year Plan period and the number of control units should be raised to 200 with a view to afford full protection to all the 200 million people living in the more malarious areas.

2. The active operational phase of each unit should be increased from three to five years before it was brought under the maintenance phase.

3. The Government of India should continue to be responsible for the supply of insecticides during the operational as well as the maintenance stage.

These proposals were examined by the Health Panel of the Planning Commission at its meeting held in October, 1955. The first two were ultimately accepted by the Planning Commission. The third proposal was accepted in a modified form, viz., that during the first three years of the maintenance period, out of the normal insecticide requirements of each unit which should not be more than 25 tons of technical D.D.T. every year, the Centre should give 2/3rd, 1/2nd and 1/3rd respectively and the balance should be provided by the State Governments from their own resources.

In order to determine the pattern of maintenance phase, the Government of India, Ministry of Health, appointed in July, 1956, a Committee of three expert malariologists*. This Committee toured certain parts of the country to assess the situation and on the basis of its findings, and the expert opinion of the World Health Organization, recommended the following criteria for interruption of spraying and reducing dosage of frequency during the maintenance phase of the programme:

The areas where for a consecutive period of two years, (*a*) spleen rate of children is less than five per cent, (*b*) parasite rate of children is less than one per cent and (*c*) infant parasite rate is nil, should be considered suitable for interruption of spraying.

The areas with the following indices should be considered suitable for a reduced dosage or frequency of spraying:—

(*a*) Spleen rate of children is between five and ten per cent;

(*b*) Parasite rate of children is between one and two per cent; and

(*c*) Infant parasite rate is nil; if it is positive, such positive findings must be restricted to only a few localities within the unit areas and in any case must not exceed one per cent in those localities.

The Committee of Experts also discussed the surveillance measures that would be necessary during the maintenance phase. When interruption of spraying is contemplated in any area, it is essential to keep strict watch to detect any early sign of possible recrudescence to enable immediate action being taken. An organization for such surveillance and the procedure to be followed by this organization in the concerned areas should be established. It should tackle the residual reservoir factor which should have been reduced to a very low level by consecutive

*1. Dr. B. Ananthaswamy Rao, Malaria Institute of India.
2. Dr. D. K. Viswanathan, Regional Malaria Adviser, World Health Organization, South-East Asia Region.
3. Dr. I. M. Puri, Malaria Institute of India. (Retired).

years of spraying. House to house visits will require to be arranged to detect suspicious malaria cases, to take blood slides of such cases, have them examined microscopically for confirmation of the diagnosis of malaria and to administer potent anti-malaria drugs like 4-aminoquinolines followed by 8-aminoquinolines to all such cases. Epidemiological investigation of positive malaria cases should also be undertaken. In addition, entomological data from certain selected villages should also be collected in order to gain an idea of the densities of the vector species.

A surveillance organization should be established one year prior to contemplated interruption of spraying and the data gathered during the first year should help to determine at its end whether spraying should be interrupted during the following season. Even after the spraying is interrupted, the surveillance staff would need to be continued for a further period of three years. Thereafter, the continuance of surveillance will become the responsibility of the regular Public Health establishments. At the end of the first year of surveillance, it should be carefully determined which of the two types of criteria laid down above has been satisfied, the surveillance staff would do the spraying at reduced frequency for a period of two years, and will continue observations thereafter for a further period of three years.

PATTERN OF SURVEILLANCE STAFF.

One worker should be able to carry out adequate surveillance in a total population of approximately 10,000 living in about 2,000 houses in areas where villages are compact. He will have to visit each house in each village approximately once a fortnight. Thus in ten days of touring in every fortnight, 2,000 houses should be visited or 200 houses per day. Where the villages are not compact, the number of houses visited will naturally be proportionately fewer. At his headquarters village, a worker will collect entomological data on one day every week. On this basis for the unit of one million population, a minimum of 100 persons will be required for purposes of surveillance.

The Second Five Year Plan provides a sum of Rs. 27 crores for a malaria control programme on the basis of a five year operational period. This is to be followed by maintenance phase, during first three years whereof the Centre will give a tapering aid. The provision of Rs. 27 crores, however, does not include the cost of surveillance.

It would thus be seen that the planning is well ahead of schedule and the administration and organization are being progressively geared to the new contingencies if and when they arise.

3. OPERATION OF THE PLAN.

(a) STAFF POSITION IN THE STATES.

The staff pattern designed for a unit was such that it could undertake the planning, execution, supervision of the spraying operations and assessment of results within the area allotted to it with a population of about a million. The normal output of indoor residual D.D.T. spraying should be approximately 2,00,000 houses

twice a year. According to the terrain, communications and density of population, which circumstances may vary within a wide range, it may be possible for a standard unit to cover the prescribed population in some areas while in others it may not be possible to do so. Anyway, the pattern suggested was not meant to be rigid and was subject to modifications according to local conditions. It would also be noticed that a specific provision was not made in the unit staff pattern for insect collectors on the presumption that such staff already existed in most of the State malaria organizations, and that the permanent superior field workers could be trained to perform the duties of insect collectors in the non-malaria season.

Without the full complement of the staff suggested for each unit at each level, neither the quantity nor the quality of work can come up to expected levels.

The single most important factor contributing to a successful malaria control programme is constant and continuous supervision of every phase of the activity. The role of supervision in the conduct of a control programme cannot be overemphasised. The supervisory staff suggested for the unit is minimal and is to be increased according to local circumstances. The State health departments concerned have to ensure that each unit is staffed at least up to the recommended strength and to consider augmentation, where necessary, after an examination of its adequacy according to local conditions.

There have been some cases of under-employment of staff. The reasons for this would appear to be many. One of them could be the time lag in obtaining competent sanction. It could in several instances be due to administrative routine when a long interval intervenes between the sanction of a post and the actual recruitment. Whatever the reason or reasons may be, it is a matter that requires the close attention of the authorities concerned for immediate rectification.

(*B*) THE SUPPLY AND USE OF INSECTICIDES.

During the three years of operation 19,736 short tons of D.D.T. 75 per cent dispersible powder and 108 short tons of dieldrin 50 per cent dispersible powder were supplied to the States (Table V). Out of these, 13,543 and 25 tons, respectively, have been used leaving a balance of 6,193 tons of D.D.T. and 83 tons of dieldrin. The quota of insecticides on the basis of 53·3 tons of D.D.T. (75 per cent dispersible powder) per unit per year was supplied to each State against the number of allotted units. The supplies for 1955-56 included one ton of dieldrin 50 per cent dispersible powder per unit in lieu of an equivalent quantity of D.D.T.

As discussions with the State authorities had revealed that the scheduled allotment of 53·3 short tons of D.D.T. 75 per cent dispersible powder to each unit had proved insufficient in certain cases to give a complete coverage to about 2,00,000 houses estimated to be occupied by one million persons, a 20 per cent extra supply (10·7 short tons) per unit was made during 1955 from a surplus stock of 3,000 short tons available with the Centre due to a margin kept for emergencies.

With the exception of Coorg, Madhya Bharat and Mysore, where more D.D.T. was used than supplied, the quantity used in the States has been less than that supplied. The difference is considerable in some states like Andhra, Delhi, Hyderabad, Madhya Pradesh, Madras, Orissa, Rajasthan, Travancore-Cochin,

Uttar Pradesh, Vindhya Pradesh and West Bengal. In the remaining States, the unused balance does not seem excessive. Dieldrin has not yet been used to any great extent.

It is not possible to analyse precisely all the factors that contributed to the difference between supply and consumption. Delay in supplies being made available to the units, is one likely reason.

The estimate for insecticides required, like the staff pattern recommended for a unit, was based on the assumption that the average square surface of a house was 1,000 sq. ft. and the quantities supplied would be sufficient to spray all the houses and animal sheds occupied by a population of one million at 100 mg./sq. ft. twice in the year. Actual experience has shown that the average surface was generally greater than 1,000 sq. ft. Even so, no acute shortage of insecticides was felt, as on account of one reason or other, the staff could not effect a complete coverage of all the houses even in the first round and reached only a small fraction in the second.

It has been indicated in the *Operations Guide** that the State organizations and the unit officers concerned should use their own discretion to plan a detailed spraying programme based on local knowledge. For instance, in certain areas the transmission season is confined to probably two to three months where a correctly timed single spray should be ample to intercept transmission. Indeed, in such areas two rounds of spray would not be justified. There are other areas where the transmission period is a little longer than six months and, depending on the duration of the residual period, either the two sprays will have to be correctly timed or three rounds of spray will be necessary, the first being of 100 mg./sq. ft. and the second and third of 50 mg./sq. ft. each, in order to keep the total annual dosage within 200 mg. of D.D.T. per sq. ft. It was also expected that the local technical personnel would continuously evaluate the result of the spraying schedules adopted and effect suitable modifications where indicated. Unfortunately, an unintended sense of uniformity crept in the operations resulting in widely varying results.

It is clear that where information based on previous surveys does not exist, it is necessary to obtain such information for realistic planning of operations. This type of survey of an area allotted to each unit has to be carried out by the unit staff or the headquarters staff of the State concerned. In many instances, the Malaria Institute staff has carried out surveys, incriminated vector species and afforded all possible assistance to the State Public Health authorities concerned.

4. ANALYSIS OF RESULTS.

(*a*) NUMBER OF HOUSES SPRAYED.

Approximately a total of 78 million houses have been sprayed, many of them once, some twice and a few thrice a year during the course of the three years. In the last two years, the majority of the States have sprayed twice during a year, three (Andhra, Madras and Coorg) have sprayed thrice and one (Bombay) has sprayed certain selected areas four times a year (Table VI). As stated in a previous

* *National Malaria Control Programme: Operations Guide.* Malaria Institute of India, Delhi.

TABLE

Distribution and consumption of insecticides

(1953 to

States	1953-1954				1954-	
	Insecticides supplied (lbs.)		Insecticides consumed (lbs.)		Insecticides supplied (lbs.)	
	D.D.T. 75 per cent	Dieldrin 50 per cent	D.D.T. 75 per cent	Dieldrin 50 per cent	D.D.T. 75 per cent	Dieldrin 50 per cent
Ajmer	63,000	...	38,827	...	71,250	...
Andhra	Data incomplete					
Bhopal	77,500	...	21,868	...	1,06,730	...
Bihar	7,46,662	...	90,886	...	14,61,000	...
Bombay	18,59,920	...	16,63,097	...	20,18,270	...
Coalfields	...	...	...	...	...	...
Coorg	35,840	...	35,840†	...	35,505	...
Delhi	2,00,500	...	1,11,869	...	2,13,365	...
Himachal Pradesh ...	44,750	...	19,899	...	53,250	...
Hyderabad	4,01,290	...	20,057	...	6,39,780	...
Jammu and Kashmir ...	...	...	...	...	1,06,650	...
Kutch	53,400	...	24,341	...	53,400	...
Madhya Pradesh ...	8,53,328	...	4,51,770	...	12,77,805	...
Madras	4,13,495	...	...	...	3,19,350	...
Manipur	45,445	...	790	...	1,06,750	...
Mysore	5,33,000	...	3,67,096	...	5,75,111	...
Orissa	2,01,710	...	2,03,897	...	5,34,630	...
Patiala and East Punjab States Union	2,94,482	...	77,958	...	2,13,350	...
Punjab	2,46,664	...	2,08,339	...	7,46,810	...
Rajasthan	...	...	...	...	2,13,350	...
Saurashtra	1,39,250	...	1,21,055	...	3,19,935	...
Travancore-Cochin ...	1,06,600	...	35,221	...	2,12,985	...
Tripura	43,600	...	7,399	...	80,000	...
Uttar Pradesh	4,93,068	...	48,418	...	4,25,400	...
Vindhya Pradesh ...	2,08,010	...	43,434	...	3,20,115	...
West Bengal	17,06,656	...	11,19,169	...	17,99,190	...
Assam	19,000	...	...	...	4,79,775	...
Madhya Bharat ...	1,01,500	...	1,01,500†	...	4,26,600	...
Total ...	90,68,670	...	48,11,730	...	1,27,99,396	...
Grand Total for D.D.T.	Supplied: 3,94,71,501 lbs. or 19,736 short tons.			Consumed: 2,70,85,597 lbs. or 13,543 short tons.		

*In addition to this quantity, Bombay State used 2,089 lbs. of Dieldrin 50 per cent from their own resources in 1955-56.

†In addition to these quantities, the States in question have used equivalents of the following quantities of D.D.T. 75 per cent wettable powder from their own resources:—

	1953-54 (lbs.)	1954-55 (lbs.)	1955-56 (lbs.)
Coorg	10,165	13,828	6,275
Mysore	...	...	15,483
Madhya Bharat ...	94,577	...	...

V.

under National Malaria Control Programme.

1956).

1955		1955–1956				Balance at the end of 1955–56 (lbs.)	
Insecticides consumed (lbs.)		Insecticides supplied (lbs.)		Insecticides consumed (lbs.)			
D.D.T. 75 per cent	Dieldrin 50 per cent	D.D.T. 75 per cent	Dieldrin 50 per cent	D.D.T. 75 per cent	Dieldrin 50 per cent	D.D.T. 75 per cent	Dieldrin 50 per cent
65,610	...	79,400	2,000	89,534	...	19,679	2,000
			Data incomplete				
1,03,066	...	1,32,200	2,000	1,02,997	...	78,499	2,000
3,06,075	...	19,51,900	22,400	16,40,966	...	16,21,635	22,400
16,76,545	...	23,72,649	22,400	20,76,716	22,400*	8,34,482	...
...	...	1,22,200	2,000	1,21,424	362	776	1,638
35,505†	...	36,750	2,000	36,750†	500	...	1,600
1,52,393	...	3,13,250	14,300	1,62,018	...	2,00,835	14,300
79,133	...	1,10,450	2,000	1,03,392	674	6,026	1,326
2,92,324	...	6,40,000	2,000	3,17,465	...	10,51,224	2,000
...	...	1,22,100	2,000	1,34,022	...	94,728	2,000
34,022	...	58,100	2,000	52,644	...	53,893	2,000
6,83,875	...	20,09,700	22,400	12,30,940	...	17,74,248	22,400
2,34,607	...	4,11,000	2,000	2,97,977	338	6,11,251	1,662
24,950	...	57,650	2,000	50,176	...	1,33,939	2,000
6,02,376	...	7,51,085	22,400	8,89,724†	14,903	...	7,497
4,62,687	...	8,13,400	2,000	6,57,682	...	2,25,474	2,000
2,56,993	...	3,11,200	2,000	2,89,398	...	1,94,683	2,000
6,10,308	...	8,29,350	22,400	10,12,656	2,171	1,71,521	20,229
...	...	4,86,965	1,000	86,487	...	6,13,828	1,000
2,86,332	...	4,97,600	2,000	5,03,791	...	45,607	2,000
46,221	...	2,51,725	2,000	96,843	435	3,93,025	1,565
49,923	...	1,06,700	2,000	1,28,789	...	44,229	2,000
2,38,927	...	8,31,640	2,000	7,71,160	...	7,45,613	2,000
1,88,200	...	3,19,955	21,400	3,93,003	3,127	2,23,443	18,273
14,99,435	...	28,22,305	11,200	16,53,870	5,713	20,44,677	5,487
...	...	6,81,900	2,000	2,86,739	...	8,93,836	2,000
3,42,272	...	5,92,250	22,400	3,68,925	...	3,07,653	22,400
87,71,779	...	1,76,03,435	2,16,300 or 108 short tons	1,35,02,088	50,623 or 25 short tons	1,23,85,904 or 6,193 short tons	1,65,677 or 83 short tons

Balance: 1,23,85,904 lbs.
6,193 short tons.

TABLE VI.

Number of houses, in thousands, sprayed in each round every year.

State	1953–54				1954–55				1955–56			
	Rounds				Rounds				Rounds			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
Ajmer	42·5	24·9	...	...	62·8	67·1	...	...	96·5	74·1	...	...
Andhra	58·1	165·3	81·0	...	121·5	414·0	257·2	...	316·0	434·9	128·4	...
Assam	...	...	...	...	...	...	...	...	...	...	...	...
Bhopal	88·6	3·8	...	...	183·1	28·6	...	...	232·7	50·5	18·7	...
Bihar	271·7	16·5	...	...	1630·2	435·0	...	...	3033·2	888·9	...	...
Bombay	3253·0	2762·0	65·5	...	3480·4	3346·8	67·4	21·1	3652·9	3422·2	89·7	21·3
Coalfields ...	...	...	...	...	...	...	...	...	...	...	...	...
Coorg	9·2	24·1	39·5	...	27·6	38·8	32·8	...	27·2	35·6	26·9	...
Delhi	119·5	76·9	...	...	176·3	150·6	...	...	205·0	108·9	2·8	...
Himachal Pradesh ...	31·4	...	...	...	160·5	2·7	...	...	187·8	17·0	...	...
Hyderabad ...	140·6	...	...	...	960·5	66·7	...	...	969·5	94·5	...	...
Jammu and Kashmir ...	...	...	...	...	...	...	...	...	75·1	55·6	...	...
Kutch	43·3	11·0	...	...	69·4	15·4	...	...	80·1	21·2	...	...
Madhya Bharat ...	...	...	...	...	...	...	...	...	...	...	...	...
Madhya Pradesh ...	1487·7	719·0	...	...	1559·0	1448·5	3·8	...	1972·7	1705·8	20·8	...
Madras	...	...	...	...	145·0	499·4	...	...	190·0	212·7	371·4	...

TABLE VI.—(*Concld.*)

State	1953-54				1954-55				1955-56			
	Rounds				Rounds				Rounds			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
Manipur	3·5	...	...	...	53·6	66·5	...	...	85·8	86·1	...	...
Mysore	687·4	38·2	...	...	510·1	635·6	...	...	1161·2	813·2	...	...
Orissa	312·0	47·5	...	...	1043·1	27·9	...	...	1219·0	478·7	...	...
Patiala and East Punjab States Union	159·2	2·3	...		347·1	62·4	...	...	466·1	45·3	...	...
Punjab	214·1	174·1	...	...	545·3	198·8	...	...	864·8	279·0	...	...
Rajasthan	...	...	...	...	...	...	...	...	257·7	28·8	...	...
Saurashtra	259·6	14·1	...	...	484·4	25·2	...	...	658·9	137·1	...	...
Travancore-Cochin ...	69·4	30·2	...	...	34·1	81·7	6·5	...	105·6	61·3	39·9	..
Tripura	27·4	16·5	...	...	120·9	48·1	...	...	317·4	44·5	...	...
Uttar Pradesh ...	158·0	63·7	...	...	302·9	383·2	...	...	784·2	689·9	11·1	..
Vindhya Pradesh ...	105·2	70·8	...	...	231·8	245·8	...	...	533·3	380·0	...	...
West Bengal	2718·4	901·1	...	...	2996·3	3179·0	332·4	...	3356·2	3499·1	...	...
Total ...	10259·8	5162·0	186·0	...	15245·9	11467·8	720·1	21·1	20848·9	13664·9	709·7	21·3
Grand Total ...	78,30,75,000 houses.											

Note:—Data from Assam, Madhya Bharat and Coalfield have not been included.

section, the number of rounds of spray during the year is purely dependent on the length of the transmission period and the period of residual activity of the sprayed insecticide in the locality.

The object of spraying premises with residual insecticides is to ensure interception of transmission. To achieve the object, it is essential that the insecticide is sprayed on the surfaces in a way that its toxicity is active throughout the transmission period. This implies that in areas where the transmission period exceeds three months and the period of residual activity is three months, the spray must be repeated a second time. Unless and until such repeated spraying is carried out, it is inevitable that some transmission would remain unintercepted through certain parts of the season. At which part of the season the transmission remains unintercepted, will depend upon the period of the first round and the interval between the subsequent rounds of spray. Therefore, unless it is ensured that the insecticide is sprayed at the required intervals to intercept transmission throughout the period in which it can take place, the population will only be partially protected, the degree of partial protection varying according to the period of exposure to transmission without the protective spray.

It would appear from Table VI that, by and large, the spraying operations in the 78 million houses carried out in the three years afforded only partial protection assuming that every one of these houses actually required two rounds for full protection, the period of residual activity of each round of spray was uniformly three months, that the dosage applied was actually 100 mg. per sq. ft. and not more. The maximum number of houses sprayed during the first round, in the first, second and third years were 10·2, 15·2 and 20·8 million respectively. One would presume that in the second round of spray the same number of houses would be covered. But it is actually seen that only 5·2, 11·5 and 13·7 million houses respectively (51, 75·6 and 66 per cent of the first round) were covered during the second round in the three years respectively. Indeed in very few States excepting Bombay, does the number of houses sprayed in the second round approximate to that sprayed in the first round. Instances of the other extreme are there, namely, Andhra, Coorg, Madras, and West Bengal where the number of houses sprayed in the second round exceeds that sprayed in the first in only one or two years of operation.

The difference in the number of houses sprayed in the third and fourth rounds, as is evident in the case of Andhra, Bombay, Coorg, Madhya Pradesh, Travancore-Cochin, may be understandable as due to certain isolated local areas in the States where the transmission period is unduly long and the population involved in such areas is a small proportion of the whole.

Judged, however, by the malariometric indices every year and the significant drop in the figures for malaria morbidity, it would appear that in many parts the season of transmission is just short enough to be largely intercepted by one single round of spray and that probably in large sections of the country the period of residual activity is much longer than the assumed three months.

The factors involved in the adequate and full coverage of a particular area are (*1*) knowledge of the local epidemiology of malaria, (*2*) changes, if any, in

insecticides on the surfaces on which they are sprayed, and (3) planning, organization, and supervision. It is understandable that there were certain defects as regards the three factors in the first year of operation, but such defects, although remediable, seem to have continued to operate in the second year and also to persist in the third year. Unless these defects are remedied, the objective of complete interception of transmission throughout the season cannot be attained. A clear understanding of the principle involved in the method of control, the period of residual activity in the locality and the length of transmission period, is essential for the successful execution of the programme.

(B) NUMBER OF HOUSES SPRAYED PER POUND OF 75 PER CENT WETTABLE D.D.T. OR ITS EQUIVALENT.

The operational index, the number of houses sprayed per pound of D.D.T., depends upon the training of the spraying crew, surface to be sprayed per house, the maintenance of equipment, and the supervision of the operation.

The officers of the Malaria Institute of India have observed during their visits that the nozzle discharge rate of the pumps in use, was much in excess of the recommended 28·6 ounces per minute. The simple procedure of routine checking the discharge rate of the nozzle tips, had in many instances been overlooked with the result that far more than 100 mg. D.D.T./sq. ft. was being sprayed. On a particular occasion, an inspector being questioned as to when the discharge rate of the nozzle was determined, replied that the discharge rate of the nozzle was normal, but 200 mg./sq. ft. were deliberately being sprayed as the area was not easily accessible more than once during the transmission period. The answer was smart and was the result of recent and fresh training received by the malaria inspector at the Malaria Institute of India. On actual testing, however, the discharge rate of the nozzle was found to exceed 50 ounces per minute. It was also confirmed from the Medical Officer of the unit that the village was not one where a 200 mg./sq. ft. dose for a single spray was planned. The technique of spraying was demonstrated to the inspector and this emphasizes the need for effective supervision.

It has also been a frequent experience during inspections that the training of the spraying crew had been defective. As a result, D.D.T. formulation was not infrequently seen to be dripping from freshly sprayed surfaces indicating an obvious excessive dosage. Training of the spraying crew does not end with the first few days of recruitment. It is a continuous process till it becomes automatic for the man to spray correctly.

The average number of houses sprayed per pound of D.D.T. per round in the States is summarized in Table VII. In general, there have been two rounds of spray each year making a total of six rounds during the three years. The index for Coorg, Madhya Bharat, Patiala and East Punjab States Union, Punjab and Tripura indicates that there is a fairly close similarity between the performance of the two rounds in any one year; in the States of Ajmer, Delhi, Himachal Pradesh, Bihar, Orissa, Saurashtra, Vindhya Pradesh and Kutch such similarity exists between only two of the three years. But in the remaining States, the index fluctuates so widely from the first to the second round that the operations appear to be defective and leave a large scope for improvement.

TABLE VII.

Number of houses sprayed per lb. of insecticide from 1953-54 to 1955-56.

States	1953-54				1954-55				1955-56				Remarks
	Rounds				Rounds				Rounds				
	I	II	III	IV	I	II	III	IV	I	II	III	IV	
Ajmer	1·5	2·9	...	...	1·9	2·0	...	...	1·9	1·8	...	...	
Andhra	5·8	4·0	4·0	...	Data incomplete				Data incomplete				
Assam	...	...	...	...	...	...	...	...	4·1	4·4	...	...	National Malaria Control Programme started in 1955-56.
Bhopal	4·3	2·8	...	...	1·9	2·6	...	...	2·9	2·6	5·0	...	
Bombay	3·8	3·7	6·5	...	3·4	5·3	6·2	6·8	3·3	3·5	5·5	6·4	
Bihar	3·0	4·2	...	...	2·6	2·4	...	...	2·4	2·3	...	...	
Coalfields ...	...	...	...	...	...	...	...	...	6·8*	6·8*	6·8*	6·8*	National Malaria Control Programme started in 1955-56.
Coorg	1·3	1·4	1·7	...	1·7	1·7	1·6	...	1·7	1·7	3·6	...	
Delhi	1·7	1·7	...	...	2·0	2·2	...	...	2·1	1·6	1·4	...	
Himachal Pradesh ...	1·6	...	...	...	2·1	2·1	...	...	1·9	2·1	...	...	
Hyderabad ...	7·0	...	...	...	3·4	5·1	...	...	3·3	3·8	...	...	
Jammu and Kashmir ...	...	...	...	...	...	...	...	...	1·1	0·8	...	...	National Malaria Control Programme started in 1955-56.
Kutch	2·1	2·3	...	...	2·6	2·0	...	...	2·2	1·86	...	...	
Madhya Pradesh ...	4·5	5·6	...	...	4·4	4·4	2·7	...	2·9	3·0	5·0	...	

TABLE VII. (*Concld.*)

State	1953–54 Rounds I	II	III	IV	1954–55 Rounds I	II	III	IV	1955–56 Rounds I	II	III	IV	Remarks
Madhya Bharat ...	1·9	2.2	1.8	...	1·4	1·4	1.7	...	1·3	1·0	...	...	
Madras	...	...	...	...	2·7	2·7	...	...	3·0	2·1	2·8	...	National Malaria Control Programme started in 1954–55.
Manipur ...	4·4	...	...	...	5·1	4·6	...	...	2·6	3·3	...	...	
Mysore	2·0	1·4	...	...	2·0	1·6	...	...	1·8	2·7	...	...	
Orissa	1·7	1·8	...	...	2·3	3·4	...	...	2·4	2·6	...	...	
Patiala and East Punjab States Union ...	2·0	2·3	...	...	1·5	1·8	...	...	1·7	1·8	...	...	
Punjab	1·7	2·2	...	...	1·2	1·2	...	...	1·1	1·2	...	...	
Rajasthan ...	...	...	...	...	...	...	...	...	3·3	2·7	...	...	National Malaria Control Programme started in 1955–56.
Saurashtra ...	2·2	2·4	...	...	1·7	1·0	...	...	1·5	1·5	...	...	
Travancore-Cochin ...	2·6	3·8	...	...	3·0	2·5	2·0	...	2·3	1·9	2·0	...	
Tripura	6·0	6·0	...	...	3·3	3·6	...	...	2·8	2·8	...	...	
Uttar Pradesh ...	4·1	4·9	...	...	3·5	2·4	...	...	2·1	2·0	3·4	...	
Vindhya Pradesh ...	3·5	5·1	...	...	2·4	2·3	...	...	2·3	2·2	...	...	
West Bengal ...	3·0	4·2	...	...	3·4	5·8	5·1	...	3·2	5·4	...	...	

*Average figure.

In this connection, an incident is of interest. The monthly technical return from a unit, when scrutinized, revealed that the average number of houses sprayed per pound of D.D.T. was identical in village after village. It seemed to be too good to be true. On further investigation, it was found that instead of reporting the actual performance, a calculated account of the same by dividing the amount of D.D.T. expended by an assumed average of three houses per pound of D.D.T. was given.

(C) THE NUMBER OF HOUSES SPRAYED PER PUMP PER DAY.

The number of houses sprayed per pump per day, is an index to assess the efficiency of operations and is used as a yard-stick to measure the daily output of work. This index reflects various aspects of the daily work as planning, organization, execution, maintenance of equipment and supervision. In interpreting the index values, allowance has to be made for the density of houses in a given area, the surface area to be sprayed per house, the communication facilities in the area and public co-operation.

In the plains where villages are compact, the average out-put of work of a well-trained team is about 50 to 60 houses per pump per day. This average has been fairly consistently reached by Bombay, Manipur and Mysore (Table VIII). The whole of the Coorg State is hilly and has an average of about 20 houses per pump per day in each of the six rounds during the three years. But in the remaining States, the index fluctuates widely from round to round and from year to year. This would seem to indicate a serious defect in supervision and calls for rectification. It should be borne in mind that this index and many others are not designed merely for purposes of filling in reports to be sent to some ultimate authority for analysis, but are for every day assessment of work accomplished and guidance in planning further operations. That such use of the index has been made, though delayed, in Ajmer, Bhopal, Himachal Pradesh, Jammu and Kashmir, Madhya Pradesh, Saurashtra, Travancore-Cochin, Vindhya Pradesh and West Bengal is apparent in the third year of operations where its fluctuations are minimal between the two rounds. If the meaning and the importance of the index are clear to the supervisory personnel, and it is used, as it should be, in the field at the end of the day's work, the defects would become obvious and their immediate correction possible.

(D) REDUCTION IN MALARIA INCIDENCE BASED ON DISPENSARY FIGURES.

The objective of any malaria control programme is to reduce the malaria incidence to such a level that the disease is no longer of public health importance. One hundred million cases suffered from malaria every year as estimated by Sinton (1935:1936). From various data available to him, he concluded that the dispensary statistics included only one in ten cases of malaria as the other nine did not or could not seek relief at the dispensaries. Although conditions have changed to some extent since that estimation in that (*a*) the number of dispensaries has increased, (*b*) there are better communications and means of transport, and (*c*) the ease with which antimalarials are available to the people resulting in the probable increase of self medication, the present estimates have also been framed on the same assumption.

TABLE VIII.

Number of houses sprayed per pump per day.

States	1953-54			1954-55			1955-56		
	Rounds			Rounds			Rounds		
	I	II	III	I	II	III	I	II	III
Ajmer	32·5	54·5	...	41·2	48·3	...	45·1	43·8	...
Andhra	19 3	21·3	18·2	16·7	23·8	23·7	45·6	23·5	16·0
Assam	...	...	...	...	...	...	...	...	...
Bhopal	47·2	47·1	...	45·3	50·4	...	45·0	44·4	38·0
Bihar	23·6	25·8	...	44·7	48·1	...	46·1	53·4	...
Bombay	...	...	62·7	58·4	60·0	...	60·7	59·2	45·5
Coalfields	...	...	...	...	...	...	47·6	60·0	...
Coorg	17·6	20·5	22·5	25·1	20·7	21·0	19·7	26·1	22·2
Delhi	24·8	...	...	...	31·8	...	37·3	32·3	21·0
Himachal Pradesh ...	18·4	...	...	25·2	7·4	...	31·0	30·3	...
Hyderabad	...	...	...	...	...	...	...	...	...
Jammu and Kashmir ...	...	...	...	...	...	...	21·0	20·0	...
Kutch	25·8	22·1	...	39·0	36·4	...	30 9	24·6	...
Madhya Bharat ...									
Madhya Pradesh ...	...	...	...	42	40	28·3	43	43·2	34·3
Madras	...	...	...	22·5	65·6	...	...	24·6	32·9
Manipur	24·4	...	...	58·3	52·9	...	63·6	72·3	...
Mysore	44·5	41·5	...	50·7	51·5	...	49·8	47·2	...
Orissa	30·1	25·4	...	36·6	41·6	...	39·7	47·3	...
Patiala and East Punjab States Union	27·6	14·8	...	40	47·2	...	46·2	35·0	...
Punjab	24·0	22	...	22·5	20·2	...	25·1	26	...
Rajasthan	...	...	...	...	...	...	39·6	41·7	...
Saurashtra	31·0	20·0	...	30·2	34·3	...	32·0	38·1	...
Travancore-Cochin ...	11·2	10·6	...	14·3	25·3	14·1	20·2	23·3	24·6
Tripura	50·2	43·6	...	38·5	34·1	...	48·8	...	...
Uttar Pradesh	...	...	...	6·0	7·6	...	15·3	14·8	8·1
Vindhya Pradesh ...	...	...	...	28·1	30·8	...	32·2	31·2	...
West Bengal	16	23·7	...	31·4	33·5	27·5	32 2	32·3	...

Sinton's estimate of 100 million cases in 1935 came down to 60·7 million in 24 States in 1953-54, when the National Malaria Control Programme began to operate, due mainly to the partition of the country and in a small measure to the anti-malaria operations undertaken patchily in so far as the entire country is concerned, but substantially in some of the States.

It will be seen from Table IX that in 16 States (Group 'A') in respect of which figures are available for the year 1955-56, the total incidence in that year was of the order of 25·5 million, recording a reduction of 19·1 million from 1953-54. In eight other States (Group 'B') in respect of which figures for the year 1955-56

TABLE IX.

Malaria cases treated in hospitals and dispensaries.

States as they existed before reorganization in 1956	Malaria cases treated in hospitals and dispensaries in 1953-54	Malaria cases treated in hospitals and dispensaries in 1955-56	Differences between malaria cases treated in 1953-54 and 1955-56
(1)	(2)	(3)	(4)
Group A			
Ajmer	12,651	4,990	(—) 7,661
Bhopal	1,44,204	96,931	(—) 47,273
Bihar	11,07,819	5,60,651	(—) 5,47,163
Bombay	2,53,006	1,64,189	(—) 88,817
Delhi	9,172	6,889	(—) 2,283
Himachal Pradesh	1,01,110	81,471	(—) 19,639
Hyderabad	1,04,776	75,330	(—) 29,446
Kutch	18,202	11,318	(—) 6,884
Manipur	65,260	44,400	(—) 20,860
Mysore	1,93,530	2,48,931	(+) 55,401*
Punjab	5,08,630	3,59,511	(—) 1,49,119
Saurashtra	47,597	20,973	(—) 26,624
Travancore-Cochin	55,012	39,515	(—) 15,497
Tripura	1,27,339	60,030	(—) 67,309
Vindhya Pradesh	94,314	36,534	(—) 57,780
West Bengal	16,18,924	7,37,634	(—) 8,81,290
Total Group 'A' States	44,61,546	25,49,297	(—) 19,12,249

*Increase due to inclusion of two districts from Madras.

TABLE IX.—(*Concld.*)

States as they existed before reorganization in 1956	Malaria cases treated in hospitals and dispensaries in 1953-54	Malaria cases treated in hospitals and dispensaries in 1954-55	Differences between Malaria cases treated in 1953-54 and 1954-55
(1)	(2)	(3)	(4)
Group B			
Andhra	31,781	25,493	(—) 6,288
Coorg	3,439	3,210	(—) 229
Madhya Bharat	1,10,638	90,782	(—) 19,856
Madhya Pradesh	5,88,715	3,32,678	(—) 2,56,038
Madras	1,56,009	1,30,454	(—) 25,555
Orissa	14,234	7,192	(—) 7,042
Patiala and East Punjab States Union	1,03,330	61,505	(—) 41,825
Uttar Pradesh	6,03,644	5,55,882	(—) 47,762
Assam	...	...	...
Coalfields	...	...	...
Jammu and Kashmir	...	...	...
Rajasthan	...	...	...
Total Group 'B' States	16,11,791	12,07,196	(—) 4,04,595

If the totals under Columns 2, 3 and 4 above are multiplied by 10 (according to Sinton's formula that the malaria cases treated in dispensaries and hospitals in India represent ten per cent of the total malaria morbidity), the respective figures will be:—

	Year	Incidence (in million)	Reduction (in million)
Group 'A' States	1953–54 1955–56	44·61 25·49	... 19·12
Group 'B' States ...	1953–54 1954–55	16·12 12·07	... 4·05

are not available the total incidence in 1954-55 was 12·1 million with a reduction of four million from that in 1953-54.

As an extreme case, it may be presumed for the sake of calculation that the incidence remained the same in 1955-56 as in 1954-55 in the eight States in Group 'B'. The total incidence (presumptive) in 1955-56 would thus be 37 million against 60·7 million in 1953-54, showing a reduction of 23·1 million which

is quite an impressive figure (The actual reduction may be confidently expected to be much more when the figures for 1955-56 in respect of the States in Group 'B' become available).

The National Malaria Control Programme, judged by this substantial reduction in incidence of the disease, has been a great success. If this reduction is translated into the economic loss prevented, the achievement becomes much more impressive.

Although effects of malaria are not as dramatic as those of small pox, cholera and plague; from the view point of the total deaths, it is the deadliest disease. Sinton (1935:1936) estimated the annual mortality directly due to malaria as one million and another one million due to malaria indirectly rendering its victims susceptible to other intercurrent infections. Taking 2 per cent as the gross malaria case mortality rate, it could be inferred that in the process of preventing 23·1 million cases due to the National Programme, approximately 4,60,000 deaths due to malaria have been prevented.

A criticism that can be made against the data on which the above mentioned discussion is based is that they are hospital returns based on clinical diagnosis. If it is borne in mind that year after year for several decades, the only largely used method that this country has had in so far as malaria is concerned is the clinical diagnosis, it would show that the figures from the dispensaries give us at least a trend of the incidence. If the impact of the National Programme has made such a spectacular effect on that trend, then the effect on the microscopically positive proportion of the clinically diagnosed cases should be either similar or more drastic, perhaps the latter.

(*b*) MICROSCOPIC CONFIRMATION OF CLINICALLY DIAGNOSED MALARIA CASES IN HOSPITALS AND DISPENSARIES.

Microscopic diagnosis of malaria in our country is still not a routine practice, and a vast majority of the cases are diagnosed clinically as malaria by the physicians in dispensaries as well as hospitals. A considerable proportion of cases are self diagnosed as malaria by the victims of fever based either on personal or hearsay experience. It is on such diagnosis that the malaria morbidity estimates have been and are continued to be based, and the picture presented may not be precise. Nevertheless, when the incidence was of the order of a 100 million cases a year, and antimalaria operations were restricted to small areas, the dispensary returns, combined with malariometric indices like spleen and parasite rates, could serve the purpose of indicating the effect of such measures in an area by the reduction of the indices. But, in the present day, when country-wide anti-malaria operations are being carried out with the defined objective to eliminate malaria as a public health problem, and the results of the operations are so dramatic, the need for microscopic confirmation of clinical diagnosis of malaria becomes one of urgency. This need becomes still greater in the proportionately small residue of cases labelled as malaria on clinical grounds when the incidence of the disease, judged from clinical diagnosis of cases, is rapidly declining. During the latter stage, the necessity to treat the individual cases adequately in order to eradicate the infection in the individuals assumes a great public health importance. A greater precision in the incidence statistics thus becomes a practical proposition unlike in the past.

Soon after D.D.T. became available for civilian use, the dramatic effects of its use on malaria incidence became obvious. Since 1948, the Malaria Advisory Committee of the Indian Council of Medical Research at their annual meetings have stressed the need for microscopic verification of the cases diagnosed clinically as malaria in the dispensaries. Table X furnishes the data of such surveys in some of the States and the results. It is seen that information for the three years is available only from six States, namely, Ajmer, Madhya Pradesh, Manipur, Mysore, Patiala and East Punjab States Union and Uttar Pradesh. States of Hyderabad, Madhya Bharat, Madras and Saurashtra have data for two out of the three years and the Coal-fields area and Himachal Pradesh have data only for one year. The remaining thirteen States have not yet furnished any data on the subject.

TABLE X.

Proportion of clinically diagnosed malaria (dispensary) cases found to be parasite positive.

States	1953–54		1954–55		1955–56		Percentage of positive		
	Estimated	Positive	Estimated	Positive	Estimated	Positive	1953–54	1954–55	1955–56
Ajmer ...	1479	151	1394	48	731	18	10·2	3·5	2·4
Andhra ...	...	...	...	...	...	...	...	...	...
Assam ...	...	...	...	...	...	...	...	...	...
Bhopal ...	...	...	...	...	...	...	...	...	...
Bihar ...	...	...	...	...	...	...	...	...	...
Bombay ...	...	...	...	...	...	...	...	...	...
Coalfields ...	...	...	...	...	11512	260	...	...	2·2
Coorg ...	...	...	...	...	...	...	...	...	...
Delhi ...	...	...	...	...	...	...	...	...	...
Himachal Pradesh ...	...	...	...	...	88	17	...	...	20
Hyderabad ...	821	253	376	62	...	...	30·3	16·5	...
Jammu and Kashmir	...	...	...	...	...	...	...	...	...
Kutch ...	...	...	...	...	...	...	...	...	...
Madhya Bharat ...	...	...	56	2	63	6	...	3·6	9·5
Madhya Pradesh ...	1135	145	263	15	570	49	12·6	5·7	8·6
Madras ...	...	...	769	64	1400	90	...	8·3	6·4
Manipur ...	21	4	612	123	203	9	19	20·1	4·0
Mysore ...	1332	167	6957	801	8482	798	12·8	11·5	9·4
Orissa ...	...	...	...	...	...	...	...	...	...
Patiala and East Punjab States Union	40	0	365	7	243	0	0	1.1	0
Punjab ...	...	...	...	...	...	...	...	...	...
Rajasthan ...	...	...	...	...	...	...	...	...	...
Saurashtra ...	39	7	...	...	840	0	18	...	0
Travancore-Cochin	...	...	...	...	...	...	...	...	...
Tripura ...	...	...	...	...	...	...	...	...	...
Uttar Pradesh ...	86	14	588	93	129	21	16·2	15·8	16·2
Vindhya Pradesh ...	...	...	...	...	...	...	...	...	...
West Bengal ...	...	...	...	...	...	...	...	...	...
Total ...	4953	751	11380	1215	24251	1368	15·1	10·6	5·2

The total number of cases examined microscopically each year in the States which have provided the data show a progressive increase from year to year. This, however, is not the case in respect of the totals for each of the States where such surveys were undertaken. In fact, as the work of the National Programme

progresses, larger proportion of the dispensary cases must be microscopically confirmed till such time as every case is so examined.

The trend in all cases except Madhya Bharat, Madhya Pradesh, and Uttar Pradesh, would appear to be that the proportion of clinically diagnosed cases, microscopically confirmed, steadily declined from year to year. This could be due to improved microscopy on the one hand and to a real decrease in the quantum of malaria transmission not reflected in the customary clinical diagnosis of fever cases as malaria on the other. This again emphasizes the need for larger proportions of the cases attending dispensaries and hospitals to be submitted to laboratory examination.

It is also true that the examination of a single blood slide from a fever case cannot eliminate a diagnosis of malaria if that single slide turns out to be negative for malaria parasites. The question of repeated examinations of the blood, to establish freedom from malaria, will automatically arise, as the residual number of malaria cases becomes smaller than even now. But till such time, the objective should be to examine at least one blood slide from every case of fever that attends a dispensary or hospital or at least every case that clinically appears to be malaria. This will become a procedure of the utmost importance in the surveillance part of the programme in the maintenance phase.

The handicap of enormous numbers of malaria cases is also an obstacle in having malaria declared as a notifiable disease. The present trends, however, indicate that notifying malaria incidence may become a public health procedure of immense importance in the next five years or so.

(*F*) THE SPLEEN INDEX.

The National Malaria Control Programme *Operations Guide*, in the section on edpidemiological assessment of results, deals with the importance of spleen and parasite indices in children between 2 and 10 years and the parasite index in infants. Units in many States have carried out surveys even in unsprayed areas, not necessarily comparable with areas where the control measures have been carried out. As, however, indices from unsprayed comparison areas generally in use through years of common practice in field investigations were not necessary in the context of the country-wide control programme, the data pertaining to unsprayed areas have not been considered in the present analysis. Nevertheless, such data are of importance as base lines for future assessment for comparison with the indices to be collected from these very areas after the introduction of control measures.

In an attempt to define minimum samples that would be adequate for the determination of different indices, certain general statements were made in the *Operations Guide*. It has been suggested that the unit area with one million population, should be classified epidemiologically into different zones based on the different types of malariogenic conditions like foot-hill, irrigation etc. In the event of the entire unit area being homogeneous in so far as the malariogenic conditions are concerned, the division should be automatic on the basis of four sub-units. In each zone, whether classified on malariogenic conditions or arbitrarily in accordance with the location of sub-units, it has been indicated that suitable number of villages should be selected to include 10 per cent of the total population in each

unit area. In general, the proportion of children between two and ten years to the total population can be taken to be 20 per cent; and the proportion of infants as three per cent.

By classification of the unit area into zones, epidemiologically or otherwise, and the selection of suitable villages, it was expected that in such villages a total of about 1,00,000 population (ten per cent of one million) would be available. In this population, there can be expected to be about 20,000 children between two and ten years and about 3,000 infants to be examined every year. In view of the difficulties in determining the infant parasite index, it has been indicated in the *Operations Guide* that the aim should be to examine at least 1,000 infant smears a year. The sampling procedures indicated in the *Guide* are by no means rigid, but indicate the minimum essential.

Information is incomplete as to whether the method described above for sampling was adopted by all the units or not. It appears more probable that the villages were not selected and the surveys were undertaken in a random fashion not necessarily in the same villages.

Table XI summarises the data available from the States regarding spleen index. Assam, Jammu and Kashmir, Rajasthan and the Coalfields area have furnished data only for the third year of operations (1955-56) when they began to participate in the National Malaria Control Programme. Madras, Travancore-Cochin and Tripura have reported for the years 1954-55 and 1955-56 only. The remaining States have furnished the data for each year of the programme.

In estimating the adequacy of the size of sample that has been examined by the units in the States, because of the absence of any definite information on the universe concerned, the calculated numbers of relevant age-groups in the population stated to have been protected by the units, have been taken into consideration. Using estimates of children and infants expected in the defined population, the actual numbers examined have been presented as percentages thereof (Table XII). On the basis of ten per cent sample being adequate, in some cases it is seen that the samples examined for spleen index are as inadequate as 0·7 per cent in Saurashtra in the first year; in others it is more than adequate, as in Mysore during every year, the figures being 35·1, 38·8 and 23·7 per cent respectively for the three years. The variation in the sample size from State to State is large. The sample size is not uniform for the same State from year to year.

The sample size for spleen index on the basis of its being ten per cent or more of the relevant universe for each of the three years, may be considered adequate in the following states: Ajmer, Coorg, Hyderabad, Mysore, Manipur and West Bengal. In Bhopal, Bihar, Bombay, Madhya Pradesh, Orissa, Rajasthan, Saurashtra, Travancore-Cochin, Tripura and Uttar Pradesh, the sample has not been uniformly adequate.

The spleen index in every one of the States, excluding those reporting for the first time in the third year, shows a downward trend successively in the three years except in Madhya Pradesh and Manipur. In the Coalfields area, Bombay, Coorg, Delhi, Mysore and Patiala and East Punjab States Union the index has been reduced to a very low level or even 0. In the remaining States, the degree of reduction varies between 56·1 per cent in the first year and 18·3 per cent in the third

year (Bihar), and 25·7 and 23·2 per cent in the same period (Andhra). In other words, the reduction of the spleen index is not of uniform degree and this is to be expected. As a general rule, very high spleen rates show a greater degree of reduction than comparatively low ones. In Ajmer, for example, the index has only slightly fluctuated around four per cent every year.

One of the factors influencing the spleen index, exclusive of the actual amount of transmission, is sampling. The adequacy of the sample size is of great importance. The spleen index seems to have been determined on different samples every year, or what has probably happened is, the samples include every year the original sample of the first year and new ones from areas subsequently included for control. Such a procedure of calculating cumulative spleen index of different samples pooled together, firstly, does not make the true reduction patent and secondly, can give rise to an apparently small reduction or even an increase. The former would seem to be the explanation for the index in the third year in Ajmer, Andhra, Hyderabad, Kutch, Manipur, Orissa and Uttar Pradesh. The latter would seem to be the explanation for the increase of the index in the second as compared to that of the first year in Madhya Bharat and Uttar Pradesh. There has been, however, apparently a spirit of complacency with the good results achieved and a critical analysis thereof does not seem to have been attempted. It would also unfortunately appear that the appraisal of the work is being carried out for the benefit of impersonal reporting to a higher authority without utilizing the same for further refinement of the local operations.

The average enlarged spleen determined and calculated according to the method of Hackett has been recommended as an index in the *Operations Guide*. Most of the units have actually reported on the index but the data available do not lend themselves to a broad analysis because the annual figures are not from the same samples. Therefore, for the present, the data have not been included in the analysis.

The spleen index is a time-honoured and tested index useful in the assessment of the results of control operations. The sensitivity of the index, for certain, will become progressively less as the control gets more and more perfect. But as yet, it would seem clear from the analysis, we are still not at the stage when the sensitivity of the index has to be questioned in the majority of the areas. The adequacy of the sample plays a large part in determining the sensitivity of the spleen index. As control is achieved in increasing degrees, the sample on which the index is determined has also got to increase. For most units, there are two more years for the operational phase of the programme to end. The spleen index along with the others will determine the success of the programme. It is, therefore, up to every unit to determine the index on adequate sample at least from the current year onwards.

Perusal of Table XI shows that the spleen indices even in the first year of the programme in the States of Ajmer, Patiala and East Punjab States Union, Punjab and Travancore-Cochin were below ten per cent; in Coorg and Delhi they were as low as 0·02 and 1·10 per cent respectively. Of these States a few, namely, Ajmer, Delhi, Patiala and East Punjab States Union and the Punjab, are well known to have large areas in them liable to cyclical epidemics of malaria and the year of commencement of the National Programme was one of the inter-epidemic years when spleen indices are expected to be low. At the

TABLE XI.

Child spleen rates in sprayed areas.

States	Number examined			Spleen rate per cent		
	1953–54	1954–55	1955–56	1953–54	1954–55	1955–56
Ajmer	8,442	14,803	11,705	4·8	4·3	4·4
Andhra	24,582	95,098	97,351	25·7	24·6	23·2
Assam	...	...	18,789	...	...	24.0
Bhopal	3,299	12,754	14,344	34·1	16·3	7·1
Bihar	1,924	1,15,255	1,79,389	56·1	23·6	18·3
Bombay	1,36,992	1,56,202	2,16,269	4·6	2·7	1·5
Coalfields	...	...	22,175	...	...	1·3
Coorg	4,440	8,694	6,721	0·02	0·02	0·0
Delhi	9,053	49,526	21,510	1·10	0·4	0·4
Himachal Pradesh	4,129	3,507	19,503	18·2	14·1	2·5
Hyderabad	39,009	2,47,407	2,16,933	8·4	4·8	5·3
Jammu and Kashmir ...	...	...	17,013	...	...	5·0
Kutch	4,498	9,512	6,457	6·0	3·2	3·4
Madhya Bharat	10,737	32,479	60,774	18·3	20·3	12·5
Madhya Pradesh	40,256	1,03,696	98,533	40·0	26·1	13·4
Madras	...	65,614	76,389	...	6·3	5·3
Manipur	561	6,773	7,963	43·1	15·5	11·1
Mysore	2,38,881	2,48,084	2,75,161	3·6	2·5	1·3
Orissa	3,096	11,838	36,118	34·1	26·4	18·2
Patiala and East Punjab States Union	26,347	27,313	27,935	4·5	1·6	0·5
Punjab	24,935	40,526	50,449	7·8	6·7	3·2
Rajasthan	...	...	6,895	...	...	37·0
Saurashtra	1,769	7,225	40,891	22·9	18·2	7·4
Travancore-Cochin ...	...	9,211	7,389	...	8·4	4·6
Tripura	...	...	2,958	...	...	18·1
Uttar Pradesh	29,045	33,476	37,851	13·6	18·1	12·7
Vindhya Pradesh	9,463	24,123	44,778	23·7	10·5	9·2
West Bengal	3,23,117	7,81,986	8,81,043	20·3	16·4	6·7

TABLE XII.

Percentage of child population examined as sample for spleen rates (sprayed area only).

States	1953–54	1954–55	1955–56	Remarks
Ajmer	20·1	17·3	12·2	
Andhra	15·4	22·6	22·1	
Assam	...	...	2·5	
Bhopal	3·7	6·9	6·2	
Bihar	0·7	7·1	5·9	
Bombay	4·2	4·5	5·9	
Coalfields	...	...	8·9	
Coorg	11·1	21·7	16·8	
Delhi	7·5	28·1	10·4	
Himachal Pradesh	13·2	2·2	10·4	
Hyderabad	27·9	30·9	21·7	
Jammu and Kashmir	...	...	26·6	
Kutch	10·2	13·6	8·1	
Madhya Bharat	4·5	11·8	17·1	
Madhya Pradesh	2·7	6·6	5·0	
Madras	...	13·1	20·5	
Manipur	14·0	11·3	9·3	
Mysore	35·1	38·8	23·7	
Orissa	1·0	1·1	3·0	
Patiala and East Punjab States Union ...	16·5	7·8	6·0	
Punjab	11·3	9·0	5·9	
Rajasthan	...	...	2·7	
Saurashtra	0·7	1·5	6·2	
Travancore-Cochin	...	9·2	7·4	
Tripura	...	...	2·5	
Uttar Pradesh	12·5	3·4	4·7	
Vindhya Pradesh	9·1	10·1	8·4	
West Bengal	11·9	24·4	25·2	

same time, antimalaria operations in Delhi urban area, Delhi rural area (in some urban and rural areas of Delhi State, hyperendemic conditions prevailed some years earlier) and in some parts of the Punjab were established earlier than the commencement of the programme. And in Delhi urban and rural areas, epidemics which occurred in similar neighbouring areas, have been averted since 1946 due to the efficient control operations.

With regard to Bombay, Coorg, Hyderabad, Mysore and Travancore, malaria is known to have been hyperendemic in the past and a considerable proportion of such areas had been under control with D.D.T. since 1948. Therefore, with the advent of the National Programme, only areas that were available for fresh control were those where malaria was hypo-endemic. The areas particularly in Coorg, Bombay, Mysore and Delhi which were once hyperendemic as seen under, had been sufficiently controlled before 1953-54 so that the spleen indices were brought down to those of hypo-endemicity when the National Programme commenced.

States	Year	Spleen rate	Citation
Bombay (Kanara and Dharwar districts ...	1942	50 to 75	Viswanathan, D. K. and Rao, T. R. (1949).
Coorg	1947	58·2	Jaswant Singh and Kariappa, C. B. (1949).
Delhi	1947	20 to 64	Jaswant Singh and Dalip Singh (1949).
Mysore	1935	21 to 73·7	Sweet, W. C. (1937).

It would appear desirable to review the position with regard to the distribution of the units in the States and the degree of endemicity in the different areas when they were included in the National Programme. Table XIII gives the frequency distribution of the units in the States according to spleen index of the unit areas before commencement of the National Programme in them. With regard to units from which pre-spray survey data are not available the data at the end of the first year have been taken. Only States having three or more units functioning during the three years, have been taken into consideration. It is seen that out of one hundred units in ten States, forty are functioning in areas which were hypo-endemic at the commencement of the National Programme. Of these forty, 17, 6 and 5 were in Bombay, Hyderabad and Mysore respectively which had controlled a considerable number of malarious areas for several years before the commencement of the programme. Of the remaining sixty units, thirty are in areas of low endemicity, twenty-seven in areas of moderate endemicity and only three in areas of high endemicity.

Although it was intended that units should first be established in hyperendemic areas, hyperendemicity alone does not seem to have been a determining factor in their location. Perhaps the spleen indices, as available for the purpose of present analysis, cannot serve as decisive guides in determining the various shades of endemicity.

Parasite rates have not been analysed for the purpose of this paper.

TABLE XIII.

Number of units functioning in the States† in areas with different spleen indices at commencement of National Malaria Control Programme.*

Range of spleen indices per cent at the commencement of National Programme	Number of units functioning in States										Total
	Bihar	Bombay	Hyderabad	Madhya Pradesh	Mysore	Orissa	Punjab	Uttar Pradesh	Vindhya Pradesh	West Bengal	
0 – 10 ...	...	17	6	1	5	...	5	3	...	3	40
11 – 25 ...	6	2	1	7	1	1	2	4	2	5	31
26 – 50 ...	9	...	...	4	...	4	...	1	1	7	26
51 and above ...	1	...	...	1	...	...	...	...	...	1	3
Total ...	16	19	7	13	6	5	7	8	3	16	100

*Only units with data available at commencement of operations are included. The samples examined for pre-spray data are not necessarily adequate.

†Only States having three or more units have been considered.

(G) ENTOMOLOGICAL DATA.

The *Operations Guide* for the National Malaria Control Programme prescribes the following entomological observations for the assessment of the results of operations:—

(*1*) Measurement of densities of vector anophelines, all anophelines and culicines in terms of man-hours of hand catches with a standardized technique in treated and untreated comparable areas.

(*2*) Estimation of 24 hour survival rates of mosquitoes caught by means of window-traps.

(*3*) Determination of residual toxicity of sprayed surfaces by means of "Wall Cage tests".

(*4*) Collection of scrapings from sprayed surfaces for estimation of D.D.T. by chemical test.

Of these, the first one is compulsory and the rest are recommended as desirable supplements wherever conditions of staff and equipment permit.

Most States, however, were not able to undertake more than the bare essential.

The data presented in the analysis have two very important features:—

(*1*) They reveal the success of operations and

(*2*) Specify indices of safe anopheline vector densities in different parts of the country.

TABLE XIV.

Overall figures for anopheline density in treated and untreated shelters during 1953-54 of National Malaria Control Programme.

States	Period	Treated shelters					Untreated shelters					Vectors
		Man-hours	Vectors	All anophelines	Ten man-hours		Man-hours	Vectors	All anophelines	Ten man-hours		
					Vectors	All anophelines				Vectors	All anophelines	
Punjab ...	Oct. 1953-Mar. 1954	5,717	335	6,284	0·585	10·99	1,107	294	7,605	2·65	68·69	*A. culicifacies*
Patiala and East Punjab States Union	Nov. 1953-Mar. 1954	1,147	3	800	0·026	6·97	440·5	2	687	0·045	15·59	*A. culicifacies*
Mysore ...	Nov. 1953-Dec. 1953	365	10	345	0·273	9·45	23·3	96	326	41·14	139·71	*A. culicifacies* *A. fluviatilis*
Bombay ...	Apr. 1953-Mar. 1954	25,425	12,496	73,069	4·914	28·73	3,086	9,339	32,798	30·26	106·27	*A. culicifacies* *A. fluviatilis* *A. stephensi*
Kutch ...	Oct. 1953-Mar. 1954	364	157	246	4·313	6·75	364	977	1,492	26·84	40·98	*A. culicifacies* *A. stephensi*
Hyderabad	Nov. 1953-Mar. 1954	15,495	1,937	4,169	1·25	2·69	2·5	35	68	140·00	272·0	*A. culicifacies* *A. fluviatilis*
Andhra ...	Apr. 1953-Mar. 1954	2,870·25	148	24,815	0·514	86·27	866	255	13,646	2·94	157·57	*A. stephensi* *A. culicifacies*
Delhi ...	Sep. 1953-Mar. 1954	298·5	0	58	0·0	1·943	...	...	...	...	...	*A. culicifacies*
Uttar Pradesh	Jan. 1954-Mar. 1954	288	380	4,017	13·196	139·47	186·5	359	2,411	19·25	129·27	*A. culicifacies* *A. fluviatilis*
Orissa ...	Oct. 1953-Feb. 1954	248·25	155	602	6·243	24·24	277	1,776	4,936	64·11	178·19	*A. annularis* *A. sundaicus*
West Bengal	Sep. and Oct. 1953-Feb. 1954	25	1	249	0·400	99·60	...	...	...	...	2	*A. fluviatilis* *A. philippinensis*
All States combined		52,249	15,662	1,14,654	2·989	21·943	6,353	13,133	63,969	20·672	100·691	

An analysis of data furnished by different States during the years 1953-54, 1954-55 and 1955-56 has consistently revealed marked reductions in the number of malaria vectors as well as in the general anopheline fauna of most of the areas. The method used for making these estimates has been based on comparison of numerical prevalence of mosquitoes in the sprayed areas with those of unsprayed areas (Tables XIV to XXI).

TABLE XV.

Comparative densities of culicines in treated and untreated shelters, 1953-54.

States	Period	Treated shelters			Untreated shelters		
		Man-hours	Total culicine	Per ten man-hours	Man-hours	Total culicines	Per ten man-hours
Punjab ...	Oct. 1953-Jan. 1954	4,805	5,158	10·734	652	1,782	27·331
Patiala and East Punjab States Union ...	Nov. 1953-Mar. 1954	1,409	1,016	7·21	554·5	491	8·854
Mysore ...	Nov. 1953-Mar. 1954	1,459·5	3,777	25·87	43·3	116	26·769
Bombay ...	Oct. 1953-Mar. 1954	10,790·5	20,959	19·423	487	5,528	113·511
Uttar Pradesh	Jan. 1954-Mar. 1954	288	1,447	50·243	186·5	1,116	62·520
Orissa ...	Oct. 1953-Feb. 1954	248	1,258	50·721	277	3,387	122·238
All States combined		19,000	33,615	17·692	2,200·3	12,470	56·666

All the States have not been able to furnish data regularly in a computable form but the reporting system has steadily improved year after year. In the year 1953-54, the entomological data were received from only 11 States while in 1954-55 and 1955-56 they were received from 19 and 21 States respectively. The data furnished by seven out of the 28 States participating in the National Malaria Control Programme were such that they could not be used for the purpose of the present analysis. For the first year of operation, data were not available from the areas influenced by *A. minimus* but in the subsequent years these areas, *viz.*, Assam, Manipur and Tripura, have been included. The data for the year 1953-54 have been presented in Tables XIV and XV. A perusal of these Tables will show that the periods of observation in the different States were variable. A significant reduction in the density of vector and all anophelines is noticed in most of the States. The reduction in Uttar Pradesh is, however, not of the same order as in the other States.

Reducing all the entomological data of the whole country to yearly average of ten man-hour density, the figures arrived at are shown in Table XVI*a*:

TABLE XVI.

Comparative anopheline densities in treated and untreated shelters during 1954-55 (Periods corresponding to the year 1953-54).

States	Period	Treated					Untreated				
		Man-hours	Vectors	All anophelines	Ten man-hours		Man-hours	Vectors	All anophelines	Ten man-hours	
					Vectors	All anophelines				Vectors	All anophelines
Punjab	Oct. 1954-Mar. 1955	11,724	537	11,523	0·46	9·82	1,630	329	6,532	2·02	40·07
Patiala and East Punjab States Union ...	Nov. 1954-Mar. 1955	3,290	8	1,425	0·02	4·02	80	0	964	0·0	120·5
Mysore	Nov. 1954-Mar. 1955	1,811	226	3,886	1·24	21·45	346	179	1,585	5·17	45·8
Bombay	Apr. 1954-Mar. 1955	30,474	9,972	70,720	3·27	23·20	2,660	9,964	23,644	37·4	88·8
Kutch	Oct. 1954-Mar. 1955	364	146	279	4·01	7·66	364	433	770	11·8	21·1
Hyderabad	Nov. 1954-Mar. 1955	35	343	4,920	98·0	1,405·7	78	4,127	11,946	529·1	1,531·5
Andhra	Apr. 1954-Mar. 1955	1,464	613	9,071	4·18	68·8	636	1,721	14,206	27·1	223·4
Delhi	Sep. 1954-Mar. 1955	109	0	0	0·0	0·0	...	...	...	...	...
Uttar Pradesh (Kheri) ...	Jan. 1955-Mar. 1955	184	159	897	8·64	48·75	184	439	2,087	23·85	113·42
Orissa	Oct. 1954-Feb. 1955	1,166	1,384	5,089	11·87	43·64	471	3,245	891	68·9	18·9
West Bengal	Sep. 1954-Oct. 1954	384	0	1,278	0·0	33·3	...	...	...	...	...
Madras	Apr. 1954-Mar. 1955	19,842	795	83,164	0·4006	41·4	14,884	6,520	1,38,068	4·3	93·4
Travancore-Cochin ...	Apr. 1954-Mar. 1955	1,441	323	1,889	2·24	13·1	307	110	811	3·6	26·1
Tripura	Apr. 1954-Mar. 1955	379	43	4,909	1·13	129·5	27	86	813	31·8	301·1
Madhya Pradesh ...	Dec. 1954-Mar. 1955	67	2	59	0·3	0·77	199	1,719	13,043	86·4	655·4
Vindhya Pradesh* ...	Jan. 1955-Mar. 1955	74	45	107	6·08	14·4	...	...	...	...	...
Saurashtra	Jan. 1955-Mar. 1955	16	54	218	32·5	136·25	16	144	454	90·0	283·8
Coalfields	Jan. 1955-Mar. 1955	177	181	1,232	6·6	69·6	159	2,347	7,519	148·0	472·8
Total ...		73,001	14,768	1,99,666	2·02	27·34	22,041	31,363	2,23,333	14·22	101·32

*One unit.

TABLE XVI*a*.

Density per 10 man-hours.

Year	Sprayed area			Unsprayed area		
	Vector	All anophelines	Culicines	Vector	All anophelines	Culicines
*1953–54	2·98	21·9	17·69	20·67	100·69	56·66
*1954–55	2·02	27·32	20·30	14·22	101·32	39·1
1954–55	2·05	28·30	28·90	11·09	92·8	45·86
1955–56	1·3	25·54	22·4	11·23	122·1	39·91

*Periods of observation in different States are variable. They are however the same in these two years.

TABLE XVII.

Comparative culicine density 1954-55 (Period corresponding to the year 1953-54) in treated and untreated shelters.

States	Period	Treated shelters			Untreated shelters		
		Man-hours	Number of culicines	Culicines per 10 man-hours	Man-hours	Number of culicines	Culicines per 10 man-hours
Punjab ...	Oct. 1954-Mar. 1955	11,724	15,774	13·4	1,630	5,155	31·6
Patiala and East Punjab States Union ...	Nov. 1954-Mar. 1955	3,290	5,371	16·3	80	60	7·5
Mysore ...	Nov. 1954-Mar. 1955	1,811	4,616	25·5	346	1,548	44·7
Bombay ...	Apr. 1954-Mar. 1955	30,474	61,630	20·2	2,660	13,825	51·9
Kutch ...	Oct. 1954-Mar. 1955	364	1,708	46·9	364	2,170	59·6
Hyderabad ...	Nov. 1954-Mar. 1955	15	2,234	1,489.3	15	3,256	2,170·6
Andhra ...	Apr. 1954-Mar. 1955	1,464	1,097	7·4	636	1,087	17·1
Delhi ...	Sep. 1954-Mar. 1955	109	540	49.5	...	...	...
Uttar Pradesh (Kheri) ...	Jan. 1955-Mar. 1955	184	1,653	89·8	184	2,637	143·3
Orissa ...	Oct. 1954-Feb. 1955	1,166	7,779	66·7	471	3,524	74·8
West Bengal ...	Apr. 1954-Mar. 1955	637	895	14.05	...	...	...
Madras ...	Apr. 1954-Mar. 1955	5,558	13,215	23·7	13,117	43,284	32·9
Travancore-Cochin ...	Apr. 1954-Mar. 1955	1,107	386	3·4	240	274	11·4
Tripura ...	Apr. 1954-Sep. 1954	219	963	43·9	27	225	83·3
Madhya Pradesh	Dec. 1954-Mar. 1955	67	75	11·2	199	812	40·8
Vindhya Pradesh	Jan. 1955-Mar. 1955	74	178	24·0	...	...	...
Saurashtra ...	Jan. 1955-Mar. 1955	16	146	91·2	16	196	122·4
Total ...		58,279	1,18,260	20·30	19,985	78,053	39·1

It may be noted that these figures exhibit a remarkable uniformity for the three years.

Culicine densities have also come down in most of the sprayed areas but not to the same extent as those of anophelines (Tables XV, XVII, XIX and XXI).

TABLE XVIII.

Anopheline density in treated and untreated shelters—April 1954 to March 1955.

States	Period	Treated shelters					Untreated shelters					Vectors
		Man-hours	Vectors	All anophelines	Ten man-hours		Man-hours	Vectors	All anophelines	Ten man-hours		
					Vectors	All anophelines				Vectors	All anophelines	
Punjab ...	Apr. 1954-Mar. 1955	17,388	1,527	23,787	0·87	14·8	1,630	329	6,532	2·0	40·0	*A. culicifacies*
Patiala and East Punjab States Union	,, ,, ,, ,,	7,051	123	10,885	0·174	15·4	953	76	5,075	0·70	53·2	*A. culicifacies*
Mysore ...	,, ,, ,, ,,	26,948	1,519	39,423	0·563	14·62	6,079	968	22,990	1·6	37·8	*A. culicifacies* *A. fluviatilis* *A. stephensi*
Bombay ...	,, ,, ,, ,,	30,474	9,972	70,720	3·27	23·20	2,660	9,964	23,644	37·45	88·8	*A. culicifacies* *A. fluviatilis* *A. stephensi*
Kutch ...	,, ,, ,, ,,	666	267	461	4·00	6·92	666	666	1,172	10·0	17·6	*A. stephensi* *A. culicifacies*
Hyderabad ...	,, ,, ,, ,,	62	445	6,428	71·77	1,036·8	102	4,650	14,931	455·8	1,463·8	*A. culicifacies* *A. fluviatilis* *A. stephensi*
Andhra ...	,, ,, ,, ,,	1,464	613	9,071	4·18	61·96	636	1,721	14,206	27·1	223·4	*A. culicifacies* *A. stephensi* *A. culicifacies*
Delhi (Rural)	,, ,, ,, ,,	220	8	6,136	0·36	278·90	...	...	...	...	...	*A. culicifacies*
Uttar Pradesh	,, ,, ,, ,,	3,490	3,216	26,567	9·2	76·12	1,209	3,586	22,950	29·6	189·8	*A. culicifacies* *A. fluviatilis*
Orissa ...	,, ,, ,, ,,	3,076	4,272	17,301	13·88	56·24	518	3,495	8,820	67·5	170·3	*A. sundaicus* *A. annularis* *A. fluviatilis* *A. varuna*

TABLE XVIII.—(*Concld.*)

States	Period	Treated shelters					Untreated shelters					Vectors
		Man-hours	Vectors	All anophelines	Ten man-hours		Man-hours	Vectors	All anophelines	Ten man-hours		
					Vectors	All anophelines				Vectors	All anophelines	
West Bengal	Apr. 1954-Mar. 1955	1,551	2	6,488	0·01	41·84	129	0	282	0·0	22·6	*A. philippinensis* *A. sundaicus*
Madras ...	,, ,, ,, ,,	19,842	4,287	82,164	2·21	41·40	14,884	9,638	1,38,068	6·47	92·8	*A. culicifacies* *A. fluviatilis*
Travancore-Cochin	,, ,, ,, ,,	1,441	323	1,889	2·24	13·10	307	110	811	3·6	26·4	*A. fluviatilis* *A. culicifacies* *A. varuna*
Tripura ...	,, ,, ,, ,,	379	43	4,909	1·13	129·52	27	86	813	31·8	301·1	*A. minimus*
Madhya Pradesh ...	Sep.-Nov. 1954-Mar. 1955	142	67	585	4·71	41·19	223	1,772	13,920	79·8	624·2	*A. fluviatilis*
Vindhya Pradesh (Nowgong)	Jan. 1955-Mar. 1955	74	45	107	6·08	14·45	...	...	...	...	...	*A. fluviatilis* *A. culicifacies*
Saurashtra ...	,, ,, ,, ,,	16	54	218	33·75	136·25	16	144	454	90·0	283·7	*A. culicifacies*
Coalfields ...	,, ,, ,, ,,	167	118	1,232	7·06	73·77	159	2,347	7,319	147·6	460·3	*A. stephensi* *A. culicifacies* *A. fluviatilis*
Bihar ...	Apr. 1954-Mar. 1955	3,867	908	24,367	2·3	63·01	595	134	3,689	2·25	62·0	*A. culicifacies* *A. fluviatilis*
Total ...		1,18,318	27,909	3,34,738	2·05	28·30	30,793	39,606	2,85,686	12·9	92·8	

TABLE XIX.

Culicine density in treated and untreated shelters—April 1954 to March 1955.

State	Period	Treated shelters			Untreated shelters		
		Man-hours	Total culicines	Per ten man-hours	Man-hours	Total culicines	Per ten man-hours
Punjab ...	Apr. 1954-Mar. 1955	17,388	43,165	24.8	3,350	19,747	58.9
Patiala and East Punjab States Union ...	,, ,, ,, ,,	7,051	15,149	21·5	953	2,453	25·7
Mysore ...	,, ,, ,, ,,	26,948	76,882	28·5	6,079	25,782	40·6
Bombay ...	,, ,, ,, ,,	30,474	61,630	20·2	2,660	13,825	59·90
Kutch ...	,, ,, ,, ,,	364	1,708	46·9	364	2,170	59·6
Hyderabad ...	,, ,, ,, ,,	42	2,862	684·4	39	4,327	1,109·6
Andhra ...	,, ,, ,, ,,	1,464	1,097	7·4	636	1,087	17·1
Delhi ...	,, ,, ,, ,,	220	3,094	140·6	...	...	...
Uttar Pradesh	,, ,, ,, ,,	3,490	8,865	25·4	1,209	11,822	96·1
Orissa ...	,, ,, ,, ,,	3,076	26,155	85·0	518	3,877	74·8
West Bengal ...	,, ,, ,,	637	895	14·05	...	...	...
Madras ...	,, ,, ,, ,,	5,558	13,215	23·8	13,117	43,284	33·0
Travancore-Cochin ...	,, ,, ,, ,,	1,107	386	3·5	240	274	11·4
Tripura ...	,, ,, ,, ,,	379	1,820	48·0	27	225	83·3
Madhya Pradesh	Sep. 1954-Mar. 1955	142	820	57·7	223	929	41·7
VindhyaPradesh	Jan. 1955-Mar. 1955	74	178	24·1	...	...	...
Saurashtra ...	,, ,, ,, ,,	16	146	91·3	16	196	122·5
Bihar ...	Apr. 1954-Mar. 1955	4,644	40,556	87·3	1,225	11,620	94·9
Total ...		1,03,074	2,98,623	28·9	30,656	1,40,618	45·86

The data for the year 1954-55 have been presented in two ways. In Tables XVI and XVII they are shown for periods which correspond with those of the previous year (1953-54), while in Tables XVIII and XIX they are shown for the complete year from most of the States. The data for the year 1955-56 are given for the whole year (Tables XX and XXI) which are comparable to those of the previous year. An examination of the data for the year 1954-55 shows that reduction in the vector and other anopheline densities in the State of Hyderabad has not been to the same extent as in other States. This may perhaps be due to some

error in recording the man-hours of work. But even in that State, although the density figures seem high yet they are significantly lower than those of the unsprayed areas. During 1955-56, the figures in Hyderabad have come down to a satisfactory low level.

The density in unsprayed areas usually provides a yard-stick in assessing the reduction in mosquito population in the controlled areas. Although it has so far served as a good index, yet it has its limitations. Many of the States have included all the areas with malaria endemicity for malaria control, and the areas that have been used for comparison purposes are with naturally low or no malaria endemicity. For this reason, many units of certain States have not been able to furnish data for unsprayed areas, while some others have altogether omitted to give such data. It is felt that in future years the data for unsprayed areas may not be available at all for the purpose of adequate comparison as more areas are rapidly included in the control operations.

CRITICAL DENSITY OF VECTORS.

In order to ensure that the density of vector species remains within safe limits, some idea of the figures which can be used as indices for gauging the risk in respect of different species is essential. The analysis of the data of past three years has helped to a great extent to arrive at these figures on the basis of yearly averages.

The critical density of *A. culicifacies* in relation to malaria transmission has been estimated by Russell and Rao (1942) to be 50 per ten man-hours. Their view is that below this figure transmission of the disease cannot take place. The validity of this has been discussed by Bhatia and Krishnan (1957). In areas where this species is the sole vector, a density below this limit will keep the transmission in check. In the areas under National Malaria Control Programme, however, it has been possible to reduce it to a much lower level in the past three years, *i.e.*, 0·9 per ten man-hours. *A. stephensi* var. *mysorensis*, which is a vector of importance in the rural areas of parts of Bombay and other States in Southern India, has been regarded by Viswanathan (1950) to behave mainly like *culicifacies*. In the rural areas, it is predominantly zoophilic, indoor rester and has breeding habits similar to those of *culicifacies*. There is thus some justification in considering its critical density at the same level as that of *culicifacies*. In Rajkot (Saurashtra) which is an urban area with more than one species acting as vectors, the highest level of density at which successful control of disease has been achieved is 33·75 per ten man-hours; species concerned being *culicifacies* and presumably type form of *stephensi*. Risk-free limit may, therefore, be safely laid down up to this figure for any of the three species, *culicifacies*, *stephensi* type form or *stephensi* var. *mysorensis* either singly or collectively. In some areas, in addition to these species, *A. fluviatilis* also takes part. In such areas, the bulk of vector collection is made up predominantly of *culicifacies* and *stephensi*. In case of *A. fluviatilis*, Viswanathan (1950) has calculated that the lowest density at which it can effectively transmit malaria is four per ten man-hours.

TABLE XX.

Comparative anopheline densities of treated and untreated areas —April 1955 to March 1956.

States	Period	Treated shelters					Untreated shelters				
		Man-hours	Vectors	All anophelines	Ten man-hours		Man-hours	Vectors	All anophelines	Ten man-hours	
					Vectors	All anophelines				Vectors	All anophelines
Punjab ...	Apr. 1955-Mar. 1956	21,681	749	18,031	0·34	8·3	4,023	601	10,338	1·49	25·7
Patiala and East Punjab States Union ...	,, ,, ,, ,,	7,969	97	7,761	0·12	10·08	180	1	2,832	0·05	157·3
Mysore	,, ,, ,, ,,	37,434	1,150	29,862	0·31	7·9	6,537	725	17,775	1·10	27·2
Bombay	,, ,, ,, ,,	29,752	8,224	93,276	2·8	31·3	2,117	6,682	18,980	30·7	91·3
Kutch	,, ,, ,, ,,	732	46	169	0·62	2·30	732	123	643	1·68	8·78
Hyderabad ...	,, ,, ,, ,,	1,223	1,389	26,193	11·36	214·2	476	5,343	16,082	112·24	337·9
Andhra	,, ,, ,, ,,	19,750	1,401	75,227	0·71	38·1	2,888	2,552	1,46,196	118·84	506·2
Delhi (Rural) ...	Jun. 1955-Nov. 1955	383	0	2,309	0·0	60·3	19	65	2,258	28·95	1,188·3
Uttar Pradesh ...	Apr. 1955-Mar. 1956	4,113	1,404	19,585	3·41	47·6	1,690	1,025	1,12,117	6·1	71·1
Orissa	Apr. 1955-Dec. 1955	4,226	2,086	14,100	4·93	33·4	60	117	399	19·50	66·5
West Bengal ...	Apr. 1955-Feb. 1956	10,356	10	36,439	0·009	35·2	503	16	4,350	0·31	86·5
Madras ...	Apr. 1955-Mar. 1956	26,026	3,664	78,073	1·41	30·0	8,954	6,089	71,602	6·80	80·0
Travancore-Cochin	,, ,, ,, ,,	1,311	69	1,918	0·53	14·6	48	2	162	0·42	33·8
Tripura ...	,, ,, ,, ,,	134	6	2,490	0·45	185·8	23	83	814	36·04	353·9
Madhya Pradesh ...	Jan. Feb. Mar. 1956	182	24	336	1·3	18·4	43	33	321	7·7	74·7
Vindhya Pradesh ...	Apr. 1955-Mar. 1956	633	331	2,299	5·22	36·31	36	8	347	2·22	96·4

TABLE XX.—(*Concld.*)

States	Period	Treated shelters					Untreated shelters				
		Man-hours	Vectors	All anophelines	Ten man-hours		Man-hours	Vectors	All anophelines	Ten man-hours	
					Vectors	All anophelines				Vectors	All anophelines
Saurashtra ...	Aug. 1955-Mar. 1956	603	387	2,092	6·42	34·7	43	255	512	59·3	119·1
Coalfields ...	Apr. 1955-Mar. 1956	2,562	510	10,763	1·98	42·0	2,371	11,833	74,205	49·91	312·9
Bihar ...	,, ,, ,, ,,	4,644	1,716	22,892	3·7	49·3	1,225	1,029	15,118	8·35	123·4
Manipur ...	,, ,, ,, ,,	90	0	135	0·0	15·0	90	4	1,340	0·44	148·9
Assam ...	Nov. 1955-Mar. 1956	483	0	548	0·0	11·3	...	...	...	...	...
Grand Total for all States ...		1,74,014	23,263	4,44,498	1·3	25·54	32,118	36,576	3,79,390	11·23	122·1

Remarks: Vector in Manipur and Assam is *A. minimus;* in other States same as in the Table XVIII.

TABLE XXI.

Comparative culicine density of sprayed and unsprayed areas, 1955-56.

States	Period	Sprayed areas			Unsprayed areas		
		Man-hours	Total culicines	Per ten man-hours	Man-hours	Total culicines	Per ten man-hours
Punjab ...	Complete year	21,681	81,323	37·51	4,023	27,994	69·58
Patiala and East Punjab States Union ...	,, ,,	7,696	26,624	34·59	84	186	22·61
Mysore ...	,, ,,	37,434	95,852	25·6	6,537	30,641	46·8
Bombay ...	,, ,,	29,752	25,629	8·6	1,854	5,162	27·8
Kutch ...	,, ,,	732	2,143	29·27	732	2,538	34·67
Hyderabad ...	,, ,,	1,223	15,995	130·8	476	7,212	151·5
Andhra ...	,, ,,	19,750	8,589	4·4	2,888	4,101	14·2
Delhi (Rural) ...	Jan. 1955-Nov. 1955	383	2,738	71·5	19	425	223·7
Uttar Pradesh ...	Complete year	4,113	6,739	16·4	1,690	5,625	33·2
Orissa ...	Apr. 1955-Dec. 1955	4,226	20,791	49·19	60	212	35·3
West Bengal ...	Complete year	10,356	38,461	37·14	503	1,666	33·12
Madras ...	,, ,,	26,026	15,154	5·82	8,954	23,873	26·66
Travancore-Cochin ...	,, ,,	1,147	812	7·1	...	...	...
Tripura ...	,, ,,	134	995	74·25	23	141	61·3
Madhya Pradesh	Jan. Feb. Mar. 1956	182	1,637	89·9	43	314	73·0
Vindhya Pradesh	Complete year	663	1,832	28·94	36	681	189·1
Saurashtra ...	,, ,,	603	457	7·6	25	73	29·2
Coalfields ...	,, ,,	2,562	1,883	7·3	2,371	2,902	12·2
Bihar ...	,, ,,	4,644	40,556	7·3	1,225	11,620	94·85
Manipur ...	,, ,,	90	182	20·22	90	575	63·9
Assam ...	Nov. Dec. 1955	483	2,188	45·30		...	...
Grand Total for all States		1,73,850	3,90,580	22·4	31,633	1,25,941	39·81

The figure of critical density has been calculated in relation to *fluviatilis* of the hill-tracts and sub-montane areas where the species is highly anthropophilic, markedly exophilic, of low density and high vectorial status. In the plain areas where it is more zoophilic, significantly endophilic, of high density with low infection

rates, its critical density must be considered at a level many times higher. In other words, it may be said that this species in plain areas behaves in many respects like *A. culicifacies*. Similarities in the resting habits, densities and infection rates of *culicifacies* and *fluviatilis* in the Visweswariah Canal area of Mysore State are borne out by the findings of Nursing, Rao and Sweet (1934). Viswanathan (1950) reported zoophilic *fluviatilis* having a very high density (350 per ten man-hours) with poor infection rate from Nira Canal Zone of Sholapur District in the Bombay State. In view of these considerations it would seem justifiable to fix the critical density of *fluviatilis* of the plain areas at a level approaching that of *culicifacies*. Nevertheless it is expedient to adopt a density figure of less than four per ten man-hours as the safe limit applicable to all *fluviatilis* areas. As long as there is no obvious explanation of why *fluviatilis* is primarily anthropophilic in some regions and zoophilic in others, it is essential not to allow any element of laxity, however, great the scope for it may be. *A. varuna* and *A. jeyporiensis* var. *candidiensis* behave in many respects like *fluviatilis* and their critical density may be regarded at the same level.

In areas where *A. minimus* is the only vector, the highest density of the species at which malaria has been effectively controlled is 1·13 per ten man-hours (Table XVI, Tripura 1954-55). One may, however, be cautious in adopting this figure in view of the findings of Bhatia (1951) in the foot-hill areas of Northern Thailand where *A. minimus* was able to carry on malaria transmission at this density. This part of Thailand is topographically and climatically similar to Southern Assam. It was only at a lower level of about 0·8 per ten man-hours that transmission ceased.

In other areas where more than one species is involved in malaria transmission, the second highest density figure with successful control is 13·88 per ten man-hours collectively of *annularis*, *sundaicus*, *fluviatilis* and *varuna* (Orissa, 1954-55). The former two species are more numerous in these areas. Habits of *A. philippinensis* resemble those of *A. annularis* with regard to domesticity, zoophily and breeding habitats and its critical density may be considered at the same level *viz.* 13·0 per ten man-hours.

Provisional critical density of vector species for the purpose of this paper may be defined as the highest limit of density up to which risk of malaria transmission is nil. The figures of critical density in respect of different species discussed above may be summarized as follows:—

A. culicifacies	33·0	per ten man-hours.
A. stephensi	33·0	,, ,, ,, ,,
A. stephensi var. *mysoransis*	33·0	,, ,, ,, ,,
A. culicifacies, *A. stephensi*	33·0	,, ,, ,, ,,
A. fluviatilis	4·0	,, ,, ,, ,,
A. jeyporiensis var. *candidiensis* ...	4·0	,, ,, ,, ,,
A. varuna	4·0	,, ,, ,, ,,
A. minimus	0·8	,, ,, ,, ,,
A. annularis	13·0	,, ,, ,, ,,
A. sundaicus	13·0	,, ,, ,, ,,

A. annularis, A. sundaicus, A. fluviatilis and *A. varuna*	13·0	per	ten	man-hours.	
A. culicifacies, A. stephensi and *A. fluviatilis*	13·0	,,	,,	,,	,,
A. culicifacies and *A. fluviatilis* ...	13·0	,,	,,	,,	,,
A. philippinensis	13·0	,,	,,	,,	,,
A. philippinensis and *A. sundaicus* ...	13·0	,,	,,	,,	,,

Even better than the above mentioned figures would be those for vector densities recorded in the sprayed areas of different States during 1955-56, as indices of successful control for the years to follow when the aim is to eradicate the disease. As long as density figures of the sprayed areas do not exceed the figures obtained during 1955-56, it may be safe to conclude that effective control has been maintained.

When the objective is changed from control to eradication of the disease, it is advisable that the States should undertake the determination of 24-hour survival rates of vector species as well as other anophelines caught from sprayed and unsprayed areas in addition to estimation of densities. This would provide the means to detect any variation in the susceptibility of the different species to the insecticide. This item was optional in the past but hence-forward should be considered an essential supplement.

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