INDIAN JOURNAL OF MALARIOLOGY

PUBLISHED UNDER THE AUTHORITY OF
THE INDIAN COUNCIL OF MEDICAL RESEARCH.

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Vol. XIII, 1959.

PUBLISHED FOR
THE INDIAN COUNCIL OF MEDICAL RESEARCH
AT
THE CAMBRIDGE PRINTING WORKS, DELHI.

Price per volume : Twelve rupees (or seventeen shillings six pence) including postage
Single copies : Five rupees (or seven shillings three pence) excluding postage.

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THE RÔLE OF DRUGS IN MALARIA ERADICATION IN SOUTH-EAST ASIA.

BY

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[January 28, 1959.]

THE Second Asian Malaria Conference (1956) stated that the ultimate aim of nation-wide malaria control programmes must be eradication of the disease. At that time it was considered that the chief place of anti-malarial drugs was to eradicate infections already present when spraying programmes were initiated although they were accepted as useful also in epidemic malaria and in the suppression of malaria in pioneer settlers and in those people whose occupations kept them out-of-doors at night. It is interesting that the Conference recorded an opinion that while the theoretical possibilities were good, yet practical difficulties confronted their use and particularly so in suppression.

During the subsequent three years, the increase in seriousness of insecticide-resistance has influenced national malaria control policies by an awareness that there might be a time limit on the effective use of insecticides and it was almost inevitable that eradication should become the objective. Any means that may reinforce residual insecticides in bringing it nearer attainment must be, therefore, examined for their usefulness, for no man knows how much time remains.

This paper attempts an assessment of the rôle of drugs in malaria eradication but does not discuss their pharmacological properties nor their dose regimés, these having been adequately described by Covell *et al.* (1955), Macdonald (1957) and in the Technical Report Series of the World Health Organization (1954; 1957) Emphasis will be chiefly on those aspects that concern the field worker, finding the cases and ensuring that the required number of tablets are taken.

In broad grouping, anti-malarial drugs may be of special use in the following situations :—

(*i*) Residual insecticides alone may be too slow in reducing an initial high incidence of malaria, or else satisfactory progress may be halted before complete interruption is achieved.

(*ii*) In India and other large countries, an eradication programme may not necessarily start simultaneously in every province. Drugs then may be useful as supportive measures to help late starting areas to catch up to the general level.

(*iii*) Drugs may have a unique place in the eradication of malaria in certain population groups whose lives, as so graphically described in a World Health Organization Technical Report (1956), "are not normally led

within four walls which can be treated with residual insecticides". These people may be fishermen or farm and forest workers or they may be nomads whose tents, so often pitched, struck and packed, possess "walls" that are not at all suitable for treatment with insecticides apart from the difficulties of finding the encampments in lonely trackless deserts.

(*iv*) There may be groups, perhaps several hundreds of thousands in number, still living a tribal existence in remote and inaccessible mountains that cannot be reached by spraying teams.

(*v*) The usefulness of drugs in checking epidemics is so obvious as to require no further discussion.

(*vi*) In the modern concept of malaria eradication, drugs assume the leading rôle.

(*vii*) There remain the urgent demands which may be made on drugs should malaria transmission no longer be controllable because of vector resistance to residual insecticides.

DRUGS IN RELATION TO SPECIAL SITUATIONS.

The ideal qualities of an anti-malarial drug have been given by Bruce-Chwatt (1956). This list specifies virtues of causal prophylaxis, suppression, rapid and complete curative action, sporontocidal effect, impossibility of creating parasite resistance, together with low toxicity, very slow excretion, palatability and low cost.

To meet most of the situations listed in this paper, the requirements may be set rather lower. Were there a drug that, while cheap and non-toxic, was also an efficient sporontocide and whose effect was so long-lasting that one—at the most two—administrations would last throughout a transmission season, then the value of drugs in eradication programmes would be in no doubt and their efficiency and widespread use would justify heroic measures. But no drug meets these requirements nor would any such one appear to be in sight.

There are a number of papers describing the use of anti-malarial drugs in mass treatment, with and without insecticide application. Farinaud and Choumara (1954), working in a hyper-endemic area of Indo-China, found that chloroquine, pyrimethamine or amodiaquine given for two or three months rapidly reduced parasite rates, though the rate subsequently rose, and only with amodiaquine did they remain low for several months. Vincke (1954) used pyrimethamine (25 mg. weekly for adults) in groups of labourers in the Belgian Congo for periods of 5 to 21 weeks and he obtained a rapid reduction of the parasite rate which remained low for six months after the drug was withdrawn. Macdonald (1957 *loc. cit.*) suggests that the persistent effect in Africa, and its absence in Indo-China, may be due to differences in the reaction of strains of the parasite. It is obviously important that the persistent effect of pyrimethamine should be estimated in the countries of Asia for the sporontocidal activities of this drug are of considerable promise in mass chemotherapy.

Turning to the groups difficult to reach, the tribes in mountainous areas, the nomads and migrants and those who are out-of-doors at night, there is a need in Asia for extensive field trials of chloroquinized salt. To Pinotti (1953) goes the credit of being the first to envisage the possibilities of mass treatment by medicating that most common addition to food, table salt. Recently, Coatney *et al.* (1958) have described the preparation of salt medicated with either chloroquine or pyrimethamine. They have shown that the substances are not affected by cooking processes and have tested them with complete success in suppressing *vivax* malaria in a small group of volunteers in Georgia, U.S.A. As, however, the drug was given before experimental infection, the conditions of the trial do not reproduce those in the field where a large proportion of the population will have been previously infected.

Before medicated salt can be introduced in any country, it will be necessary to investigate whether all sources of salt production can be controlled. If small-scale production goes on over a wide area, control may be difficult or even impossible ; certainly legal enactments will be necessary to give the required powers.

In addition, the average quantity of salt consumed must be measured so that the dose of drug can be adjusted. Instances are known where mountain people exist with very little salt for many months and then consume large quantities in a short time, when they come in contact with supplies. With pyrimethamine-salt, parasite resistance may be expected and with chloroquine-salt resistance cannot be dismissed as impossible, though there is little likelihood of its occurrence.

In an entirely different category to the above-described situations is the use of drugs in the surveillance stage of malaria eradication programmes, i.e., when malaria transmission has been terminated by residual insecticides and the campaign has been directed towards the discovery of parasite carriers and their complete treatment. In the first stages when cases may be numerous, single-dose treatments with chloroquine or amodiaquine, followed by courses of primaquine in *vivax* infections, may be all that is possible. As case treatment becomes increasingly successful, it should then be possible to give the smaller numbers full 3-day treatments. Both regimés described by Covell *et al.* (1955 *loc. cit.*), Macdonald (1957 *loc. cit.*), and in the World Health Organization Technical Reports, may need variations to suit local conditions and the described regimés cannot be considered as fixed for all time and all countries.

THE DIFFICULTIES OF DRUG ADMINISTRATION.

In a review of the place of drugs in a malaria eradication programme, the emphasis is on cases of malaria and administration of drugs. In the actual use of drugs, the viewpoint changes from the impersonal to the personal, from "cases" to men, women and children, and from "drug administration" to swallowing the prescribed number of tablets. The change introduces entirely new problems that are incompletely understood, unpredictable and may even be insoluble.

Firstly, there are the difficulties of discovering parasite-positive cases. No general answer can yet be given to the question, what proportion of actual malaria cases is found positive in a single thick blood smear ? Then again, people whose blood is positive may not feel ill and therefore will not offer a chance of blood examination by seeking treatment.

Should parasitaemia be discovered in a sampling survey, another set of problems arises because it must not be assumed that a man who does not feel sick will take a course of treatment unless obliged to do so ; to him it would seem purposeless, still more so if the course consisted of primaquine for fourteen days.

Again, a person may feel ill, may request treatment and may have a blood smear taken, but if his symptoms have disappeared before the blood is examined and the result reported to the treatment unit, the factors described above will again operate.

Formidable as are these difficulties, they are dwarfed by those confronting continuous drug administration. Ray (1948), working with controllable tea estate labourers in India, and taking all possible care to ensure continuous and regular drug administration, found that only 70-80 per cent of his people never missed a single dose in six months.

Gilroy (1952) in Assam used urine tests to check the administration of proguanil and found that only 56 per cent of tea estate labourers, said to have been given their tablets, had actually swallowed them. If these difficulties are encountered with disciplined people, accessible and readily supervised, to what extent will they increase when drug administration is applied to people whose numbers may not be known, who live in distant villages difficult of access and who are, perhaps for the first time, encountering mass chemotherapy ?

Why people resist attempts to make them swallow pills week after week, is not known. It is quite certainly a complex problem that would justify investigation by social anthropologists. The attitude may perhaps be glimpsed if we imagine ourselves lined up outside our houses, or made to visit treatment centres, every week, to swallow a tablet whose purpose is not entirely clear to us and hence is suspect. Health education will certainly help but it is a slow process, perhaps too slow to fit in the time schedule of malaria eradication programmes.

Legal power to enforce drug administration has been suggested. To anyone who is experienced in the lengths to which people will go to evade swallowing medicine and who has seen the growth of consumer-resistance, legal sanction would seem certain to convert guile and indifference into sullen opposition.

CONCLUSIONS.

Anti-malarial drugs are of unquestionable importance in the surveillance phase of malaria eradication programmes. In limited areas they have effected a rapid, though temporary, reduction of parasite rates. They may be the method of choice

in eradicating malaria in problem groups such as nomads, migrants and dwellers in mountains and forests.

Their theoretical and experimental virtues, however, encounter formidable obstacles in practical application, and to underestimate or minimize them does eradication a dis-service and sets the goal further off. Too often it becomes apparent that the greatest hindrance to malaria eradication, and the greatest challenge to the malariologist is, in fact, man himself.

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INSECTICIDE RESISTANCE IN SOME ANOPHELES LARVAE.

BY

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[April 6, 1959.]

INTRODUCTION.

THE response to insecticides of Anopheles larvae has been studied by means of a technique devised primarily for *A. gambiae* Giles (Elliott, 1958); the present account refers to the extension of this method to two other species.

METHOD.

Batches of about 15 larvae in the early and middle part of the fourth instar were exposed to aqueous dilutions of toxicant for one hour, removed to clean water, fed, and examined for mortality after another five hours. The temperature was controlled at 27°C. (80°F.).

A. SUNDAICUS RODENWALDT.

Larvae hatching from eggs sent to Lagos from two of the Ross Institute colonies of this species were tested. The two strains originated in Malaya and Java, the former showing normal susceptibility and the latter DDT resistance in the adult state (Davidson, 1957). The response to DDT is shown in Graph 1.

The median lethal concentrations (LC50) for DDT (in parts per million) were :—

(with confidence limits for 95 per cent probability obtained by the method of Litchfield and Wilcoxon, 1949)

Malaya	...	...	0·35 ppm (0·26—0·47).
Java	...	...	25 ppm (19—33).

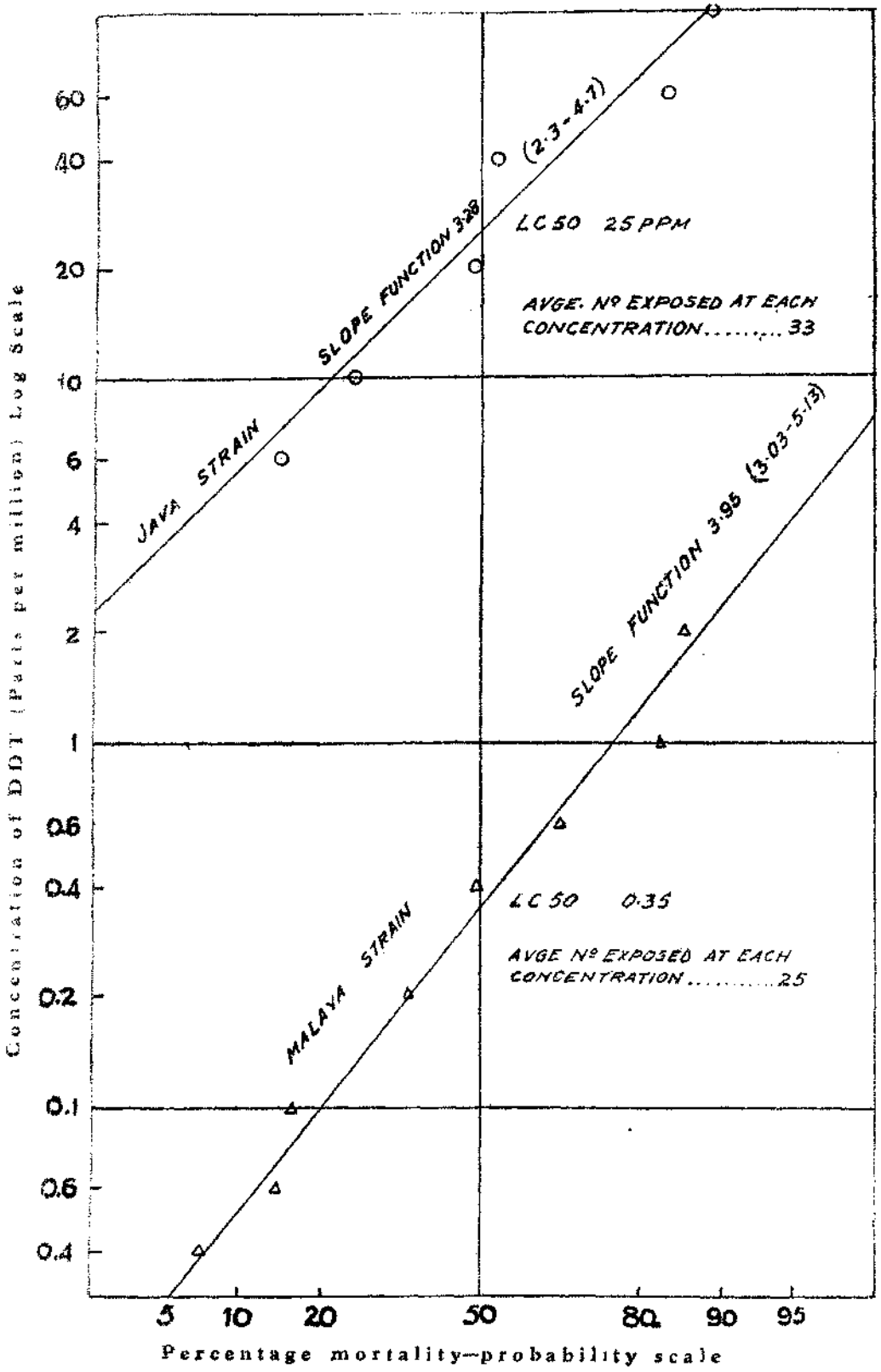
For dieldrin, the LC50 appeared to be approximately 0·025 ppm for both strains, but could not be accurately determined with the number of larvae available.

A. STEPHENSI LISTON.

Of this species three strains from the Ross Institute were tested ; a susceptible strain from India, a strain selected from this by breeding for 20 generations from larvae surviving exposure to DDT, and a resistant strain from Iraq.

GRAPH 1

Response of A. Sundaicus larvae to DDT.



The results of exposure to DDT are shown in Graph 2. The LC50 values for DDT and dieldrin, (with confidence limits as before), were :—

	DDT	Dieldrin
Indian "S"	... 11·5 ppm (7·7—17·25)	0·084 ppm (0·058—0·122)
Selected	... 22·5 ppm (15·0—33·5)	0·17 ppm (0·094—0·31)
Iraq "R"	... 150 ppm (111—202)	0·04 ppm (0·029—0·055)

The selected strain was about twice as resistant to DDT as the susceptible strain from which it was derived, but the resistant field strain was thirteen times resistant. It will be noted also that the selected strain shows a barely significant enhanced resistance to dieldrin, suggesting that the result of selection has been to induce a non-specific 'vigour tolerance'. The slopes of the three curves do not depart significantly from parallelism.

DISCUSSION.

Since the same physiological mechanisms may be expected to operate throughout the life history of the resistant or susceptible mosquito, some correlation between adult and larval levels of susceptibility may be looked for. Figures applying to both adult and larval material of resistant and susceptible strains are available for three species, namely, *A. gambiae* (Davidson, 1956 ; Elliott, 1958) and the two species considered here, whose adults have been studied by Davidson (1958). Table I shows the LC50 values obtained for adults using the Busvine-Nash (1953) method, and for the corresponding larvae using the technique of Elliott (1958).

TABLE I.

Median lethal concentrations of adult and larval Anopheles.

Species	Toxicant	Susceptible strains		Resistant Strains	
		a larvae (ppm)	*b* Adults (per cent)	*c* larvae (ppm)	*d* Adults (per cent)
A. gambiae	BHC	0·045	0·007	3·7	0·21
A. sundaicus	DDT	0·35	0·30	25·0	9·0
A. stephensi	DDT	11·5	1·9	150·0	11·4

The relationship might be expressed by the formula :—

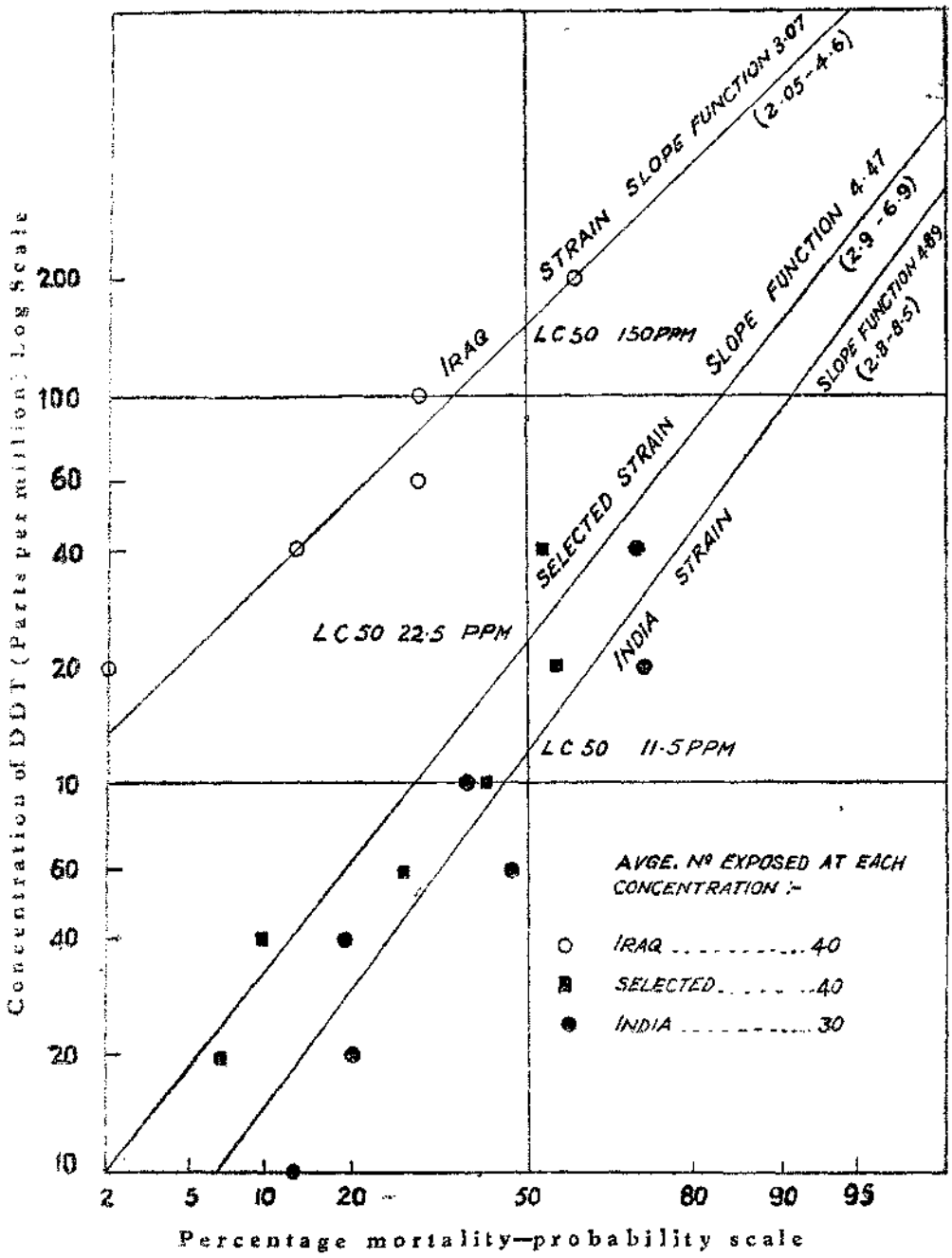
$$\frac{\text{LC50 R larvae}}{\text{LC50 S larvae}} = K \frac{\text{LC50 R adults}}{\text{LC50 S adults}}$$

or

$$\frac{c}{a} = K \frac{d}{b} \text{ (Table I).}$$

GRAPH 2

Response of A. stephensi larvae to DDT.



The values of K from Table 1 are then :—

A. gambiae	...	...	...	K = 2·7 (1·4—4·2).
A. sundaicus	...	...	...	K = 2·85 (1·6—5·0).
A. stephensi	...	...	...	K = 1·82 (0·9—3·6).

The figures in brackets indicate extreme values of K obtained from the 95 per cent confidence intervals of the estimates of larval LC50 values. It is clear that as yet no correlation is proven, but in view of the value of a formula which could be used to predict information about adult susceptibility from observations on larvae, and *vice versa*, the calculation is put forward in the hope that further figures may be forthcoming.

SUMMARY.

1. The method of Elliott (1958) for examination of the response to insecticides of Anopheles larvae has been extended to larvae of two strains of *A. sundaicus* and of three strains of *A. stephensi*.

2. In both species, there was evidence of a correlation between the degree of susceptibility shown by the larvae and that of the corresponding adults.

3. An attempt was made to obtain an arithmetical relationship between larval and adult susceptibilities to insecticides, comparing the present results with observations published on the corresponding adults and with similar figures applying to *A. gambiae*.

ACKNOWLEDGEMENTS.

The work was carried out in the Insecticide Resistance Reference Laboratory of the Malaria Service; the laboratory is supported jointly by the Government of the Federation of Nigeria and by the World Health Organisation. The author has to thank Dr. C.M. Norman-Williams, Chief Medical Adviser to the Federation, for permission to publish these results, and Mr. G. Davidson of the Ross Institute for supplying the experimental material.

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NOTES ON THE FEEDING AND EGG-LAYING HABITS OF *FICALBIA (MIMOMYIA) CHAMBERLAINI*, LUDLOW 1904. (DIPTERA, CULICIDAE).

BY

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AND

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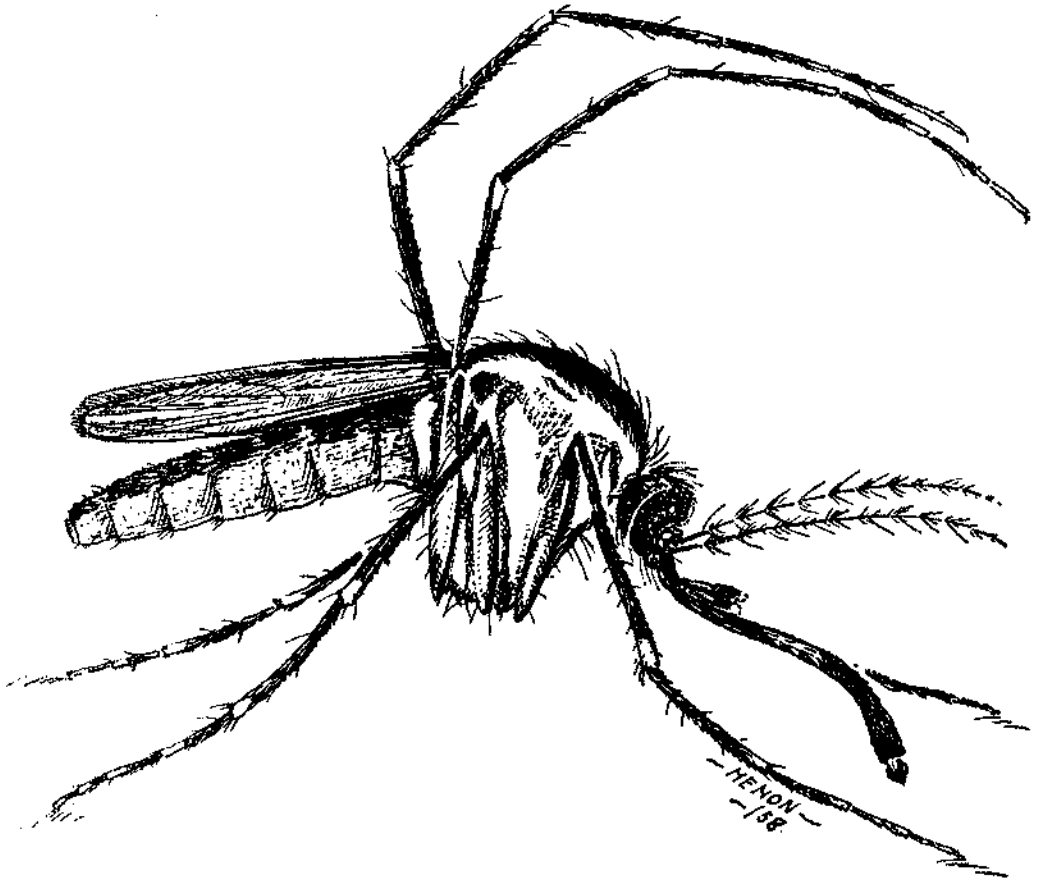
[March 14, 1959.]

Not much is known about the habits and bionomics of the *Ficalbias*. In these notes, the feeding habits of *Ficalbia (Mimomyia) chamberlaini* are described for the first time together with notes on its hitherto unknown egg.

Barraud (1934) has recorded 5 species of the genus *Ficalbia* from India. Of these, three species namely, *F. (Ficalbia) minima*, *F. (M.) hybrida* and *F. (M.) chamberlaini* are common in the Travancore region of Kerala (Iyengar 1935*a* : 1935*b*). All these three species are found in association with the water lettuce, *Pistia stratiotes*. However, *F. chamberlaini* differs from the other two species mentioned in that presence of *Pistia* is not essential for its development, for it has been observed to breed in ponds with little or no *Pistia* (Iyengar, 1935*a* : 1935*b loc. cit.*).

While on a routine reconnaissance of breeding places of culicines, in connection with the Filariasis Control Programme in Trivandrum City, the authors came upon a large tank with plenty of aquatic vegetation, mainly *Pistia*. The margin of the tank was fringed with *Colocasia* plants. A search for wild mosquitoes resting among the leaves of these plants, yielded a large number of males and females of *F. chamberlaini*. They could easily be made out by the characteristic yellow colour of the pleurae and the yellow bands on tarsi, in distinct relief against the dark green of the leaves. Many adults were also caught resting on the leaves of the *Pistia* in the tank. Even though the mosquitoes definitely preferred shade, it was not unusual to find individuals resting in the sunlight. The peculiar manner in which they carried the hind legs arched forwards over the body (Fig. 1) while at rest, was also an unmistakable mark of their identity. The identity of the mosquitoes was further confirmed by subsequent systematic study. The tank, on inspection, showed considerable breeding of the larvae of *F. chamberlaini*, which can easily be distinguished by the dark, almost blackish colouration of the head (Barraud, 1934 *loc. cit.*). Other species breeding in the tank were *F. hybrida*, *Taeniorhynchus annuliferus*, *Culex vishnui*, and some anophelines.

FIGURE 1.



Characteristic resting pose of *Ficalbia (Mimomyia) chamberlaini.*

FEEDING HABITS OF *F.* (*M.*) *CHAMBERLAINI.*

On closer scrutiny of the collection of *F. chamberlaini*, it was found that many of the females had blood-feeds. This was an unexpected finding. According to Barraud (1934 *loc. cit.*), the *Ficalbias* 'do not appear to attack man'. Nor has there been any known record so far of any species of this genus having been found engorged with blood. Indeed, even though these mosquitoes are common enough in this area, they hardly come indoors and are seldom found in indoor collections. This has been substantiated by the conspicuous absence of the species in the collections taken from the dwellings in the close vicinity of the tank in question. Furthermore, they are weak fliers and, apparently, they do not move far from the breeding sites. Considering these facts, it was presumed that the source of the blood meal seen in the engorged specimens of *F. chamberlaini* was some animal frequenting the tank or its

immediate neighbourhood. The bull frog (*Rana tigrina*) which inhabited the tank, appeared to be the most likely host of this species.

Starting from this presumption, an attempt was made to trace the source of the blood meal in the engorged specimens, in the manner described below :—

More collections of *F. chamberlaini* were made on the following days from the same habitat. The fully engorged specimens were separated, and their stomach contents isolated by dissection in saline on clean slides. The blood clot was teased fine and mixed well with the saline on each slide, to form a homogeneous drop. This was then spread into a thin oval film. The dry film was fixed in methyl alcohol, stained in 5 per cent Giemsa's stain for 15 minutes, and was examined under the microscope for any possible cellular characteristics which might give a clue to the identity of the blood. Of several smears thus examined, 8 showed considerable numbers of large oval nucleated red corpuscles as well as numerous naked nuclei and nuclear remnants of partially digested red blood corpuscles. The leucocytes appeared to be less affected by the digestive processes. Those red blood corpuscles which still retained their original morphology were measured and the average length and breadth determined. Among the 50 cells measured, the length varied from 19 μ. to 24 μ. and the breadth from 14 μ. to 16 μ., giving an average red cell size of 21 μ.×15 μ.

The average measurements of the red cells from the blood meals of *F. chamberlaini* were then compared with those given by Ecker (1889) for the frog, *Rana temporaria*, and by Prosser *et al.* (1950) for birds, reptiles and amphibians, in all of which the red cells are nucleated. The average measurements of the blood cells fall within the range given for amphibians by these authors as indicated in the Table I below :

TABLE I.

Comparative sizes of the red blood corpuscles of some amphibians.

[After Ecker (1889) and Prosser *et al.* (1950).]

Author	Animal	Red cell size in microns. (length×breadth)
Ecker (1889)	*Rana esculenta*	25·5×17·0
-do-	*Rana temporaria*	23·5×14·5
Prosser *et al.* (1950)	*Rana temporaria*	19·7×13·3
-do-	*Rana esculenta*	22·8×15·7
-do-	*Bufo vulgarias*	20·5×13·3

The possibility of the blood meal being reptilian or avian in source could be ruled out because in either case the red cells are definitely smaller. It was thus presumed that the mosquitoes had fed on frog's blood.

On a subsequent visit to the tank, the authors were able to confirm this premise by observing some females of *F. chamberlaini* feeding on frogs (*Rana tigrina*) both on land, and in water with their head just raised above the water surface.

Two female specimens of *F. chamberlaini* were caught while in the act of feeding on frogs and smears of their stomach contents were made on the spot. The microscopic picture of the blood meal in these cases was quite clear and was confirmatory of the identity of the blood meals examined of the engorged resting specimens.

The area around the frog's eyes seemed to be the preferred feeding site for these mosquitoes. Apart from being comparatively soft, this area is the most easily accessible when the frog is afloat. The frogs are apparently callous to the bites as evidenced by their undisturbed attitude when the mosquitoes feed on them. As the mosquitoes were found to feed during day time, it would appear that *F. chamberlaini* is a diurnal species*.

Whether or not *F. chamberlaini* exclusively feeds on frog-blood is yet to be determined. But in the blood-fed specimens whose stomach contents were examined and in which the cellular characteristics of the blood meal were discernible, nothing has been found so far to indicate that it feeds on other animals.

In view of the fact that frogs have been known to be naturally infected with the Filarioid, *Foleyella sp.* (Causey, 1939; Wehr and Causey, 1939), a number of female specimens (72) of *F. chamberlaini* were dissected for filaria larvae; but none was found infected.

THE EGGS OF *F. (M.) CHAMBERLAINI.*

The eggs of *F. chamberlaini* have not hitherto been known. Some 15 gravid females were made to oviposit in a Petri dish full of water, placed inside a closed glass jar. Under these conditions, after four days, only two of these laid, a total of 33 eggs. On a second occasion, another 15 mosquitoes were kept for oviposition within a large glass jar, 12 inches in diameter, containing water and a few *Pistia* plants. Numerous eggs were obtained this time, indicating that the presence of *Pistia* is a desirable factor in their breeding.

The eggs (Plate I, Fig. 1) on study were found to be very similar in morphology, appearance and diamensions to those of *F. (M.) hybrida*, described by Menon (1938). The egg is 0·7 mm. × 0·2 mm. (average size), boat shaped and its upper side is demarcated into a "deck" by a striated frill which surrounds it. The frill is broader at the apices than at the sides of the egg. The exochorion is decorated with minute refractile polygonal reticulation visible in incident light. The egg dehisces by a transverse incomplete cleavage just behind the anterior end. In the empty egg shell, the apical portion remains like a hinged operculum (Plate I, Fig. 2).

Menon (1938 *loc. cit.*) pointed cut that the egg of *F. hybrida* is the nearest approach among culicines to the *Anopheles*-type of egg. The finding of identical eggs in *F. chamberlaini* suggests that the anopheline features seen in the eggs of these two species are possibly characteristic of their subgenus, *Mimomyia*.

* *Night time observations need to be carried out to confirm this.—Editor.*

PLATE I.

FIG. 1.

FIG. 2.

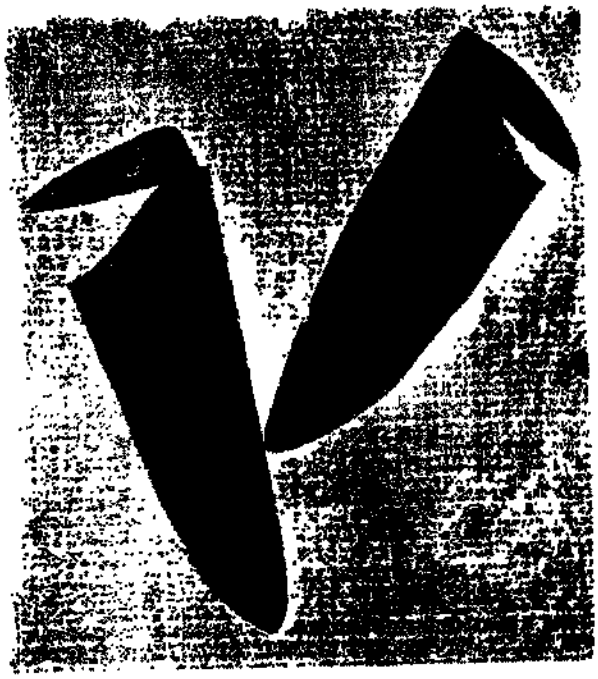


FIG. 4.

FIG. 3.

FIG. 1. Eggs of *Ficalbia* (*Mimomyia*) *chamberlaini*. Lateral view. (×100).
FIG. 2. Unhatched eggs of *Ficalbia* (*Mimomyia*) *chamberlaini*. Lateral view. (×100). The frill has peeled off.
FIG. 3. Microscopic picture of blood-meal smear from an engorged specimen of *Ficalbia* (*Mimomyia*) *chamberlaini* caught resting. (×1,100).
FIG. 4. Microscopic picture of 'on-the spot' blood-meal smear from a specimen of *Ficalbia* (*Mimomyia*) [illegible] caught while feeding on [illegible].

SUMMARY.

1. By studying the cellular characteristics in smears of the blood meals of specimens of *Ficalbia* (*Mimomyia chamberlaini* and by actual observations in the field, it is established that this species habitually feeds on frogs.

2. The egg of *F.* (*M.*) *chamberlaini* is described. It is identical with that of *F.* (*M.*) *hybrida*. The anopheline-type of egg seen in these two species is probably a distinctive feature of the subgenus *Mimomyia* to which they belong.

ACKNOWLEDGMENTS.

The authors are thankful to Dr. C. Joseph, Assistant Director of Health Services (Malaria and Filariasis), Trivandrum, for permission to publish this paper. They are indebted to Dr. M. Thankavelu, Principal, Medical College, Trivandrum, for facilities to take the photomicrographs and to Professor Ananthanarayanan, Department of Zoology, University College, Trivandrum, for library facilities.

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STUDIES ON INSECTICIDE RESISTANCE IN INSECTS OF PUBLIC HEALTH IMPORTANCE IN WEST BENGAL, INDIA.

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[November 17, 1958.]

INTRODUCTION.

Resistance to insecticides is not a new thing in insects, but the phenomenon has assumed importance in public health insects only recently. True resistance among the insects came to be known in housefly first and then in *Culex* and *Anopheles* among mosquitoes from countries far and wide like Italy, Greece, India, Indonesia, Australia during the years 1947 to 1953. The problem has come to be appreciated in the insects of medical importance since the introduction of synthetic insecticides. In many agricultural insect pests, on the other hand, resistance has been reported even with lime-sulphur and arsenic sprays long before the synthetic insecticides of the chlorinated group appeared.

Valuable reviews on insecticide resistance among insects of public health importance have appeared from time to time, and the latest by Brown (1958) gives a complete picture in an admirable way on the subject.

In the present communication, however, an attempt has been made to record our experience with the different insects in this respect in this part of the world and how far they responded to the susceptibility tests with the different chlorinated hydrocarbon insecticides and others.

METHODS.

Susceptibility tests in the insects discussed here were mostly carried out by the Busvine-Nash technique (World Health Organization Tech. Rep. Ser. 80) by utilising filter papers impregnated locally with different concentrations of insecticides to be tested unless otherwise stated, the solvents used being ether and white oil. The insects experimented on were exposed to these papers for one hour or for a definite time period and the mortality recorded after 24 hours as a routine measure. Controls were kept and they behaved normally. Tests were carried out under laboratory conditions through the various seasons during the period October, 1956 to October, 1958.

Houseflies.—The houseflies belonging to *Musca d. vicina*, either wild-caught or laboratory-bred, were subjected to various dosages of different insecticides and the result of the susceptibility tests is shown in Table I. The result is based on 5 to 8 replications of eight flies each.

TABLE I.

Tolerance level of houseflies to different insecticides.

Insecticides.	Dosage (Per cent).	Mortality (Per cent).
DDT ...	2·0	4·7
DDT ...	3·0	44·0
DDT ...	4·0	50·0*
Dieldrin ...	1·0	91·6
Dieldrin ...	0·5	51·5
Dieldrin ...	0·1	42·5
Diazinon ...	0·5	100·0
Diazinon ...	0·25	82·5
Diazinon ...	1·125	80·0
Malathion ...	1·0	100·0
Malathion ...	0·5	100·0

*After 4 hours exposure.

It will become apparent that even at a high dosage of 3·0 per cent DDT in one hour's exposure the mortality in the houseflies was not more than 44 per cent. When the dosage was raised to 4·0 and that also after 4 hours' exposure the LD_{50} was obtained, proving that highly resistant strains of flies exist in Calcutta.

Musca vicina nebulo has proved resistant to DDT and BHC in other parts of the world as in northern districts of Israel (Brown, 1958) and the sub-species *vicina* to DDT only in Tadzhik (U.S.S.R.) and in South-Eastern Italy, and to BHC in Egypt (Gahan and Weir, 1950) and Northern India (Khan, 1958) but not to DDT. Physiological resistance of *vicina* to chlorinated hydrocarbons has been confirmed by sufficient data not only from Africa and the Middle East, but also from the Far East as well as the South Pacific and Hawaii (Quaterman and Schoof, 1958).

The Calcutta flies we worked with, although showed evidence of a positive resistance to DDT, these were still susceptible to Dieldrin, Malathion and Diazinon, as experienced in the flies of South Pacific and Hawaii (Byers *et al.*, 1956) but not so in Lativia (Italy) where the flies proved resistant to Diazinon also (Brown, 1958).

Flies raised in the laboratory from survivals of 1 per cent Dieldrin-treated specimens gave a much higher tolerance level. In the first generation the mortality was found to be only 35 per cent as opposed to 91 per cent of the original strain treated with the same dosage (1 per cent) (Table II), and only 82 per cent in second

TABLE II.

Tolerance level of houseflies subjected to dieldrin pressure through generations.

Dosage.	Generation.					
	F_1		F_2		F_3	
	Number exposed	Mortality (per cent)	Number exposed	Mortality (per cent)	Number exposed	Mortality (per cent)
1 per cent ...	40	35·0	...	...	...	...
2 per cent ...	...	...	44	81·8	...	...
3 per cent ...	...	...	...	...	24	81·9

generation with twice the original dosage (2 per cent). A similar rise in the tolerance level up to third generation in *nebulo* was obtained with DDT in Northern India (Pal *et al.*, 1952). Our flies were resistant to knock-down as well, which the latter workers did not notice in theirs. Although the selection pressure could raise the tolerance level in the Calcutta flies, their fecundity was retarded in the third generation as recently reported in certain strains of Australian flies (*M. domestica*) (Kerr and Venables, 1957), and we could not proceed further as the brood died.

ANOPHELINES.

Anopheles annularis.—Tests with DDT indicated that the species is highly susceptible to the insecticide. The mortality in the mosquitoes collected from the fields in neighbouring districts outside Calcutta, not subjected to residual sprays, was nearly cent per cent even in 0·5 per cent DDT when tested in the laboratory (Table III). Even if the contact time was reduced to half-an-hour, the mortality was just as complete in the higher dosages of 2 per cent or 1 per cent (Sen, 1957), although in 15 minutes' contact a few may withstand the dosages. The result was obtained from 3 to 7 replicates of eight mosquitoes each.

TABLE III.

Susceptibility of A. annularis *females to insecticides tested.*

Insecticides.		Dosage (Per cent).	Mortality (Per cent).
DDT	...	2·0	100·0
DDT	...	1·0	100·0
DDT	...	0·5	95·0
Dieldrin	...	0·3	100·0

The calculated LC_{50} of the species, 0·26 per cent DDT, in Bengal specimens would appear nearly half of that obtained for Rajasthan area at 0·56 (Sharma *et al.*, 1957*a*). This difference probably lies in the mosquitoes of the latter place, being collected from sprayed areas.

Anopheles pallidus, A. philippinensis.—Both these species have become extremely rare and from the limited tests carried out the LC_{50} of the species from Hooghly District worked out to be 0·3 per cent DDT as recorded by Talibi (1958); a much lower figure of 0·125 for *philippinensis* quoted by Brown (1958) we failed to corroborate. *A. annularis* appeared highly susceptible to dieldrin, and in North India to BHC as well, with the LC_{50}, 0·42 per cent and 0·039 per cent respectively (Sharma *et al.*, *loc. cit.*).

A. hycranus nigerrimus, A. barbirostris.—These two species, like the others discussed above, also proved highly susceptible to DDT and, when tested against 1 per cent to 2 per cent dosages, induced a mortality of over 80 per cent. These species exhibited equally low tolerance to gamma-BHC and dieldrin (Table IV). The species *A. hyrcanus nigerrimus* is of special significance in the State as a natural vector of filariasis (Sen, 1957*c*).

TABLE IV.

Susceptibility of A. hyrcanus *and* A. barbirostris *to different insecticides tested.*

(Results from 3 replicates of eight mosquitoes each.)

Insecticides.		Dosage (Per cent).	Mortality (Per cent): Sprayed.	Unsprayed.
A. hyrcanus	DDT	2	100	100
		1	86	92
	BHC	0·5	87	100
		0·1	100	...
A. barbirostris	DDT	1	83	...
	BHC	0·025	100	...
	Malathion	0·1	100	...

Although in respect of mortality, *A. hyrcanus* behaved almost similarly in sprayed and unsprayed villages, its knock-down effect differed at the lowest dosage (0·5 per cent DDT) which brought about a 53 per cent knock-down in sprayed area against 87 per cent in unsprayed area.

A. subpictus.—This species from unsprayed areas in 24-Parganas (West Bengal) proved on tests highly susceptible to the chlorinated insecticides, just as much as the other species discussed above, on the observation from 4 to 10 replications of eight mosquitoes each (Table V). The LC_{50} of the species with DDT was 0·36.

TABLE V.

Susceptibility of A. subpictus *to different chlorinated insecticides.*

Insecticides.		Dosage (Per cent).	Mortality (Per cent): Unsprayed	Sprayed.
DDT	...	2·0	100·0	93·8
		1·0	72·6	81·2
		0·5	69·8	66·8
BHC	...	0·1	90·0	...
		0·05	77·0	...
Dieldrin	...	0·3	87·5	...
		0·1	62·5	...

When *A. subpictus* from the sprayed areas in Hooghly District were similarly tested with DDT alone, the mortality in 24 hours' interval after one hour's exposure was almost of the same order, indicating that unlike the mosquito from Delhi villages where the LC_{50} of the species rose to 3·1 per cent for a 24 hours' exposure after about six years' residual spray as against the normal LC_{50} of 0·4 per cent for one hour exposure from unsprayed villages of Uttar Pradesh, the species failed to acquire higher tolerance or resistance in Bengal villages on repeated sprays for a

similar period (Sharma and Krishnamurthy, 1957). The species has also become resistant in parts of Java recently.

But comparing the species of the sprayed areas with that of the unsprayed areas, some palpable difference in their knock-down effect was noticed. While in the mosquito from unsprayed areas, the knock-down percentage in the three dosages of DDT (2 per cent, 1 per cent, 0·5 per cent) was of the order of 83 per cent, 28 per cent and 20 per cent respectively, in the mosquito from sprayed areas the corresponding figures of knock-down were 56 per cent, 23 per cent and 18 per cent, indicating a selective effect in resisting the knock-down by the species as a result of sprays.

Similar variation in the knock-down of *A. hyrcanus* from the sprayed and unsprayed areas was also recorded, but the gradients were not so regular in the three dosages mentioned above as in the *A. subpictus*. The corresponding percentages in the two sets of observations were 75 per cent, 50 per cent and 87 per cent in an unsprayed area, as opposed to 92 per cent, 77 per cent and 53 per cent in the sprayed area, indicating that at the lowest dosage (0·5 per cent DDT) the sprayed area mosquito was better able to resist the knock-down but at the higher dosages (1 per cent or 2 per cent DDT) there was a reversal and the species was more adversely affected at these dosages. Why this should happen is difficult to explain at this stage ?

In *A. subpictus*, the gamma-BHC susceptibility has not been suspected in any part of the Indian region so far ; but with respect to dieldrin, in spite of its strong susceptibility in Delhi State with the LC_{50} at 0·16 (Sharma and Krishnamurthy, 1957 *loc. cit.*) as also in Bengal where the LC_{50} came to 0·08 nearly half of Delhi, resistance to this insecticide has been reported from Bombay State as quoted by Brown (1958 *loc. cit.*) where LC_{50} had gone as high as 6 per cent. With BHC the LC_{50} worked out to 0·03 in West Bengal.

A. sundaicus.—The species *A. sundaicus*, despite intensive antilarval measures with DDT wettable powder (50 per cent) at 0·3 lb per acre of water surface for a continuous stretch of over 5 years, is still susceptible to DDT; the area is only lately placed under residual spray after a few years' suspension of antilarval measures. The LC_{50} of the species is more or less the same as in *A. subpictus*, although in Java (Crandell, 1954) and in parts of Burma (Brown, *loc. cit.*) resistance to DDT has been reported as a result of large-scale spray operations. The resistance developed after three years of spray in Java and in Akyab (Burma) after about five years. Adult *A. sundaicus* in Java was not sitting on sprayed surfaces and avoided contacts with DDT. But the species did not avoid dieldrin sprayed surface, and has been found susceptible to dieldrin and BHC.

In Bengal although we had no area where adult pressure was acting, but as already stated, the larval pressure had no effect in selecting resistant strains as in the countries mentioned above. The susceptibility tests are recorded below (Table VI), from specimens collected on the outskirts of Calcutta and observations based on 3 replications.

TABLE VI.

Susceptibility of adult A. sundaicus *to different dosages of DDT.*

Dosage (Per cent).	Mortality (Per cent).
0·25	31
0·5	60
1·0	97
2·0	100

The LC_{50} of the species was 0·4 which would appear almost the same as in *A. subpictus*. From other close-by unsprayed areas the LC_{50} of the species had proved to be 0·5 (Ganguli *et al.*, 1958) confirming its susceptibility to DDT.

A. minimus.—In field tests *A. minimus* in Duars proved to be highly susceptible to DDT. At 1 or 2 per cent DDT, one hour's exposure would induce a cent per cent kill. The mortality at 0·5 per cent DDT was found to be 69 per cent which is almost a close approximation of the percentage mortality recorded by Bertram (1950) at 2·2 gm/m².

A. stephensi.—In Calcutta, laboratory tests carried out through different seasons indicate that the local *stephensi* is highly susceptible to hydrocarbon and organophosphorus insecticides. In the local strain when subjected to 2 per cent or 1 per cent DDT treatment, a complete kill almost invariably followed. The kill was not insignificant even at the considerably reduced dosage of 0·5 per cent DDT, being nearly 65 per cent.

Similarly, BHC 0·1 per cent produced a mortality of over 80 per cent ; this was, however, less than 50 per cent at 0·5 per cent BHC. (Table VII).

TABLE VII.

Susceptibility of stephensi *adults to different insecticides.*

Insecticide.	Dosage (Per cent).	Number exposed.	Mortality (Per cent).
DDT ...	2	46	100
	1	38	94·7
	0·5	44	63·6
BHC ...	0·1	38	81·6
	0·05	40	32·5

The LC_{50} of DDT was found to be 0·39 (Sen, 1957*b*) while for that of BHC, 0·06 (Sen, 1958). Both the figures prove conclusively that the species in Calcutta is not only not refractory to DDT but the susceptibility is of a high order to the insecticides most frequently in use in this country.

The species, however, withstand a larger dosage in South India where the tolerance of DDT is very much pronounced, and the Mysore strain from sprayed areas requires nearly 4 times the dosage used in Calcutta to get the LC_{50} (Shama Sastry

et al., 1956). Although the species in Mysore has not yet exceeded the threshold of susceptibility, a close watch on the situation is warranted in order that a condition like the Persian Gulf zone, where a strong resistance has developed in the species recently as a result of extensive spray operations in the countries, Saudi Arabia, Iraq and Iran, is not set up.

In Calcutta, although the adults were not from areas subjected to DDT pressure, the larval pressure as a result of breeding control with antimalarial oil already existed, for nearly 20 years. During this period, since 1951, occasional treatments with DDT or BHC water dispersible powder have been used no doubt, but mostly against culicine breeding.

Larval control with DDT, however, is known to enhance the tolerance of the species, as happened in Erode Town (Madras) where after 6 to 7 years of larvicidal application the mosquito has become highly resistant to DDT in the larval stage (Rajagopalan *et al.*, 1956).

We have not yet seen how the *stephensi* larvae behave on DDT pressure in Calcutta, neither have we any information on the adults in Erode behaving under DDT application.

The progeny bred out from survivals at the highest dosage showed a slight rise in tolerance up to three generations which confirms the finding of the Ross Institute, London, working on Delhi strain. But the increased tolerance soon disappeared on the withdrawal of the pressure. The experiments further indicated that the oviposition after third generation was much retarded, and the brood emerging failed to take a blood feed. The result of these experiments will be discussed in detail in another paper.

CULICINES.

Culex fatigans.—This species in Calcutta as reported elsewhere (Sen, 1957*a*) possesses a natural resistance to DDT, but the specimens from outlying areas, 10 to 15 miles beyond, were still susceptible at the top concentration of 2 per cent which nearly gives the LD_{50}. At this concentration, the kill in the insectary strain at the Calcutta School of Tropical Medicine was 25 per cent only. The observations were based on 8 to 12 replications of eight mosquitoes each. The mortality of 70 to 80 per cent at the dosage of 4 per cent DDT mentioned in an earlier communication (Sen, *loc. cit.*), referred to an overall rate where strict separation of the susceptible strain was not possible.

In the Calcutta strain, the LC_{50} was nearly 3 per cent DDT in 4 hours' exposure, or at 4 per cent in 3 hours' exposure. One hour's exposure in these two dosages hardly induced a mortality exceeding 31 per cent (Table VIII).

In Calcutta, control of culicine larvae had been carried on for over 20 years with larvicidal oil, and very seldom with DDT or gammexane water suspension in the fringe areas under special breeding conditions in the last 5 or 6 years. Unless

the oiling had some effect in increasing the tolerance of *fatigans* in Calcutta, the stray application of DDT, as mentioned above, would not make the big difference reported here.

TABLE VIII.

Susceptibility of Culex fatigans *adults to different dosages of DDT.*

Outside strain.		Insectary strain.
Dosage (Per cent).	Mortality (Per cent).	Mortality (Per cent).
1	18	12
2	48	25
3	56·5	31·8
3	...	47*
4	...	27
4	...	53‡

* 4 hours' exposure. ‡ 3 hours' exposure.

DDT resistance in *fatigans* has also been reported from Delhi State after 6 years of residual spray, and from other parts of India like Khurda in Orissa State (Krishnan, 1956), as well as from other global regions. In Malaya, for instance, even with the maximum dose of 200 mg/sq. ft. on wall surface, not more than 10 per cent of the *fatigans* were killed (Wharton, 1951). The *fatigans* on sprayed walls become greatly irritated and leave the room as revealed by window trap collections.

The species did not prove refractory to BHC, however, like the DDT. In the insectary bred out strain of Calcutta, a higher tolerance was no doubt pre-existent in the absence of any regular adult pressure, and a cross tolerance of dieldrin also became apparent from filter-paper tests (Table IX). The LC_{50} with latter insecticide was obtained only at as high a dose as 1 per cent which exceeds many times the dose required for anophelines.

TABLE IX.

Susceptibility of Culex fatigans *adults to insecticides other than DDT.*

(From 6 to 8 replicates of 8 mosquitos each.)

Insecticide.	Outside strain:		Insectary strain:
	Dosage (Per cent).	Mortality (Per cent).	Mortality (Per cent).
BHC	0·4	61·8	41·0
Dieldrin	0·2	...	12·3
	0·5	...	23·0
	1·0	...	52·0
Malathion	0·2	75·0	67·5
	0·15	37·5	32·6
Diazinon	0·5	...	100·0
	0·25	...	97·9
	0·1	...	100·0
	0·05	...	83·0

Cross resistance to dieldrin appeared in Kumbakonan (Madras State), as with BHC, after 20 months of weekly larvicidal treatment by gammexane (BHC) (Rajagopalan *et al*,, 1955).

Adults in the Calcutta laboratory were subjected to DDT pressure for a few generations but no enhanced resistance over the parent stock was noticed, as obtained in Venezuella (Blasquez and Maier, 1951) where the *fatigans* (*quinquefasciatus*) exhibited an increased tolerance by 20 to 30 per cent in the F_2 generation under DDT pressure. In our experiments (Table X), while with 3 per cent dosage in 4 hours' exposure the mortality rate remained almost constant up to F_2 generation, at 4 per cent dosage in 3 hours' exposure a progressive rise in the kill occurred.

TABLE X.

Susceptibility of C. fatigans *adults to DDT under adult pressure up to* F_2 *generation.*

(Based on 6 to 8 replicates of eight mosquitoes each.)

	Dosage (Per cent).	Time exposed.	Mortality (Per cent).
F_1	3	4 hours	65
	4	3 hours	53
	3	4 hours	62·5
	4	3 hours	70·8
F_2	3	4 hours	67
	4	3 hours	81

The *fatigans* of Calcutta was, however, very strongly susceptible to organophosphorus compounds like Malathion and Diazinon.

Mansonia (Mansonioides) annulifera.—This mosquito from Dum-Dum area in 4 tests each with the dosages of 1 per cent or 2 per cent DDT, each replicate having 8 mosquitoes, proved equally effective and caused a complete kill in both. Owing to paucity of materials, other dosages could not be tried.

Aedes aegypti.—Tests carried out with the laboratory-bred strains indicated that the *aegypti* of Calcutta was highly susceptible to both hydrocarbon and organophosphorus compounds (Table XI) basing the observations on 4 to 8 replicates of eight mosquitoes each.

The LC_{50} would appear to be 0·3 for DDT, nearly 0·2 for BHC and 0·05 for Malathion. The normal LC_{50} of *aegypti* adults in respect of DDT, according to Busvine (1956), lies at 1 per cent, between that of *Culex* and *Anopheles*. In our observations, however, the LC_{50} with this insecticide was more or less the same as found in most *Anopheles* we have tested. The Calcutta strain would thus appear to be nearly three times more susceptible than the *aegypti* from Delhi, Poona, Karachi or Lagos (Nigeria) (Busvine and Harrison, 1953).

The specimens from the last-named country when, treated with BHC, showed the LC_{50} as 0·02 per cent which, comparing with our finding of 0·2 per cent recorded above, stresses that the Calcutta strain is nearly ten times as resistant as the Nigerian strain in respect of gamma-BHC.

TABLE XI.

Susceptibility of Aedes aegypti *to different insecticides.*

Insecticide.	Dosage (Per cent).	Mortality (Per cent).
DDT ...	0·5	77
	1·0	84
BHC ...	0·05	30
	0·1	36
	0·2	47
	0·3	69
	0·5	93·7
Malathion ...	0·5	100
	0·25	100
	0·1	90
	0·05	52

Strong resistance of *aegypti* to DDT, however, has been detected in recent years from the Americas and Trinidad, but nowhere so far in respect of the other chlorinated hydrocarbons.

Application of adult pressure with DDT over generations in the Calcutta laboratory indicated that the tolerance may be slightly increased in F_8 generation, but this soon reverts to normal on suspension of pressure.

PHLEBOTOMINAE.

Phlebotomus argentipes.—The susceptibility of this species to the chlorinated insecticides has not been in record so far. All that we know is that the species was eliminated in an experimental area of Bengal by the use of 750-1,500 mg. dosage of DDT per sq. ft. (Ghosh, 1950) which is too large a dose for any nematoceran species. But a clear evidence of effective control of the species in rural areas by the residual spray of 50 mg. or 100 mg./sq. ft. is reflected in the reduction of kala-azar. (*Annual Report, Calcutta School of Tropical Medicine*, 1955-56, 1956-57).

The species in laboratory tests also proved to be highly susceptible to DDT and BHC. This observation was based on 4 to 6 replicates of six adults each which were bred in the insectary.

The different tests of DDT with 1 per cent, 0·5 per cent and 0·25 per cent dosages produced a complete or near complete kill (95 per cent to 100 per cent) in one hour's exposure, while BHC induced a complete wiping out of the species at 0·5 per cent, and about 90 per cent kill at the dosage of 0·025 per cent. All the dosages experimented with proved too strong for the delicate insect, and to keep the lone survivors in proper condition for 24 hours' observation in assessing mortality was still more difficult. With *argentipes*, the knock-down would probably give a more ready index of the dosage tests. The knock-down was found to be 70 per cent in 1 per cent DDT, 46 per cent in 0·5 per cent DDT, and 37 per cent only in 0·25 per cent DDT. The highest knock-down (70 per cent) observed in

1 per cent DDT was attained in the filter paper tests with BHC at 0·05 per cent and the lowest of 37 per cent in the tests with 0·25 per cent DDT was obtained at 0·025 per cent BHC.

LC_{50} for DDT in respect of *P. papatasii* recorded as 0·28 per cent in Greece (Brown, 1958) would, therefore, appear to be a much too strong a dose for *argentipes*.

Xenopsylla cheopis.—Since the appearance of some unconfirmed reports of the occurrence of resistance in the ratflea, *Xenopsylla cheopis*, to DDT in Ecuador (Vera, 1953), and in the human flea *Pulex irritans* in Greece, suspicion prevailed as to the extent of susceptibility of the species to DDT in India. Plague, and consequently *X. cheopis* fleas, however, have been successfully controlled in Bombay State, and in Dakar and parts of South America by the field application of DDT either as 10 per cent dust or as residual spray in the dosages used for malaria control.

Laboratory tests carried out in Calcutta with bred out *X. cheopis* also indicated that the species is susceptible not only to DDT but to many others, BHC, dieldrin and Malathion (Table XII). Six replicates, each of 8 fleas, were utilised for computing the result.

TABLE XII.

Susceptibility of X. cheopis *adults to different insecticides.*

Insecticide.	Dosage (Per cent).	Mortality (Per cent).
DDT ...	1·0	83
	0·5	60
BHC ...	0·1	83
	0·05	42
Dieldrin* ...	0·5	100
	0·3	96
Malathion ...	0·25	89
	0·1	34

*Result computed from 4 replicates.

The LC_{50} of the above insecticides, also reported earlier (Sen, 1958*a*), was found to be for DDT—0·4 per cent, BHC—0·06 per cent and Malathion—0·15 per cent indicating that *X. chcopis* is highly susceptible. Observations with dieldrin also show that the species is equally vulnerable to the dosages tested.

The susceptibility of the fleas *X. cheopis* to various insecticides used in dust form was also tested in the laboratory with bred out specimens. The tests were carried out in large sized tubes, 5 inch × 1 inch or in specimen jars, 8 inch × 4½ inch × 3½ inch, the bottom of which was dressed with a measured quantity of the insecticide to be tested on top of column of sand, rabbit faeces, saw-dust or bran.

The result computed from 3 to 6 replicates, each of 8 fleas, of both sexes, showed that at 1 per cent DDT, the kill was absolute, and with 0·5 per cent to 0·1 per cent DDT, 24 hours' mortality after 1 hour's exposure, varied from 77 per cent to 96 per cent, depending on the insecticide carrier introduced at the bottom of the test chambers.

The lowest kill was recorded in association with saw-dust, and largest in sand grains or rabbit faeces. The LC_{50} with DDT, when used as dust, worked out to 0·32 per cent which was not very much different from the LC_{50} of 0·4 per cent with DDT in solution as mentioned above.

Diazinon as 10 per cent dusting powder, when used in a substratum of sand, gave a mortality of 56·6 per cent ; and with 5 per cent dust, the mortality was less than 50 per cent. The unfed fleas, 48 hours old, when tested at the room temperature of 109° F., showed a mortality of 75 per cent even at the last mentioned dosage of 5 per cent Diazinon, indicating the importance of testing fed fleas only for comparable result.

Cimex hemipterus.—The bed-bug in Calcutta has been reported resistant to DDT (Sen, 1957*a*) in a preliminary note, and this occurrence would appear to be natural in the species *Cimex hemipterus*, since no DDT selection existed in the city. A series of observations have been carried out in the laboratory with not only DDT, but with other hydrocarbon and organophosphorus compounds as well, to assess the level of susceptibility to these insecticides (Table XIII). The specimens were collected from infested houses or animal room in and around the local Medical College compound. Ten replicates, each of 8 bed-bugs, were taken for DDT, Dieldrin and Diazinon, five for BHC, and three replicates only for Malathion.

TABLE XIII.

Tolerance of Cimex hemipterus towards different insecticides.

Insecticide.	Dosage (Per cent).	Mortality (Per cent).
DDT ...	2·0	26
	4·0	24
BHC ...	0·3	32
Dieldrin ...	0·3	40
	0·5	57
	1·0	86

A strong resistance of bed-bugs *hemipterus* to DDT at the top dosage which would kill an entire population of any susceptible insect, has been revealed from the above tests. The mortality even at 4 per cent dosage was 24 per cent and was not very different from that obtained with 2 per cent DDT. Resistance of the bed-bug *C. hemipterus* to DDT has also been reported from Bombay State (Rao and Halgeri, 1956) and from other parts of the Far East (Hong-Kong), South Pacific

(Formosa), and in *C. lectularius* in Israel (Quarterman and Schoof, 1958), but not BHC resistance. Dieldrin resistance has also been mentioned from Pala in *C. rotundatus*, but not DDT resistance (Hamon *et al.*, 1957).

The tests carried out not only confirmed that the Calcutta strain of the bed-bug *Cimex hemipterus* was resistant to DDT but it also exhibited low susceptibility to BHC, as compared to London strain of *C. tectularius* (Busvine, 1958), and also a good tolerance to Dieldrin, where the mortality was no higher than 30 per cent to 40 per cent at 0·3 per cent dosage. The LC_{50} of Dieldrin worked out to nearly 0·4 per cent.

The bed-bug *hemipterus* showed a fair tolerance of the organo-phosphorus insecticides in laboratory tests (Table XIV).

TABLE XIV.

Tolerance of Cimex hemipterus *to organo-phosphorus insecticides.*

Insecticide.	Dosage (Per cent).	Mortality (Per cent).
Malathion ...	2	100
	1	87
	0·5	83
	0·25	43
Diazinon ...	0·125	16
	0·25	20
	0·5	72

LC_{50} of Malathion and Diazinon worked out to 0·3 and 0·35 respectively.

DISCUSSION.

Resistance in insects of public health importance to chlorinated hydrocarbon insecticides as a result of long residual programme has been known from various countries, and their list is on the increase. The development of the phenomenon has been attributed in many such instances to the very high degree of selection pressure from the insecticides. The larval pressure has also been known in certain instances to possess a greater influence in selecting resistant strains. There is also a good volume of evidence to indicate that the resistance may be linked up with the genetics of a species, and the phenomenon in some insects has come to be regarded as natural since the potentiality to resist the ill-effects of the insecticides pre-existed in the individuals.

As indicated above, the present investigation brought out convincingly three clear instances of resistance in local insects, that of the housefly *vicina*, the mosquito *fatigans* and the bed-bug *hemipterus*, and all to DDT. The anophelines and the *Aedes* mosquitoes worked with proved susceptible to the insecticides tested, both chlorinated and organo-phosphorus insecticides ; so were the fleas *cheopis*. Even in the resistant insects mentioned above, one or the other chlorinated insecticide is still available for control. The laboratory strain of the sandfly *P. argentipes* is also highly susceptible, succumbing to the smallest dose of DDT or BHC.

Unless larval pressure of larvicidal oil (Malariol) which has been extensively used in Calcutta for a long period and the occasional use of DDT and Gammexane as antilarval spray is contributory to the development of DDT resistance in the Calcutta *fatigans*, no other apparent reason may be offered.

The existence of a susceptible strain of *fatigans* outside the zone of larvicidal activity makes us contemplative. In the absence of DDT adult pressure, one would be justified to suggest that the phenomenon in the three instances cited above is primarily a natural development.

ACKNOWLEDGEMENT.

The author thanks Sri T.C. Biswas and Sri D.B. Seal for the faithful assistance rendered by them in taking down the observations, and maintenance of records. Thanks are also conveyed to several workers in the State Health Services and at the School of Tropical Medicine, Calcutta, for the facilities received from them.

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Indian Journal of Malariology, **13**, 1, March, 1959.

FIRST YEAR'S RESULTS OF A PILOT STUDY OF MALARIA SURVEILLANCE MEASURES IN MYSORE STATE, INDIA.

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[June 13, 1958.]

INTRODUCTION.

MALARIA control operations in Mysore State, by the use of residual insecticides, were commenced during 1946 in certain hyperendemic areas of the State and were progressively extended to other areas. The activities in the State merged with the Indian National Malaria Control Programme (N.M.C.P.) implemented in April, 1953. The N.M.C.P. (Jaswant Singh, 1953) envisaged an attack phase of 3 years, later extended to 5 years. Thereafter the plan included a maintenance phase, during which, depending on certain criteria, either the spraying operations would be withdrawn or reduced in frequency or dosage.

The Expert Body*, appointed by the Government of India, visited in July, 1956 certain areas in Mysore as well as other States which had been under continuous D.D.T. operations for over five years, in order to determine if any of these were ready for the maintenance phase and also to formulate the maintenance procedures. The experts selected certain areas in the State as well as in the neighbouring States of Madras, Coorg and Bombay as being fit for the institution of maintenance measures.† Since the reorganisation of the States, however, all the areas chosen for experimental surveillance in Coorg, Madras, Bombay and Mysore States became part of the re-organised Mysore State. Vectorially these areas can be divided into two zones, *viz.*, one under the influence of *A. fluviatilis* and the other under *A. culicifacies*.

*The Expert Body consisted of (1) Dr. B. Ananthaswamy Rao, Director, Malaria Institute of India, Delhi, (2) Dr. D.K. Viswanathan, Regional Malaria Adviser, World Health Organization, South-East Asia Region, New Delhi, and (3) Dr. I.M. Puri, Retired Deputy Director, Malaria Institute of India.

†The experts considered that maintenance measures fell into two types, (1) where the spraying was withdrawn and (2) where the dosage or frequency of spraying is reduced. The criteria to determine the type of maintenance measures suited to an area were :

(a) Interruption of spray—areas where for two consecutive years, prior to the interruption, the child spleen index is less than 5 per cent, the child parasite index less than 1 per cent and infant parasite index nil.

(b) Reduction of dosage or frequency of spraying—areas where for two consecutive years, prior to changing dosage or frequency, the child spleen index is less than 10 per cent, child parasite index less than 2 per cent and infant parasite index nil or, if positive, such positive findings are restricted to only a few localities within the unit area and in any case does not exceed one per cent.

The objective of the study in the above mentioned areas, however, has altered since the time of its commencement. As mentioned previously, the original objective was to formulate procedures for a maintenance phase at the end of a Malaria Control Programme. Since the Government of India accepted and sanctioned a malaria eradication programme for the country, in December, 1957, the current objective of the studies that are being continued is to determine and test the working procedures of the surveillance formulated for the last year of the three years' attack phase and the subsequent three years of surveillance phase of the six year eradication programme commencing from the fiscal year 1958.

The pilot surveillance studies in Mysore State lend themselves to appraisal of intrinsic differences in the organisation, administration and procedures suited to the existing different patterns of public health services. Therefore, it is considered useful to deal with the distinct aspects of the studies separately in different sections bringing out the differences.

A main organisational difference between the areas of study in the erstwhile Mysore State and the integrated districts of Coorg, Bombay and Madras States is that in the former the study areas are covered with a net work of primary and secondary health units, while in the latter there is no such health organisation. Fundamentally, the ease with which the surveillance measures could be organised is different in the two types of areas. Statement 1 summarises the variations in organisation, pattern and procedure of surveillance measures and Statement 2 gives the population under surveillance in each area in the erstwhile Mysore State.

The *fluviatilis* areas in the erstwhile Mysore State selected for maintenance work are of two types in accordance with the criteria already mentioned ; the first, consisting of Shikaripur, Sorab, Sagar, Hosanagar, Tirthahalli Taluks* in Shimoga District†; Saklespur, and Koppa and Sringeri Taluks of Hassan and Chikmagalur Districts repectively, was selected for total withdrawal of spraying (Map 1). The pilot study and its results during the first year are described in Section 2. The second area consisting of Mudigere Taluk of Chikmagalur District and Belur Taluk of Hassan District was found to be ready for reduced frequency of spraying. The pilot study and its results during the first year are described in Section 3.

The *A. culicifacies* area selected for maintenance phase also was found ready only for reduced frequency of spraying. The study and its results in the first year are described in Section 4.

The pilot study organisation for the *fluviatilis* area of Bombay State, North Kanara District, now included in Mysore State since Re-organisation of States in November 1956, has already been described by Patel, Rao and Paranjpay (1957). One year's observations during the period November, 1956 to 1957 in that area are reported in Section 5.

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*Taluk is a revenue Sub-Division of a District.

†District is a revenue Division of the State.

STATEMENT 1.

Variations in organisation, pattern and procedure of surveillance measures.

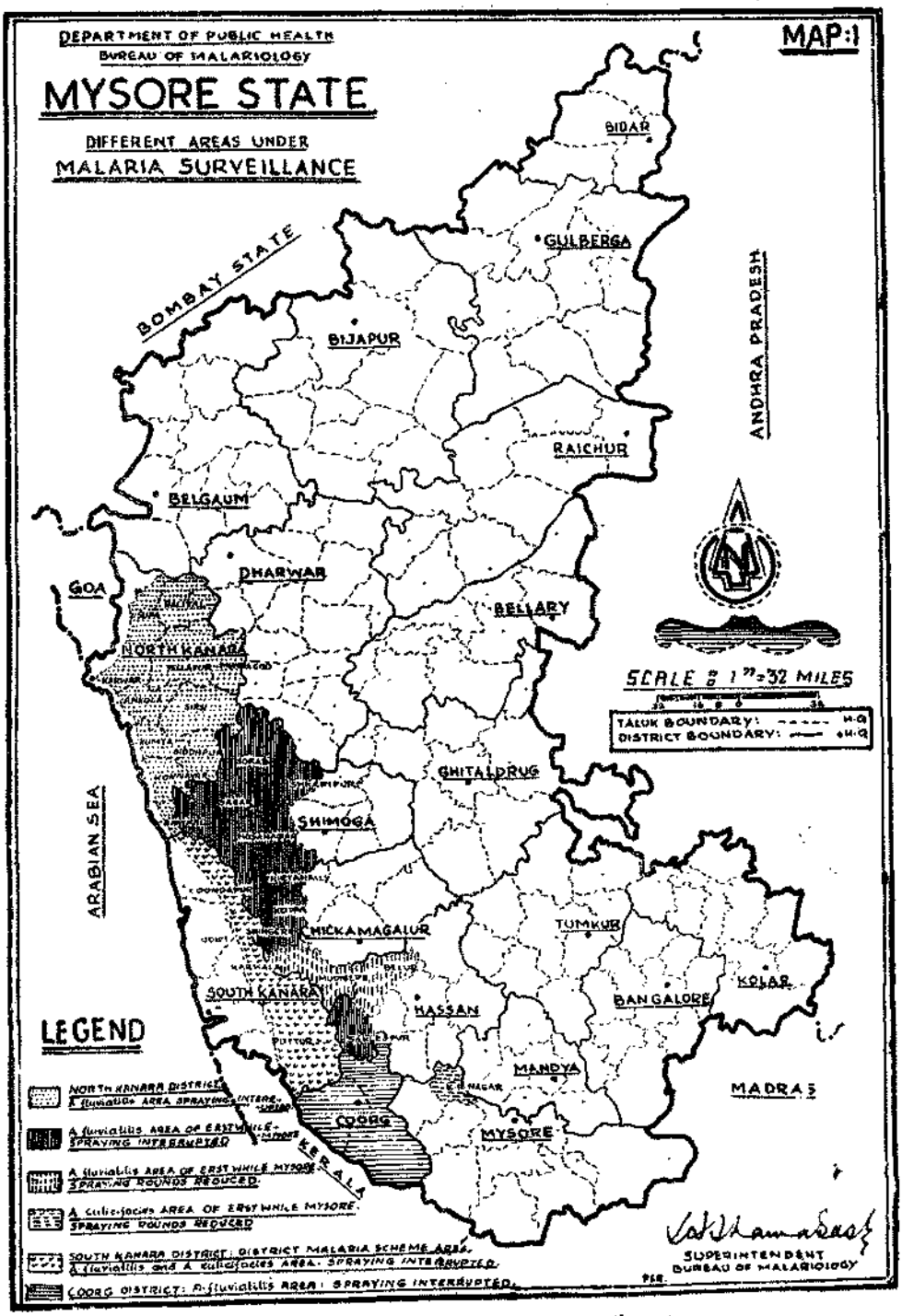
Areas.	Surveillance carried out		Houses visited		Transport		Municipal population		General public health activities and surveillance		Clinical malaria and fever cases		Parasite positive cases investigated by malaria officer and treated by		Remarks.
	Throughout the year.	Only during transmission season.	Once a fortnight.	Once a month.	Provided.	Not provided.	Included.	Not included.	Integrated.	Not integrated.	Differentiated.	Not differentiated.	Medical Officer.	Health surveillance workers.	
1. Sorab, Sagar, Hosanagar Tirthahalli, Saklespur, Koppa, Sringeri (*A. fluviatilis area*)	Yes		Yes			No	Yes		Yes		Yes		Yes		
2. Mudigere and Belur (*A. fluviatilis* area; one round of spraying)	Yes		Yes			No	Yes		Yes		Yes		Yes		
3. K. R. Nagar (*A. culicifacies area*; one round of spraying.)	Yes			Yes		No	Yes		Yes		Yes		Yes		
4. North Kanara (Garden tract)		Yes Dec.-May.	Yes		Yes*			No	Yes†			No		Yes	
5. North Kanara (Rice tract)		Yes June-Nov.	Yes		Yes*			No	Yes†			No		Yes	
6. Coorg	Yes					No		No‡	Yes		Yes			Yes	
7. Puthur (S. Kanara)	Yes					No	Yes§			No	Yes			Yes	

* Boats also provided.
† General public health staff, malaria staff and medical staff of adjacent districts also included.
‡ Compact population of 30,000 around dispensaries and hospitals not included.
§ Panchayat population included.

STATEMENT 2.

Population of the Taluks and the number of health units with sanctioned staff in the study areas of erstwhile Mysore State.

Serial number.	Name of Taluk.	Population (1951 Census)	NUMBER OF HEALTH UNITS : Secondary Centre.	Primary Health Unit.	Modified Health Unit.	Gap Areas.	STAFF SANCTIONED : Sr. Health Inspector, Secondary Centre.	Reserve Junior Health Inspector.	Jr. Health Inspector, Primary Health Unit.	Malaria surveillance Workers.	Senior Malaria Inspector.	Junior Malaria Inspector.	Remarks.
			PART I. *A. fluviatilis* Area—Spraying Interrupted.										
1	Shikaripura ...	64,320	1	4	2	–	1	1	10	3	1	1	Senior and Junior Malaria Inspectors are available for surveillance work only during non-spraying season.
2	Sorab ...	65,388	1	9	1	–	1	1	18	–	–	–	
3	Sagar ...	71,580	1	10	1	1	1	1	21	4	1	1	
4	Hosanagar ...	37,514	1	8	–	1	1	1	16	2	–	–	
5	Thirthahalli ...	60,083	1	9	3	–	1	1	21	3	–	–	
6	Koppa and Sringeri	51,688	1	7	2	–	1	1	16	3	–	–	
7	Saklaspur ...	53,398	1	8	–	1	1	1	16	3	–	–	
	Totals	4,03,971	7	55	8	3	7	7	118	18	2	2	
			PART II. *A. fluviatilis* Area—Spraying Rounds Reduced.										
1	Belur ...	70,487	1	7	2	1	1	1	16	2	1	1	Senior and Junior Malaria Inspectors are available for surveillance work only during non-spraying season.
2	Mudigere ...	49,026	1	9	1	1	1	1	19	3	–	–	
	Total	1,19,513	2	16	3	2	2	2	35	5	1	1	
			PART III. *A. culicifacies* Area—Spraying Rounds Reduced.										
1	K.R. Nagar ...	1,07,895	1	7	–	–	–	1	7 (SHIs.)	2	1	1	Senior and Junior Malaria Inspectors are available for surveillance work only during non-spraying season.


DEPARTMENT OF PUBLIC HEALTH
BUREAU OF MALARIOLOGY
MAP:1
MYSORE STATE
DIFFERENT AREAS UNDER
MALARIA SURVEILLANCE
BIDAR
GULBERGA
BOMBAY STATE
BIJAPUR
ANDHRA PRADESH
RAICHUR
BELGAUM
GOA
DHARWAR
BELLARY
NORTH KANARA
SCALE 8 1"=32 MILES
TALUK BOUNDARY: H.Q
DISTRICT BOUNDARY: H.Q
CHITALDRUG
SHIMOGA
ARABIAN SEA
TUMKUR
CHICKAMAGALUR
KOLAR
SOUTH KANARA
BANGALORE
HASSAN
LEGEND
MANDYA
MADRAS
COORG
MYSORE
KERALA
SUPERINTENDENT
BUREAU OF MALARIOLOGY

In Section 6 and Section 7, malaria surveillance work organised in Coorg District and the District Malaria Scheme area, Puttur, are briefly stated. The discussion of the results achieved is set out in the final Section 8. The duties of the malaria surveillance workers are shown in Appendix I.

2. A. *FLUVIATILIS* AREAS OF ERSTWHILE MYSORE STATE WHERE SPRAYING IS TOTALLY INTERRUPTED.

The study areas, part of Malnad* tracts of the State, were hyperendemic for malaria (Usman, 1928; Sweet, 1933) [*Annual Report Public Health Department* (1947), p. 62].

Though all the 26 species of anophelines recorded in the State are found in the study area, only *A. fluviatilis* has been incriminated as vector (Nursing *et al.*, 1934).

No part of the year was climatologically unfavourable for malaria transmission, but the actual seasons of transmission were determined mainly by the facilities existing for the continued breeding of the vector species which in turn were determined by the topography and rainfall. Though the chief breeding place of *A. fluviatilis* was streams, a small percentage were found in tanks, channels and river bed pools. The transmission season, subject to local conditions, ranged from October to June, (Bhombore *et. al.*, 1954-55).

HISTORY OF MALARIA CONTROL.

Malaria control by indoor residual spraying with D.D.T. was commenced in this area during December, 1948. A 25 per cent DDT-Aromex emulsion prepared locally, or a ready-made emulsion and 50 per cent Geigy's wettable powder were used depending on availability of material. Spraying rounds repeated once in 8 weeks during transmission period, with an application of 56 mg. per square foot, were changed to 100 mg. per square foot applied at intervals of 4 months during 1951. From 1953 onwards, under the National Malaria Control Programme spraying at 100 mg. per square foot was carried out twice a year by using 75 per cent DDT wettable powder during the transmission season.

RESULTS OF CONTROL.

The highly satisfactory results of the malaria control measures, as seen from the spleen rate, infant parasite rate, child parasite rate and the percentage of malaria cases treated in the dispensaries and also the significant change in the demographic indices, *viz.*, the birth rate, death rate, infant mortality rate and maternal mortality rate are shown in Statement 3.

The effect on vector densities has also been spectacular. *A.fluviatilis* has practically disappeared from human and mixed dwellings and only few specimens were collected from cattle-sheds. The man-hour density during the control period had never exceeded the assumed critical density in the area of more than 4 per 10 man-hours.

*Malnad is a region of foot-hills, forests, and heavy rainfall.

INTEGRATION OF SURVEILLANCE WORK WITH HEALTH UNIT ACTIVITIES.

The area under study is covered with a net work of Primary and Secondary Health Units. Map 2 shows the extent of area under surveillance and the area covered by Health Units. Each Primary Health Unit has a population varying from 3,000 to 10,000 divided into two convenient divisions and is provided with two junior Health Inspectors each of whom is in charge of a division. The area and population of the divisions are not uniform as the divisions have been made on the basis of terrain and density of population. The Health Inspectors working in these divisions and whose duties require them to visit all the villages in their respective divisions once during the month for collection and verification of vital statistics and attending to other health works, were with advantage entrusted with malaria surveillance work. In addition, surveillance workers were also appointed to cover the population not coming under Health Unit jurisdiction. The expenditure for appointing surveillance workers was met out of funds sanctioned for appointing temporary field staff under N.M.C.P., and now saved in areas of interruption or reduced rounds of spraying.

RESULTS.

The surveillance work was started during October, 1956, in the Health Unit areas by entrusting the work to the Health Unit staff. However, from May, 1957 onwards, surveillance work in the non-Health Unit areas was also started by entrusting the work to malaria surveillance workers, specially appointed.

The spraying has been interrupted in these areas since October, 1956, except for the Sharavarthy valley project area in Sagar Taluk and the Ambligola project area in Shikaripur Taluk in Shimoga District and the villages within a radius of five miles from these project areas where spraying is continued in view of the aggregation of labour drawn from several areas of varying endemicity of malaria outside Mysore State and probably not included under National Malaria Control Programme.

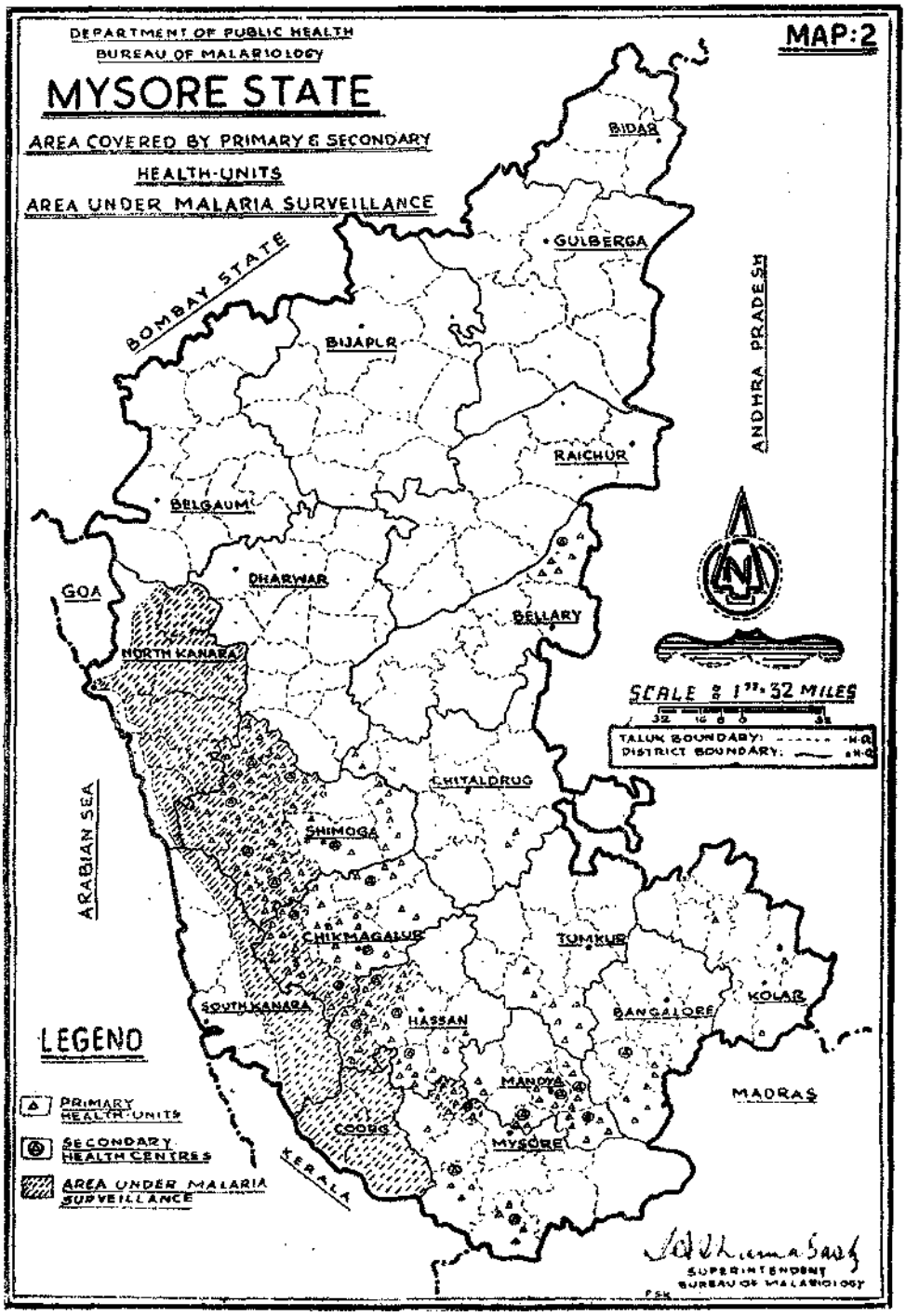
The Statement 2 (Map 2) shows the number of Health Units in each Taluk with the sanctioned staff and the population to be surveillanced. Statement 4 furnishes the details of malaria surveillance work from October, 1956, to September, 1957. From the Statement, it can be observed that the population targetted for surveillance in 8 taluks was 4,03,971. But the population actually covered during each month ranges from 2·9 lakhs to 3·9 lakhs. This fluctuation in the population surveillanced is due to (*i*) vacancies among the sanctioned posts, not being filled up (*ii*) there being not enough substitutes to be posted when permanent staff proceeded on leave or were deputed for other urgent health works, and (*iii*) frequent resignations from candidates appointed as surveillance workers in view of the arduous nature of work. Out of the sanctioned posts of 118 Junior Health Inspectors and 18 Surveillance Workers, the number working in each month varied from 87-108 and 8-14 respectively.

STATEMENT 3.

Malariometric indices and vital statistics of Taluks under experimental surveillance in erstwhile Mysore State showin the results of malaria control.

Year.	Taluk.	Spleen index (in April)	Child parasite index.	Infant parasite index.	Percentage of malaria to total attendance in dispensaries.	Birth rate.	Death rate.	Infant mortality rate.	Maternal mortality rate.	Remarks.
1940	Shikaripura	50·6	N.D.	N.D.	29·6	34·6	11·1	114·2	27·9	
1956		0·7	0·0	0·0	0·3	47·3	12·3	66·5	4·3	
1946	Sorab	34·5	N.D.	13·1	34·2	27·6	29·1	134·6	31·1	
1956		0·22	0·0	0·0	0·3	45·2	12·1	74·3	5·0	
1946	Sagar	68·9	N.D.	22·7	16·8	29·1	13·01	93·8	21·7	
1956		0·28	0·0	0·0	0·3	48·7	11·2	78·7	7·7	
1947	Hosanagar	60·9*	N.D.	7·5	25·8	18·2	25·6	124·0	15·5	October spleen survey.
1956		0·6	0·0	0·0	0·5	45·2	8·6	56·1	7·5	
1947	Tirthahalli	36·3	N.D.	N.D.	42·4	24·0	24·8	132·3	48·2	
1956		0·7	0·0	0·0	2·4	43·5	9·7	46·1	4·2	
1946	Koppa	62·6	N.D.	N.D.	27·2	12·9	10·6	71·1	77·1	
1956		0·9	0·0	0·0	0·4	36·6	8·3	62·1	5·8	
1946	Sringeri	78·2*	N.D.	N.D.	31·3	15·9**	2·5**	36·7	48·8	*1944
1956		0·4	N.D.	0·0	1·9	47·2	3·2	35·5	2·4	**1947
1946	Saklespur	82·47	N.D.	3·7*	20·3	22·5	21·7	158·1	40·9	*1948
1956		0·4	0·0	0·0	0·04	46·1	11·6	63·2	8·4	
1949	Mudigere	36·4	N.D.	N.D.	24·6	22·6	11·0	127·3	21·7	
1956		0·6	0·29	0·0	0·9	45·0	13·7	88·8	5·6	
1949	Belur	60·3	N.D.	N.D.	26·2*	10·4*	10·0*	60·4*	33·2*	*1946
1956		0·1	0·0	0·0	0·4	44·1	9·7	82·8	9·9	
1946	K.R.Nagar	60·5	N.D.	8·5 to 30*.	38·6	21·6	17·8	86·9	28·0	1946 to
1956		0·2	0·0	0·0	0·13	37·9	13·2	71·6	7·3	1948

*N.D. =No Data.


DEPARTMENT OF PUBLIC HEALTH
BUREAU OF MALARIOLOGY
MAP:2
MYSORE STATE
AREA COVERED BY PRIMARY & SECONDARY HEALTH-UNITS
AREA UNDER MALARIA SURVEILLANCE
BOMBAY STATE
ANDHRA PRADESH
BIDAR
GULBERGA
BIJAPUR
RAICHUR
BELGAUM
DHARWAR
GOA
BELLARY
NORTH KANARA
CHITALDRUG
SHIMOGA
ARABIAN SEA
CHIKMAGALUR
TUMKUR
KOLAR
SOUTH KANARA
HASSAN
BANGALORE
MANDYA
MADRAS
COORG
MYSORE
KERALA
SCALE 1" = 32 MILES
TALUK BOUNDARY:
DISTRICT BOUNDARY:
LEGEND
PRIMARY HEALTH-UNITS
SECONDARY HEALTH CENTRES
AREA UNDER MALARIA SURVEILLANCE
SUPERINTENDENT
BUREAU OF MALARIOLOGY

STATEMENT 5.

Details of the positive blood smears collected under malaria surveillance programme—(June 1956 to September 1957).

Serial number.	District.	Taluk.	Primary Health Unit.	Village.	Case: Age.	Case: Sex.	Date of taking the smear.	Result.	History and movement.	Treatment.	Remarks.
				Erstwhile Mysore State.							
1.	Hassan	Saklaspur	Hethur	Kodarasthe Bagedahalli.	40	Male	April 25, 1957	*P. vivax* rings.	Fever and rigor often. Frequent visits to Kateri village in Coorg District, 9 miles from Somwarpet. No spleen.	6 Paludrine 0·1 gm. on April 25, 1957. 4 Resochin on June 1, 1957.	Fever case.
							June 1, 1957	*Negative.*			
2.	Shimoga	Hosanagar	Nagar	Floating population working at Nagar bridge construction.	Not furnished	Female	May, 1957	*P. vivax.*	Whereabouts not known.	Not mentioned.	Clinical.
3.	Hassan	Saklaspur	Kadmane estate pocket area.	Heggade	19	Female	June, 1957	*P. vivax.*	Not furnished.	Not furnished.	Fever case.
4.	Hassan	Saklaspur	Hettur	Kodarasthe	28	Male	July, 1957	*P. falciparum* crescents.	Not known.	Treated with Primaquine.	Fever case.
				Smears taken during spleen survey of April, 1957.							
1.	Shimoga	Sagar	Talaguppa	Talaguppa	1½	Female	April, 1957	*P. vivax* gametocytes and presegmenting schizonts.	History not furnished. Born at Talaguppa and residing there. When 3 months old baby was taken to Honnali.	¼ tablet of Camoquine, every alternate day for a week.	
2.	Shimoga	Sorab	Anavatti	Thoravanda	12	Male	April, 1957	*P. falciparum* rings and crescents.	Reported to be indigenous.	Prior to taking the smear for the third time, two courses of Avlochlor tablets at an interval of a fortnight. Instructions issued to treat with 8 Amino-quinolines.	
							July 31, 1957	*P. falciparum* crescents only.			
							September 12, 1957	Negative.			

STATEMENT 5 (*Concld.*)

Serial number.	District.	Taluk.	Primary Health Unit.	Village.	Case: Age.	Case: Sex.	Date of taking the smear.	Result.	History and movement.	Treatment.	Remarks.
North Kanara District—Rice Tracts (June, 1956 to November, 1956.)											
Three blood smears were found positive for M. P. for the period from June, 1956 to November, 1956 of which 2 blood smears were reported as positive for *P. vivax* and another for *P. malariae* already reported by Patel, Rao and Paranjapey (1957). One case was treated with Primaquine and the other two could not be treated as they had left the village.											
North Kanara District—Garden Tracts (December, 1956 to May, 1957.)											
1.	North Kanara	Yellapur	North Kanara East	Baraballi	9	Female.	April 16, 1957	*P. vivax* light infection.	Had fever now and then for a period of six months prior to April 16, 1957.	Chloroquine on April 16, 1957. Primaquine on May 29, 1957.	Clinical or fever case not specified.
2.	North Kanara	Siddapur	North Kanara East	Kavenchur	4	Female.	May 10, 1957	*P. falciparum* rings.	Frequent visits to Tyarasi village of Siddapur Taluk. Two months prior to taking blood smears, she had fever for 4 to 5 days.	Chloroquine on April 16, 1957. Primaquine on May 29, 1957.	Clinical or fever case not specified.
3.	North Kanara	Bhatkal	North Kanara West	Hosapatna	25	Male.	April 23, 1957	*P. vivax* trophozoites and gametocytes.	Had rigor and fever 8 days prior to smear taking.	Avlochlor on April 23, 1957. Primaquine on May 29, 1957.	
North Kanara District—Rice Tract (June, 1957 to September, 1957.)											
1.	North Kanara	Haliyal	North Kanara West	Kumbarkop	25	Female.	October 16, 1957	*P. vivax*	Imported cases from Vellore, Madras State.	Primaquine from November 14, 1957.	
2.	North Kanara	Haliyal	North Kanara West	Kumbarkop	25	Male.	October 16, 1957	*P. vivax*		Primaquine from November 14, 1957.	
3.	North Kanara	Haliyal	North Kanara West	Kumbarkop	7	Female.	November 14, 1957	*P. vivax* and *falciparum*		Primaquine treatment February 6, 1958.	

Fever morbidity.—A total of 1,186 clinical malaria cases and 9,703 fever cases were investigated in a population of 2·9 to 3·9 lakhs during the period of one year. The average number of clinical malaria and fever cases per 1,000 during the first fortnight ranged from 0·52 to 2·04 and during the second fortnight from 0·42 to 1·97. For the month, the average number of clinical malaria and fever cases per 1,000 population ranged from 0·97 to 3·59. The number of clinical malaria cases investigated formed 24·2 per cent of the fever cases investigated during the same period. From June onwards, there was a slight rise in the incidence of fever cases. The influenza epidemic in the State, prevalent during June, might have also contributed to the rise.

Blood smear examination.—Blood smears were stained with J.S.B. Stain and a smear was declared negative after examining 100 fields of thick smear in 3'-5'. The smears are examined at the laboratories attached to the Bureau of Malariology, Bangalore ; Malaria Investigation Centre, Mandya ; Malaria Field Station, Saklaspur and District Health Offices and Secondary Centres.

815 blood smears from clinical malaria cases and 9,213 smears from fever cases were collected and examined during the year. Four smears were found to be positive, three for *vivax* and one for *falciparum*. Of the four positives, 3 were among fever cases and one among clinical malaria cases. Incidentally, during the half-yearly spleen and parasite survey conducted in April, 1957, among the blood smears collected and examined, an infant aged 12 months with a history of movement was found positive for *vivax* infection and another positive was in the case of a boy aged 12 years with *falciparum* infection. Particulars of investigations in respect of parasite-positive cases are detailed in Statement 5. The positives were among blood smears collected during April to July.

Drug distribution.—A total of 2,110 tablets to clinical malaria and 29,459 tablets to fever cases were distributed during the period of one year.

Entomological data.—Entomological data collected in selected villages round about Sagar, Saklaspur and Shikaripur where collection of all anophelines and vectors were made from October, 1954 to September, 1957, are presented in Tables I, II, III and IV.

TABLE I.

Collection of anophelines and vector species in areas selected for malaria surveillance.

(Man-hours search per station ranged from 1277.5 to 2775.25).

	INTERRUPTED AREA *A. fluviatilis*			INTERRUPTED AREA *A. fluviatilis*		
	Sagar.			Shikaripur.		
	1954-55	1955-56	1956-57	1954-55	1955-56	1956-57
All anophelines	697	844	13,988	1,479	1,423	5,505
A. culicifacies	0	0	91	2	4	24
A. fluviatilis	0	0	23	4	2	19
Per man-hour density of all anophelines	0·38	0·42	5·03	1·15	0·69	2·47

TABLE II.

Shelters from which the vectors have been collected and their density.

(Man-hours search per station ranged from 606·3 to 1871·3).

	Interrupted area—*A. fluviatilis*.											
	Sagar.						Shikaripur.					
	1954–55		1955–56		1956–57		1954–55		1955–56		1956–57	
	HD & MD	CS	HD & MD	CS	HD & MD	CS	HD & MD	CS	HD & MD	CS	HD & MD	CS
A. culicifacies	0	0	0	0	26	65	0	2	1	3	8	16
A. fluviatilis	0	0	0	0	6	17	0	4	2	0	13	6
Per man-hour density of *A. culicifacies*	0·0	0·0	0·0	0·0	0·13	0·071	0·0	0·004	0·0007	0·004	0·0055	0·02
Per man-hour density of *A. fluviatilis*	0·0	0·0	0·0	0·0	0·003	0·018	0·0	0·009	0·0014	0·0	0·008	0·008

HD = Human dwellings.

MD = Mixed dwellings.

CS = Cattle-sheds.

TABLE III.

Collection of anophelines and vectors in Saklaspur Area for the calendar years 1955, 1956 and 1957 (up to end of September 1957).

(Man-hours search per station ranged from 22.53 to 636).

	A. fluviatilis area (spraying interrupted).								
	1955			1956			1957		
	High rainfall area	Low rainfall area.	Intermediate rainfall area.	High rainfall area.	Low rainfall area.	Intermediate rainfall area.	High rainfall area.	Low rainfall area.	Intermediate rainfall area.
All anophelines	49	96	83	80	135	349	44	161	853
A. culicifacies	0	0	12*	0	0	6	1	7	226
A. fluviatilis	1	2	0	0	2	0	2	3	3
Per man-hour density of all anophelines	0·34	2·1	0·1	0·4	0·9	1·7	1·91	3·35	9·42

* 11 *A. culicifacies* were from unsprayed cattle-sheds.

In Sagar area, the per man-hour density of all anophelines is 5·03 during 1956-57 (Table I) and the *A. fluviatilis* density is 0·003 in HD and MD (Human Dwellings and Mixed Dwellings) and 0·018 in C.S. (Cattle-sheds) (Table II). Whereas during the two years prior to interruption the all anopheline density was 0·38 during 1954-55 and 0·42 during 1955-56 and the vector density was 0·0 (Table I) ; both *A. fluviatilis* and *A. culicifacies* have re-appeared since interruption, though in low densities. Both the species were encountered in all types of shelters.

TABLE IV.

Shelters from which the vectors have been collected and their density in Saklaspur Area for the calendar years 1955, 1956 and 1957 (up to end of September, 1957).

(Man-hours search per station ranged from 22.53 to 636).

	1955						1956						1957					
	High rainfall area.		Low rainfall area.		Intermediate rainfall area.		High rainfall area.		Low rainfall area.		Intermediate rainfall area.		High rainfall area.		Low rainfall area.		Intermediate rainfall area.	
	HD & MD	CS	HD & MD	CS	HD & MD	CS	HD & MD	CS	HD & MD	CS	HD & MD	CS	HD & MD	CS	HD & MD	CS	HD & MD	CS
A. culicifacies	0	0	0	0	0	12*	0	0	0	0	0	6	0	1	0	7	0	236
A. fluviatilis	0	1	0	2	0	0	0	0	0	2	0	0	0	2	0	3	0	3
Per man-hour density of *A. culicifacies*	0	0	0	0	0	0·019	0	0	0	0	0	0·03	0	0·043	0	0·145	0	2·91
Per man-hour density of *A. fluviatilis*	0	0·007	0	0·009	4	0	0	0	0	0·01	0	0	0	0·087	0	0·062	0	0·038

HD = Human dwellings.
MD = Mixed dwellings.
CS = Cattle-sheds.

* 11 *A. culicifacies* were from unsprayed cattle-sheds.

In Shikaripur area, the per man-hour density for all anophelines was 2·47 during 1956-57 (Table I). *A. culicifacies* density is 0·0055 and 0·02 in HD and MD, and C.S. respectively and 0·008 for *A. fluviatilis* both in HD and MD, and C.S. (Table II). During the two years prior to interruption the all anopheline density was 1·15 (1954-55) and 0·69 (1955-56) (Table I) and the vector density for *A. culicifacies* was 0·004 in C.S. only during 1955 and 0·0007 and 0·004 in HD and MD, and C.S. respectively during 1956 (Table II). For *A. fluviatilis*, the man-hour density during 1954-55 was 0·009 in C.S. only, and 0·0014 in HD and MD only during 1955-56 (Table II). In this area the very low densities of the vector species prevailing during the period prior to interruption, have increased since interruption. They are collected in all types of shelters.

In Saklaspur area, there was a rise in all anopheline density in all the three rainfall areas* from 1955 to 1957 but markedly so in the intermediate rainfall area from 0·1 and 1·7 in 1955 and 1956 respectively to 9·42 in 1957. In the high and low rainfall areas, *A. culicifacies* was absent all through 1955 and 1956 calendar years. It re-appeared during 1957 in both the rainfall areas and its density in the intermediate rainfall area rose from 0·019 during 1955 to 2·91 in C.S. only during 1957. *A. fluviatilis* though absent in intermediate rainfall area but present in other rainfall areas during 1955 and 1956, re-appeared in low densities in cattle-sheds in all the areas.

3. *A. FLUVIATILIS* AREA OF ERSTWHILE MYSORE STATE, WHERE THE FREQUENCY OF SPRAYING WAS REDUCED TO ONE ROUND A YEAR.

Mudigere Taluk of Chikmagalur District and Belur Taluk of Hassan District were selected for reduction in the number of spraying rounds. The Expert Committee recommended only one round of spraying with 112 mg. dose per square foot to be carried out in October-November in Mudigere Taluk and during May-June in Belur Taluk. These taluks are also covered by a net work of primary and Secondary Health Units (Map 2). The surveillance work was commenced in these taluks during October, 1956.

There are 16 Primary and 3 modified Health Units in this area with a sanctioned staff of 35 Junior Health Inspectors and 5 Malaria Surveillance Workers (Statement 2). The total population of the two taluks is 1,19,513 (1951 census) and the average population surveillanced per day by each worker was 300. The pattern of surveillance is similar to one described under Section 2 of this paper.

Work turned out so far.—Statement 6 furnishes the details of malaria surveillance work done from October, 1956 to September, 1957.

Fever morbidity.—A total of 24 clinical malaria and 1973 fever cases were encountered in a population of 0·98 lakhs to 1·2 lakhs. The average number of

*The high rainfall area, the intermediate rainfall area, and the low rainfall area are the areas with annual rainfall above 200 inches, between 80-100 inches, and 40-50 inches, respectively.

clinical malaria and fever cases per 1,000 population during the year works out to 0·018 and 1·52 respectively. The maximum number of fever cases were investigated during February, 1957. The proportion of clinical malaria to fever cases works out to 1·2 per cent of the fever cases investigated.

Blood smear examination.—24 blood smears from clinical malaria and 1,779 smears from fever cases were collected and examined and all were found negative for malaria parasites.

Drug distribution.—A total of 56 and 7,190 tablets were distributed to clinical malaria and fever cases respectively.

Entomological data.—Tables V and VI, show the collections of all anophelines and vectors from October, 1956 to September, 1957. There is a rise in the all anopheline density from 0·94 during 1954-1955 to 4·8 during 1956-1957. While

TABLE V.

Collection of anophelines and vectors in areas selected for malaria surveillance.

Man-hours search per station ranged from 326 to 1478·30.

Reduced round area—*A. fluviatilis.*

BELUR :

	1954-1955	1955-1956	1956-1957
All anophelines	873	1,436	7,154
A. culicifacies	4	0	61
A. fluviatilis	1	0	15
Per-man-hour density of all anophelines	0·94	1·4	4·8

TABLE VI.

Shelters from which the vectors have been collected and their density.

Man-hours search per station ranged from 275·5 to 1031·5.

Reduced round area—*A. fluviatilis.*

BELUR :

	1954-55		1955-56		1956-57	
	House-dwellings and mixed dwellings.	Cattle-sheds.	House-dwellings and mixed dwellings.	Cattle-sheds.	House-dwellings and mixed dwellings.	Cattle-sheds.
A. culicifacies	0	4	0	0	7	54
A. fluviatilis	0	1	0	0	1	14
Per-man-hour density of *A. culicifacies*	0·0	0·014	0·0	0·0	0·006	0·12
Per-man-hour density of *A. fluviatilis*	0·0	0·003	0·0	0·0	0·0009	0·031

A. fluviatilis was absent in house-dwellings and mixed dwellings during 1954-1955, it has re-appeared in very low densities during 1956-1957. The man-hour density of *A. fluviatilis* was 0·003 in cattle-sheds during 1954-1955 and it has increased to 0·031 during 1956-1957. *A. culicifacies* was absent in house-dwellings and mixed dwellings during 1954-1955 and the same has re-appeared in low densities in house-dwellings and mixed dwellings. Its man-hour density in cattle sheds during 1954-1955 was 0·014 and the same has increased to 0·12 during 1956-1957.

4. *A. CULICIFACIES* AREA OF ERSTWHILE MYSORE STATE.

Krishnarajnagar Taluk (Mysore District), with an area of 231 square miles and a population of 1,07,895 (1951), is a gently rolling country and is situated in that part of the State which receives an average of 25 to 40 inches of rain per annum. Practically the whole of the Taluk is under paddy cultivation through irrigation channels drawn from the Cauvery River. Paddy is grown between June 15 and the end of the year, so that during these months channels of all sizes run full continuously and the fields are under slowly flowing water. Between January and the middle of June, the fields lie fallow and the channels dry up.

The whole of the taluk, excepting few of the non-irrigated villages, was highly malarious. The spleen rate during the October, 1948 survey was 59·8 per cent. All the three species of plasmodia were prevalent. Of the 165 positive blood smears collected from October, 1948 to December, 1949, *P. vivax*, *P. falciparum* and *P. malariae* formed 89·8, 7·8 and 2·4 per cent respectively. The infant parasite rates (quarterly) varied from 8·5 per cent to 50 per cent (*Annual Report of the Public Health Department, Mysore State*, 1949, pp. 183).

A. culicifacies is the chief vector in the area, *A. fluviatilis* being a secondary vector. The distributaries and the numerous field channels, the margins of the tanks, and the valleys are the chief breeding places of *A. culicifacies* and *A. fluviatilis*. The transmission season extends from June to December.

RESULTS OF MALARIA CONTROL.

In a substantial part of the Taluk, the D.D.T. spraying programme has been under operation since 1948. The entire Taluk has been brought within the scope of the programme since 1953. In conformity with the National Malaria Control Programme, two rounds of spraying were carried out in this Taluk between May and November. The results of malaria control measures are shown in Statement 3.

The data available (Statement 3) indicated the reduction to one round of spraying. The Expert Body recommended one round of spraying with 112 mg. per square foot during May-June.

Surveillance work was started in the area during October, 1956 by availing of the services of the Health Unit Staff. There are seven Primary Health Units with a Secondary Centre and only one Senior Health Inspector is provided for each Primary Health Unit (Statement 2). The pattern of surveillance in this area

STATEMENT 7.

Details of surveillance work done during the period October, 1956 to September, 1957 in Krishnarajnagar Taluk, Mysore District.

A. culicifacies area where frequency of spraying is reduced to one round from June, 1956.

Population : 1,07,895. (1951 Census)

Serial Number.	Month and Year.	Population.	Number of Surveillance Workers.		Cases Investigated.		Blood smears collected.		Results of blood smears.		Anti-malaria pills issued		Number of cases investigated per 1,000 population.	
			Health Inspectors.	Surveillance Workers.	C.	F.	C.	F.	C.	F.	C.	F.	C.	F.
1.	October, 1956	1,08,000	5	...	...	36	...	36	...	Neg.	...	62	0·0	0·33
2.	November, 1956	1,08,000	6	...	...	30	...	30	...	Neg.	...	112	0·0	0·27
3.	December, 1956	1,08,000	5	...	...	40	...	40	...	Neg.	...	146	0·0	0·37
4.	January, 1957	1,08,000	7	...	...	21	...	21	...	Neg.	...	77	0·0	0·19
5.	February, 1957	70,971	7	...	...	22	...	22	...	Neg.	...	82	0·0	0·31
6.	March, 1957	52,577	6	...	...	27	...	27	...	Neg.	...	80	0·0	0·5
7.	April, 1957	85,616	5	...	...	38	...	38	...	Neg.	...	113	0·0	0·44
8.	May, 1957	71,690	5	1	...	45	...	45	...	Neg.	...	131	0·0	0·62
9.	June, 1957	1,09,692	5	1	...	58	...	58	...	Neg.	...	201	0·0	0·52
10.	July, 1957	1,09,692	5	2	...	58	...	58	...	Neg.	...	201	0·0	0·52
11.	August, 1957	1,06,581	6	1	...	70	...	70	...	Neg.	...	241	0·0	0·65
12.	September, 1957	1,08,118	7	1	...	98	...	98	...	Neg.	...	327	0·0	0·91
Grand Total :					...	543	...	543	...	...	...	1,773	0·0	0·47

C = Clinical malaria cases.
F = Fever cases.

was confined to one visit to all the villages during the month. No transport was provided. For a total population of 1,07,895, in addition to seven Health Inspectors, two Malaria Surveillance Workers were posted during April, 1957. To cover the assigned population in a period of 10 working days during each month, the average population per day comes to 1,200 per worker.

Statement 7 furnishes the details of work done from October 1956 to September, 1957.

Fever morbidity.—A total of 543 fever cases were investigated in a population of 0·5 to 1 lakh surveillanced during the period of one year. The average number of fever cases per 1,000 population varies from 0·19 to 0·91. During May to September there is a rise in fever cases investigated. This perhaps was partly due to the influenza epidemic in the State.

Blood smear examination.—543 blood smears were taken from fever cases and all were found negative for M.P.

Drug distribution.—A total of 1,773 tablets were distributed to clinical, malaria and fever cases.

Entomological data.—*A. culicifacies* was not found in the collections during two years prior to the reduction of rounds of spraying. It has made its appearance during 1956-1957 in low densities. (Tables VII and VIII).

TABLE VII.

Collection of anophelines and vectors in areas selected for malaria surveillance.

Man-hours search per station ranged from 775·5 to 2025·3.

Reduced round area—*A. culicifacies*			
	KRISHNARAJANAGAR :		
	1954-55	1955-56	1956-57
All anophelines	117	664	2,975
A. culicifacies	0	0	29
A. fluviatilis	0	0	0
Per-man hour density of all anophelines	0·15	0·53	1·46

5. MALARIA SURVEILLANCE WORK IN NORTH KANARA DISTRICT.

After completion of spraying for the tenth year in succession, D.D.T spraying was suspended in North Kanara District with effect from June, 1956. An organisation was simultaneously started to study the after-effects of interruption of spraying. From August 1956, a pilot surveillance programme was taken up in the villages of North Kanara District. It was planned to restrict surveillance to the transmission season, in the eastern rice tracts of the district till the end of November, 1956 and to carry out similar programme in garden tracts of the district

TABLE VIII.

Shelters from which the vectors have been collected and their density.

Man-hours search per station ranged from 205·25 to 1438.

Reduced round area—*A. culicifacies.*

KRISHNARAJNAGAR :

	1954-55		1955-56		1956-57	
	House dwellings and mixed dwellings.	Cattle-sheds.	House dwellings and mixed dwellings.	Cattle-sheds.	House dwellings and mixed dwellings	Cattle-sheds.
A. culicifacies	0	0	0	0	9	20
A. fluviatilis	0	0	0	0	0	0
Per-man-hour density of *A. culicifacies*	0·0	0·0	0·0	0·0	0·006	0·034
Per-man-hour density of *A. fluviatilis*	0·0	0·0	0·0	0·0	0·0	0·0

from December to June next. The results of the work in rice tract up to October 31, 1956 have already been described by Patel, Rao and Paranjpey (1957).

Malaria surveillance in garden tracts.—The garden tracts comprise a population of 2,76,017 in Sirsi, Yellapur, Supa and Siddapur Taluks of North Kanara East Division and Karwar, Ankola, Kumta, Honnawar and Bhatkal Peta of Kanara West Division. The malaria surveillance work was organised in the garden tracts from December, 1956 with the available staff. However the entire population in the garden tract, excluding that under spraying and that of certain towns *viz.*, Ankola, Kumta, Gokarna, Honnavar and Bhatkal, was surveillanced commencing from February 1957 when the malaria staff from adjacent districts were also posted. Boats were also hired for the purpose of malaria surveillance in riverine villages in Honnavar and Bhatkal Taluks. The number of villages and total population allotted to each team are given below.—

Team.	Number of persons in each team.	Taluk.	Number of Villages allotted.	Total population allotted.
1	3	Sirsi	144	29,693
2	4	Yellapur	125	19,112
3	1	Supa	59	6,288
4	4	Siddapur	198	41,777
Total for Kanara East Division	12		526	96,870
5	3	Karwar	32 *plus* 1 town	49,658
6	3	Ankola	58 villages	30,271
7	3	Kumka	71 ,,	31,038
8	3	Honnavar	53 ,,	43,074
9	3	Bhatkalpeta	58 ,,	25,106
Total for Kanara West Division	15		272 *plus* 1 town	1,79,147

In addition four insect collectors were posted for routine fortnightly mosquito collection work, each working in 10 villages.

During the period, D.D.T. spraying with 112 mg. dose per square foot was carried out once in the hitherto unsprayed hypoendemic villages in the coastal belt and twice in the villages situated in 10 mile belt adjacent to Goa border, in Supa and Karwar Taluks and in Dandeli mining area covering a total population of 1,13,711 so as to create a "CORDON SANITAIRE".

Results.—Statement 8 furnishes the details of malaria surveillance work from December, 1956 to May, 1957.

Fever morbidity cases.—A total of 2,103 fever cases were investigated in 3,33,430 house visits. The number of fever cases per 200 house visits (at the rate of 5 persons per house for 1,000 population) was 1·26.

Blood Smear examination.—Out of the total number of 2,090 blood smears taken and examined, three were positive, two for *vivax* and one for *falciparum*. Statement 5 furnishes details of investigation conducted in respect of positive cases. All the three were given 8-aminoquinolines.

Entomological data.—Entomological collections were arranged in selected villages in Sirsi and Siddapur area of Kanara East and in Karwar Kumta, and Honnavar Taluks of Kanara West Division. The entomological data for the calendar years 1954, 1955 and 1956 and up to end of November 1957 is detailed in Tables IX and X. Eight *A. fluviatilis* were collected from cattle-sheds yielding a man-hour density of 0·016. This is far below the assumed critical density for this area.

MALARIA SURVEILLANCE WORK IN THE RICE TRACT.

The malaria surveillance work in the rice-tract was resumed for the second time from June, 1957. The surveillance organisation consisted of 4 Malaria Supervisors, 2 Sanitary Inspectors and 2 Sanitary Sub-Inspectors. The Malaria Supervisors and Sanitary Inspectors worked as investigators throughout the week but the Sanitary Sub-Inspectors worked only for three days in a week and attended to their routine vaccination work on other days. Among the insect collectors, two were on mosquito collection duty in selected villages on all days of the week. The whole staff was required to cover a total population of 81,890 in rice-tract and the work was concluded in November, 1957. The number of villages and population allotted to each team is as follows :—

Team.	Number of persons in each team.	Taluk.	Number of villages allotted.	Total population allotted.
1	2	Sirsi	99	29,011
2	2	Mundagod	68	15,871
3	3	Haliyal	79	36,002
4	1 (part-time)	Yellapur	2	1,006
Total	8		248	81,890

STATEMENT 8.

Details of malaria surveillance work done in garden tracts of North-Kanara District for the period December, 1956 to May, 1957.

A. fluviatilis area : Spraying interrupted from June, 1956.

Total population under surveillance in garden tract : 2,76,017.

Serial number.	Month and year.	Number of village visits.	Number of house visits.	Number of fever cases investigated.	Number of fever cases treated.	Number of blood smears taken.	Results of blood smears.	Remarks.
1	December, 1956	38	2,644	46	34	51	Neg.	...
2	January, 1957	456	32,326	292	227	292	Neg.	...
3	February, 1957*	821	53,158	505	450	492	Neg.	...
4	March, 1957	993	70,977	515	487	504	Neg.	...
5	April, 1957	1,330	95,221	395	392	398	2 pos.	1 *P. vivax* rings, 1 *P. vivax* trophozoites.
6	May, 1957	1,139	79,104	350	329	353	1 pos.	*P. falciparum* rings.
	Grand total	4,777	3,33,430	2,103	1,928	2,090	3 pos.	

*From February, 1957, nine teams comprising a total of 27 workers were posted to cover the entire population.

STATEMENT 9.

Details of malaria surveillance work done in rice tracts of North Kanara District for the period June, 1957 to November, 1957.

A. fluviatilis area : Spraying interrupted from June, 1956.

Total population under surveillance in rice-tracts : 81,890. Total number of villages : 248.

Serial number.	Month and Year.	Number of village visits.	Number of house visits.	Number of fever cases investigated.	Average number of fever cases per 200 houses	Number of fever cases treated.	Number of blood smears taken.	Results of blood smears.	Remarks.
1	June, 1957	266	20,142	282	2·8	279	282	Neg.	...
2	July, 1957	324	24,373	401	3·2	345	401	Neg.	...
3	August, 1957	210	17,410	194	2·26	194	194	Neg.	...
4	September, 1957	170	14,405	220	3·14	220	220	Neg.	...
5	October 1957	195	15,275	165	2·16	165	165	163 Neg./2 pos.	(*P. vivax*)
6	November. 1957	299	17,050	265	3·10	256	265	264 Neg./1 pos.	(*P. vivax* and *P. falciparum*)
	Grand Total	1,464	1,08,655	1,527	2·9	1,459	1,527	1,524 Neg./3 pos.	

TABLE IX.

Collection of anophelines and vectors in North Kanara District for the period January, 1954 to end of November, 1957.

	North Kanara East :		Rice-tract :		North Kanara West :		Garden tract :	Remarks.
	January to December 1954	January to December 1955.	June to November 1956.	June to November 1957*.	January to December 1955.	January to December 1956.	December 1956 to May 1957.	
All anophelines	1,142	654	8,283	4,556	562	53	3,089	
A. culicifacies	16	0	0	4	99	7	38	
A. fluviatilis	0	0	1	4	0	0	8	
Man-hours-spent	1,044	657	Data not furnished.	1,306	670	272	1,896	
Per-man-hour density	1.008	0·96	...	3·48	0·83	0·15	1·62	

* Data in respect of rice tracts from January 1956 to May 1956 and January 1957 to May 1957, is not available.

TABLE X.

Shelters from which the vectors have been collected and their density in North Kanara District.

	North Kanara East :				Rice tract :				North Kanara West :				Garden tract :		Remarks.
	January to December 1954.		January to December 1955.		June to November 1956.		June to November 1957*.		January to December 1955.		January to December 1956.		January to May 1957.		
	HD and MD.	C.S.	HD and MD.	C.S.	HD and MD.	C.S.	HD and MD.	C.S.	HD and MD.	C.S.	HD and MD.	C.S.	HD and MD.	C.S.	
A. culicifacies	0	16	0	0	0	0	1	3	0	99	7	0	0	38	
A. fluviatilis	0	0	0	0	0	1	0	4	0	0	0	0	0	8	
Man-hours spent	541	503	342	315	Data not furnished.		990	317	335	335	136	136	1,418	478	
Per-man-hour density of *A. culicifacies*	0	0·03	0	0	...	...	0·001	0·009	0	0·29	0	0·051	0	0·079	
Per-man-hour density of *A. fluviatilis*	0	0	0	0	...	...	0·00	0·01	0	0	0	0	0	0·016	

* Data in respect of rice-tracts from January 1956 to May 1956 and January 1957 to May 1957 is not available.
HD and MD = House dwellings and Mixed dwellings.
C.S. = Cattle-sheds.

Results.—Statement 9 furnishes the details of malaria surveillance work done from June, 1957 to November, 1957.

Fever morbidity.—A total of 1,527 fever cases were investigated in 1,08,655 house visits. The number of fever cases per 200 house visits (at the rate of 5 persons per house, for 1,000 population) was 2·8. The incidence of fever cases was maximum during July.

Blood smear examination.—Out of 1,527 blood smears collected and examined 3 were found positive, 2 for *vivax* and one for mixed infection. Particulars of investigation in respect of parasite positive cases are furnished in Statement 5.

Entomological data.—The entomological data for the calendar years 1954 and and 1955 in respect of Kanara East Division and for the years 1955 and 1956 in respect of Kanara West Division and for the surveillance period are presented in Tables IX and X. *A. fluviatilis* was absent in Kanara East Division in 1954 and 1955 and in Kanara West Division during 1955 and 1956. During the period June to November, 1956, one *A. fluviatilis* was collected from cattle-shed in rice-tract. Again, 4 *A. fluviatilis* have been collected in the rice-tract during the period June to November, 1957.

6. MALARIA SURVEILLANCE WORK IN COORG DISTRICT—*A. FLUVIATILIS* AREA—SPRAYING INTERRUPTED.

Coorg District comprising an area of 1,593 square miles and a population of 2,29,405 (1951 census) is a mountainous country, situated on the summits and the eastern and western slopes of the western hill ranges of Peninsular India. The southern portion of the district is covered by one stretch of forest. In the north, the country becomes open towards the east, while to the west and north the country rises to high peaks measuring from 3,800 feet to 5,729 feet above the sea level. The district gets both the south-west and north-east monsoons. The monsoon usually starts at the end of May. The western part of the district receives an annual rainfall ranging from 120 to 250 inches and the eastern portion, which is in the nature of a plateau, from 50 to 65 inches ; while in the middle tract running north to south the rainfall varies from 70 to 90 inches. Cultivation of rice is carried out in most of the district except in the eastern and north-eastern villages where dry farming is more common. The malaria transmission season extends from October to May and the vector is *A. fluviatilis*. The pre-control spleen rate was 58·2 per cent in 1947 and the anti-malaria work in the district, with residual D.D.T. spraying, was commenced in 1947 (Jaswant Singh and Kariappa, 1949). The spleen rates for the years 1953, 1954 and 1955 have been less than one per cent. Even in the absence of information regarding child parasite rate and infant parasite rate either before or after the commencement of the control and as the confirmed malaria cases were extremely few since 1951 and nil in 1956, the Expert Committee recommended interruption of spraying in the entire district.

Malaria surveillance work in Coorg District was started with effect from July 1, 1957 with the assistance of Health Inspectors and Health Sub-Inspectors. General

health work has been integrated with malaria work. There are eight sanctioned posts each of Health Inspectors and Health Sub-Inspectors. Each Health Inspector or Health Sub-Inspector is assigned a population of 10,000 on an average. He is required to visit the assigned population once during the month, in 20 work days. Case finding, record maintenance and reporting are in accordance with erstwhile Mysore pattern. A population of 30,000 concentrated around the dispensaries and hospitals is excluded from malaria surveillance work. Work turned out up to the end of September, 1957 is as follows :—

Month (1957)	Population surveillanced.	Number of surveillance workers.	Fever cases.	Blood smears.	Result.	Tablets issued.
July	96,104	11	40	40	Negative	142
August	94,209	13	127	116	Negative	371
September	1,21,958	14	142	137	Negative	429
			309	293		942

7. MALARIA SURVEILLANCE IN DISTRICT MALARIA SCHEME AREA, PUTTUR (SOUTH KANARA DISTRICT).

The District Malaria Scheme was started (Madras State) in 1948 to cover 44 villages in Puttur Taluk of South Kanara District. The number of villages under control increased year after year and the scheme now extends to the entire Malnad areas of the South Kanara District, comprising 191 villages having 2,80,175 population in five Taluks of Puttur, Belthangady, Karkal, Coondapur and Udipi. Except Udipi, all the other four Taluks do not touch the coastal areas. The scheme area which is 15 to 20 miles from the coastal margin, comprises the foot-hill regions covered with thick forests, deep valleys with steep ridges, with swiftly flowing streams. The terrain merges with the coastal plain as one proceeds west. The height varies from 300 feet rising to 2000 feet along the Ghat regions,* studed with peaks like Kodachadri, Kuduremukha and Amedikal, etc. The average annual rainfall in Puttur and Belthangady is about 150 to 180 inches respectively. The Ghat regions receive about 250 inches rainfall and over, the maximum precipitation being from June to September through south-west monsoon, the north-east monsoon being weak. The maximum and minimum temperature during summer and winter being 94°F-82°F and 88°F-74°F.

Malaria transmission season extends from December to June. *A. fluviatilis* and *A. culicifacies* are the vectors (Ramakrishnan *et al.*, 1948). Table XI shows the results of malaria control.

On the basis of the spleen rate, parasite rate and infant parasite rate, for the three years, 1953 to 1955, the Expert Committee recommended interruption of spraying and institution of malaria surveillance work.

*The Western Hill-Range.

TABLE XI.

Results of malaria control.

Year.	Spleen rate. (Per cent.)	Parasite rate. (Per cent.)	Birth rate per mille.	Death rate per mille.	Fever death rate per mille.	Malaria death rate per mille.
1949	39·0	3·1	34	21	9	...
1950	23·5	1·0	41	12	4	...
1951	9·5	0·5	41	15	4	0·5
1952	4·6	0·2	44	14	3	0·1
1953	3·6	0·2	46	14	4	0·1
1954	2·0	0·01	46	14	4	0·1
1955	1·2	0·01	43·5	12·5	4	0·0

Hospital statistics (Percentage of malaria cases to total cases treated).

Name of the Dispensary.	PERCENTAGE OF MALARIA CASES TO TOTAL CASES TREATED : YEAR						
	1949	1950	1951	1952	1953	1954	1955
L.F. Kadaba	46	25	23	20	25	22	20
R.D. Uppinangady	32	27	10	9	18	14	21
L.F. Belthangady	36	27	14	17	6	8	6
L.F. Sullia	22	18	14	18	25	31	8
Subramania	17	12	7	6	3	3	2·5

ORGANISATION OF MALARIA SURVEILANCE WORK.

The surveillance work was started during the month of September, 1957. The staff consisted of 6 Health Inspectors, 26 Field Assistants, 2 Assistant Entomologists and 2 Laboratory Assistants under the control of whole time Malaria Health Officer. All the Field Assistants, many of whom have undergone Sanitary Inspectors' training, were trained in technique of blood smear collection and investigation of malaria and fever cases. Each one of the Field Assistants was assigned a population of 10,000. He was instructed to visit each house in his area once a month. No transport was provided. Of the 26 Field Assistants, 23 were detailed for surveillance work and 3 were detailed for entomological work. The work of 4 Field Assistants was supervised by one Health Inspector ; the entomological work of Field Assistants was supervised by Assistant Entomologist by actual test collections in the villages. The blood smears were examined at the laboratory attached to the Health Office, Puttur. Test checking was done at the Central Laboratory, Bangalore.

It may be of interest to note that as D.D.T. spraying in Puttur area was carried out by field staff without any transport, the sanctioned staff came to be very handy for organising malaria surveillance work. During September, 97 clinical malaria cases and 679 fever cases were investigated. Of 679 blood smears collected and examined, all were found negative.

DISCUSSION.

From the previous Sections, it can be made out that the following patterns of malaria surveillance, in the selected areas of the Mysore State, are being tried.

I. *FLUVIATILIS* AREA OF ERSTWHILE MYSORE STATE. (SPRAYING COMPLETELY INTERRUPTED OR FREQUENCY OF ROUNDS REDUCED).

(*a*) Two visits to all the houses in the entire area during the month, at fortnightly intervals.
(*b*) Surveillance all through the year.
(*c*) No transport is provided to Health Inspectors or Malaria Surveillance workers.
(*d*) Municipal population is also included for surveillance.
(*e*) General public health activities and malaria surveillance work are integrated.
(*f*) Clinical malaria and fever cases are differentiated.
(*g*) Parasite positive cases are investigated by the Assistant Medical Officers of Health or the Medical Officers of Health and treated.

II. *CULICIFACIES* AREA OF ERSTWHILE MYSORE STATE. (FREQUENCY OF SPRAYING REDUCED TO ONE ROUND).

The pattern of surveillance is similar to the one explained above except that all houses in the area are visited once during the month at monthly intervals.

III. NORTH KANARA (GARDEN AND RICE TRACTS).

(*a*) Two visits to each of the houses in the area allotted to each team during the month at fortnightly intervals.
(*b*) Surveillance is confined only to the transmission season of six months from December to May, in garden tracts and June-November in rice-tracts.
(*c*) General public health staff and malaria staff of the district and malaria staff of the adjacent districts are also mobilised for surveillance work.
(*d*) Transport is provided to each team.
(*e*) General public health and malaria surveillance work are integrated.
(*f*) Clinical malaria and fever cases are not differentiated.
(*g*) Parasite positive cases are investigated by Medical Officer of Health and treated by non-medical personnel.
(*h*) Municipal and Panchayat Board population excluded from surveillance.

IV. COORG DISTRICT.

(*a*) One visit during the month to each house. Each Health Inspector or Sub-Inspector is allotted a population of about 10,000.
(*b*) No transport is provided to the surveillance workers.
(*c*) General public health work and malaria surveillance work are integrated.

(*d*) Surveillance is carried out all through the year.

(*e*) Clinical malaria and fever cases are differentiated.

(*f*) Compact population of 30,000 around dispensaries and hospitals is excluded from malaria surveillance work.

(*g*) Parasite positive cases are investigated by Assistant Public Health Officer and treated by Health Inspectors or Malaria Surveillance workers.

V. DISTRICT MALARIA SCHEME AREA—PUTTUR.

(*a*) Only one visit to each house in the area during the month.

(*b*) No transport is provided to the Field Assistants or Health Inspectors. Each field Assistant is allotted a population of 10,000.

(*c*) Surveillance is carried out all through the year.

(*d*) Panchayat Board population is also included.

(*e*) Public Health activities are not integrated with malaria surveillance work.

(*f*) Clinical malaria and fever cases are differentiated.

(*g*) Parasite positive cases are investigated by Health Officer and treated by Health Inspectors and Field Assistants.

From a review of the work turned out in the several areas, it can be made out that in *A. fluviatilis area* (both interrupted and the reduced round areas) of erstwhile Mysore State, comprising a population of 5,23,484 and served by a chain of Primary Health Units, each with a sanctioned staff of two Junior Health Inspectors and surveillance workers specially appointed for area not covered by health units, the population to be surveillanced by each worker is about 300 per day and the allotted population is visited each fortnight. The work turned out so far shows that the Health Inspectors and surveillance workers are able to cover the allotted population in each fortnight. The fluctuation of population surveillanced, as per Statements 4 and 6, in each month is for reasons already enumerated.

In the North Kanara District, one season of surveillance work carried out in garden tracts with a population of 2,76,017 and resumed surveillance work in rice tract during June to November 1957 with a population of 81,890 where the population to be surveillanced daily by each worker is 1,020, have shown that the surveillance workers are not able to cover the assigned population *vide* Statements 8 and 9, for the following reasons :

1. The assigned population of 1,020 per day per worker in such hilly tracts apparently is too much and therefore some assigned villages are omitted during daily itineration.

2. When the staff either proceed on leave (earned leave or holidays) or deputed for other health work, the non-availability of substitutes results in the assigned population not being surveillanced.

3. Limited utility of transport and frequent break-downs, due to bad communications, result in omission of certain population during daily surveillance.

4. Some of the villages could not be visited on account of floods.

5. The malaria staff, deputed from the adjacent districts, have to stay for six months outside their Headquarters and most of them like to avoid this arduous work.

In the *A. culicifacies* area of erstwhile Mysore State, comprising a population area, of 1,07,895, in plain country, the population to be surveillanced daily by each worker is 1,200, and it is seen that the surveillance workers have been able to cover the entire population, once during the month.

In the other two areas, *viz.*, Coorg District and the District Malaria Scheme area, Puttur, the pattern of surveillance has been organised on the experience gained in erstwhile Mysore and the North Kanara District. The work turned out will be presented after one season of surveillance.

It can be made out that, in *A. fluviatilis* area of erstwhile Mysore State (Statements 4 and 6), during each fortnight almost equal number of clinical malaria and fever cases are investigated, and the monthly average per 1000 population has gone up to 3·58.

Whereas in *A. culicifacies* area (Statement 7), where surveillance is restricted only once a month, the number of cases investigated is also less and the monthly average has gone up only to 0·91. From this it looks that by two visits to each house during the month, more cases are investigated.

Positive blood smears.—So far 13 positive blood smears have been collected from June, 1956 to November, 1957. Of these, 4 are from erstwhile Mysore State, 9 from North Kanara District (including one *P. malariae* already reported by Patel, Rao and Paranjpey (1957) and one *P. vivax* and one mixed infection collected during November, 1956 in rice-tracts). All these cases have been investigated and 8 cases were treated with 8 amino-quinolines. Three patients could not be traced and two others treated with only schizonticides. Though the number of positives that could not be traced is three only, this aspect has to be kept in view in large scale malaria surveillance work, when inter-state reporting of movements of positive cases may be of great help.

The positive findings in a child of four years (*vide* Serial number 2 under North Kanara District Garden Tract in Statement 5) of *P. falciparum* is interesting as the child is stated not to have moved out of Siddapur Taluk.

The positive smears hitherto collected were found during the months of April, May, June and July 1957 in erstwhile Mysore State ; April and May in the garden-tracts and October and November in rice-tracts.

The Expert Body estimated that 60,000 tablets of 4-amino-quinolines may be required for one N.M.C. Unit during a period of one year. During the period of one year under report, in erstwhile Mysore State, 40,588 tablets of 4-aminoquinolines (as against 37,883 tablets as per expert body estimate) were distributed in a population of 6,31,379.

Since the interruption of spraying, there is an increase in all anopheline density in all the areas. The vectors have not yet reached their presumed critical densities. In the light of this, it is too early to predict the future course of malaria in the area.

CONCLUSION.

1. Malaria surveillance work, carried out in selected areas of Mysore State from October, 1956 to September, 1957, is presented.

2. The organisation and distinct patterns of surveillance have been discussed and suggestions made.

3. The patterns of surveillance tried lend themselves for adoption in other areas during the surveillance phase of the Malaria Eradication Programme to come round during 1960-61.

4. It is too early to predict the future course of malaria in areas under malaria surveillance.

ACKNOWLEDGEMENTS.

Grateful thanks are due to the Director of Public Health in Mysore, Bangalore, for permitting to publish this paper and to Dr. B. Ananthaswami Rao, Director, Malaria Institute of India, Delhi, for many useful suggestions in writing this paper. The faithfull co-operation of the members of the General Public Health Service and of Malaria Organisation of Mysore State has rendered it possible to accumulate these data.

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APPENDIX I.

Duties of the Health Inspectors and Malaria Surveillance Workers :

The surveillance procedure and the duties assigned to the staff were :

1. House to house visit once a fortnight in their divisions and to list all active cases of fever during their house visits or those with a history of fever in between the visits and to take from each of those a thick and thin blood smear for microscopic diagnosis. All those cases with clinical signs and symptoms of malaria to be recorded as malaria cases and the rest as fever cases.

2. To administer a single dose of Chloroquine 0·6 gm. base or its equivalent to each adult person suffering from fever.

3. To treat all the parasite positive cases with Primaquine for five days administering 7·5 mg. base per adult twice a day for radical cure, and

4. To epidemiologically investigate the positive cases to determine the source of infection.

INDIAN COUNCIL OF MEDICAL RESEARCH

Applications are invited for the following posts in the Indian Council of Medical Research to reach the Director not later than 15th December, 1959.

(1) **Deputy Director (Administration).** Scale of pay Rs. 1,300-60-1,600 *plus* allowances as admissible under the rules. The applicants should be between 35 and 50 years of age.

Qualifications and experience.—A degree in medicine recognised under the Indian Medical Council Act, 1956 and a post-graduate qualification in medicine or Public Health. At least five years experience of administration as head of an Institution or Department. Post tenable initially for five years, period being extendable thereafter.

(2) **Deputy Director (Technical).** Scale of pay Rs. 1,300-60-1,600 *plus* allowances as admissible under the rules. The applicants should be between 35 and 50 years of age.

Qualifications and experience.—A degree in medicine recognised under the Indian Medical Council Act, 1956, and a post-graduate qualification in any branch of medical sciences. At least seven years experience in research in the medical and allied fields. Post tenable initially for five years, period being extendable thereafter.

(3) **Assistant Editor for the Indian Journal of Medical Research.** Scale of pay Rs. 900-50-1,200 *plus* allowances admissible under the rules. The applicants should be between 35 and 50 years of age, lower age limit being relaxable in otherwise suitable candidates.

Qualifications and experience.—A degree in medicine recognised under the Indian Medical Council Act, 1956 or a degree in allied sciences, such as Biochemistry, Physiology, Microbiology, Pharmacology, etc., with experience in research and medical journalism. Other things being equal, preference will be given to medical graduates. The selected candidate will be required to work at Kasauli for a few months. Appointment will be on five years' contract, renewable thereafter.

General.—Selected candidates for all the three posts will be on probation for one year. Benefits of Contributory Provident Fund admissible subject to Rules of the Fund. Private practice or compensation in lieu thereof not allowed. Candidates called for interview will be granted one return first class rail fare. Only concessional rail fare, if available, will be allowed. No travelling allowance admissible for joining appointment or on termination of appointment.

Applications on the prescribed form, obtainable from the Director, Indian Council of Medical Research, Medical Enclave, New Delhi, should be sent to him accompanied by a crossed postal order for Rs. 7-50 (Rs. 1-87 only in case of Scheduled Castes/Tribes and other backward classes candidates) made out in the name of the Indian Council of Medical Research. Applications without postal orders will not be considered.

INDIAN COUNCIL OF MEDICAL RESEARCH.

NOTICE

MEDICAL RESEARCH—NEW PROPOSALS FOR 1960-61.

The Indian Council of Medical Research invites applications for grants-in-aid for carrying out research during 1960-61 (from 1st April, 1960 to 31st March, 1961) on fundamental and applied aspects of any of the problems listed below in Institutions where adequate laboratory and other necessary facilities for conducting research are available :—

A. *Communicable Diseases*
   1. Tuberculosis
   2. Cholera
   3. Leprosy
   4. Infantile Diarrhœa
   5. Typhoid
   6. Filariasis
   7. Poliomyelitis
   8. Diptheria
   9. Smallpox

A. 1 *Virus Diseases*
   1. Respiratory Viruses
   2. Enteric Viruses

B.
   1. Malnutrition
   2. Venereal Diseases
   3. Helminthic Infections
   4. Amœbiasis
   5. Dental Health
   6. Cardio-vascular Diseases
   7. Metabolic Disorders

C. Environmental Sanitation

D. Industrial Health

E. Trials of Indigenous Drugs.

Applications in triplicate on the prescribed application form, with 120 copies of a note giving details of lines of work proposed to be undertaken on the enquiry, should be submitted through proper channel to the Director, Indian Council of Medical Research, New Delhi, so as to reach him not later than **20th November, 1959.** Application forms can be supplied on request by the Director, Indian Council of Medical Research.

N.B.—It may please be noted that schemes on the subjects mentioned against 'A' to 'E' in the list referred to above, received from research workers for financial support in 1959-60 and which were recommended by the Scientific Advisory Board of the Council for acceptance, would be reconsidered at the annual meetings of the Council in December, 1959, and that it is not necessary for those workers to submit those schemes over again for grant of funds in 1960-61.

Indian Journal of Malariology, **13**, 2/3, June/September 1959.

THE EFFECT OF SMALL DOSES OF PRIMAQUINE UPON MALARIA INFECTIONS.

BY

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[June 12, 1959.]

PLASMOCHIN, the first 8-aminoquinoline drug used against malaria, was shown to have a rapid sporontocidal effect in small doses by Barber, Komp, and Newman (1929). They demonstrated that a single dose of 5 mg. sterilized the gametocytes of *Plasmodium falciparum* within 24 hours. Because of its toxicity, however, plasmochin has not been widely used.

Recently, new members of this series of drugs have been discovered which are less toxic. One of these, primaquine, has the ability to eradicate the tissue phases of malaria when given at the rate of 15 mg. daily for 14 days. Recently small doses of this drug have been tried to determine the effects upon induced malaria infections, especially the sporogonous cycle. The results are given in this report.

MATERIALS AND METHODS.

Plasmodium vivax or *P. falciparum* was induced in neurosyphilitic patients. All of the patients were Negroes except Nos. 1314 and 1315 who were Caucasians. When the gametocytemias appeared, or, in some cases of *P. falciparum* were expected to appear, mosquitoes were allowed to feed upon the patients daily. After several days primaquine treatment was begun either weekly, twice weekly, or daily. The drug was given following the feeding of the mosquitoes.

The mosquitoes used were *Anopheles quadrimaculatus* (Q-1 strain) and *A. freeborni* (F-1 strain). After the infective feedings, they were maintained at 76°F.±2°F. and at a high relative humidity for 16 to 18 days. Usually 20 mosquitoes were dissected to determine the presence of infection. On the eighth to tenth day of incubation 10 mosquitoes were dissected to determine the density of oöcysts ; if the infection was light or absent, an additional 10 were dissected. After about two weeks' incubation, 10 more mosquitoes were dissected to determine the presence of sporozoites. The other details in handling the infected mosquitoes have been described elsewhere (Burgess and Young, 1944).

When the term "infected mosquitoes" is used in this report it means the presence of sporozoites or normal appearing oöcysts capable of producing sporozoites, unless otherwise noted.

*The author gratefully acknowledges the aid of Mr. Jimmie C. Skinner.

Daily determinations were made of the blood for parasite densities. The temperatures of the patients were recorded routinely every four hours, and hourly when elevated. Examinations, usually weekly, were done as follows : blood—hemoglobin levels and white cell counts ; urines—albumin, sugar, specific gravity, pH reaction, and microscopic.

RESULTS.

The results are summarized in Table I and some are shown graphically in Figures 1 and 2. Seven cases of *P. falciparum* and three cases of *P. vivax* were given primaquine in one or more regimens. Thirty mg. weekly (Patient Number 1310) and 15 mg. twice weekly (Patient Number 1311, second regimen) exerted full sporontocidal effects against *P. falciparum* infections within 48 hours after the first dose and continuously for as long as the drug was given. In the first trial against *P. falciparum* in Patient Number 1311, 15 mg. twice weekly did not have full sporontocidal effect until 48 hours after the second dose, or the sixth day following institution of therapy.

Against *P. vivax*, sporontocidal effects occurred within 24 hours after 30 mg. (Patient Number 1314) and after 15 mg. (Patient Number 1315). The latter dose given twice weekly prevented mosquito infection. Fifteen mg. given weekly had intermittent sporontocidal effect (Patient Number 1305). The 30 and 15 mg. doses had variable effects upon the asexual parasites and clinical symptoms ranging from quick clearance (Patient Number 1314) to little, if any, modification (Patient Number 1305).

A daily dose of 5 mg. was sporontocidal against *P. vivax* (Patient Number 1315) three days later and subsequently. The fevers disappeared after four days and the parasite density gradually lessened until they disappeared on the ninetieth day of this regimen. Three mg. daily had sporontocidal effects within 48 hours (Patient Number 1314) and cleared the parasitaemia on the fifth day.

In the *P. vivax* infections, the administration of primaquine once weekly, twice weekly, or daily, did not prevent the presence of gametocytes in the blood stream.

Two cases of *P. falciparum* received the drug either before mosquitoes were becoming infected or before gametocytes were present. Case Number 1319 started receiving 3 mg. primaquine daily on the sixth day of gametocytemia (Table I). Mosquitoes fed on five days preceding the drugging did not become infected nor did mosquitoes become infected during the drugging period of 58 days ; although the drug did not prevent the development of the waves of gametocytes, it may have reduced their number and perhaps prevented their becoming infective to mosquitoes.

The patient was followed for an additional 99 days after drugging was stopped. Of the 58 attempts to infect mosquitoes during the latter period, sporozoites occurred only once, on patency day 79, or 21 days after the last dose of 3 mg. of primaquine.

In Case Number 1320, 3 mg. of primaquine daily was begun on the first day of gametocytemia. Gametocytes increased to a maximum of 1862 per cmm. on the eighth day of medication, at which time mosquito feedings were begun. No mosquitoes were infected during the next 20 days. The drug was given for 146 consecutive days during which time waves of gametocytes, of low density, appeared.

Three mg. daily against *P. falciparum* (Patient Number 1321) was sporontocidal within 72 hours and subsequently. The gametocytes disappeared on patency day 35, the seventeenth day of drugging, but reappeared briefly on two occasions. In Patient Number 1316, the same daily dose was sporontocidal within five days and remained so for the 45 days it was tested. Several waves of gametocytes appeared during the drugging period. The density of asexual parasites fluctuated and clinical symptoms occasionally occurred.

The schizontocidal effect of the small doses of primaquine was inconsistent but in general not pronounced. In a few instances, for example Patient 1314, the parasitologic and symptomatic responses were rapid, but in other cases, namely Patients 1316 and 1305, there appeared to be little, if any, effect.

Regular blood and urine determinations on these patients did not reveal any toxic manifestations attributable to the drug.

TABLE I.

Summary of sporontocidal effects of primaquine.

Patient number.	Drug (mg.)		Percentage Mosquitoes Infected: Days after first drug dose.									Mosquitoes Negative Until Day.	Days blood Exam.
	Amt.	Frequency.	−1	0	1	2	3	4	5	6	7		
Plasmodium falciparum—Panama strain:													
1,310	30	wk.×4	80	100	80	0	0	0	0	0	0	27**	94
1,311	15	bi-wk.×8	100	100	60	83	89	100*	60	0	0	28**	56
1,311	15	bi-wk.×5	100	100	80	0	0	0	0	0	0	15	154
1,312	15	wk.×5	0	0	0	0	0	0	0	0	0	11**	38
1,316	3	daily×127	0	100	100	100	100	100	0	0	0	45†	170
1,319	3	daily×58	0	0	0	0	0	0	0	0	0	137 except day 79‡	157
1,320	3	daily×146	0	0	0	0	0	0	0	0	0	35**	211
1,321	3	daily×30	100	90	80	100	0	0	0	0	0	70§**	76
Plasmodium vivax													
1,314	30	once	..	100	0	0	P.D.	..	..	..	..	..	39
1,315	15	bi-wk.×6	100	100	0	0	0	0	0	0	0	Between drug day 18 and 26	29
1,315	5	daily×93	100	100	100	60	0	0	0	0	0	9**	189
1,314§§	3	daily×14	..	100	100	0	0	0	P.D.	..	..	..	93
1,305	15	wk.×3	100	80	0	0	0	0	60	80	80*	..	62

Note: 1,314 was St. Elizabeth strain; 1,315 and 1,305 were Chesson.

wk. = weekly; bi-wk. = bi-weekly.
* = subsequent dose of drug.
** = last day tested.
† = no trials days 21 through 33 or after day 45.
‡ = 16 mosquito feedings from day 0 to day 27 and 59 feedings from day 58 to day 137.
§ = feedings 3 times weekly days 41 through 70.
P.D. = Parasitemia disappeared.
§§ = infection from reinoculation, S.E. strain.

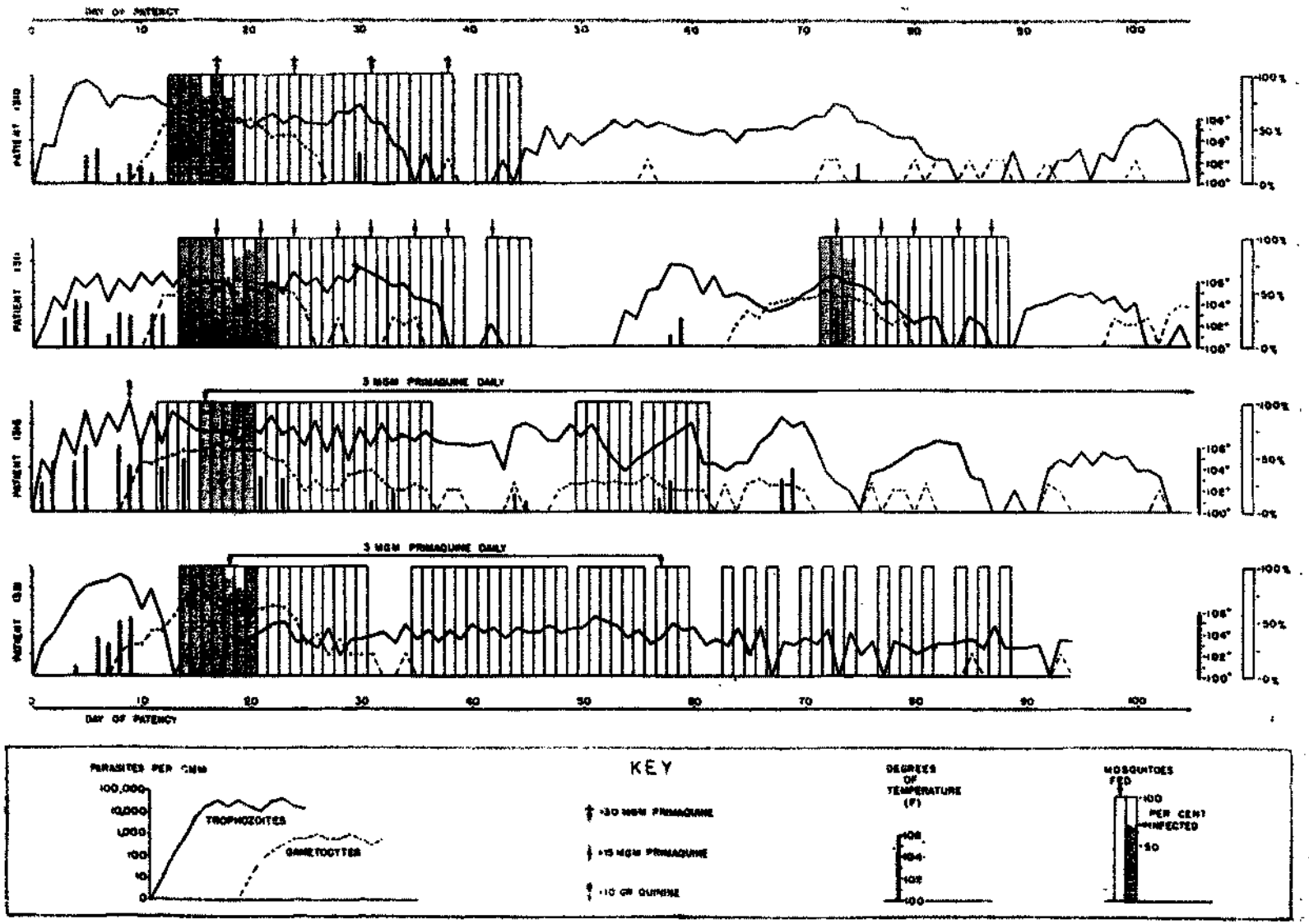

Figure 1. Response of *Plasmodium falciparum* to primaquine.

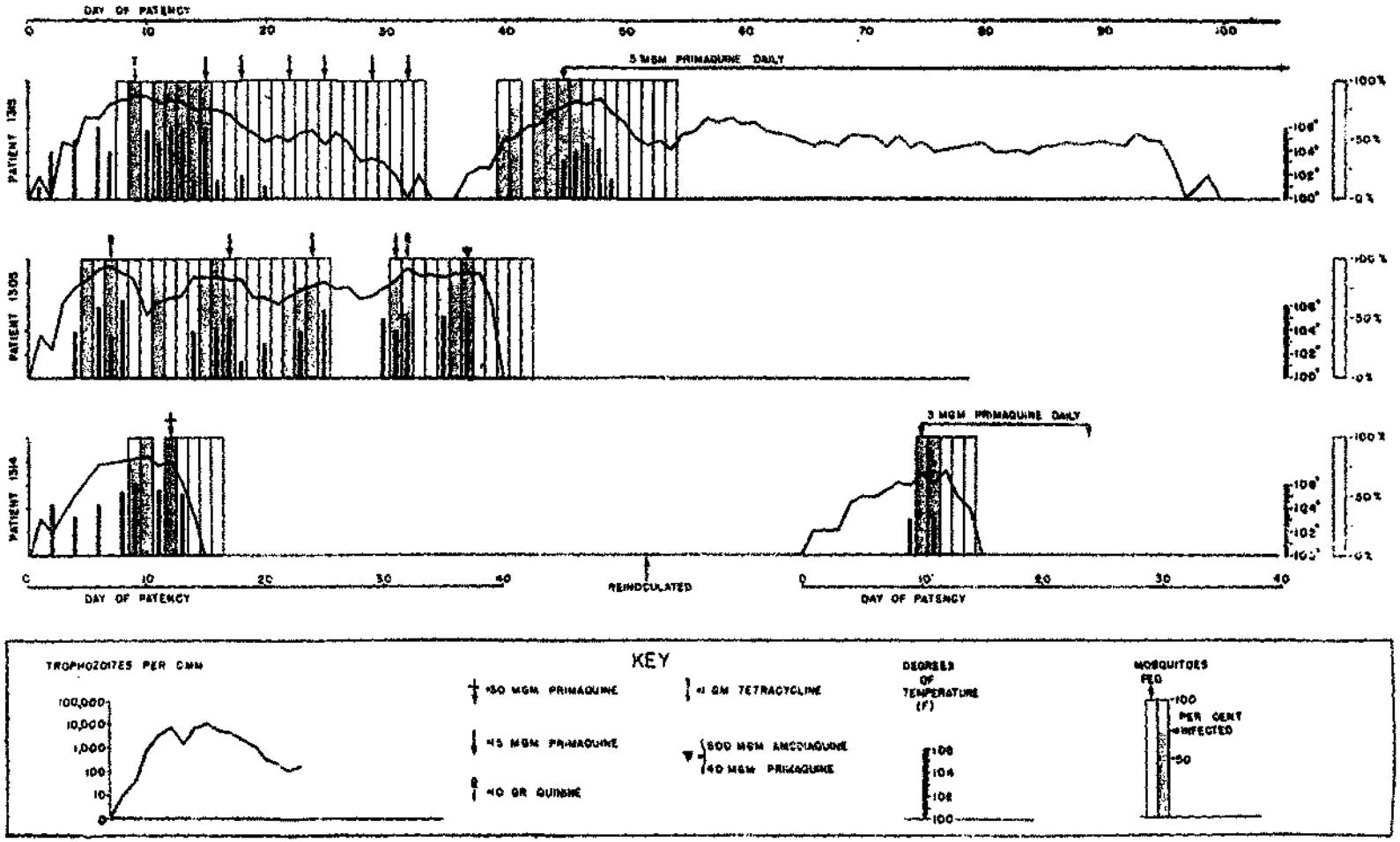

Figure 2. Response of *Plasmodium vivax* to primaquine.

DISCUSSION.

These results indicate that mosquito transmission of malaria can be interrupted and prevented by the administering of small weekly, bi-weekly, or daily doses of primaquine. It appears that more than 15 mg. single dose weekly is required for continuous prevention. However, the daily dose of 3 mg. was sporontocidal and it is possible that even smaller daily doses would be sufficient. Although there was no consistent schizontocidal effect, there was no evidence of resistance to primaquine. In our previous work with the resistance of malaria to pyrimethamine, the gametocytes became resistant at the same time as the asexual forms with subsequent infection of mosquitoes in the presence of the drug (Young and Burgess, 1959 ; Burgess and Young, 1959). The resistance was apparent in the relapses following the initial exposure to pyrimethamine. In the present work, there was no evidence of resistance during the primary attack or the relapse by either the asexual or sexual parasites under continuous long exposure to primaquine.

The drug did not prevent the development of the waves of gametocytes, especially *P. falciparum*, but it may have reduced the number of gametocytes as they never reached the usual densities. Once the drug became effective against the gametocytes, those present in the blood or those which appeared later during the drug period never were able to complete the sporogonous cycle in the mosquito. The persistence and reappearance of the gametocytes during drug therapy, and in some cases early in the therapy, indicate that primaquine in the amounts given was not gametocytocidal. The term sporontocidal appears to be more definitive.

SUMMARY AND CONCLUSIONS.

Small doses of primaquine given to cases of *Plasmodium vivax* and *P. falciparum* exerted sporontocidal effects. Thirty mg. weekly, either in a single dose or in two doses and 5 or 3 mg. in daily doses usually prevented the transmission of malaria. Gametocytes were present or appeared in waves during continued drug treatment but were not able to complete the sporogonous cycle in the mosquito. The schizontocidal action was inconsistent but usually ineffective.

Five mg. was given daily for 93 days continuously and 3 mg. daily for 30, 14, 127, 58, and 146 days respectively without any apparent toxicity.

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Indian Journal of Malariology, **13**, 2/3, June/September 1959.

STUDIES ON THE BIONOMICS OF MOSQUITO VECTORS WHICH TRANSMIT FILARIASIS IN INDIA.

1. ATTACHMENT OF *MANSONIA ANNULIFERA* AND *MANSONIA UNIFORMIS* LARVAE TO HOST PLANTS OCCURRING IN *PISTIA* TANKS IN KERALA, SOUTH INDIA.

BY

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[September 14, 1959.]

THIS is the first of several anticipated papers relating to the bionomics of mosquitoes which transmit filariasis in India, chiefly *Culex fatigans* and several species of *Mansonia*, subgenus *Mansonioides*, particularly *M. annulifera* (Theobald) and *M. uniformis* (Theobald). The present study was carried out in Palluruthy and Ernakulam, Kerala State, South India in April and May, 1959. In so far as possible, observations were made in the field, supplemented by laboratory study. For the purpose of recording the activities of the larvae either at the tank or in the laboratory, the host plants were placed into a rectangular, clear glass jar containing 200 to 300 third and fourth instar larvae of either *M. annulifera* or *M. uniformis*. Attachment attempts and behaviour were studied by means of a detached upper portion of a stereoscopic entomological miscroscope placed horizontally. To supplement such observations, those portions of the host plant to which attachment is effected, such as roots, were placed into a petri dish, permitting behaviour of the larvae to be studied in detail under high magnification. The larvae did not seem to be aware of the changed orientation of the roots from the vertical to the horizontal position, and carried on their attachments as is usual in nature.

A "tank" as used in India, refers to a small pond usually located near a house or hut, and which furnishes water for household use. Generally it is between 10 and 20 feet in diameter, but may be larger in some instances. In Kerala, such tanks are never used for buffalo or cattle wallowing. The water is often covered with *Pistia stratiotes* or water lettuce, which is said to keep the water clean and cool. *Pistia* removal is therefore objected to by the householders. The tanks are used for soaking of coconut husks to produce coir fibres which are used in making mats, rope, and carpets. The prolonged soaking of the husks over a period of five to six months yields an infusion which probably helps increase *Mansonioides* breeding.

All of the close-up photographs shown here were taken with an Edixa (German) single-lens reflex 35 mm. camera having a Takumar (Japanese) f. 2·2, 55 mm. lens and close-up extension rings. The microscope photography was done with the same camera body minus lens, using the microscope ocular and objective to replace the

*Assigned to Filariasis Division, Malaria Institute of India, Delhi and Ernakulam (India).

camera lens, with a two-inch adapter for fitting the camera body to the drawtube of the microscope. The films used were Adox KB 14 and Kodak Panatomic X. Since no histological equipment was available at the time of the study, all cross sections of roots were done with the naked eye, using a new razor blade. Although this method did not give very thin sections, it provided sections which were adequate enough for showing the cross sectional anatomy.

Since it is practically impossible to photograph the actions of larvae under water in the actual *Pistia* tanks, it must be assumed that the detailed behaviour of *Mansonioides* larvae as recorded here, based on observations in a deep glass jar and in a petri dish, follows fairly closely the actual field behaviour. This assumption is justified by the fact that in most cases such attachments in the glass jar took place instantly. The rectangular jars were brought to the site of the tank, and just as soon as the larvae were collected they were placed into the jar containing the host plant in tank water ; the only difference between the actual tank conditions and the experimental conditions was the presence of the glass jar. In practically every case, except as mentioned otherwise, the larvae attached or began to attach within one minute of being introduced to the host plant.

Pistia stratiotes, family Araceae, called water lettuce, is the chief host plant for *Mansonioides* in Kerala State, for the simple reason that it is more numerous than any of the other tank plants. Although water hyacinth, *Eichhornia speciosa* Kunth, family Pontederiaceae, occurs extensively in very large tanks or water areas, it is not as widespread as *Pistia* in Kerala.

The present study reveals conclusively that *Mansonioides* attaches as readily to *Eichhornia* as to *Pistia*. Preliminary observations have indicated that wherever *Pistia* and *Eichhornia* occur in the same area, if there are larvae and pupae attached to *Pistia*, they will generally also be found attached to water hyacinth in the same area, either in the same or in other tanks. In one case, however, where water lettuce and water hyacinth occurred side by side in the same tank containing soaking coconut husks, only the water hyacinth had attached larvae of *M. annulifera*, but *Pistia* did not, despite much searching.

The other plants described herein were found growing around the margins of *Pistia* tanks in Palluruthy near Cochin. Some occur more frequently than others. *Azolla pinnata* is invariably found associated with *Pistia* and water hyacinth (Fig. 1), and in Shertallay, Kerala, it occurred in overwhelming numbers in *Azolla* tanks as such (Fig. 40). *Lemna minor*, or duckweed, is likewise found associated with *Pistia* and *Eichhornia* in small numbers, or may completely cover the surface of a tank by itself. *Marsilea quadrifoliata*, which resembles a four-leaf clover on a long stalk, occurs rather frequently in *Pistia* tanks, and likewise *Bacopa monnieri* and *Commelina salicifolia*. On the other hand, *Trapa bispinosa* and the lotus plants *Nelumbo* and *Nymphaea* may occur in tanks with or without *Pistia* or *Eichhornia* (Fig. 2). Other plants are known to occur in these tanks, but owing to the heavy monsoon at the time of the present study, they could not be sought at this time. All plant identifications were made with the aid of the "*Handbook of Common Water and Marsh*

PLATE I.

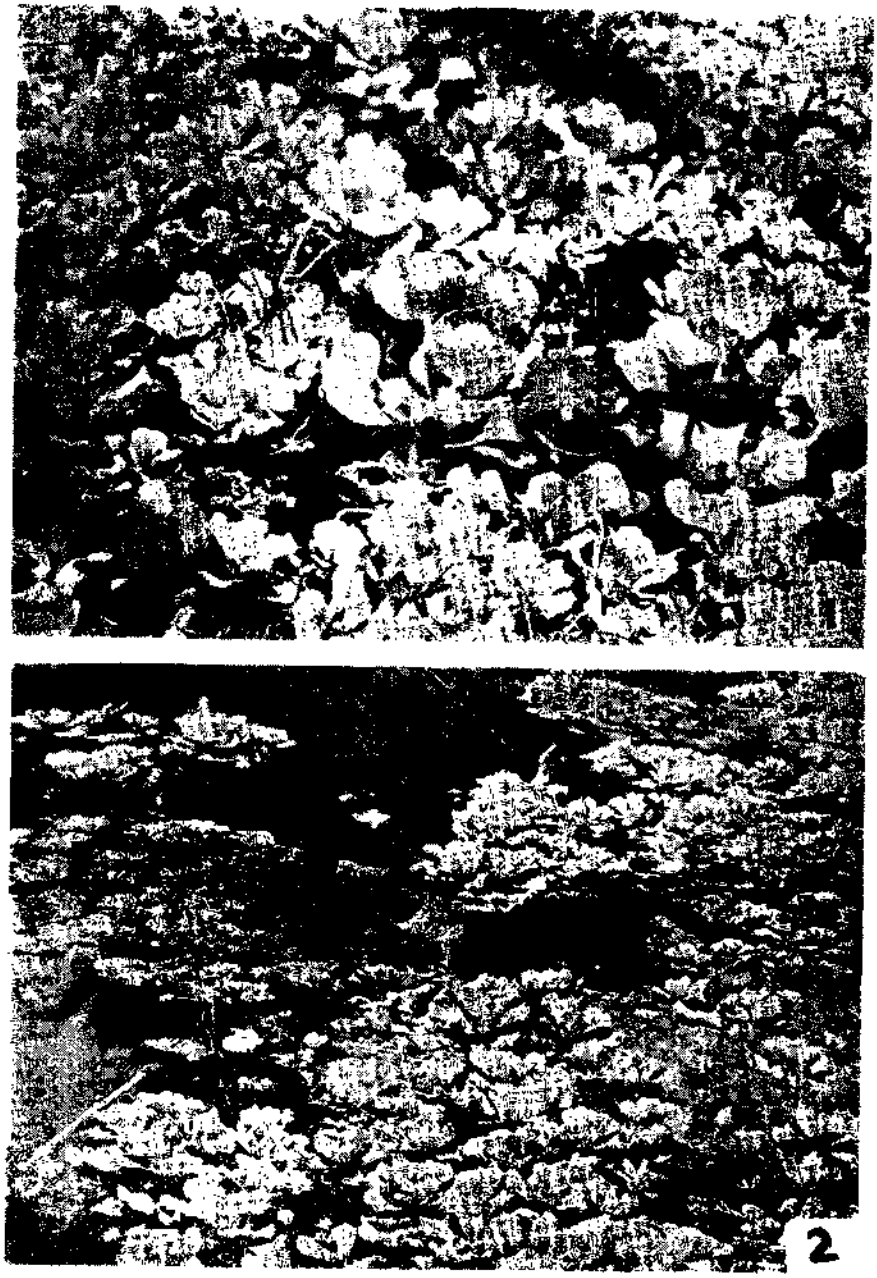


FIG. 1. Ecological association of *Pistia stratiotes, Eichhornia speciosa* and *Azolla pinnata*.
FIG. 2. Ecological association of *Trapa bispinosa, Pistia stratiotes, Azolla pinnata*, and *Marsilea quadrifoliata*.

PLATE II.

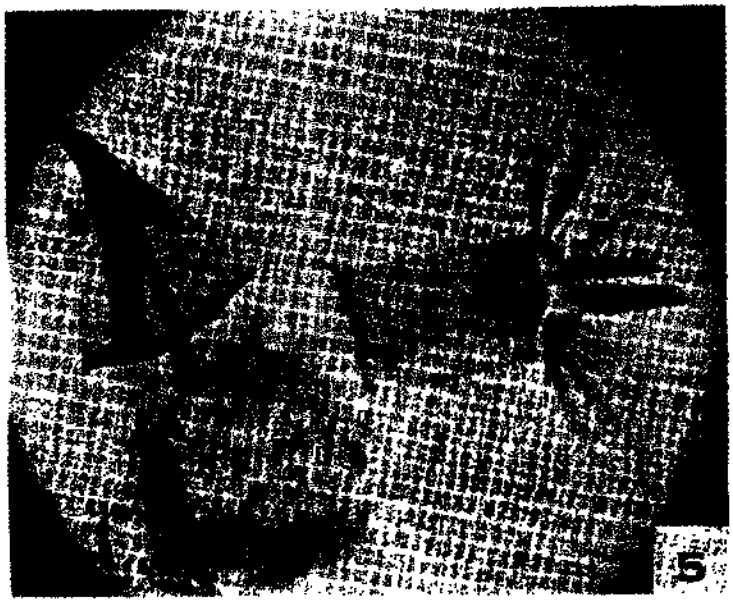


FIG. 3 (Insert) Head on view of tip of siphon of *Mansonia annulifera* larva.
FIG. 4. Lateral aspect of terminal portion of siphon of *Mansonia uniformis* larva.
FIG. 5. Terminal segments of fourth-instar larva of *Mansonia uniformis*.

Plants of India and Burma, 1936" by K. Biswas and C. C. Calder, second edition, 1955. Two of the specimens were sent to the National Herbarium in Calcutta for more positive identification of genus and species, and turned to be *Commelina salicifolia* and *Limnophila gratioloides.*

GENERAL OBSERVATIONS CONCERNING ATTACHMENT.

The attachment of *Mansonioides* larvae to roots or other parts of a plant is a mode of behaviour that is instinctive, constant, and deliberate. Most larvae seem to sense that a particular structure will or will not be penetrable. The action is, of course, inherited, and one would almost think that a larva was aware of the cross sectional anatomy of a root or leaf. The purpose of an attachment is to obtain oxygen and excrete carbon dioxide, and the larva must penetrate through the outer layers (epidermis and cortex) in order to reach the air spaces in the root or leaf.

Based on the continual observations involved in the present study, it can be said that, in general, the larvae attach instinctively to parts of tank plants which contain internal air spaces for gaseous exchange. Perhaps some attractant or repellent chemical is associated with attempts or non-attempts to attach. In some cases attachment takes place in random positions (Fig. 26), but in other cases swift penetration takes place with unerring accuracy (Fig. 51). In a few cases some larvae attempt to penetrate organs that have a tough cortex; there is air-containing tissue within, but it cannot be reached, although the attempt is made (Fig. 54).

It is possible that oxygen pressure within a root or leaf has some bearing on the attachment activity. The chitinous plates comprising the terminal part of the siphon (thought by various investigators to be equivalent to the valves of non-attaching culicines) probably permit oxygen to enter the body only when the insertion provides the necessary reflex.

The extreme end of the siphon of *Mansonioides* larvae was studied head-on in both living and in preserved specimens which had been instantly killed in hot water. In every case the aperture at the extreme end of the siphon was found to remain open, regardless of circumstances (Fig. 6, 7, and 8), even when the larva lay flat on the bottom of the vessel. One explanation for this phenomenon may be that, even though the terminal portion is always open, there appears to be a cut-off valve behind, in the respiratory passageway (Fig. 6), thus preventing the intake of oxygen unless the proper circumstances permit.

A number of observers have noted the eventual death of *Mansonioides* larvae in the absence of the host plant. This was verified in several longevity experiments, all yielding similar results. No matter how many micro-organisms were available as food, no *Mansonia annulifera* fourth instar larva survived beyond 60 hours in the absence of the plant. There were individual variations in longevity, as might be expected. The experimental results are shown in Table I.

It appears that, in the absence of a host plant, the maximum survival time of a fourth instar larva is between 2 and 3 days, no pupation taking place. Of course, if

TABLE I.

Mansonia annulifera 4th instar larvae.

Number of larvae used.	NUMBER OF LARVAE STILL ALIVE AFTER VARIOUS HOURS IN NATURAL TANK WATER WITHOUT HOST PLANT :						
	12 hours.	18 hours.	24 hours.	36 hours.	48 hours.	51 hours.	60 hours.
10	10	10	10	6	2	0	...
100	80	40	25	25	10	5	0
50	42	28	16	13	6	3	0

no host plant is present to begin with, no eggs would be laid. Such mortality probably also occurs in first through third instar larvae. *Mansonioides* evidently could not develop to the adult stage in the absence of the plant unless some other adaptation for obtaining oxygen were developed or effected.

When the larva gets ready to attach, it backs in toward the root in an upside-down position, the saw-toothed edge ventrally placed, and the anal segment facing outwards and dorsally. The older larvae never attach to a rootlet, but always to a main root, to the sympodial mass from which the leaves and roots arise (Fig. 17), or to a leaf which happens to lie horizontally in the water (Fig. 12, 14, 42 and 49). In *Pistia* and *Eichhornia* the attachment to a main root generally occurs between the node where a rootlet originates and a distance up to one-third of the way down the internode. Many attachments take place in the axil of a rootlet. A study of cross sections along an internode did not reveal any significant difference in air spaces within the internode. First instar larvae also attach to rootlets.

Preliminary study indicates that the pair of sensory hairs at the base of the saw (Fig. 4 and 5) probably assist in selection of the place of penetration. As the larva backs in, it makes between 5 and 7, sometimes fewer as in the case of *Lemna*, seldom more, short, rapid sawing or jabbing movements into the tissue which it is trying to penetrate. These movements stop when the air space within has been reached. The serrated portion is not inserted completely, as the air spaces lie just inside the cortex of the root or leaf. Generally only about one-third or less of the black pigmented valvular portion of the siphon is inserted into roots. A corrugated, transparent membrane opposes the serrated edge (Fig. 4) and probably acts as a rasp or file during penetration. Two pairs of bilobed sensory sacs at the base of the anterior prolongation of the saw probably receive and transmit sensory impulses from pads at the bases of the fringed, sensory hairs, thus activating the saw in order to effect penetration (Fig. 4).

As soon as attachment is completed, the larva, which normally lies in an upside-down position when attached to *Pistia* and *Eichhornia*, moves its mouth-brushes rapidly, creating a powerful suction which draws into the mouth all matter from a considerable distance ahead. Food particles or organisms are ingested, and unwanted matter is ejected from the buccal cavity. Excretion from the anus takes place frequently as ingestion continues. Fig. 5 shows the orientation of the siphon and the anal segment for a root penetration.

PLATE III

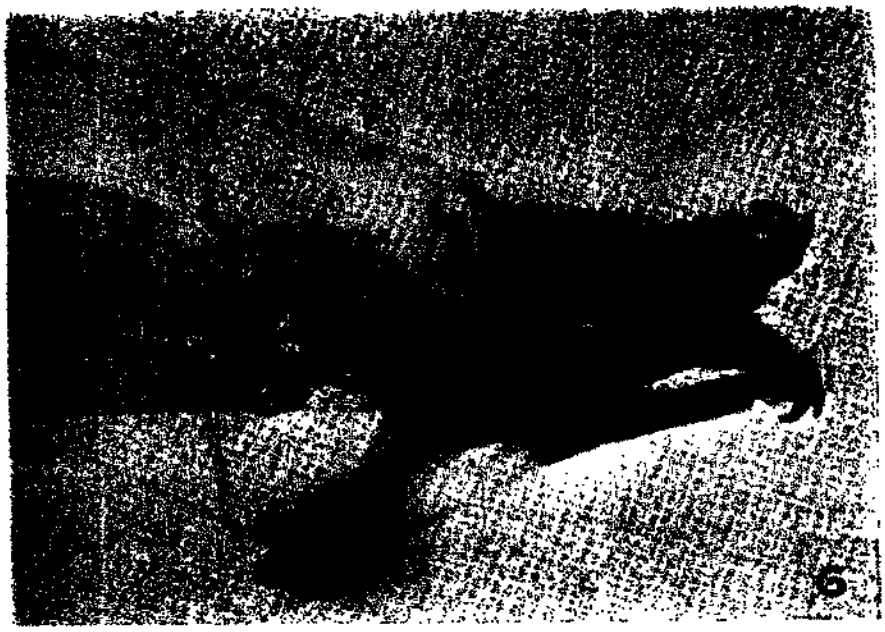


FIG. 6. Terminal portion of siphon of *Mansonia annulifera* larva showing plates and respiratory passageway.

FIG. 7. Ventral aspect of *Mansonia annulifera* siphon showing terminal hooks and tip of serrated plates.

FIG. 8. Penetration of *Mansonia annulifera* siphon tip into main root of water hyacinth.

PLATE IV.

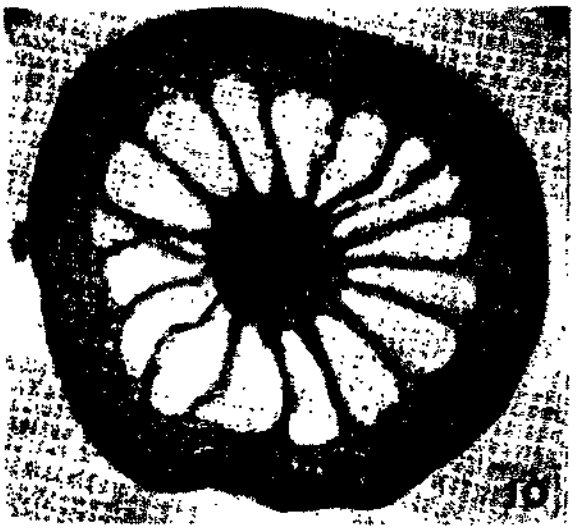


FIG. 9. *Pistia stratiotes* plant unit showing root system and *Mansonia annulifera* larvae attached to main roots. (×3/4)

FIG. 10. Cross section through main root of *Pistia stratiotes* showing air chambers.

Once attached, a larva may remain for several days or longer in the very same position as long as adequate and suitable food is available within reach of the mouth brushes. The angle of attachment is usually between 30 and 45 degrees to the vertical root (Fig. 24), but occasionally larvae are seen to have the axis of the body horizontal or actually sloping upwards in the reverse position (Fig. 23 and 25). *Mansonia uniformis*, which attached very tenaciously, has been observed to remain attached for the entire duration of the third and fourth-instars. The fourth-instar larvae detach at pupation, and the pupae are more often found attached high up near the origin of the roots, possibly because they would have only a short distance to rise to the surface prior to emergence of the adult, or because there is less chance of their being disturbed by some predator while hidden beneath the leaves. *Mansonia annulifera* larvae do not attach as tenaciously as those of *M. uniformis*, possibly because it may cut a wider hole in the plant tissue, permitting a quick detachment. When an attached *M. annulifera* larva is moderately disturbed, it usully detaches, but the *M. uniformis* larva remains attached even when the plant is shaken violently unless the shaking is continued rather strongly. On the other hand, the pupae of both species remain attached quite tenaciously, probably because the gaseous exchange is so vital for conversion of the internal pupal tissues into that of the adult.

A larva that has detached does not usually reattach to the same place. When a *Mansonia annulifera* larva is detached it prefers to swim around for a while before reattaching, often rising to the surface of the water to hang for some time (Fig. 11, 25, and 38), or else it may go to the bottom and lie motionless in a horizontal position, appearing to be dead. On the other hand, when a *M. uniformis* larva becomes detached, it prefers to go to the bottom where it remains for a long time either swimming or lying in the mud.

Experiments were conducted to test the tenacity of attachment of *M. uniformis* larvae by putting a known number of larvae into a jar containing a water hyacinth plant, waiting until all larvae had attached, and then shaking the plant very violently in the water in order to dislodge the larvae. It took many shakings to dislodge only half the number, and it was difficult to dislodge every single one originally placed into the vessel. Likewise, if *M. uniformis* larvae are introduced into a wide, white enamelled pan containing a layer of mud at the bottom and no plant at all, it takes considerable, patient searching to recover all of them, as they bury themselves into the mud. These two factors may have accounted for failure to find *M. uniformis* often on water hyacinth in the past.

The actual swimming movements of *Mansonioides* larvae, when not attempting to penetrate a root, are not very many as compared with those of a normal culicine larva. The larva is probably less attractive to a predator while it is attached and motionless, while it lies quietly at the surface, or while lying quietly and invisibly on the bottom. Predators generally react to motion, feeding more readily on animals that exhibit movement, probably because this indicates that the food desired is alive and probably edible.

Attachment to floating leaves takes place in accordance with the penetrability of the cuticle of the leaf. Most leaves have an aerenchyma or spongy parenchyma containing air spaces within which the larva can effect gaseous exchange. If the larva can penetrate the leaf, it will remain attached for a long time, as in *Lemna minor* and *Azolla pinnata* (Fig. 42 and 48). In *Pistia* and *Eichhornia* attachment to leaves occurs only when they lie horizontally in the water, probably because with the leaf in this position the protective, waxy cuticle can be penetrated more easily (Fig. 12 and 14).

Mansonioides larvae hang from the surface film almost vertically, the ventral aspect uppermost, the head slightly arched upwards, and the mouth brushes working continuously (Fig. 11 and 38). It has already been noted that, in the absence of the host plant, no larva remained alive beyond 60 hours. Although hanging at the surface like *Culex fatigans* or some other culicine, *Mansonioides* evidently cannot respire in the normal fashion, although the terminal part of the siphon remains open. Reference has already been made to the apparent presence of a subterminal valve among the pigmented plates, as seen in Fig. 6. Muscular contractions probably regulate the opening and closing of the internal valve. This is being studied in detail. As suggested above, a successful attachment appears to initiate respiratory activities. The anal gills probably do not have a very prominent rôle in respiration, except possibly in the case of *M. uniformis* larvae which lie buried in dense mud at the bottom for hours. The pair of triple hooks at the tip of the siphon, seen in Fig. 6, 7, and 8, evidently helps in anchoring the siphon in the plant tissue, being buried in the cortex of the root or leaf. From the appearance of the cross sections of *Pistia* and *Eichhornia* (Fig. 10 and 19), the serrated portion of the siphon does not push deeply into the air space, otherwise there would not be adequate anchorage. It is sufficient that the terminal end go past the cortex just up to the air space, the hooks being buried within the cortex. This is indicated in Fig. 8. Other smaller pairs of hooks are also grouped peripherally and terminally, several being visible in Fig. 3.

The various attachments to host plants will be presented in the order in which the plants were studied.

1. *PISTIA STRATIOTES* LINN., WATER LETTUCE (FAMILY ARACEAE).

Pistia stratiotes is a small floating plant which reproduces vegetatively by stoloniferous means so as to form a mat across the surface of tanks in Kerala. The sessile leaves are rather obovate or somewhat wedge-shaped, forming a sort of cup. Botanically the plant mass is called a *symposium*, being made up of a group of secondary axes having short internodes. The lower surface of each leaf is composed of spongy, air-containing fissures which serve as a float. Many main roots having fine, lateral rootlets originate from the sympodial mass. These rootlets arise in whorls which are irregular, not forming a complete circle, but are alternately staggered. The basic pattern can be seen more readily at the terminal portion of a root where the rootlets are beginning to grow.

PLATE V.

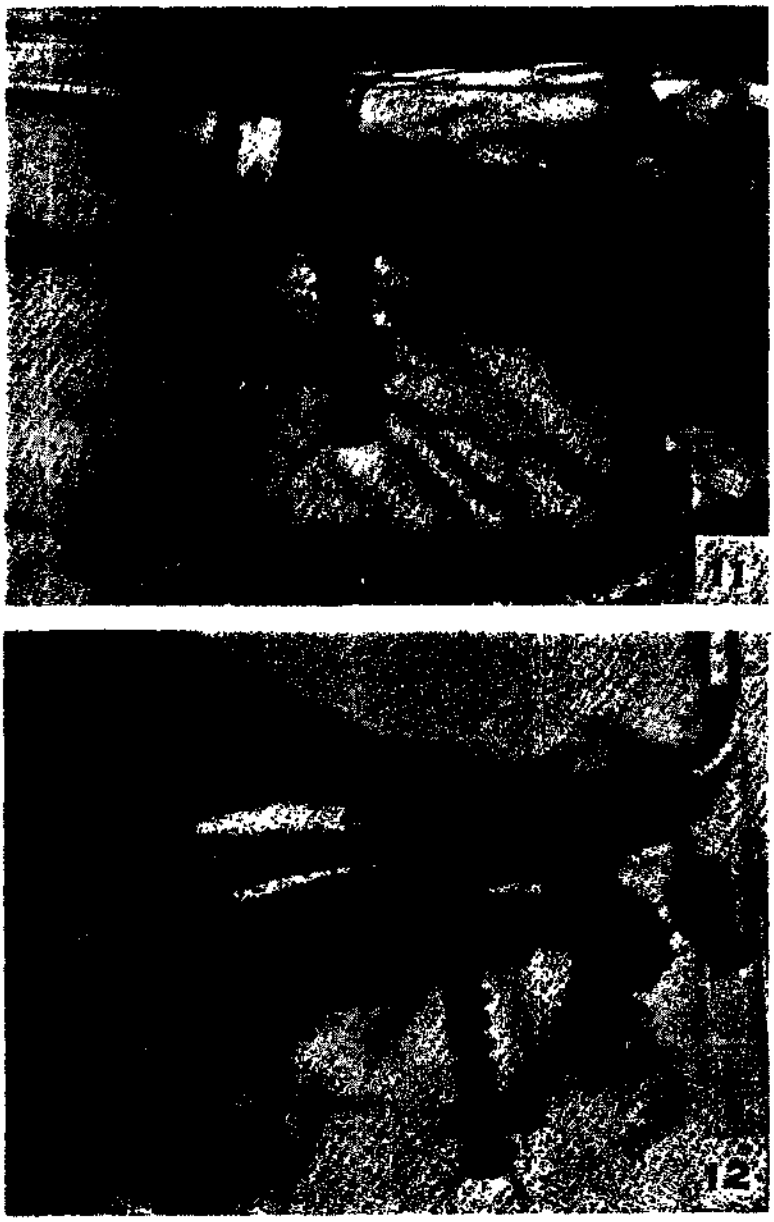


FIG. 11. *Mansonia annulifera* larvæ ranging from surface film

FIG. 12. One larva and three pupæ of *M. annulifera* attached to horizontal leaf of *Pistia stratiotes*.

PLATE VI.

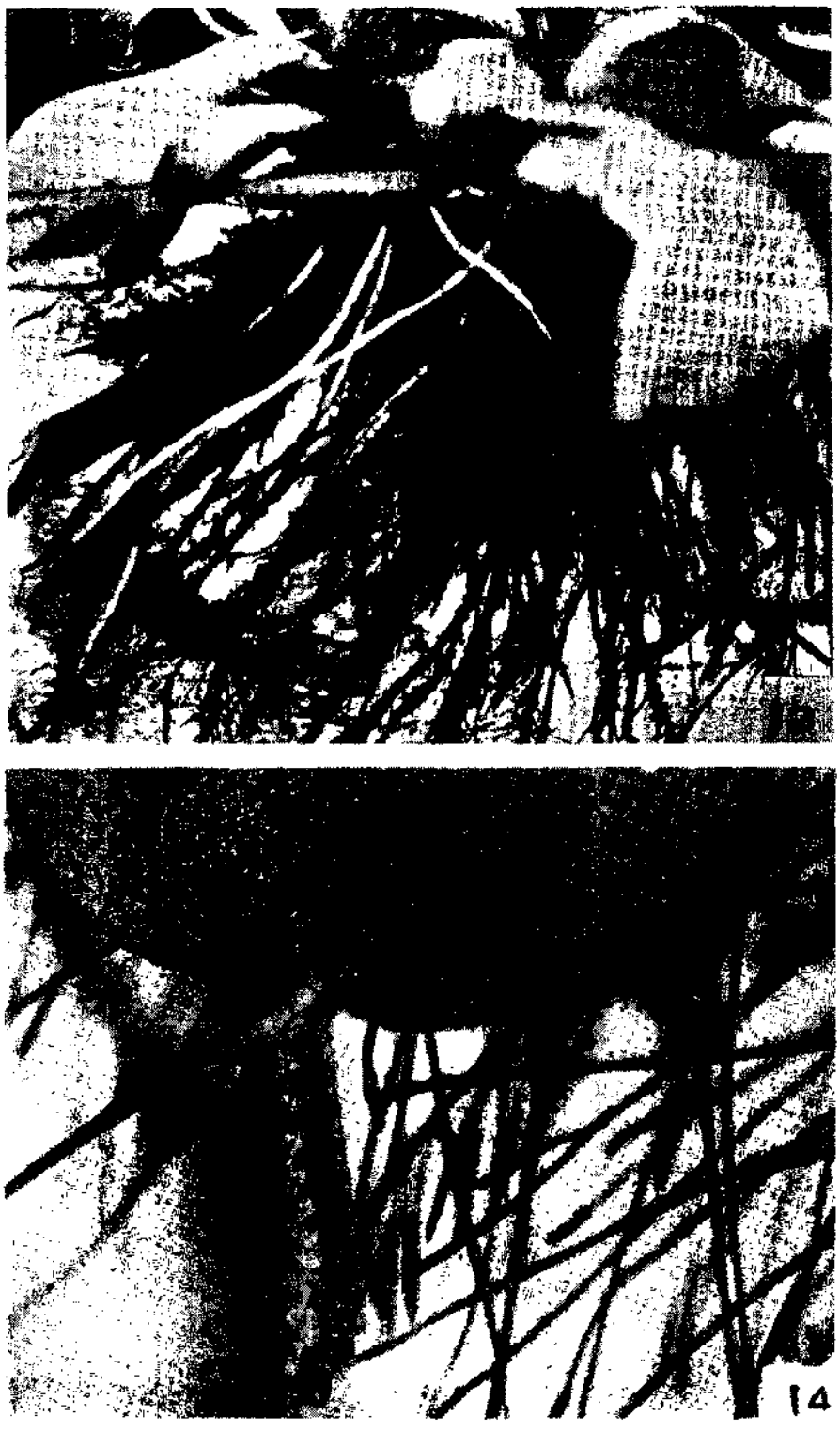


FIG. 13. Water hyacinth (*Eichhornia speciosa*) roots encrusted with mud.

At a node or place where the rootlet originates there is a slight prolongation or protuberance. Although rootlets occur more frequently in pairs, there may be only one or three. Under the microscope the main root appears to be five or six times the diameter of a rootlet. A clear area is seen at the periphery of the root, representing the cortex and epidermis combined. In the cross section of a main root of *Pistia* shown in Fig. 10, there are 17 air locules or chambers separated by narrow septa, so that no matter where the larva punctures the root it will reach one of the air chambers. The epidermis and cortex together comprise one-fourth of the radius of the root. The xylem and phloem, or conducting vessels of the plant, are in the very centre of the root. *Mansonia annulifera* larvae will attach at any point from beneath the leaves down to the tip of the main root (Fig. 9 and 15). *M. uniformis* will attach in the same manner, but tends to prefer being higher up on the root, as seen in Fig. 17. Figures 12 and 14 show *Mansonia annulifera* larvae and pupae attached to *Pistia* leaves which are horizontal in the water. Note the extent of penetration of the siphon into the leafy tissue, at least one-half of the serrated portion being inserted.

In the field the presence of attached larvae of *Mansonia annulifera* can be rather easily ascertained, since in general they are quite easily detached by vigorous swirling of the roots in a water-containing vessel. When the *Pistia* plant is suddenly yanked out of the water, the larvae remain attached for the most part, and may or may not detach when the plant is then placed into some vessel, detaching more often than not. Pupae, however, less often detach during vigorous shaking, and it is necessary to immerse the plant into a tall, clear glass container in order to see the attached pupae.

2. *EICHHORNIA SPECIOSA* KUNTH, WATER HYACINTH (FAMILY PONTEDERIACEAE).

Mansonia annulifera and *Mansonia uniformis* attach to the main roots of water hyacinth just as readily as to roots of *Pistia*. In order to test this, about 200 third and fourth-instar larvae of *M. annulifera* were placed into a rectangular jar, after which small plants of *Pistia* and *Eichhornia* were introduced simultaneously into the jar. Most of the larvae headed for one or the other, and attachment took place depending on which type of roots were closest to the larva (Fig. 15). There did not seem to be the slightest preference for one or the other. It was observed that those that attached remained in place for many hours. *M. uniformis* behaves likewise.

Eichhornia speciosa may either be floating or rooted in the mud, and forms a much denser mat in tanks than does *Pistia*. Figure 13 shows a typical water hyacinth plant with its dense mass of roots and expanded portions of the petioles which act as floats. A typical water hyacinth tank next to Sastavinparambil Temple in Palluruthy, Kerala, is shown in Fig. 18. Note the packed condition of the plants. Here the water is shallow, and the dense, fibrous masses of roots penetrate the bottom mud. The leaves, which are obovate or sometimes broadly rounded, form a rosette. The spiked flowers are hyacinth-like and pale violet in colour. Vegeta-

tive reproduction proceeds from the submerged rhizome. The leaves, usually one to two feet high, have a long or short petiole which is inflated near the middle so as to form a float. Those plants which are not rooted in the bottom mud or soil may be blown for considerable distances. When formed, the seeds sink to the bottom, where they have been reported to remain dormant for up to 5 to 7 years.

The attachment of *Mansonia annulifera* to water hyacinth follows just about the same pattern as on water lettuce. *M. annulifera* larvae do not appear to have any more tenacity on *Eichhornia* than they have on *Pistia*. Attachment locations are about the same (Fig. 15, 16, 20, 21) and habits of movement, when not attached, are also the same as with *Pistia*. The larvae of *Mansonia uniformis* are stouter than those of *annulifera*, particularly in the fourth-instar. *M. uniformis* shows a definite preference for attaching high up on the roots in the sympodial portion beneath the leaves (Fig. 17). The attachment is very tenacious, and the larvae remain in the same, exact position for days, whether on *Pistia* or on water hyacinth.

When attached *M. annulifera* are lifted up suddenly together with the plant, and plunged into hot water just short of boiling, many of them lose their grip on the tissues and fall off after being moderately disturbed, probably due to the nature of the attachment. On the other hand, if a water hyacinth plant having many attached *M. uniformis* larvae is suddenly lifted up and plunged into hot water, practically every attached larva and pupa remains firmly fixed to the roots. Thus permanent preparations showing larvae and pupae in position on the plant can be made more easily with *M. uniformis*. It has been possible to make a similar preparation using *M. annulifera* larvae and pupae, but they have to be fixed in the hot water longer (2 to 3 minutes), and the preparation then has to be handled very carefully. The plants should then be kept in a preservative containing 86 parts water, 10 parts of glycerine, and 4 parts of 40 per cent formaldehyde.

When the non-attachment habits of *M. uniformis* were studied, it could be seen why there had been no report previously of *M. uniformis* on water hyacinth in nature in India. First of all, when a larva is not attached, it does not prefer to go to the surface to hang from the film as does *annulifera*, but instead drops into the bottom mud and lies there horizontally for many hours if necessary, making sinuous movements. *Mansonia annulifera*, likewise has been observed to rest on the bottom of the vessel for some time, but is much more restless and soon swims up to attach. For some reason the bottom-resting ability of *M. annulifera* is less than that of *M. uniformis*; the latter does not appear to suffer from lack of oxygen when buried deep in the bottom mud.

Secondly, attachment of *M. uniformis* larvae and pupae often takes place very high up on the roots beneath the leaves, so that even when a water hyacinth plant is placed into a tall, clear, water-filled jar, the larvae and pupae cannot be seen. Such high-up attachments can also take place on water lettuce, but on *Pistia* they can more often be shaken loose when the plant is swirled violently back and forth in water. It takes very rough and continued shaking to loosen *M. uniformis* larvae from their high attachments on water hyacinth roots.

PLATE VII.

FIG. 1a. *Mansonia annulifera* mcae attach equally to *Pistia stratiotes* (left) and *Eichhornia speciosa* (right). (×0·85)

FIG. 16. *Mansonia uniformis* larvae attached to main roots of water hyacinth.
FIG. 17. *Mansonia uniformis* larvae attached in dense, sympodial mass beneath *Pistia* leaves.

For the reasons given above, water hyacinth has previously been reported as being negative for *Mansonioides* larvae and pupae, since the specimens are comparatively non-detachable or else buried down in the bottom mud, particularly in the case of *M. uniformis*, or else the jet black muddy water makes the larvae and pupae invisible. It is recommended, therefore, that when investigating water hyacinth, shaking of plants be continued over and over again, and the bottom mud should be examined at the same time. In a sludgy-bottomed tank such as that next to Sastavinparambil Temple in Palluruthy, the water hyacinth roots are all heavily encrusted with black mud (Fig. 13). Shaking even a small plant in a white enamelled pan soon turns the water so black that the larvae can be seen only when a little of the black water is greatly diluted with clean water. This is a laborious procedure, but it appears to be the only sure way of finding *M. uniformis* larvae on water hyacinth in a muddy tank. In Cochin, Kerala, *M. uniformis* larvae attach to water hyacinth in far greater numbers than those of *M. annulifera*.

Figure 1 shows an association of *Pistia*, *Eichhornia*, and *Azolla* in a tank in the fourth ward, south side, Thallupu area of Palluruthy. This tank had numerous coconut husks soaking in it. For some reason only *Eichhornia* had attached *M. annulifera* larvae, while *Pistia* and *Azolla* were both negative.

From Fig. 19 it can be seen that the cross sectional anatomy of a main root of water hyacinth is very similar to that of *Pistia*. The nature of the air-conducting locules or chambers is the same, and as in *Pistia* an air chamber can be pierced no matter from which angle the larva penetrates. This explains why, when the two plants are suddenly introduced into a jar containing *Mansonioides* larvae, there is no preference shown for one or the other plant, as either one is suitable for effecting gaseous exchange. Also note the similarities of the cortical and central portions of the roots of *Pistia* and *Eichhornia*. The section of *Eichhornia* shows 45 air chambers, whereas the one of *Pistia* in Fig. 10 shows only 17.

From Fig. 21 and 22 a very interesting observation can be made concerning larval orientation of the body with respect to the plant part to which it is attached. The very long main roots at the right coil around to the left and end halfway up against the left edge of the jar. The larvae had been placed into the jar *before* the water hyacinth plant was introduced. It appears as if the body axis of the larva has some correlation with the direction of the *rootlets*, because at the left the larvae angle *upwards*, as the photograph is viewed normally. This means that the larvae are not aware that the direction of the root has been reversed. Preliminary studies indicate that the pair of fringed, sensitive hairs or setae attached near the base of the serrated portions of the siphon and projecting dorsally in the same aspect as the saw itself (Fig. 4 and 5), are of some importance in the determination of the place and angle of attachment; tactile sensations are probably transmitted from the hairs to the basal pads, thence to the paired, bilobed sacs which connect with the proximal, long, prong-shaped portion of the saw.

The above observation is supported by the fact that when a piece of either *Pistia* or *Eichhornia* root is placed horizontally in a water-filled Petri dish, the larvae

go through exactly the same motions in attaching that they do when the root is vertical. It has already been mentioned that most of the larvae attach themselves either in the axil of a rootlet or up to one-third of the distance down the internode from the axil. This is clearly shown in Fig. 23 in which every larva is attached either at, or immediately below, the origin of a rootlet. Another bit of supporting evidence for this idea is seen in Fig. 25 and 26, where *Mansonia annulifera* larvae on *Bacopa monnieri* roots have no rootlets to guide them or orient them, and therefore hold the body in any position, i.e. downwards, upwards, and horizontally.

No attachments were observed on the air-filled expansions of the petioles, probably because the outer tissues were too thick for penetration. In addion to the attached larvae and pupae, egg masses of *Mansonia uniformis* were found on water hyacinth in nature in Kerala, thus indicating that complete development can take place on water hyacinth alone.

3. *BACOPA MONNIERI* PENNELL, (FAMILY SCROPHULARIACEAE).

This succulent, aquatic, creeping herb has small, sessile, entire leaves which are $\frac{1}{2}$ to $\frac{3}{4}$ inch long, and may be obovate or spatulate. It is eaten in India, being of some medical importance. The leaves are opposite, almost veinless. The roots are white, except for a reddish hue at the proximal end where they are attached to the stem. There is a main root portion, and also roots which lie along the creeping stem from one to two inches apart. When this plant is introduced to *Mansonia annulifera* in a glass jar, there is instant attachment to the roots, as seen in Fig. 25 and 26. The cross section of a *Bacopa* root shown in Fig. 27 explains the ready attachment. The root diameter is about the same as that of *Pistia*, and the section shows 30 air chambers. In Fig. 26, two of the larvae are oriented horizontally, while four are angled upwards. Because of the large number of air chambers penetration may be effected from any angle; the absence of rootlets may be a factor in this behaviour pattern, as already noted. Note the clusters of larvae at the proximal portions of the roots in Fig. 25, at the lower centre, centre right and upper right. A few of the larvae penetrated the stem, but the number was negligible; quite a few sawing motions were necessary for penetration of the stem, which has a tougher epidermis than that of the root. It is interesting that, although this is not a floating plant of the nature of *Pistia* and *Eichhornia*, the larvae directed themselves instantly towards the roots and penetrated very easily. Once the penetration was made, the larvae remained attached in the same position and place for many hours.

4. *COMMELINA SALICIFOLIA* ROXBURGH (FAMILY COMMELINACEAE).

This is a slender, prostrate herb with somewhat linear, lanceolate, sessile leaves having parallel veins and a membranous sheath at the base. The leaves reach a length up to six inches and up to 5/8 inch in width, and originate from a horizontal rhizome. Fig. 28 and 29 show attachments to main roots. Attachments were noted on secondary roots and sometimes to the underside of horizontally oriented leaves, but after a while they left these attachments and transferred to

PLATE IX.

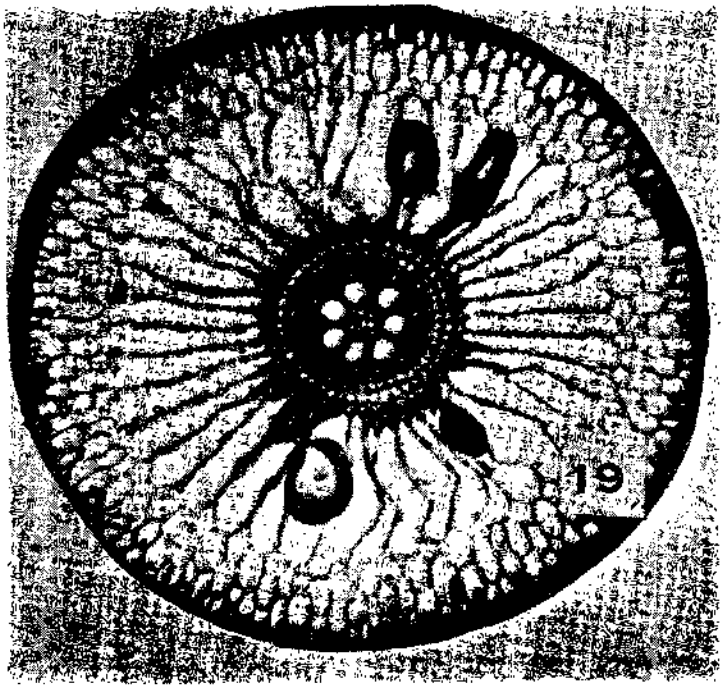


FIG. 18. Large water hyacinth tank in Palluruthy, Kerala, containing considerable *M. uniformis* breeding. No *Pistia* was found in this tank.

FIG. 19. Cross section through main root of *Eichhornia speciosa* showing air chambers.

PLATE X.

FIG. 20. *M. annulifera* larvae attached to main roots of water hyacinth.
FIG. 21. Small water hyacinth plant showing *M. annulifera* larvae attached to main roots.
FIG. 22. Close-up showing orientation of *M. annulifera* larvae on upward directed water hyacinth root.

PLATE XI

FIG. 23. *M. uniformis* larvae attached to main root of water hyacinth.
FIG. 24. *M. uniformis* larvae showing upside down attachment at usual angle on water hyacinth root.

PLATE XII.

FIG. 25. *Mansonia annulifera* larvae attached to roots of *Bacopa monnieri*.

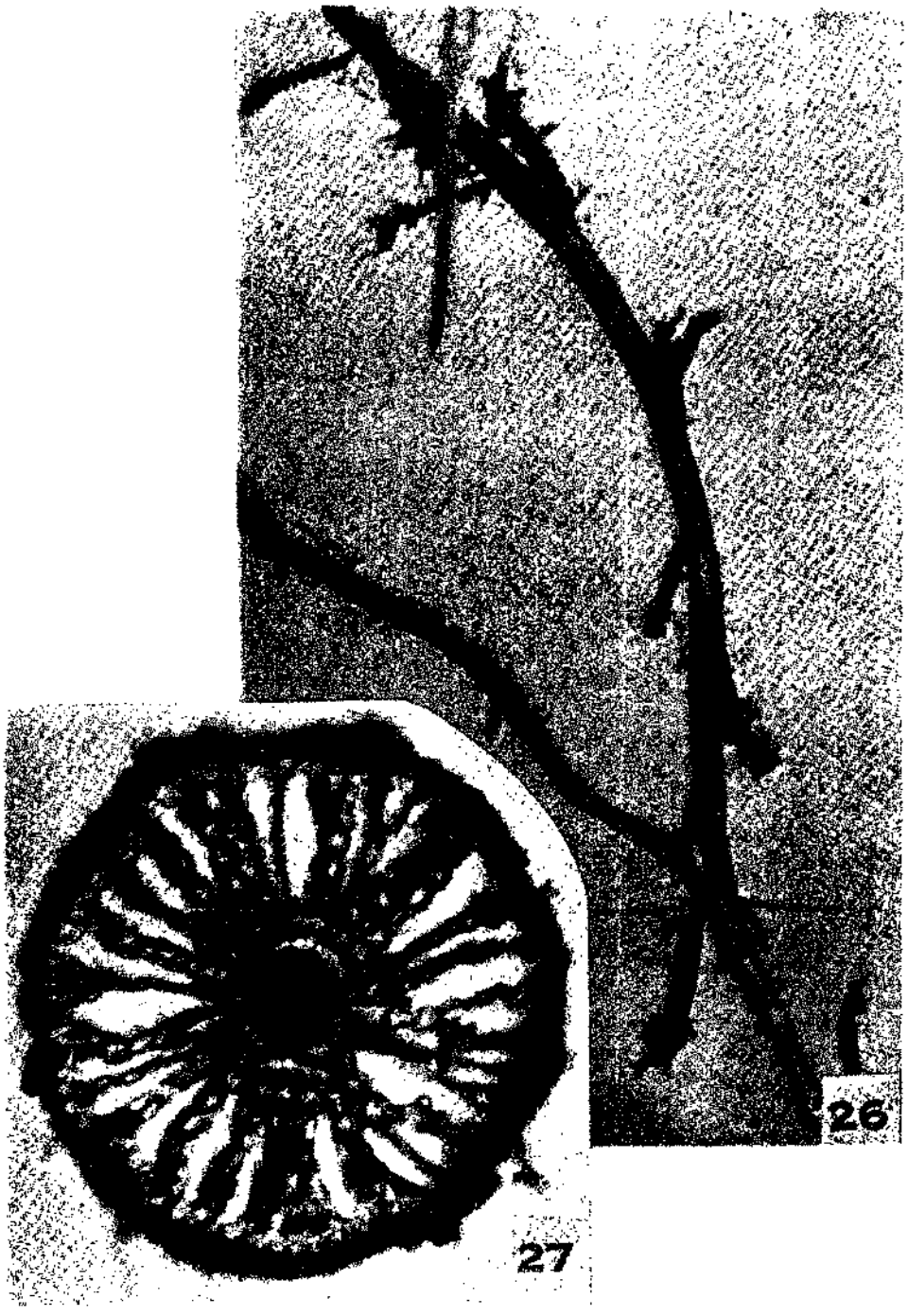


FIG. 26. Close-up showing attachment positions of *Mansonia annulifera* larvae on root of *Bacopa monnieri*.

FIG. 27. Cross section through root of *Bacopa monnieri*, showing air chambers.

PLATE XIV.

FIG. 28. *Mansonia annulifera* larvae attached to roots of *Commelina salicifolia*. (×0·7)
FIG. 29. Close-up showing *M. annulifera* larvae attached to roots of *Commelina salicifolia*.

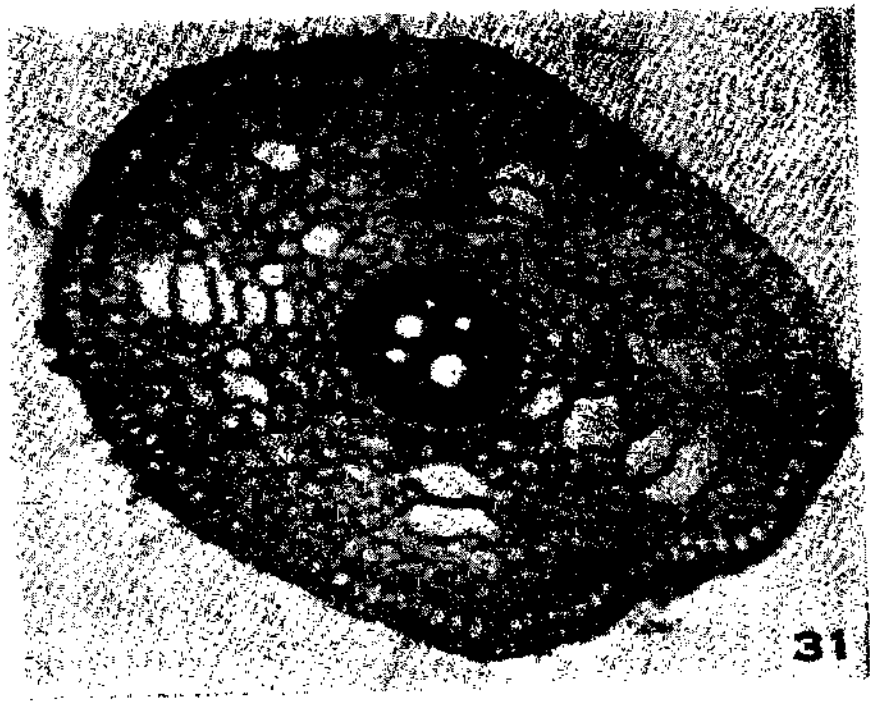


FIG. 30. Close-up showing details of attachment of *M. annulipes* larvae to roots of *Commelina diffusa*.

FIG. 31. Cross section through root of *Commelina diffusa* showing nature of air chambers.

PLATE XVI.

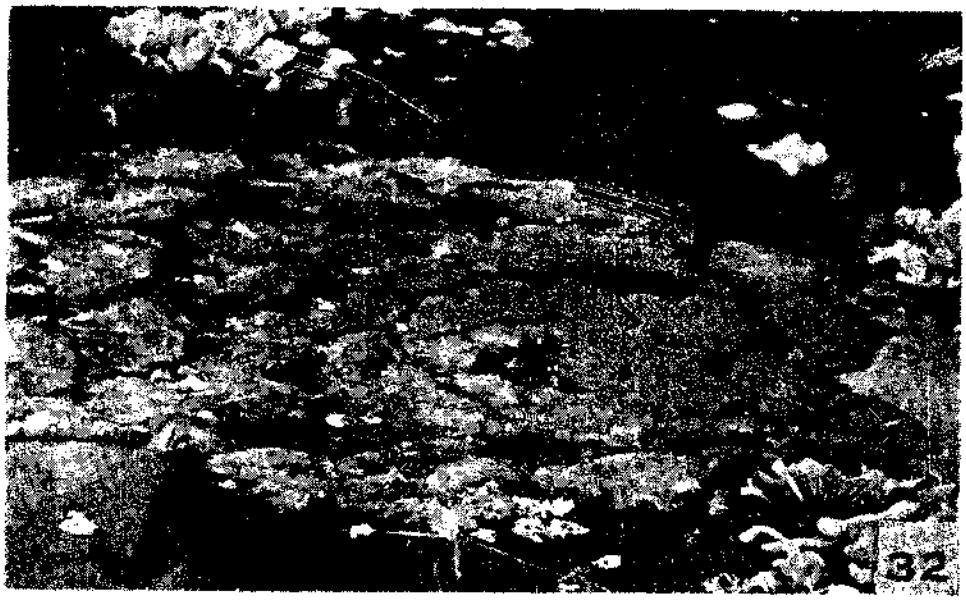


Fig. 32. *Trapa bispinosa* leaves at surface of Pistia tank.

Fig. 33. Complete plant of *Trapa bispinosa*.

the main roots after numerous sawing movements into the latter. A cross section of a main root, seen in Fig. 31, indicates that the number of large air chambers is limited, not being in a radiating pattern such as in the three previously considered roots, hence the numerous sawing movements in order to enter a satisfactory chamber for gaseous exchange. The epidermal and cortical layers are also densely packed with cells. Once attached, the larvae remain in the same position for many hours. Note that the larvae are oriented in various directions, perhaps because the rootlets or secondary roots form a random pattern. Note also that many of the larvae are attached not far below an axil. The random positions may be due to the fact that there are not many large air chambers, and the larvae dig in until they have reached one. Fig. 30 shows the larvae hanging both upside down and right side up.

5. *TRAPA BISPINOSA* ROXBURGH (FAMILY ONAGRACEAE).

This is a widely-spreading, floating and partly submerged aquatic plant having the toothed and somewhat triangular leaves forming a large rosette on the surface, about 15-18 inches wide (Fig. 2 and 32). The petiole of each leaf has a bulbous, spindle-shaped inflated portion which maintains the buoyancy of the leaves. The stems are long and stoloniferous, going down to a considerable depth, the exact nature of the entire plant being shown in Fig. 33. Arising from the stem are thick masses of assimilatory fine roots and stouter, smoother other roots. The specimen pictured in Fig. 33 was over 36 inches long. *Mansonia annulifera* attached readily to the main roots arising from the stem. Fig. 34 shows one leaf, a portion of the stem, and a portion of the root mass. Note the branched roots and the slender, unbranched roots which arise at the nodes along with the branched roots. Fig. 35 shows an enlarged portion of the root mass at the bottom of Fig. 34. Very careful study of this figure will reveal at least 15 larvae hidden partially by rootlets. A few larvae attached to the smooth, unbranched roots. Many larvae attempted to penetrate the undersurface of the leaves, into the prominent leaf veins, but the epidermis was too impenetrable. No attachments were noted either on the stem or on the air-filled expansions of the petioles.

6. *LIMNOPHILA GRATIOLOIDES* BR. (FAMILY SCROPHULARIACEAE).

This is delicate, erect, low herb with 3-6 finely divided or pinnatified leaves arranged in a whorl at each node. The submerged, creeping stem sends up simple branches bearing the leaves. The latter have minute, transparent dots beneath. *Mansonia annulifera* larvae attached at once to the larger roots, as shown in Fig. 36 at the upper left and the right. The latter attachments are shown in Fig. 37. Some attachments to horizontal leaves were also noted at the surface of the water, indicated in Fig. 36. A few larvae attached directly to the underwater stem, as seen in centre of Fig. 36. The larvae have the body oriented into various planes, and the siphons are rather deeply embedded into the roots.

7. *MARSILEA QUADRIFOLIATA*, LINN., A WATER FERN (FAMILY MARSILEACEAE).

Resembling a stalked four-leaf clover, this aquatic herb has four sessile leaflets which are $\frac{1}{2}$ to $\frac{3}{4}$ inch long, the petioles being 3 to 8 inches in length. The rhizome is either creeping or floating. The petioles of the leaves arise from the upper side of each node, and the roots from the lower side. The plant is eaten as a vegetable and also has medicinal uses. Attachment of *Mansonia annulifera* takes place very readily, the larvae remaining attached very tenaciously for many hours in the same position and place. Fig. 38 shows the nature of the plant and the intermingling of the roots and rootlets. Fig. 52 shows the plant erect in a *Pistia* tank. In Fig. 39 a number of larvae are seen to be attached to main roots. The plants of this family have a stalked sporocarp, thereby closely resembling terrestrial leptosporangiate ferns.

8. *AZOLLA PINNATA* R. Br., A WATER FERN (FAMILY SALVINIACEAE).

The fern-like nature of this plant is shown in Fig. 41. It is a small, free-floating, branched aquatic plant having fine, feathered roots. The frond is $\frac{1}{2}$ to 1 inch long, with many crowded branches. The leaves are sessile, somewhat alternate and slightly overlapping, about $\frac{3}{32}$ inch in length, and are somewhat fleshy and convex, with numerous tiny, rounded, fleshy protuberances above. The spongy tissue within each leaf is green in colour due to minute chloroplasts; a blue-green colour is also seen under the microscope because of the presence of a symbiotic, beadlike, blue-green alga, *Anabaena azollae*. Transparent scales are attached below the leaves. Fig. 44 shows a leaf dissected so as to show the soft, cushiony portion in relation to the scale.

Mansonia annulifera attaches immediately and frequently to the undersurface of the leaves, remaining so attached for many hours. No larvae were seen to be attached to the very fine roots. Since there is a symbiotic blue-green alga within each leaf, the alga carries on its process of photosynthesis which increases the oxygen supply within the leaf, in addition to that formed from the chloroplasts.

In an earlier discussion, it was made clear that *Mansoniodes* tends to attach to leaves that fall or float horizontally on the surface of the water. Whereas a leaf attachment in *Pistia* or *Eichhornia* is rather secondary, in the case of *Azolla pinnata* and *Lemna minor* such an attachment is of primary importance because these plants do not have roots which are penetrable by reason of their diameter in relation to the diameter of the black tip of the siphon. The exact nature of this attachment to the blade of *Azolla* is shown progressively in Fig. 42, 43, 45 and 46. Note that for the most part *Mansonia annulifera* larvae hang right side up while attached, just as a normal culicine would hang from the surface film. Sometimes the body is twisted, however, and the head arched upwards, as in Fig. 45.

In some parts of the frond the imbricated leaves do not touch the water, but sit arched, instead, on the top of the floating scales; in other portions of the plant

PLATE XVII.

FIG. 34. Single leaf and portion of rhizome and roots of *Trapa bispinosa*. × [illegible]

FIG. 35. Close-up showing *M. annulifera* larvae, partially hidden by rootlets, attached to a main root of *Trapa bispinosa*.

PLATE XVIII.

FIG. 36. *Neureclipsis annulifera* larvae attached to roots of *Limnephila gratioloides*. (×0·7)

FIG. 37. Close up showing some *N. annulifera* larvae attached to roots of *Limnephila gratioloides*.

PLATE XIX.

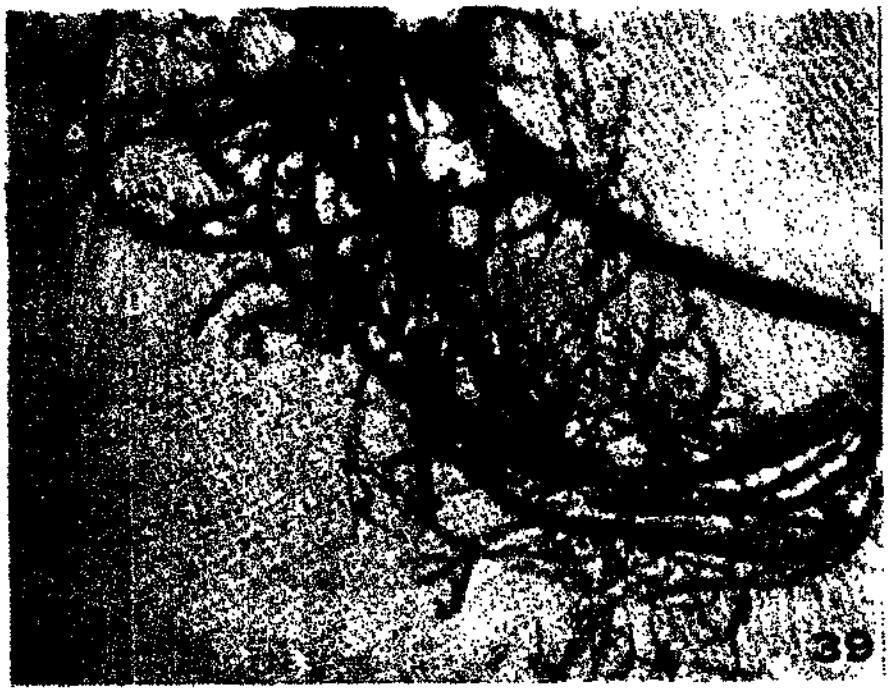


FIG. 38. *Mansonia annulifera* larvae attached to roots of *Marsilea quadrifoliata*. (×67)

FIG. 39. Close-up showing *M. annulifera* larvae attached to main roots of *Marsilea quadrifoliata*.

PLATE XX.

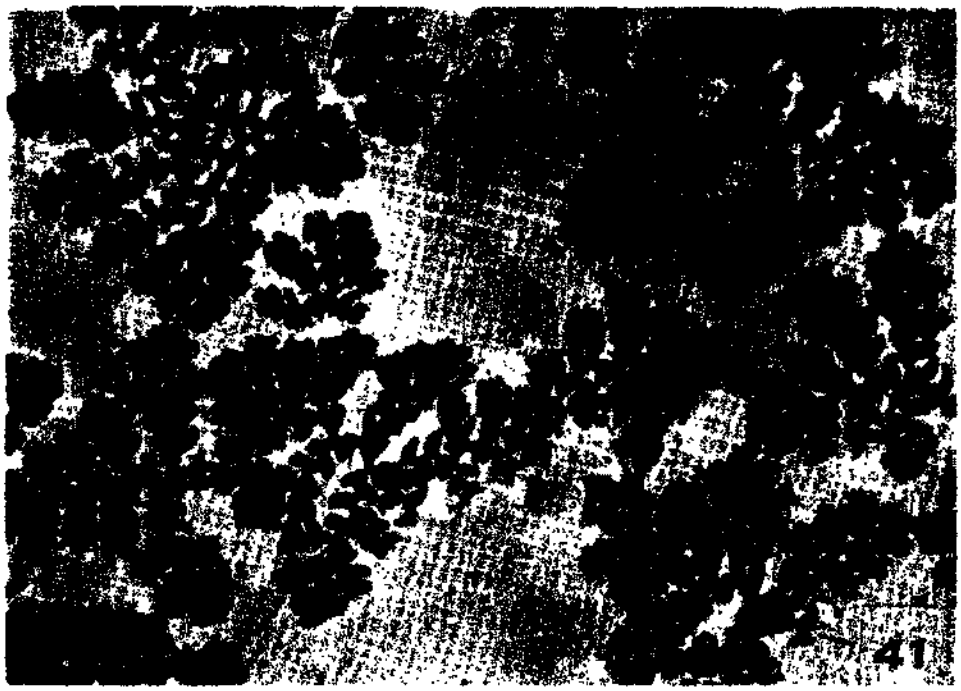


FIG. 40. Portion of tank in Shertallay, Kerala, covered with *Azolla pinnata*.

FIG. 41. Close-up showing fern-like nature of *Azolla pinnata*. (About natural size).

PLATE XXI.

FIG. 12. *Mansonia annulifera* larvae embedded in leaves of *Azolla pinnata*.
FIG. 13. Closer view of *M. annulifera* larvae attached to *Azolla pinnata*.

PLATE XXII.

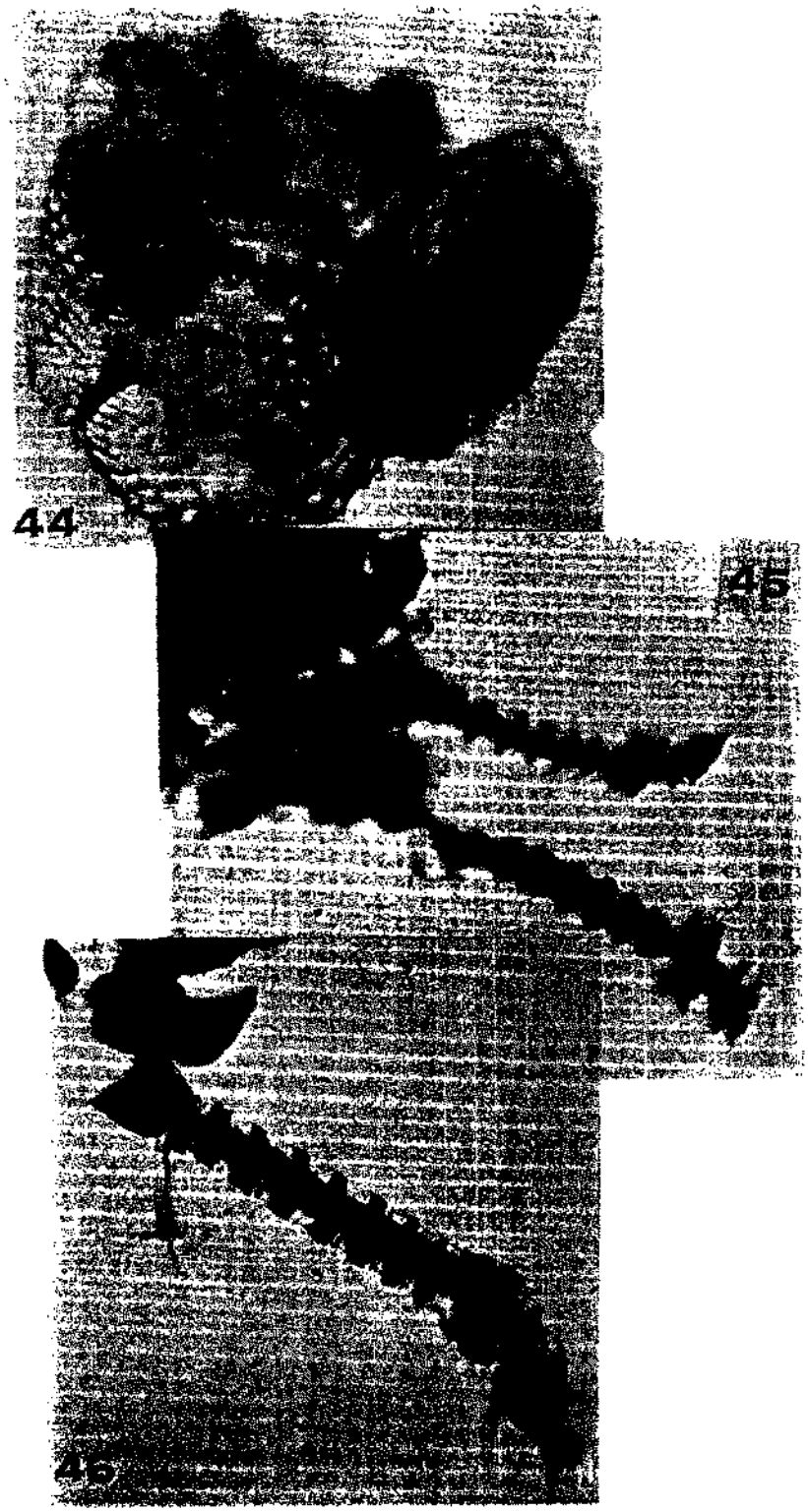


FIG. 44. Single end unit of *Azolla pinnata* showing dissected blade and transparent scale beneath.
FIG. 45. Two *M. annulifera* larvae attached to *Azolla pinnata*.
FIG. 46. Single *M. annulifera* larva showing siphon tip deeply embedded in *Azolla pinnata* leaf

the fleshy stem is on the surface and so are the leaves. Both the stem and leaves have prominent papillae. Due to the extreme softness of the cushiony leaves, the siphon often penetrates for ½ to the entire length of the serrated plate (Fig. 46). In some cases penetration takes place through the floating scale and up into the leaf above, and in other cases directly into the leaf or blade. The attachment is so deep that the larva may then be swung around and around by manipulating the *Azolla* frond with a needle, and it will remain attached very tenaciously despite this whirling movement. Larvae have been noted to remain attached for 24-48 hours or more to the same place without dropping off at all, provided sufficient food is available, as in natural tank water. Probably the reason for this strong attachment is that the wind could then easily blow the frond across the surface of the water, making food available in different locations. There was not sufficient time during the present investigation to follow through the complete life cycle on *Azolla* or *Lemna*, but it is hoped to see whether this is possible at some later date. There is obviously sufficient air-containing tissue to provide for gaseous exchange for a long time, coupled with the depth of the attachment and a long period of time during which the larva (and pupa ?) remains attached.

The close association of *Azolla* fronds, plus rapid reproduction, gives rise to a very heavy mat of *Azolla* in many layers, often covering the surface of a tank completely, as indicated in Fig. 40 and 42. Under the microscope it can be seen that many organisms, including various types of adult and larval insects and roundworms, find shelter among such layers of *Azolla*.

9. *LEMNA MINOR* LINN., DUCKWEED (FAMILY LEMNACEAE).

Lemna minor is a small, floating, aquatic herb having a frond about ⅛ inch wide by ⅛ to ¼ inch long. The green floating blade is really the stem to which a single fine rootlet is attached. Branches arise from a groove in the posterior portion of the blade, under the edge, and may remain attached to the parent or separate later on. Thus two to five blades or more may be seen adhering together, as in Fig. 47. Air chambers within the blade can be seen under the microscope, appearing as white dots in the photographs shown here. Crowded masses of *Lemna* form a protective mat over the surface of the water, similar to that of *Pistia*, *Eichhornia*, and *Azolla* (Fig. 48).

Attachment of *Mansonia annulifera* to *Lemna* in nature was found in Palluruthy, Kerala. The larva not only attaches readily, inserting the tip of the siphon from one-half to almost its full length, usually near the origin of the rootlet (Fig. 48 and 51), but as in *Azolla*, remains attached to the same frond for many hours and also for days, in the exact same position with mouth brushes whirling continuously. Fig. 49 and 50 show the attachments in greater detail. In Fig. 48 at least 6 *Mansonia annulifera* larvae can be made out in perspective view, having been photographed from below. Fig. 51 shows three foreshortened attachments, with the respective siphons deeply embedded in just about identical distances from the fine rootlet. The act of attaching is performed with only one to three sawing or jabbing

movements, because of the softness of the tissue. Very often two and sometimes three larvae will attach to a single blade, as seen in Fig. 49 and 50. As in *Azolla* the larva may hang in the normal culicine fashion, or else twist the body so that the head is upside down. In one case 6 larvae were seen attached to a single leaf, but they dispersed before they could be photographed.

10. *NYMPHAEA* SPECIES, WATER LILY OR LOTUS (FAMILY NYMPHAEACEAE).

The species of *Nymphaea* collected and observed was either *N. lotus* or *N. rubra*, but no flower was seen so the exact species could not be determined. This is a perennial water plant rooted in the mud. The leaves are borne on long petioles, the margins being sharply sinuately toothed. They are smooth and shining above and downy underneath. Of 200 *Mansonia annulifera* fourth-instar larvae introduced into a large jar containing the complete plant, only three attached to the roots but did not remain long, and none attached to the petiole; however, 26 attached themselves to the edge of the large, floating leaf, inserting the siphon laterally into the very edge, penetrating probably into the spongy parenchyma of the leaf which contains air spaces. No attachments were made from below. The leaf was torn into smaller pieces, and then 75 larvae were noted to attach into the edge. Fig. 53 shows this type of attachment in the case of a *Nelumbo nucifera* leaf. Since the larvae remained so attached for many hours, it appears as if they are getting sufficient oxygen in this way. A cross section of a *Nymphaea* root, as seen in Fig. 54, shows considerable air-containing tissue internally, but the epidermis and cortex are much too tough for the larva to effect easy penetration.

11. *NELUMBO NUCIFERA*, GAERTN., INDIAN LOTUS, SACRED LOTUS (FAMILY NYMPHAEACEAE).

As in *Nymphaea* the roots and rhizome are embedded in the mud, and long petioles bear large, almost round leaves which are slightly flattened at the poles and may be one to three feet in diameter. Unlike with *Nymphaea*, several attachments were made from below by *Mansonia annulifera* larvae, but the majority of them attached directly into the edge of the leaf, as shown in Fig. 53. Also, when the leaf was cut into smaller pieces, many more attachments were made into the edge. This type of attachment is indeed an unusual one, but the larvae apparently spare no effort to find air-conducting tissue from which they can take up oxygen and into which they can excrete carbon dioxide, apparently not being able to respire normally just by the hanging from the surface film. In order to make sure that the siphons were actually inserted into the tissue of the leaf, and not merely resting against the edge of the leaf, the latter was depressed and pushed under the water with a needle; the larvae were depressed beneath the surface along with the leaf. Microscope study revealed the actual insertion of the serrated edge into the plant tissue for $\frac{1}{8}$ to $\frac{1}{4}$ its length.

12. *COLOCASIA ANTIQUORUM* SCHOTT (FAMILY ARACEAE).

Colocasia antiquorum is a tall, coarse herb with a tuberous rhizome from which spring rather wide main roots $\frac{1}{8}$ inch in diameter or wider, and finer rootlets. The

PLATE XXIII.

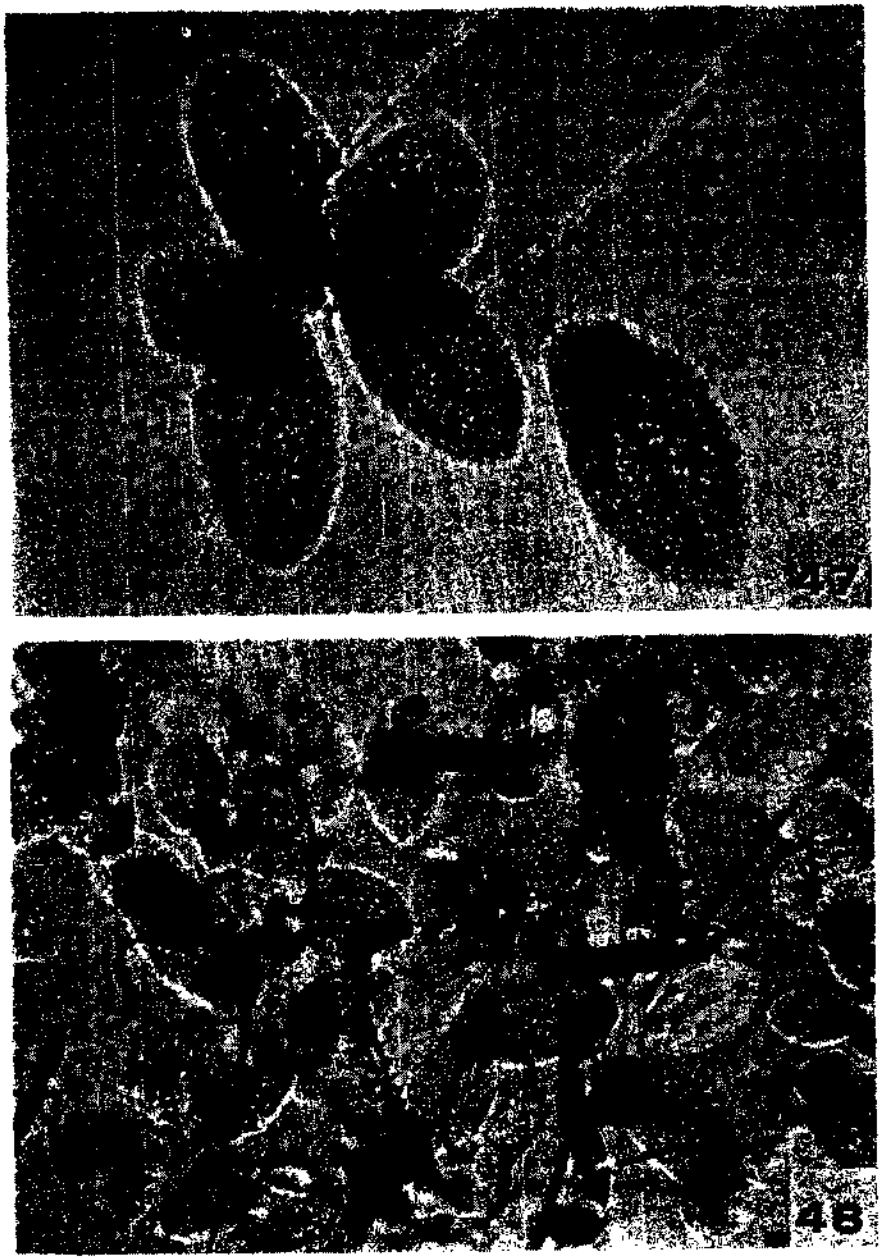


FIG. 47. Branching and single blades of *Lemna minor*.

FIG. 48. View from below showing *Mansonia annulifera* larvae attached to blades of *Lemna minor*.

PLATE XXIV.

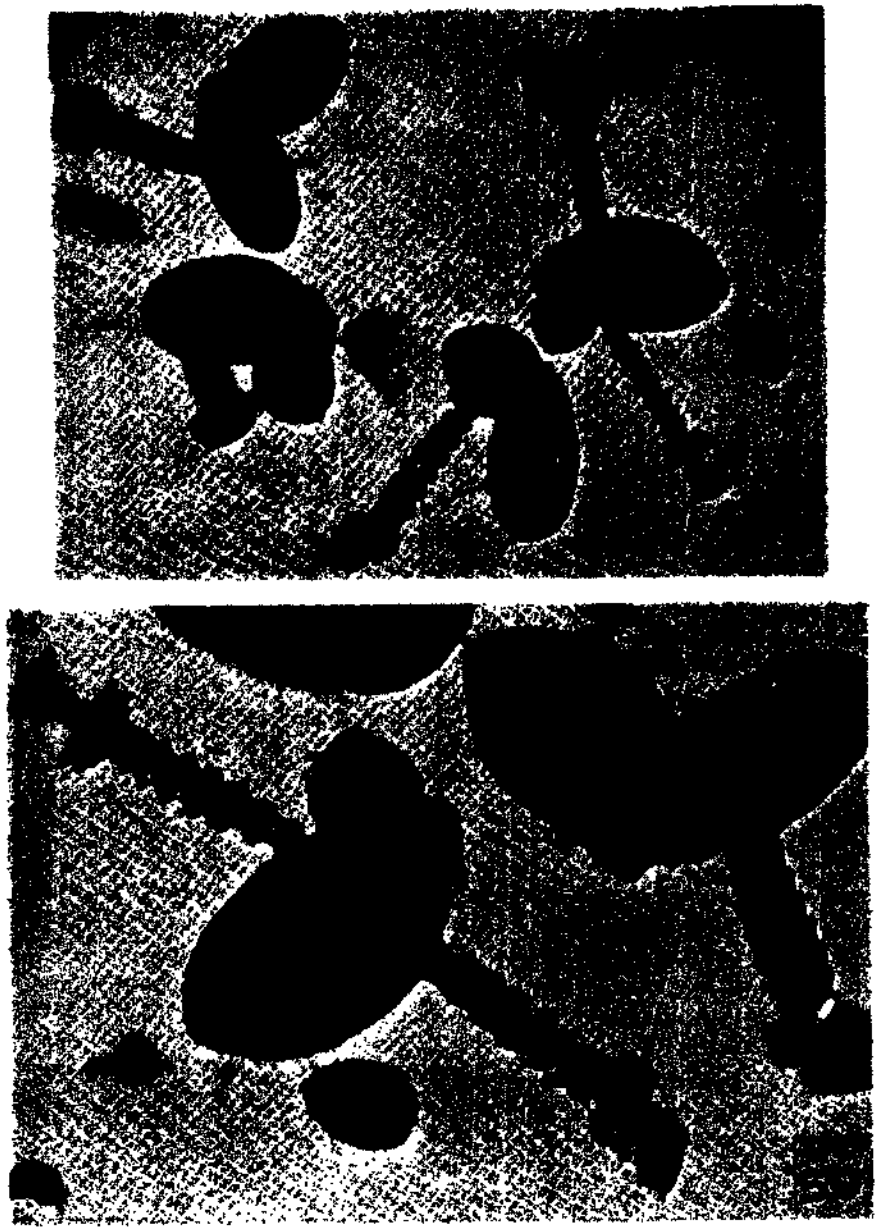


FIG. 4*b*. *Mansonia annulifera* larvae attached to blades of *Lemna minor*.

FIG. 4*c*. Close-up showing *M. annulifera* larvae attached to blades of *Lemna minor*.

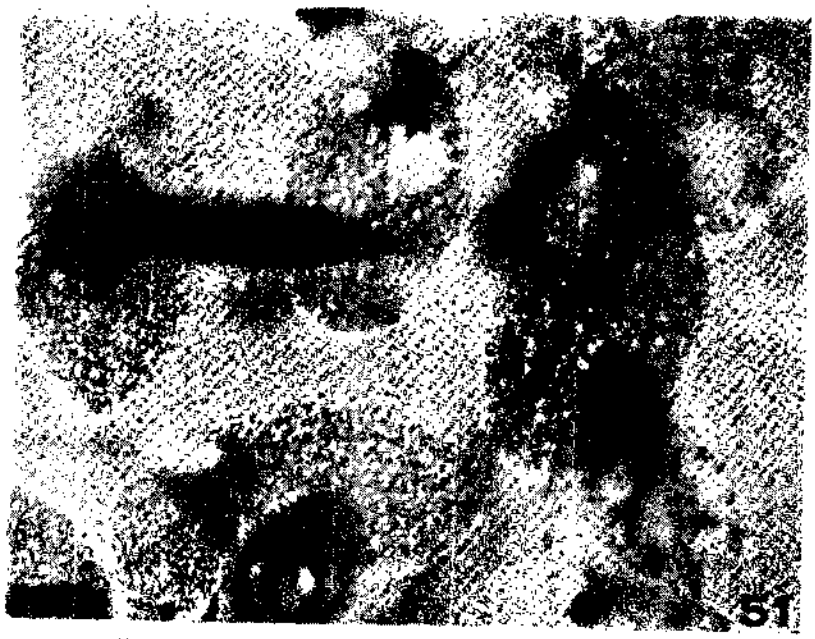
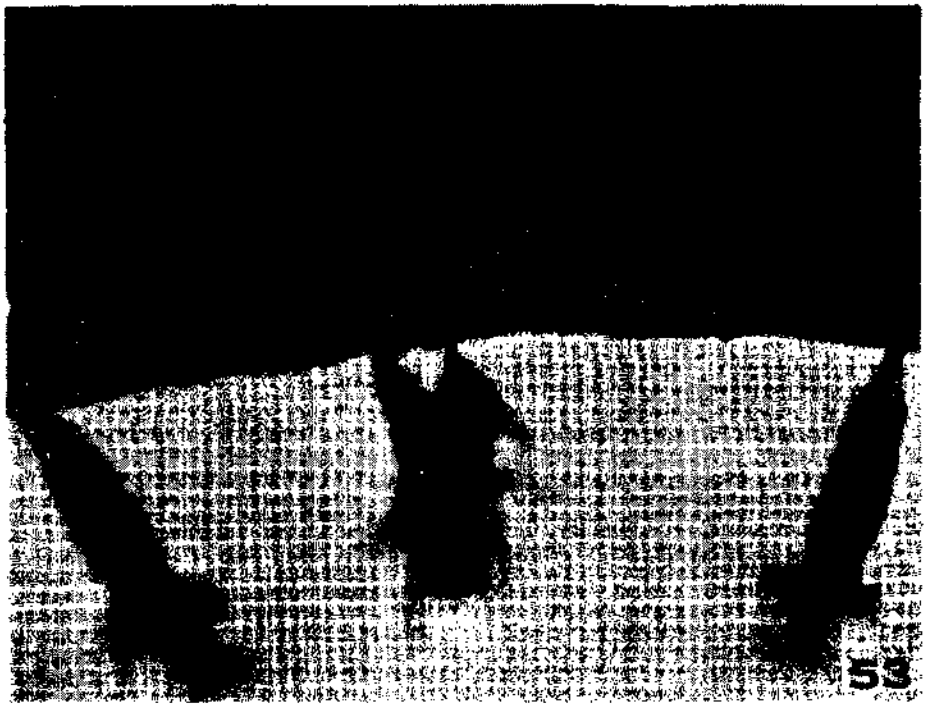


FIG. 51. Base of three foreshortened *M. quadrifoliata* larvae with siphon tips embedded in almost identical places within blades of *Lemna minor*.

FIG. 52. Erect *Marsilea quadrifoliata* plants in Pistia tank.

PLATE XXVI.



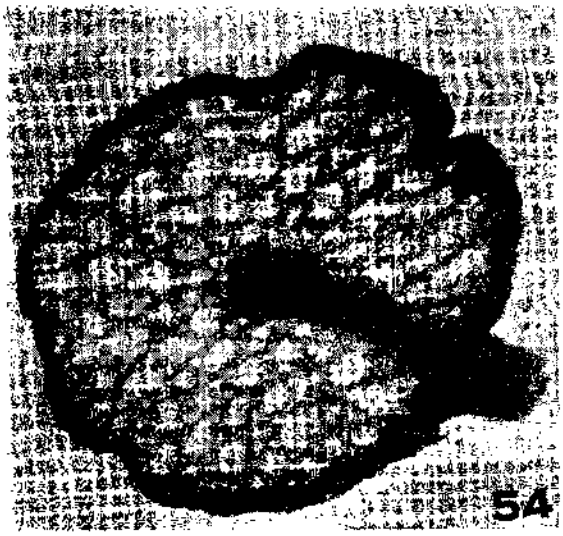


FIG. 53. *Mansonia annulifera* larvae with siphon tips embedded into edge of *Nelumbo nucifera* leaf.

FIG. 54. Cross section through root of *Nymphaea* species showing air chambers within and dense, tough epidermis and cortex.

heart-shaped or arrowhead-shaped leaves are at the end of long, stout peduncles. Some two hundred *Mansonia annulifera* larvae were introduced with the plant, but there were only three temporary attachments to roots, and none at all to the leaves. Although many attempts were made to penetrate the roots, they were almost all unsuccessful, indicating too thick an epidermis and cortex. Attachments from below and into the edge of the leaf were also unsuccessful for the same reason. This plant is therefore of no importance in a consideration of host plants to which *Mansonioides* larvae and pupae attach, based on present observations with *Mansonia annulifera*.

SUMMARY.

The attachment of *Mansonioides* larvae to 12 species of plants growing in tanks or ponds in Kerala was studied in detail. *Mansonia annulifera* larvae attached readily to roots of *Pistia stratiotes*, *Eichhornia speciosa*, *Bacopa monnieri*, *Commelina salicifolia*, *Trapa bispinosa*, *Marsilea quadrifoliata*, and *Limnophila gratioloides*. Considerable attachment to horizontal leaves at the surface of the water was noted in *Pistia stratiotes*, *Azolla pinnata*, *Lemna minor*, *Limnophila gratioloides*, *Nelumbo nucifera*, and *Nymphaea* species. Practically no attachment occurred in *Colocasia antiquorum*. *Mansonia uniformis* was found attached to water hyacinth in large numbers in nature, and comparative studies revealed that *Mansonia annulifera* and *Mansonia uniformis* attached with equal ease to both *Pistia* and *Eichhornia*, with *uniformis* being much more tenacious. Attachment of larvae took place wherever the plant tissue was easily penetrable and contained air chambers for effecting gaseous exchange involved in respiration and excretion, the actual act of attachment being an instinctive type of behaviour. Fifty-four photographs illustrate host plants and the attachment habits of *Mansonia annulifera* and *Mansonia uniformis* larvae.

Indian Journal of Malariology, **13**, *2/3, June/September 1959.*

FILARIASIS IN KERALA STATE.

Part V.

FILARIA SURVEY OF EDAPALLY PANCHAYAT (ERNAKULAM DISTRICT).

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[January 2, 1959.]

EDAPALLY, recorded as a highly filarious area in the erstwhile Travancore State (Iyengar, 1938), borders the town of Ernakulam and lies to its north. It is an extensive rural tract stretching over an area of 32 square miles with a population of 12,393 (1951 census). It is traversed by several channels extending from the back waters on the western side.

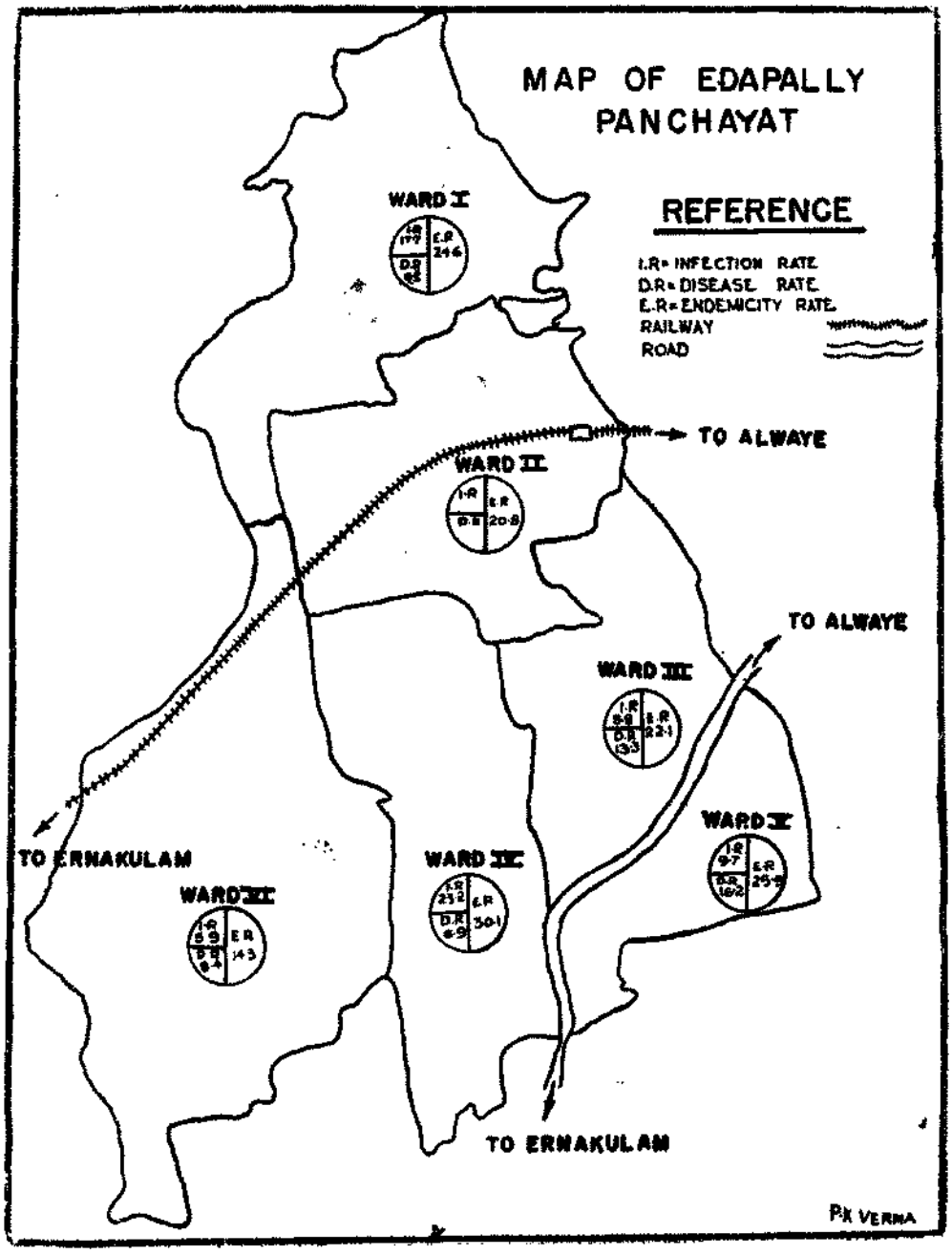
The soil in most parts is sandy and the level of subsoil water is high. Annual rainfall is between 105 and 110 inches and the temperature during a greater part of the year remains between 70°F and 90°F. The humidity is high throughout the year.

The houses are scattered, each house having a compound and one or two ponds overgrown with pistia plants. The people depend mainly on the ponds for the domestic water. Most of the houses are made of plaited cocoanut leaves. There is practically no planned drainage; the porous sandy soil helps absorption of the domestic waste water. The Panchayat has been divided into six wards for administrative convenience (Map 1).

HISTORY OF FILARIASIS AND PREVIOUS SURVEYS.

The entire coastal strip of Kerala is notorious for its filarial endemicity. Edapally Panchayat, with its topographical conditions ideally suited for *W. malayi* endemicity, was surveyed by Iyengar (*1938 loc. cit.*). He recorded a microfilaria rate of 15·7 per cent and a disease rate of 11·04 per cent among the residents, the infection prevalent being exclusively *W. malayi*. He also observed that presence of *Pistia stratiotes* and the local practices associated with the coir industry, like soaking of cocoanut husk in the ponds, were responsible for the prevalence of the vector. These practices still continue.

MAP 1.


MAP OF EDAPALLY PANCHAYAT
REFERENCE
I.R= INFECTION RATE
D.R= DISEASE RATE
E.R= ENDEMICITY RATE
RAILWAY
ROAD
WARD I
I.R 11·7
E.R 24·6
D.R 6·8
TO ALWAYE
WARD II
I.R 6·8
E.R 20·8
TO ALWAYE
WARD III
I.R 9·8
E.R 22·1
D.R 13·3
WARD IV
I.R 9·7
E.R 25·9
D.R 16·2
TO ERNAKULAM
WARD V
I.R 23·2
E.R 30·1
D.R 6·9
WARD VI
I.R 5·9
E.R 14·3
D.R 8·4
TO ERNAKULAM
P.K VERMA

PRESENT SURVEY.

Edapally was surveyed by a team from the Malaria Institute of India during January-February, 1957 with a view to find out if there had been any change in the prevalence of this disease in the course of last two decades since Iyengar (1938 *loc. cit.*) surveyed it. The area covered during the present survey corresponded to the locality referred to as "Edapally North" by Iyengar (1938 *loc. cit.*).

Random representative samples from all the six wards of the Panchayat, covering all age-groups and both sexes, were examined. The examination was made between 8·30 and 11·30 p.m. by a house-to-house visit. About 20 c.mm. of blood from each person from a pricked finger was made into a thick smear and examined, after staining, the next day. The disease manifestations (history of filarial fever, lymphangitis, lymphadenitis, chyluria and elephantoid swelling of the different parts of the body) in these persons were also recorded at the same time. Cases of filarial fever, lymphangitis or lymphadenitis were, however, not taken into consideration for working out the disease rate.

Entomological survey was conducted during the morning hours by collecting adult mosquitoes from houses in the different wards and dissecting all females. Many water collections were examined for detecting the breeding places of vector mosquitoes.

SURVEY FINDINGS.

(*1*) *Microfilaria rate.*—631 persons (5·1 per cent of the total population) were examined during the present survey. The details of the observations are given in Tables I to III. The microfilaria rate ranged from 5·9 per cent to 23·2 per cent in the different wards of the Panchayat. The average for the whole community was 11·7 per cent (Table I). *W. malayi* infection was found in all positive cases except in one case which showed *W. bancrofti*.

TABLE I.

Microfilaria, disease and endemicity rates by wards.

Ward number.	Total population.	Number examined.	Number with microfilaria.	Percentage with microfilaria.	Persons with disease manifestation.	Percentage with disease manifestation.	Endemicity rate.	Average infestation per 20 c.mm.
I	1,818	130	23	17·7	9	6·9	24·6	13·9
II	1,706	172	18	10·4	18	10·4	20·8	5·5
III	2,886	45	4	8·8	6	13·3	22·1	28·25
IV	..	43	10	23·2	3	6·9	30·1	15·4
V	1,900	123	12	9·7	20	16·2	25·9	11·0
VI	4,083	118	7	5·9	10	8·4	14·3	18·8
Total	12,393	631	74	11·7	66	10·4	22·1	12·7

Infection in the different age-groups for the two sexes is indicated in Table II. The overall infection rate for males was 13·5 per cent and for females 10·0 per cent. Infection in children up to 5 years was definitely lower than that recorded in other

TABLE II.

Microfilaria and disease rates by age and sex.

Age group.	BOTH SEXES :					MALES :					FEMALES :				
	Number examined.	Number with Mf.	Percentage with Mf.	Number with disease.	Percentage with disease.	Number examined.	Number with Mf.	Percentage with Mf.	Number with disease.	Percentage with disease.	Number examined.	Number with Mf.	Percentage with Mf.	Number with disease.	Percentage with disease.
Up to 5 years	24	1	4·1	0	0	8	0	0	0	0	16	1	6·2	0	0
6—10	55	7	12·7	0	0	25	3	12	0	0	30	4	13·3	0	0
11—20	135	16	11·8	5	3·7	76	11	14·4	2	2·6	59	5	8·9	3	5
21—30	149	19	12·7	13	8·7	74	9	12·1	9	12·1	75	10	13·3	4	5·2
31—40	118	12	10·1	17	14·4	75	10	13·3	11	14·6	43	2	4·4	5	13·9
41—50	69	7	10·1	13	18·8	32	3	9·3	7	21·8	37	4	10·8	6	16·2
Above 50	81	12	14·8	18	22·2	42	8	19	9	21·4	39	4	10·2	9	23
Total	631	74	11·7	66	10·4	332	44	13·5	38	11·4	299	30	10·0	28	8·4

Mf. = Microfilaria

age-groups. There is a general tendency for the infection rate to rise gradually up to the age of 20 or 30 in both the sexes. Afterwards, it remains more or less fluctuating. This is in accordance with the observations recorded earlier by Iyengar (1938 *loc. cit.*). The youngest age showing microfilaria was in a girl of five-years old.

Average microfilarial infestation per 20 c.mm. of blood for both sexes of the different age-groups is recorded in Table III. Average infestation in the whole community was 12·7. There is more or less a general tendency for the infestation to rise gradually from the lower age-group to higher age-group up to 31-40 years, following which a fall is observed. Highest count recorded was 74 in a male aged 31 years.

TABLE III.

Average infestation by age-group and sex.

Age group.	BOTH SEXES :		MALES :		FEMALES :	
	Number with Mf.	Average infestation.	Number with Mf.	Average infestation.	Number with Mf.	Average infestation.
Up to 5 years	1	1	0	0	1	1
6—10	7	6·5	3	6·6	4	6
11—20	16	7·8	11	8·1	5	7·2
21—30	19	14·5	9	22·6	10	6·3
31—40	12	27·1	10	31·6	2	3
41—50	7	13·2	3	19·6	4	8·5
Above 50	12	7·7	8	9·8	4	3·5
	74	12·7	44	17·7	30	5·9

(2) *Filarial disease.*— 10·4 per cent of the people examined had disease manifestations in the form of swelling of the lower or upper extremity or both. A few cases of hydrocele were also recorded; these persons had swelling of the legs in addition.

The incidence of the disease in the different age-groups is presented in Table II. No disease manifestation was recorded below the age of 10. There was a progressive rise in the disease rate in the higher age-groups. The youngest age at which swelling of the leg was observed was in a girl of 12 years. Classification of the elephantoid condition, according to the parts affected, is given in Table IV below.

TABLE IV.

Classification of the elephantoid condition according to the parts affected

Parts affected.	NUMBER :		Total.
	Unilateral.	Bilateral.	
Leg ...	38	13	51
Hand ...	5	3	8
Both extremities ...	..	..	7
Hydrocele ...	..	..	2

No person with disease manifestation was found to harbour microfilaria among those examined in the present survey.

(*3*) *Endemicity.*—The filarial endemicity was 22·1 per cent for the whole area. The rate for the different wards varied from 14·3 to 30·1 per cent (Table I).

(*4*) *Vector.*— 253 adult mosquitoes were collected from the different wards of the Panchayat. The following species were recorded :

1. *M. annulifera*
2. *C. gelidus*
3. *C. lutzia*
4. *C. sitiens*
5. *C. vishnui*
6. *A. subpictus*
7. *Armegeres obturbans.*

205 of these mosquitoes, representing all the species, were dissected but developing stages of filarial infection were found only in *M. annulifera*. Out of 31 females of this species dissected, 4 (12·9 per cent) showed filarial infection. Two mosquitoes showed second-stage larvae, one mosquito showed third-stage and one showed fourth-stage larvae. The infectivity rate thus worked out to 3·2.

M. annulifera appears to be the only vector in the area.

Breeding of *Mansonioides* sp. was noted in ponds with *Pistia stratiotes* with organic pollution.

DISCUSSION.

Edapally lies adjacent to Ernakulam Municipality. The latter place was surveyed by Jaswant Singh *et al.* (1956) who concluded that the infection in the thickly populated central part of the town was purely *W. bancrofti*, whereas in the outskirts either *W. malayi* or mixed infections of *W. malayi* and *W. bancrofti* were prevalent. The present survey indicates that in the rural areas, immediately bordering this town, the infection is purely *W. malayi*. One case of *W. bancrofti* infection as also two cases of filarial scrotum, a lesion generally associated with *bancroftian* filariasis, were also recorded during the survey. All these three persons on enquiry, gave a history of having lived in Ernakulam for a long time.

A comparative statement of the findings recorded by Iyengar (1938 *loc. cit.*) and those recorded during the present survey is given in Table V.

TABLE V.

Filariasis survey data—Edapally Panchayat.

Year.	Number examined.	Microfilaria rate per cent.	Disease rate per cent.	Reference.
1938	471	15·7	11·04	Iyengar (1938)
1957	631	11·7	10·4	Present survey.

The results of the present survey indicate that the incidence of filariasis in the area has not altered to any marked degree during the past 25 years. The disease rate has remained almost the same; though a slight fall (not statistically significant) was recorded in the microfilaria rate. No control measures have been in force during these years.

SUMMARY.

1. Edapally Panchayat, with a population of 12,393 was surveyed during January-February, 1957. 631 persons were examined.

2. The microfilarial, filarial disease and endemicity rates for the area were 11·7, 10·4, and 22·1 per cent respectively. The average infestation was 12·7 per positive smear of 20 c.mm. of blood.

3. *W. malayi* was the predominent species of infection in the area.

4. The vector incriminated was *M. annulifera* with an infection rate of 12·9 per cent and an infectivity rate of 3·2 per cent.

ACKNOWLEDGMENT.

The authors wish to record their grateful thanks to the President of the Edapally Panchayat for help during the survey and to Mr. S.S. Nair, M.Sc., Deputy Asstt. Director, Malaria Institute of India, for statistical analysis.

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Indian Journal of Malariology, **13**, 2/3, June/September 1959.

DEVELOPMENT OF INCREASED TOLERANCE TO D.D.T. IN *A. CULICIFACIES* GILES IN THE PANCH MAHAL DISTRICT OF BOMBAY STATE (INDIA).

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[September 30, 1959.]

DURING the course of malaria survey of different areas of the Bombay and Rajasthan States, an increased preponderance of *A. culicifacies* was noticed in the villages of Khunpeer and Sindbhai Mehta of the Taluka Halol of the district of Panch Mahal in Bombay State. The villages are situated within one mile from each other. This area was under continuous indoor residual spraying of D.D.T. since the year 1950. It had received three rounds of spraying with a dosage of 56 mg. per square foot every year for the first three years which was followed by two rounds of spryaing each year with a dosage of 112 mg. per square foot excepting for the years 1956 and 1957 when only one round of spray was given with a dosage of 112 mg. per square foot. The first round of spraying for the year 1959 was carried out in these villages on July 10, 1959. The attention of the team was specially drawn to this area due to the fact that, even after ten years of indoor residual spraying, low grade transmission of malaria was still going on, as revealed by the infant parasite survey. The sudden increase in the density of *A. culicifacies* after the recent heavy rain-fall made it imperative to determine its susceptibility status.

Two experiments, each consisting of four tests, were therefore carried out in this area according to the World Health Organization technique during the period from September 19 to 26, 1959. The mosquitoes were collected in the morning hours by the aspirators from cattlesheds of typical rural mixed dwellings. Selective type of collection was made in which only freshly-fed adult female *A. culicifacies* were chosen. The average man-hour density was 180. The actual experiments were carried out in a room of the Taluka Dispensary, Halol, about two miles away from the place of mosquito collections. The impregnated paper of the World Health Organization Kit, used for the experiments, were valid up to February 15, 1961. The temperature of the room, where the experiments were performed, varied between 76°F., and 94°F., and the relative humidity was between 87 and 91 per cent. The results of these two experiments are given in Tables I and II.

TABLE I.

Result of Experiment I—Village Sindbhai Metha (from September 19 to 23, 1959).

D.D.T. concentration (per cent).	Test 1:			Test 2:			Test 3:			Test 4:			Grand total:			Percentage of mortality.
	A.	D.	Total.	A.	D.	Total.	A.	D.	Total.	A.	D.	Total.	A.	D.	Total.	
0·5	18	2	20	19	1	20	18	2	20	18	2	20	73	7	80	8·8
1·0	18	2	20	16	4	20	19	1	20	19	1	20	72	8	80	10
2·0	13	7	20	10	10	20	7	13	20	13	7	20	43	37	80	46·3
4·6	7	13	20	3	17	20	5	15	20	4	16	20	19	61	80	76·3
Control: (oil alone)	20	0	20	19	1	20	20	0	20	18	2	20	77	3	80	3·8

A. = Number alive D. = Number dead.

TABLE II.

Result of experiment II—Village Khunpeer (from September 24 to 26, 1959).

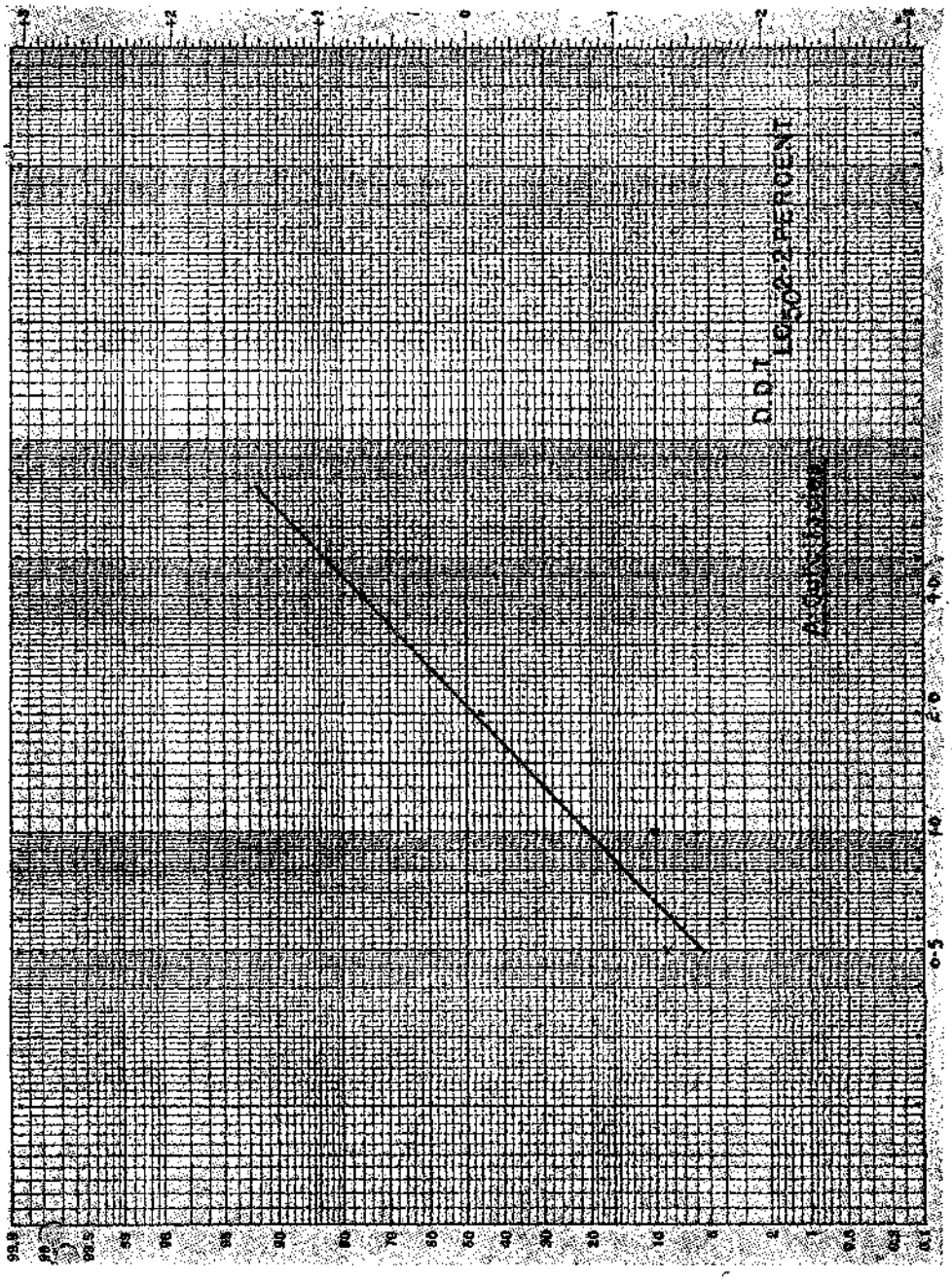
D.D.T. concentration. (per cent)	Test 1:			Test 2:			Test 3:			Test 4:			Grand Total:			Percentage of mortality.
	A.	D.	Total.	A.	D.	Total.	A.	D.	Total.	A.	D.	Total.	A.	D.	Total.	
0·5	18	1	19	18	2	20	20	0	20	18	2	20	74	5	79	6·3
1·0	13	7	20	13	7	20	10	3	13	19	1	20	55	18	73	24·7
2·0	8	12	20	14	6	20	11	10	21	10	10	20	43	38	81	46·9
4·0	5	15	20	1	18	19	6	14	20	5	13	18	17	60	77	77·9
Control: (oil alone)	18	1	19	19	1	20	21	0	21	20	0	20	78	2	80	2·5

A. = Number alive D. = Number dead.

The results, as plotted out in the logarithmic probability paper, for the experiments I and II showed approximately the LC_{50} at 2·2 per cent and 2·05 per cent respectively (Charts 1 and 2).

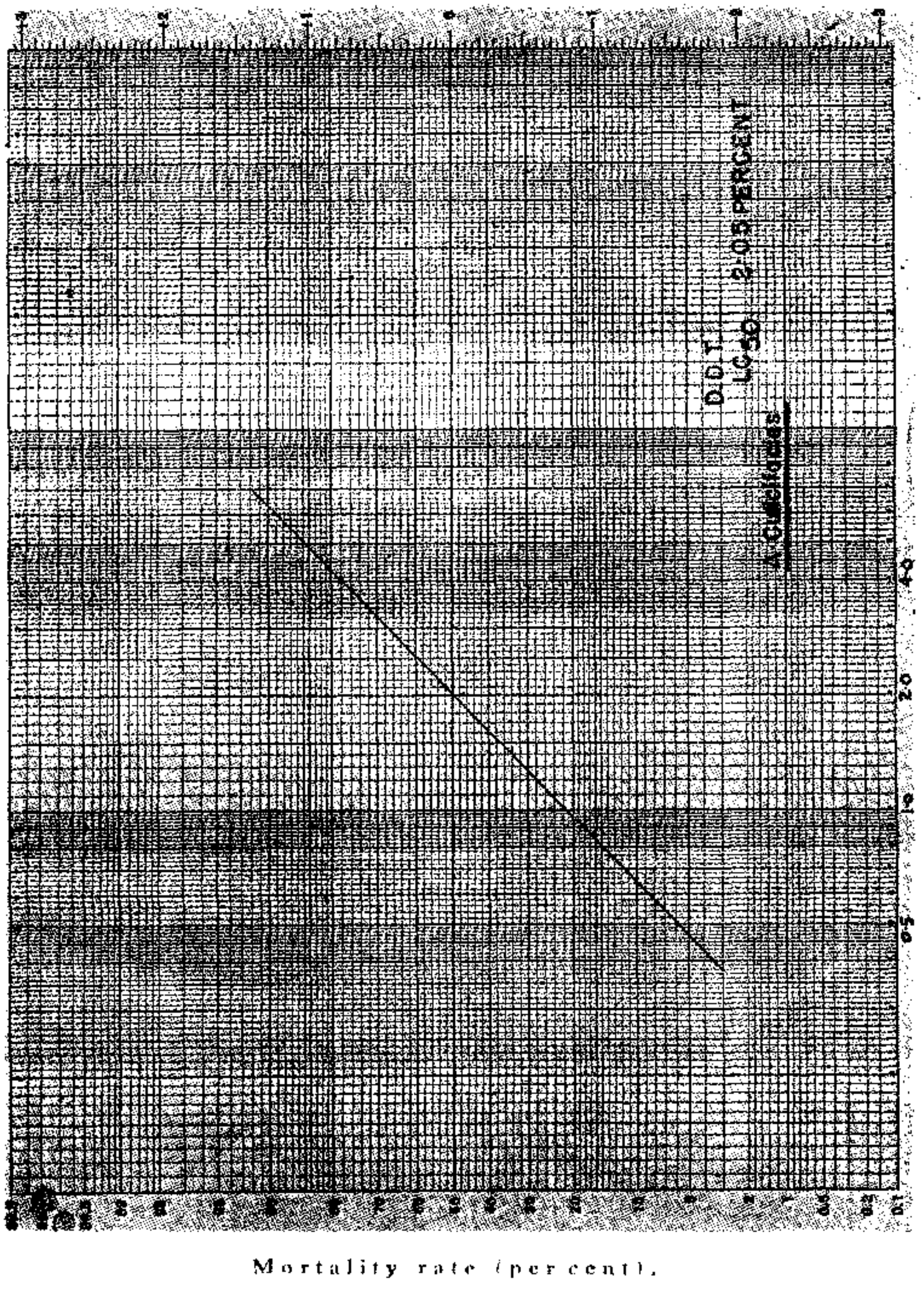
It is significant that the experiments on susceptibility status of *A. culicifacies* which were carried out independently by Dr. A.M. Shalaby, Entomologist, World Health Organization, A.T.M.E. No. 2 at village Motipura of Taluka Baria of the same district at a distance of about 30 miles from the place of these experiments, showed more or less similar results.

It, therefore, shows that LC_{50} has at least increased 3 to 11 times during the last three years than that previously reported by other workers as summarised in Table III. This increased LC_{50} indicates that tolerant strain might be gradually building up.


D.D.T. LC50-2-PERCENT
Concentration (per cent.)
Mortality rate (per cent).

Graph 1.

CHART 2.


D.D.T.
LC 50 2.05 PERCENT
4.0
2.0
1.0
0.5
Concentration (per cent).
Mortality rate (per cent).

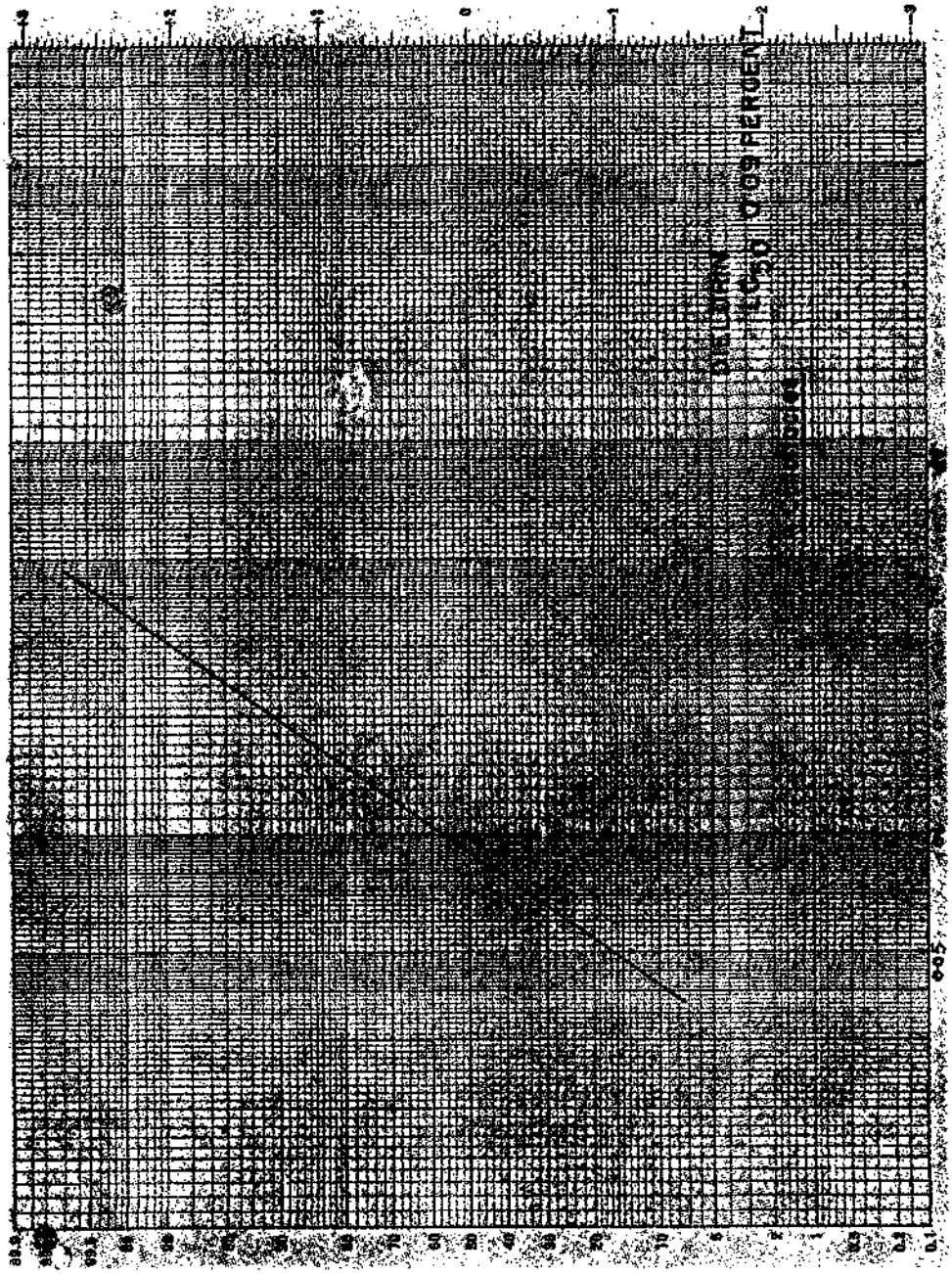

LC50 0.09 PERCENT
Concentration (per cent).
Mortality rate (per cent).

CHART 3.

TABLE III.

LC_{50} of adult female A. culicifacies *in respect of D.D.T. as determined by other workers during the years 1956 to 1958.*

Year of experiment.	Place.	Name of workers.	LC_{50} of D.D.T.
1956	(*a*) Bombay State.	Ramachandra Rao and Bhatia.	0·5–0·6 Per cent
	(*b*) Revti, Bombay State.	Ramachandra Rao and Bhatia.	0·41 Per cent
1957	(*a*) Arthala, Utter Pradesh.	Sharma *et al.*	0·43 Per cent
	(*b*) Kailashpuri, Rajasthan.	Sharma *et al.*	0·19 Per cent
Sep. 1957	Mandya (Mysore).	Sundar Rao, Sitaraman and Rama Rao.	0·25 Per cent
Oct. 1958	Potgaon (Bombay State).	Patel *et al.*	0·84 Per cent

It is of interest to note that the tests performed on the susceptibility status of *A. culicifacies* to dieldrin in the village Khunpeer gave LC_{50} of 0·09 per cent whereas in the Thana District of Bombay State it was reported to be over 1·6 per cent in 1958 (Patel *et al.*, 1959). *A. culicifacies* in the former area, therefore, though tolerant to D.D.T., was susceptible to dieldrin. (Table IV and Chart III).

TABLE IV.

Village Khunpeer (September 12 to 17, 1959).

Dieldrient concentration (per cent).	Test 1:			Test 2:			Test 3:			Test 4:			Grand Total:			Percentage of mortality.
	A.	D.	Total.	A.	D.	Total.	A.	D.	Total.	A.	D.	Total.	A.	D.	Total.	
0·05	16	4	20	16	4	20	17	3	20	20	0	20	69	11	80	13·8
0·1	11	9	20	6	14	20	7	13	20	7	13	20	31	49	80	61·3
0·2	2	18	20	1	19	20	1	19	20	1	19	20	5	75	80	93·8
0·4	1	19	20	0	20	20	0	20	20	0	20	20	1	79	80	98·8
Control: (oil alone)	19	1	20	20	0	20	19	1	20	20	0	20	78	2	80	2·5

A. = Number alive D. = Number dead.

As this species of mosquito is a major vector of India, the evidence of its increased tolerance to D.D.T. is an alert signal for all areas which are under its influence. In view of the fact that the country has entered into the second year of the attack phase of the National Malaria Eradication Programme, the urgency and importance of carrying out larger number of susceptibility tests all over the country cannot be overemphasised.

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Indian Journal of Malariology, **13**, 2/3, June/September 1959.

STUDIES ON PARTICLE SIZE DISTRIBUTION IN RELATION TO DISPERSIBILITY.

Part II

75 PER CENT DIELDRIN WATER DISPERSIBLE POWDERS AND GENERAL CHARACTERISTICS OF INSECTICIDE WATER DISPERSIBLE POWDERS.

BY

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[January 31, 1959.]

STABILITY of aqueous suspensions, prepared from insecticide water dispersible powders, is mainly governed by the size of the particles constituting the powder, quality and quantity of wetting and dispersing agents used and electro-kinetic state of the aqueous suspension. Size of the powder particles is, however, considered the most important. Bami and Cheema (1957) studied the particle size distribution of D.D.T. and clay constituents of several commercial samples of 50 per cent D.D.T. water dispersible powder with varying suspensibility values (World Health Organization, 1956). Particle size distribution of D.D.T. and clay (inert diluents) in these cases was determined, both before and after subjecting the material to accelerated tropical storage (World Health Organization, 1956) by sedimentation method as adopted by Thomas (1956). Particle size distribution of pure clay, as separated from the formulation, was also determined separately. Further extension of the above work by Bami, Cheema and Dhatt (1958) led to the study of 75 per cent D.D.T., 50 per cent B.H.C. and 50 per cent dieldrin water dispersible powders. In the present paper, studies on composition, physical properties and particle size distribution of a commercial formulation of 75 per cent dieldrin water dispersible powder have been reported. Based upon the data obtained so far in the case of above type of insecticide water dispersible powders, certain relationships between suspensibility, as determined by the World Health Organization specification method (1956) and particle size distribution of the insecticide constituent, have been established and their significance discussed.

75 PER CENT DIELDRIN WATER DISPERSIBLE POWDER.

A commercial sample of 75 per cent dieldrin water dispersible powder was analysed for its composition and physical properties, especially suspensibility (Table I). Data on particle size distribution of dieldrin and inert clay constituents

have been presented in Graph 1 in a manner adopted previously (Bami, Cheema and Dhatt, 1958). Composition of 75 per cent dieldrin water dispersible powder, was found to be similar to that of 75 per cent D.D.T. water dispersible powder with the added advantage that suspensibility values were above 90 per cent in the former case. Particle size distribution of dieldrin also revealed that nearly 90 per cent by weight of the dieldrin content was below 10 microns particle size. This observation confirmed the previous findings that suspensibility value could directly represent percentage weight of the insecticide below 10 microns particle size. From Graph 1, it was also evident that fineness to which dieldrin was ground, almost approached that of the clay (inert carrier). Apparently, high suspensibility value in this case had been mainly achieved by means of extremely fine micronisation of dieldrin. Accelerated tropical storage treatment (World Health Organization, 1956) also did not change the particle size distribution picture (Graph 1) as supported by absence of any change in suspensibility values (Table I).

TABLE I.

Analysis of 75 per cent dieldrin water dispersible powder.

Serial number.	Property tested.	Results.
1	*Composition* (per cent)	
	(*a*) Dieldrin content ...	74·8
	(*b*) Clay (inert carrier content) ...	15·0
	(*c*) Wetting and dispersing agents (including other water soluble ingredients like stabilizers) ...	10·0
2	*Physical Properties* :	
	(*a*) pH ...	Neutral
	(*b*) Suspensibility (per cent) ...	91·1
	(*c*) Suspensibility after accelerated tropical storage (per cent) ...	90·6
	(*d*) Sieve analysis after tropical storage (per cent retained on 74 micron sieve) ...	0·0

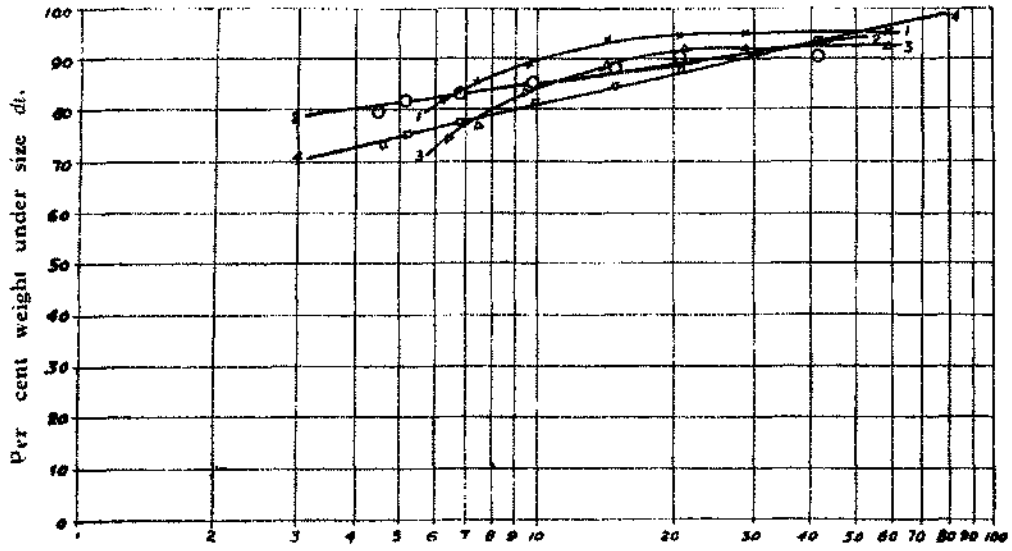
INSECTICIDE WATER DISPERSIBLE POWDERS.

A large number of different commercial samples of D.D.T., B.H.C., dieldrin and dieldrin-resin water dispersible powder, have been analysed for their suspensibility and particle size distribution of their active and inert ingredients both before and after accelerated tropical storage. In order to draw a broad correlation between suspensibility (World Health Organization, 1956) and size of the insecticide particles constituting a powder, all the available data, (Bami, *et al.*, 1957 ; 1958) has been plotted in Graph 2. In this graph, percentage weight of the insecticide of particle sizes below 10, 20 and 40 microns have been separately plotted against suspensibility value of each of the formulations. Within limits of analytical methods used, Graph 2 would indicate the particle size distribution picture of any insecticide

GRAPH 1.

75 per cent dieldrin water dispersible powder.

1. Dieldrin in the formulation.
2. Clay in the formulation.
3. Dieldrin after accelerated tropical storage.
4. Clay after accelerated tropical storage.

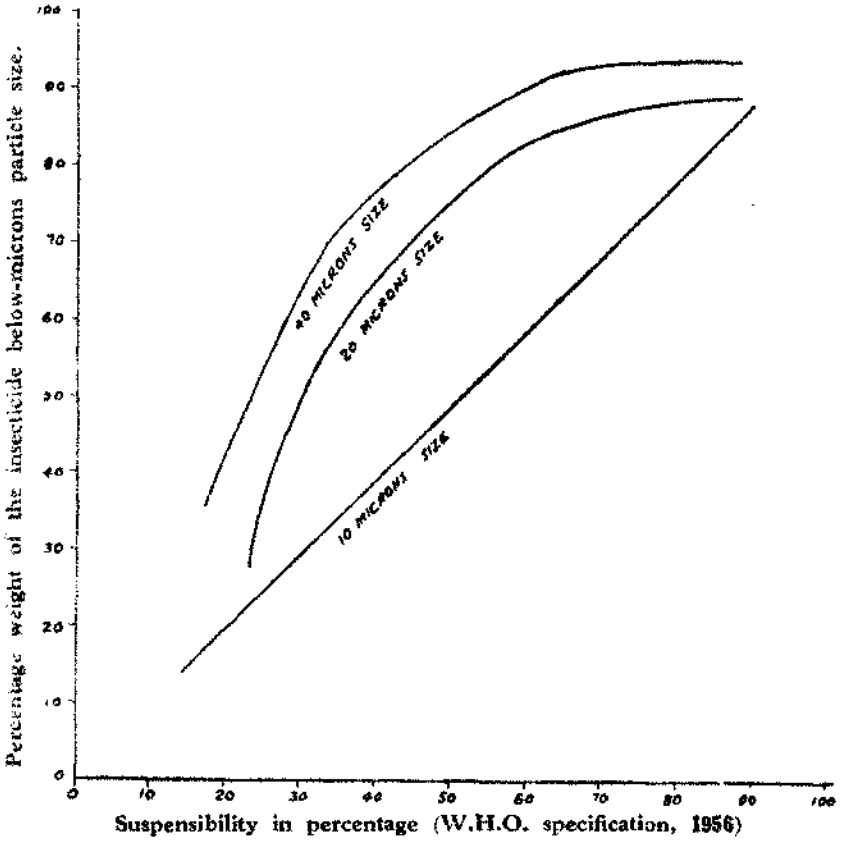


which has been formulated into a water dispersible powder of a given suspensibility. Some of the broad observations, which have been based on the available data presented in this paper (Graphs 1 and 2) and the previous communications (Bami, 1957 ; 1958 ; Bami and Cheema, 1957 ; Bami, Cheema and Dhatt, 1958), are summarised below :—

(*a*) Except in case of dieldrin water dispersible powder, constituents of D.D.T. and B.H.C. powders were generally found to be coarser than their clay (carrier) components. Dieldrin alone appeared to be ground to a fineness approaching that of the inert carrier itself. If suspensibility values (both before and after tropical storage treatment) of these powders were compared with percentage weight of the insecticide in the formulation below 10 microns particle size, they were found to be nearly equal. This meant a linear relationship (within the limits of accuracy of the analytical methods used) between suspensibility and percentage weight of the insecticide below 10 microns particle size. Percentage weight of the insecticide below 20 and 40 microns particle size in the case of a standard water dispersible powder (suspensibility above 50 per cent) would be usually more than 75 and 85 per cent respectively (Graph 2). Obviously, as there was a limit to which the dispersing agents alone could increase the suspension stability, their functions have been conveniently and economically replaced by ultra fine grinding of the active ingredient along with highly adsorbent inert carriers (Graph 1).

GRAPH 2.

Relationship between suspensibility and size of insecticide particles in case of D.D.T., B.H.C. and dieldrin water dispersible powders.



(*b*) Effect of tropical storage on the particle size distribution of insecticide constituent of products conforming to the Indian Standards Institution and the World Health Organization specifications, was also usually very small. In other words, in order to meet the specifications, the products should not only have a high initial suspensibility but also a limited tendency to agglomerate (increase in particle size of clay and insecticide constituent) which was otherwise a major cause of loss of suspensibility. In case of products below the World Health Organization specifications on suspensibility, the insecticide particles had not only been coarse initially but there was also an excessive agglomeration of these particles on tropical storage pre-treatment, resulting in considerable loss of suspensibility. However, general relationship between suspensibility and percentage weight of insecticide below 10 microns particle size was maintained even in these cases.

(*c*) Particle size distribution of pure clay (inert carrier, bulking material etc., as separated from the formulation) in all cases, indicated that 100 per cent of this

constituent had a particle size of below 40 microns, while nearly 80 per cent of it was below 10 microns. The carrier used in the case of 75 per cent dieldrin water dispersible powder (Graph 1), was found to be even finer than those used in other insecticide formulations.

(*d*) Particle size distribution of clay in the formulation, indicated it to be slightly coarser when compared to that of the same clay separated from the formulation. This was due to some adsorption and agglomeration of the insecticide constituent with clay particles during grinding. Tropical storage treatment (World Health Organization, 1956) of the formulations, however, resulted in slight to no increase in the size of clay particles. This could be correlated with limited loss of suspensibility displayed by these formulations when treated similarly. Use of fine and highly adsorbent carrier with low bulk density have generally offered products which have very limited tendency for agglomeration of fine particles with consequent little loss of suspensibility.

(*e*) In the present investigations, wetting and dispersing agents, stabilizers and other water soluble materials have been estimated together and amounted to nearly 10 per cent in most cases. No attempt was made to qualitatively compare these products in different formulations. However, next to fine particle size of the clay and insecticide constituents, the quality and quantity of these chemicals was very important for initial dispersion of the powder in the aqueous phase and subsequent stability of the suspension. Loss of suspensibility on accelerated tropical storage, if any, was mostly due to agglomeration of powder particles. However, loss of suspensibility of a product on long term actual tropical storage could not be explained on the basis of increase of particle size of the powder alone and was at least partly due to deterioration of the dispersing agents.

(*f*) Products meeting the World Health Organization specifications (1956) on suspensibility, were generally neutral or well within the indicated limits of alkalinity and acidity. There has hardly ever been a case where a product was unacceptable on this ground only.

DISCUSSION.

For efficient use of insecticide water dispersible powders, it was essential that these materials should offer a stable aqueous suspension and the material should also be fine enough not to clog the spraying equipments. These two requisites have been adequately met in the case of powders which come up to specifications of the World Health Organization or of the Indian Standards Institution for different insecticide water dispersible powders. However, the present overall studies on physical characteristics of different insecticide water dispersible powders obtained from various commercial sources have made it clear that for standard products, particle size distribution picture was nearly indetical. Quality and quantity of the wetting and dispersing agent used, though important, was only second to the extent to which the constituents had been ground.

From the present data, it was quite evident that high quality water dispersible powders would mean insecticide particles of extremely low micron size. This has to be viewed from the background of sorption of fine insecticide particles on adsorbent mud surfaces. Studies on loss of insecticide through sorption on mud-surfaces have already shown (Hadaway and Barlow, 1952*a*, 1952*b*; Sharma, *et al.*, 1957) that the finer the size of the insecticide particles, the higher the loss on active mud surfaces. In fact, particles below 10 microns size were very extensively and rapidly lost on mud surfaces while similar loss was also quite appreciable even up to particles of 20 microns size. On the other hand, it has been also proved that nearly 50 per cent of the insecticide in a standard water dispersible powder (50 per cent suspensibility) would have a particle size of less than 10 microns and these particles would be prone to excessive loss under these circumstances. Evidently, to meet the minimum standard for suspension stability, a certain degree of micronisation of insecticides was unavoidable. However, suspensibility values much above the minimum standard of 50 per cent, which could only be achieved at the expense of reducing the particle size still further (up to 90 per cent being below 10 microns particle size), were not wholly desirable especially in view of the greater risk of loss of such fine particle through sorption. In fact, the present minimum requirement of 50 per cent suspensibility (Indian Standards Institution, 1955; World Health Organization, 1956) was a good compromise in these two respects.

SUMMARY.

Particle size distribution of dieldrin and clay constituents of 75 per cent dieldrin water dispersible powder was determined by sedimentation method. Corresponding to a suspensibility of 90 per cent, nearly 90 per cent of the dieldrin content by weight was found to be below 10 microns particle size.

Based upon previous and present observations, general characteristics of different commercial D.D.T., B.H.C. and dieldrin water dispersible powders have been outlined. Particle size distribution of the active toxicant had a direct relationship with suspensibility, and high degree of suspension stability in these cases was mainly achieved through excessive grindings of the insecticides.

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Indian Journal of Malariology, **13**, 2/3, June/September 1959.

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

Part XXVI.

THE MINIMUM DURATION OF PATENT PRIMARY PARASITAEMIA IN ALBINO RATS FOR THE DEVELOPMENT OF IMMUNITY TO RESIST RE-INFECTION.

BY

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[June 30, 1959.]

A PORTION of adult rats infected with *P. berghei* succumb to the infection during the course of primary patent parasitaemia. The remainder that survive the primary episode, develop a latent infection which lasts for varying periods during which they are immune to homologous superinfection (Ramakrishnan, Satya Prakash and Krishnaswami, 1951). The object of the study presented in this paper was to determine the minimum period of patent primary parasitaemia necessary for the animal to become immune against homologous re-infection.

MATERIAL AND METHODS.

The albino rats used in the investigation were adults from the colony‡ maintained at the Malaria Institute of India, Delhi. In the majority of the series, the two sexes were equally represented. A total of 124 animals were used for the study. A minimum of 8 and a maximum of 20 animals were inoculated each time to determine the minimum duration of primary parasitaemia which can confer sufficient immunity after terminating the infection to resist the challenge inoculation.

The method and dose (one million) of inoculation, daily examination of blood smears from the animals, staining technique and enumeration of parasites were the same as described by Ramakrishnan and Satya Prakash (1950).

*The strain of *P. berghei* used in the experiment is being maintained in rats at the Institute since 1952 and was originally obtained from London through the courtesy of Brig. J.S.K. Boyd of the Burroughs Wellcome Laboratories.

†This paper forms part of a thesis for the doctorate degree of the Punjab University.

‡The colony diet consists of :

Whole wheat flour	...	...	72 parts.
Skimmed milk powder	...	...	23 parts.
Brewers yeast dry	...	...	3 parts.
Calcium carbonate	...	...	1 part.
Common salt	...	...	1 part.

There were certain departures from routine methods that were considered necessary for the study. In order to test the response to re-inoculation, it was necessary to terminate the primary parasitaemia progressively on each day of its course and to prove that the cure affected was a radical one.

The dose of sulphadiazine determined to be necessary for radical cure of the infection, was a single dose of 1·2 mg. per 20 gm. body weight of the animal, administered intraperitoneally (Ramakrishnan *et al.*, 1951*b*). In order to be certain beyond any doubt that the cure effected was radical, each animal was administered 1·2 mg. sulphadiazine per 20 gm. body weight once a day intraperitoneally for 3 consecutive days, i.e. three times the required curative dose. Each animal so treated was rested for at least seven days before being subjected to any further experimental procedure.

It was necessary to demonstrate that the treatment resulted in sterilising the host of its infection. The normal isodiagnostic procedure employed in routine experimental malariology, of inoculating a small quantity of blood from the animal under observation into susceptible clean animals, has been shown to be inadequate by Sergent (1954). A negative inoculation of this nature does not rule out the existence of latent infection in the former. A total infectivity test was described by him in which he inoculated into susceptible animals the total mass of blood of the animal under investigation, sacrificed by exsanguination and also to include in the inoculum homogenised internal organs of the donor animal. In the present study, this procedure of total infectivity test was adopted to confirm that the animal underwent a radical cure.

This test was terminal and the same animal could not be used to determine the immunity status conferred on the animal by the respective duration of the primary patent parasitaemia. It was, therefore, necessary to utilise sufficient number of animals in order to subject half of them to the total infectivity test to confirm the absence of any latent infection in them ; and to subject the remaining half, similar in all other respects, to demonstrate the presence or absence of immunity to a challenge homologous infection.

For the total infectivity test, inoculations were made into *R. rattus* specimens (Ramakrishnan and Satya Prakash, 1950) obtained from the Delhi City rat catches made by the municipal authorities. Approximately six specimens were used to sub-inoculate the blood and homogenised internal vicera of each cured albino rat. The blood of the inoculated animal was examined every day for a minimum period of 10 days. Over 300 *R. rattus* were utilized in these tests.

RESULTS.

Of the 124 albino rats experimented upon, 21 died before the final stages of the experiment. The remaining 103 animals were cured on specific progressive days of primary parasitaemia. The cure was effected at intervals ranging from 2 to 15 days of patent parasitaemia. The interval between the commencement of the

cure and the subinoculation test for infectivity or challenge infection varied from 11 to 24 days. The total infectivity test proved negative in each of the 51 animals submitted to it, proving that the chemotherapy proved successful and every time effected a radical cure, sterilising the infection in the treated animals.

Table I gives the protocols of the animals used in the study.

TABLE I.

Protocols of albino rats cured from first to fifteenth day of parasitaemia, showing the results of challenge infection and total sub-inoculation.

Number of animals used.	Sex : Male.	Sex : Female.	Day of patent parasitaemia on which treatment was commenced.	Date of inoculation.	Date of commencement of treatment.	Number of animals : (a) For challenge.	Number of animals : (b) Sub-inoculation.	Result, number positive/negative : (a) Challenge infection.	Result, number positive/negative : (b) Sub-inoculation.	REMARKS.
8	4	4	2	May 30, 1955	June 1, 1955	3	4	3/0	0/4	One animal died.
8	4	4	3	May 30, 1955	June 2, 1955	3	3	3/0	0/3	Two animals died.
8	4	4	4	June 4, 1955	June 8, 1955	4	4	4/0	0/4	..
8	8	..	5	June 6, 1955	June 21, 1955	4	4	4/0	0/4	..
10	5	5	6	Aug. 9, 1955	Aug. 15, 1955	5	5	5/0	0/5	..
10	5	5	7	Aug. 17, 1955	Aug. 24, 1955	4	4	4/0	0/4	Two animals died.
8	1	7	8	June 11, 1956	June 19, 1956	4	3	3/1	0/3	One animal died.
8	4	4	9	Jan. 6, 1956	Jan. 15, 1956	4	4	0/4	0/4	Two experiments.
8	5	3	9	July 24, 1956	Aug. 2, 1956	5	2	3/2	0/2	One died.
8	4	4	10	Jan. 16, 1956	Jan. 26, 1956	2	3	0/2	0/3	Two experiments.
10	10	..	10	Sept. 3, 1956	Sept. 13, 1956	4	4	4/0	0/4	Five animals died.
10	5	5	11	Oct. 22, 1956	Nov. 2, 1956	5	5	0/5	0/5	Two experiments.
10	10	..	11	Oct. 25, 1956	Nov. 5, 1956	3	2	0/3	0/2	Five animals died.
10	5	5	15	Nov. 1, 1955	Nov. 16, 1955	2	4	0/2	0/4	Four animals died.
124	74	50				52	51	33/19	0/51	

The remaining fifty two animals were submitted to a challenge homologous re-infection. Twenty-three of this group were cured on days 2, 3, 4, 5, 6 and 7 of the primary parasitaemia ; every one of these developed the infection following the challenge showing that up to the seventh day of primary parasitaemia, the acquired immunity was insufficient to resist the challenge re-infection. Of the 4, 9 and 6 animals that were cured on the eighth, ninth and tenth day of primary parasitaemia respectively, 1, 6 and 2 animals gave a negative response to the challenge re-infection. It appeared that the immunity, developed during 8-10 days of parasitaemia, was sufficient to resist the challenge re-infection in a proportion but not all of the animals. All the 8 rats cured on the eleventh and two rats cured on the fifteenth day of primary parasitaemia, were able to totally resist the challenge infection. It would thus appear that a primary parasitaemia of 11 days or more

was sufficient to confer adequate immunity in the albino rats to resist a challenge re-infection.

The blood smears of the experimental animals were obtained only at 24 hour intervals. The available data on the prepatent periods, after the primary and the challenge inoculations compared in 52 animals cured on different days of primary patent parasitaemia, is contained in Table II.

TABLE II.

Average prepatent period after primary and challenge inoculation in albino rats radically cured of the primary parasitaemia of different intervals.

Number of animals.	Average prepatent period in days after primary inoculation.	Day of patent parasitaemia on which treatment was commenced.	Interval in days between completion of cure and challenge inoculation.	Average prepatent period in days after challenge inoculation.
3	0·0	2	At least 8 days	0·0
3	0·0	3	-do-	0·0
4	0·0	4	-do-	0·0
4	0·0	5	-do-	2·5
5	0·0	6	-do-	1·2
4	0·0	7	-do-	2·5
4	0·0	8	-do-	3·6*
4	0·0	9	-do-	3·6†
6	0·0	10	-do-	4·0‡
8	0·0	11	-do-	All negative to re-inoculations.
2	1·0	15	-do-	All negative to re-inoculations.
52				

*One animal was negative to challenge infection.
†Only three animals were positive to re-inoculation.
‡Only four animals were positive to inoculation.

It is seen from the data in Table II that the average prepatent period, after the primary inoculation, varied from 0 to 1 day. It was similar in the case of the challenge re-infection in the 7 animals that were cured on 2, 3 and 4 days respectively, indicating that the acquired immunity conferred by the respective durations of primary parasitaemia was little or nil. The prepatent period for the challenge re-infection of animals cured on the fifth to the tenth day, showed a progressive increase from an average of 2·5 to 4 days.

The duration of patent parasitaemia, following challenge re-infection, also shows a definite correlation with that of primary parasitaemia in the respective animal. It was observed that animals which ran an uninterrupted course of primary parasitaemia for periods of 11 and 15 days and were cured thereafter, failed to respond to the challenge dose. The duration and the average intensity of daily parasitaemia, following challenge, were also observed to decline, with progressive increase in the duration of primary patency preceding curative treatment (Table III).

TABLE III.

Daily average parasitaemia in animals of primary infection and challenge inoculation after radical cure.

Cured on day.	Number of animals used.	Daily average parasitaemia in primary infection :															Rest period.	Daily average parasitaemia in challenge infection :									
																		Day.									
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		1	2	3	4	5	6	7	8	9	10
2nd	3	P	5	N	N	N	N	N	N	N	N	N	N	..	..	..		N	P	12	78	196	326	117	58	26	8
3rd	3	P	9	60	79	56	9	P	N	N	N	N	N	..	..	..		9	48	304	580	253	82	41	11	P	P
4th	4	P	3	32	88	11	N	N	N	N	N	N	N	N	..	..		7	55	637	940	360	2	P	N	N	N
5th	4	1	2	17	37	66	17	3	N	N	N	N	N	N	N	..		N	N	42	250	140	35	24	P	N	N
6th	5	7	25	83	252	340	483	264	57	P	N	N	N	N	N	N		N	7	13	235	199	11	N	N	N	N
7th	4	P	9	106	421	601	767	1,332	368	21	N	N	N	N	N	N		N	N	3	18	6	1	N	N	N	N
8th	4	P	12	138	182	247	275	533	1,343	280	P	N	N	N	N	N		N	N	P	P	5	7	N	N	N	N
9th	9	1	19	149	330	652	1,015	1,855	1,987	1,962	403	P	N	N	N	N		N	N	P	1	15	8	1	N	N	N
10th	6	1	18	130	403	916	1,255	1,384	1,777	1,975	1,750	289	N	N	N	N		N	N	17	28	3	P	N	N	N	N
11th	8	P	12	120	234	433	839	1,298	1,411	1,718	1,233	1,110	735	3	N	N		N	N	N	N	N	N	N	N	N	N
15th	2	N	5	55	344	530	858	1,390	1,602	1,008	584	520	370	230	174	98		N	N	N	N	N	N	N	N	N	N

P = positive.
N = negative.

DISCUSSION.

Although it was aimed to have at least 10 animals for each experiment, it was not always possible due to difficulty in obtaining the requisite number of animals of the same age. In no case, however, was the sample less than 8. For experiments considered crucial, as for instance the determination of the immunity status conferred on the ninth, tenth and eleventh days of primary parasitaemia, the experiments were repeated to confirm the results.

The period of observations of the *R. rattus* that were sub-inoculated for the total infectivity test, was arbitrarily fixed at 10 days. This would seem to be justified, however, in the light of previous observation on the prepatent period of *P. berghei* in *R. rattus* (Ramakrishnan and Satya Prakash, 1950). Occasionally, at the end of the observation period the test-inoculated *R. rattus* were submitted to the total infectivity test in a second series of animals and the absence of infection, determined by the examination of blood on ten consecutive days, was confirmed.

With the above mentioned experimental limitations, the results would seem to be clear cut. The absence of any difference in the average prepatent period after primary and challenge inoculations in the animals cured of the infection after the second, third and fourth days of primary parasitaemia, indicates that no measurable immunity is conferred within the period (Table II). This is of particular interest in the light of the hypothesis of Macdonald (1957). He postulates that in the case of hyper-endemic areas where P. *falciparum* predominates in the first few years of life, at least one infection with *P. falciparum* makes no material alteration to the probability of another during its course and that fresh infections during this time are marked by a fresh onset of parasitaemia materially unaffected by the previous one. In the case of *P. berghei* in rats, however fresh infections can manifest as distinct episodes of parasitaemia only during a very short period, during the course of the first 10 days at the most of the primary infection.

The period of primary *P. berghei* parasitaemia in albino rats which does not make any alteration to the immunity status of the host, with reference to re-infection, would seem to be relatively short and limited to three days. In the present study, the infections dealt with are single blood-induced episodes. It should be of interest to study multiple infections and observe whether the duration of primary parasitaemia, required for conferring measurable immunity, is shortened further and whether the total parasitaemia and the duration of the primary patent episode materially differ from the patterns of single infections.

The total resistance to challenge re-infections seem to manifest in a proportion of animals only after the eighth day of primary parasitaemia. Progressively, in animals cured after the ninth and tenth day of primary parasitaemia, the proportion of such animals increases. One hundred per cent of the animals cured after the eleventh day onwards of primary parasitaemia, totally resist re-infection. The analysis is shown in Table IV.

TABLE IV.

Results of challenge re-infection and total infectivity test after radical cure of primary parasitaemia on successive days after the primary inoculation.

Number of rats inoculated.	Day of patent parasitaemia on which treatment was commenced.	Interval in days between commencement of treatment and challenge inoculation/ total infectivity test.	Positive/Negative.		REMARKS
			Challenge inoculation.	Total infectivity test.	
8	2	11	3/0	0/4	1 animal died.
8	3	11	3/0	0/3	2 died.
8	4	11	4/0	0/4	
8	5	11	4/0	0/4	
10	6	11	5/0	0/5	
10	7	24	4/0	0/4	Two died.
8	8	21	3/1	0/3	One died.
16	9	18	3/6	0/6	One died (Two experiments).
18	10	17	4/2	0/7	Five died (Two experiments).
20	11	18	0/8	0/7	Five died (Two experiments).
10	15	12	0/2	0/4	4 died.
Total 124			33/19	0/51	

All possible care was taken to ensure that in no case was the challenge inoculation a superinfection. The radical cure of the primary infections have been amply proved by Sergent's total infectivity test (Sergent, 1954). In every case a minimum of 8 days elapsed between the radical cure and the challenge re-infection. This raises the question whether immunity to *P. berghei* infections in albino rats is premunition (Sergent, 1954) or true immunity (Corradetti *et al.*, 1954) persisting after complete recovery from the primary infection. The latter based his conclusion of recovery on the absence of relapses in splenectomised animals at different intervals after the primary patent parasitaemia. It would appear that acquired immunity in a measurable degree was conferred only after a minimum of 4 days of primary patent parasitaemia. This immunity could be shown to increase quantitatively up to the tenth day of primary parasitaemia after which, from the eleventh day onwards, sufficient immunity is developed to totally resist the challenge re-infection. The development of such immunity is intimately related to and is proportional to the duration of primary parasitaemia. The time interval allowed between the cure and administration of the challenge re-infection was short. Data allowing longer intervals between the cure and challenge re-infection will form the subject of another publication.

SUMMARY.

Studies were carried out to determine the minimum period of parasitaemia (*P. berghei*) in albino rats necessary for conferring immunity as detected by challenge re-infection.

It was found that no significant immunity was conferred by parasitaemia of a patent duration of less than 5 days, beyond which immunity was detectable.

The degree of immunity built by a patent course of 11 days or more was adequate to totally resist homologous re-infection.

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Indian Journal of Malariology, **13**, 4, December 1959.

METABOLIC STUDIES WITH 1-p-BROMOPHENYL-2 : 4-DIAMINO-1 : 6-DIHYDRO-6 : 6-DIMETHYL-1 : 3 : 5-TRIAZINE (M.I.S. 11) IN MONKEYS*.

BY

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[June 22, 1959.]

STUDIES on metabolic degradation of N^1-p-chlorophenyl-N^5-isopropyl biguanide (proguanil, I ; X=Cl) in both man and rabbits indicated the formation of 1-p-chlorophenyl-2 : 4-diamino-1 : 6-dihydro-6 : 6-dimethyl-1 : 3 : 5-triazine (II ; X=Cl) and a small amount of an isomeric compound, viz., 2-chloro-anilino-4-amino-1 : 6-dihydro-6 : 6-dimethyl 1 : 3 : 5-triazine (III, X=Cl) (Carrington *et al.*, 1951 ; Crowther and Levi, 1953). The first mentioned triazine was highly active against experimental malaria infections while the latter compound showed no such activity (Carrington *et al.*, 1951 ; Crounse 1951 ; Modest *et al.*, 1952 ; Schmidt *et al.*, 1952 ; Crowther and Levi 1953, Krishnaswami *et al.*, 1953). Crounse (1951), while studying the same problem in monkeys, could only isolate the inactive triazine (III ; X=Cl) and p-chlorophenyl biguanide as the two major breakdown products of proguanil. Considering the fact that active triazine (II) was very labile and got readily converted into its isomeric derivative (III) when exposed to heat or alkali, it was probable that during the process of isolation and characterisation, the primary metabolite (II ; X=Cl) got isomerized into (III ; X=Cl) and as such escaped detection by Crounse (1951). Even in the case of studies by Crowther and Levi (1953), the amount of isomeric triazine (III ; X=Cl) isolated, was negligibly small and could have easily originated from the active triazine (II ; X=Cl) during isolation procedure. Metabolic degradation studies with N^1-p-bromophenyl-N^5-isopropyl-biguanide (Bromoguanide, I ; X=Br) in monkeys led to the isolation of 1-p-bromophenyl-2 : 4-diamino-1 : 6-dihydro-6 : 6-methyl-1 : 3 : 5-triazine (II ; X=Br) from the urine in 5 per cent yield. In this case, however, formation of isomeric 2-p-bromoanilino-4-amino-1 : 6-dihydro-6 : 6-dimethyl-1 : 3 : 5-triazine (III ; X=Br) could not be proved (Bami, 1953).

1-p-bromophenyl-2 : 4-diamino-1 : 6-dihydro-6 : 6-dimethyl-1 : 3 : 5-triazine (M.I.S. 11; II ; X=Br) and a large number of dihydro-triazines of this class have also been synthesised and their physico-chemical properties and biological activities evaluated (I.C.I. patent, 1951 ; Modest *et al.*, 1952, Basu *et al.*, 1952 ; Loo, 1954 ; Carrington *et al.*, 1954 ; Bami, 1955 ; Modest, 1956 ; Modest and Levine, 1956). Amongst these, M.I.S. 11 (II ; X=Br) was one of the few compounds which

* (a) A preliminary report of this work was published in *Proc. Indian Sci. Congress*, 1957, **44**, Part III, p. 150.

(b) M.I.S. represents Malaria Institute Survey number.

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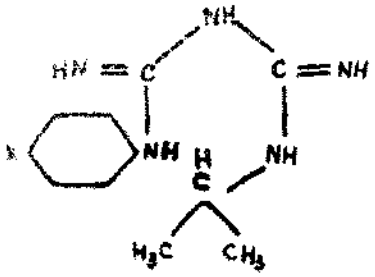
displayed high degree of antimalarial activity coupled with low toxicity when tested against experimental malaria infections like *P. gallinaceum*, *P. lophurae*, *P. berghei* and *P. cynomolgi* (Krishnaswami *et al.*, 1953 ; Hewitt *et al.*, 1954 ; Jaswant Singh *et al.*, 1954 ; Ray *et al.*, 1954). Preliminary clinical trials with this compound also revealed it to be very promising when compared to drugs like chloroquin and it was very well tolerated at the dosage tried (Ray, Bami and Basu, 1956). Similarly, pharmacological studies with M.I.S. 11 indicated that it had very low acute toxicity (LD_{50} for rats and monkeys on oral feeding, 1500 and 800 mg. base/kg. body weight respectively) (Sirsi and Bami, 1958). Low toxicity, associated with this highly soluble and active triazine (II ; X=Br), led to the present investigations on its metabolic degradation in monkeys. In view of the above background, it was also to be ascertained if any further isomerisation of M.I.S. 11 took place *in vivo* and what was the nature of other metabolic breakdown products originating from it.



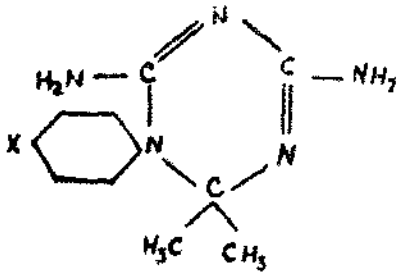
(I)

X=Cl (proguanil).

X=Br (bromoguanide).

N¹-p-substituted phenyl-N⁵-isopropyl-biguanide.

(H_2O 225 mμ Max.



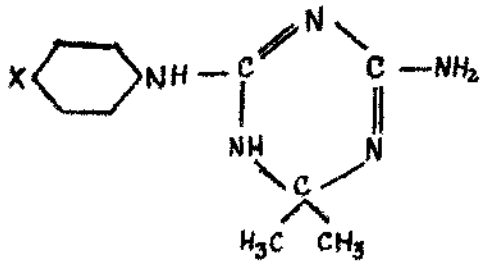
(II)

X=Cl (active proguanil metabolite).

X=Br (M.I.S. 11-active bromoguanide metabolite).

1-p-substituted phenyl-2:4-diamino-1:6-dihydro-6:6-diamethyl-1:3:5-triazine.

(H_2O 240 mμ Max.



(III)

X=Cl or Br.

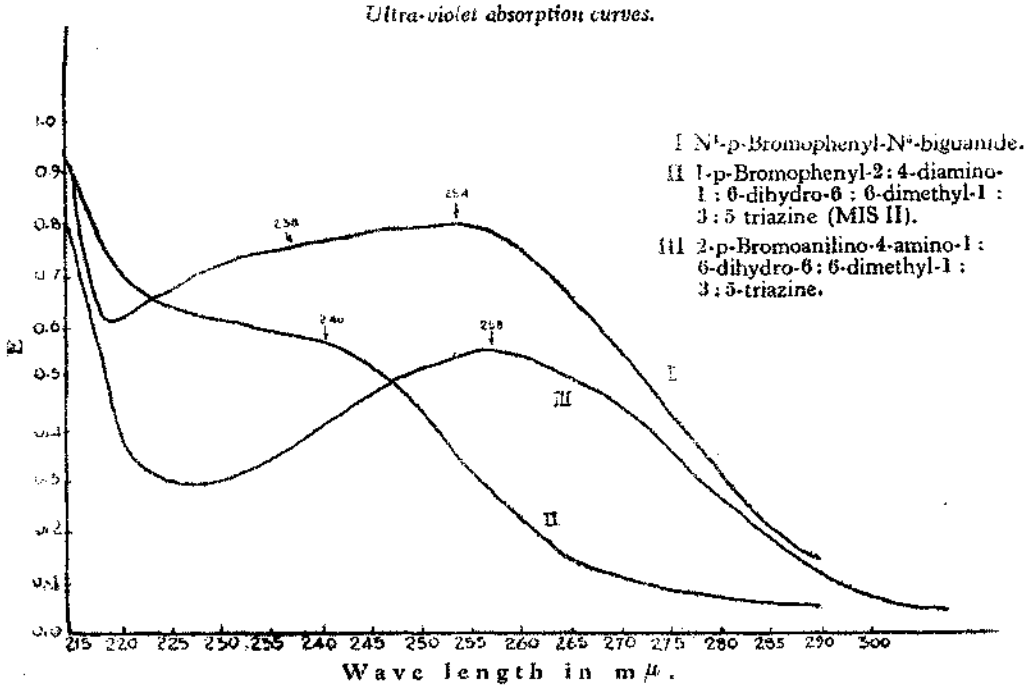
2-p-substituted-anilino-4-amino-1:6-dihydro-6:6-dimethyl-1:3:5-triazine.

(H_2O 258 mμ Max.

EXPERIMENTAL.

A pure sample of 1-p-bromophenyl-2 : 4-diamino-1 : 6-dihydro-6 : 6-dimethyl-1 : 3 : 5-triazine hydrochloride (II ; X=Br), required for the present studies, was synthesised and crystallised twice from water (Bami, 1955). It was isomerised into III (X=Br) by heating it in an alkaline solution. In order to aid in identification, ultra-violet absorption spectra of M.I.S. 11 (II ; X=Br), isomerised triazine (III ; X=Br) and N^1-p-bromophenyl biguanide, was determined in 0·01 N-hydrochloric acid with a Hilger UV spectrophotometer (Graph 1). Similarly, infra-red spectrograms of II (X=Br) and III (X=Br) were obtained through the courtesy of Messrs. S.P. Sadler & Son Inc., U.S.A. (Graphs 2 and 3).

GRAPH I.
Ultra-violet absorption curves.



M.I.S. 11 hydrochloride was given orally as a single daily dose of 20 to 30 mg. base/kg. body-weight because these dosages were very well tolerated by monkeys. A typical experiment to characterise the metabolites has been recorded below :—

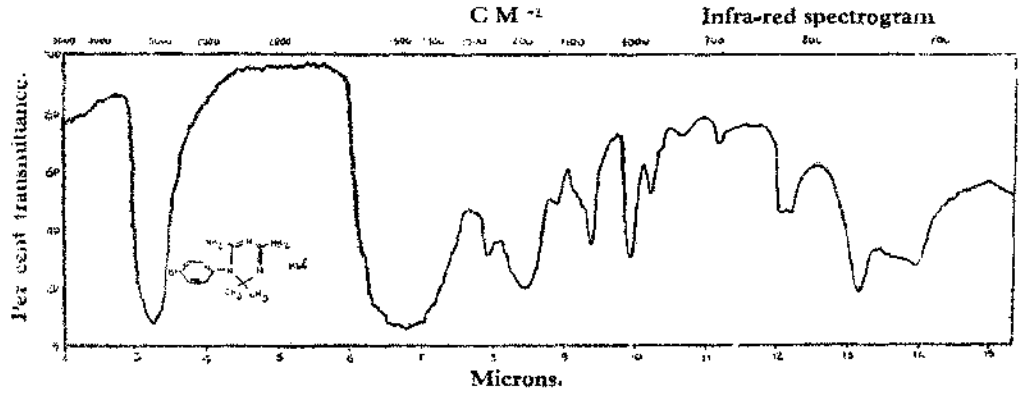
(*a*) *Isolation of metabolites from monkey urine.*—Healthy normal monkeys (weighing 5 to 6 kg.) were fed orally once daily at 30 mg. base/kg. body-weight dose of 1·0 per cent solution of M.I.S. 11 hydrochloride. Administration of drug was continued for 15 days. Urine (preserved over chloroform) and faeces (kept in methanol) were collected daily and stored in a refrigerator. Suitable quantities of urine and faeces were taken as required.

Urine (2 litres) was treated with lead acetate till no more of the proteins were precipitated. The mixture was filtered through kiselguhr. The clear filterate was

extracted with butanol (1·5 litre) three times. Butanol extract was concentrated under reduced pressure and the soluble metabolites taken into a lead-free aqueous phase according to the technique described by Crowther and Levi (1953) and Bami (1953). The aqueous extract (50 ml.) was made alkaline and quickly extracted with ether three times (100 ml., 50 ml., 50 ml.) at 0°C. The alkaline extract was neutralised in cold, and extracted with butanol twice (50 ml. each time). The butanol extract was again evaporated under reduced pressure to get the material back into aqueous phase. The aqueous extract at this stage, was directly treated with alcoholic picric acid. The resultant crude picrate was collected and crystallised from ethanol as fine silky yellow needles, yield 100 mg. m.p. 201°C. Mixed m.p. with an authentic sample 201°C. (found C, 38·7 H, 3·3 N, 21·35, $C_{11}H_{14}N_5Br.$, $C_6H_3O_7N_3$ require C, 38·8, H, 3·2, N, 21·3 per cent).

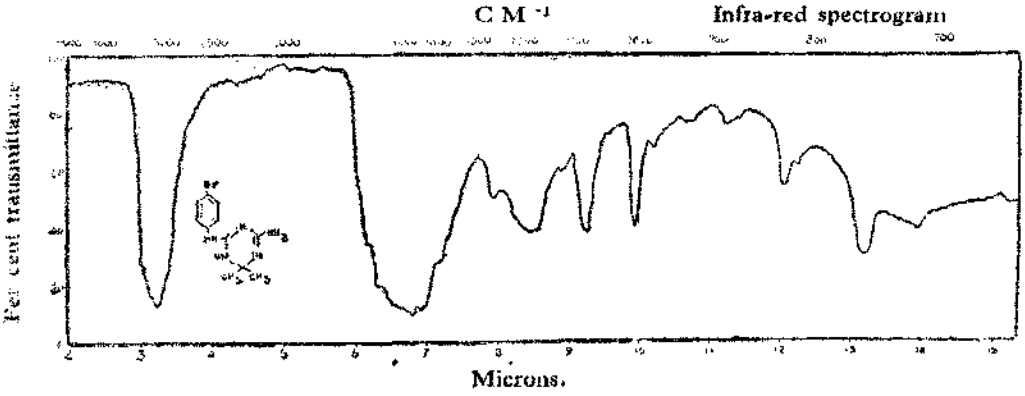
GRAPH 2.

1-(p-Bromophenyl)-4, 6-diamino-1, 2-dihydro-2, 2-dimethyl-s-triazine, hydrochloride ($C_{11}H_{14}BrN_5HCl$, Mol. Wt. 332·65) (0·5 mm, 0·5 per cent in KBr wafer).



GRAPH 3.

1-amino-6-(p-bromoanilino)-4, 2-dihydro-2, 2-dimethyl-s-triazine ($C_{11}H_{14}BrH_5$, Mol. Wt. 296·19) (0·5 mm, 0·5 per cent in KBr wafer).



Ether extract obtained above, was evaporated to dryness and the residue triturated with dilute hydrochloric acid (10 ml.). A portion of this solution was treated with ammonical copper sulphate solution but there was no red precipitate indicating the presence of any biguanide derivative. Similarly, no picrate was formed when remaining of the acid solution was treated with picric acid. This indicated the absence of any ether soluble basic compounds.

(*b*) *Isolation of metabolites from monkeys' faeces.*—Faeces (500 gm.) obtained from monkeys receiving the drug orally, were shaken with methanol (500 ml.) for one hour and then filtered, using celite filter aid. The filterate was concentrated to a small volume (50 ml.) by blowing air at its surface at room temperature and then diluted with water to 500 ml. The solution was worked up as the urine sample described above. Only a small quantity of 1-p-bromophenyl-2 : 4-diamino-1 : 6-dihydro-6 : 6-dimethyl-1 : 3 : 5-triazine picrate was recovered finally as silky needles m.p. 200°C.

Crounse (1951) had studied the nature of breakdown products of alkaline and acidic hydrolysis of proguanil and dihydrotriazines (II and III) using p-phenyl-azo-benzoyl chloride as a reagent. In order to see if smaller metabolic fragments of M.I.S. 11 could also be detected by this technique, the following experiment was undertaken.

(*c*) *Characterisation of metabolites through their p-phenylazo benzoates.*—Urine (2 litres) from monkeys receiving M.I.S. 11 orally was extracted with butanol and the butanol phase replaced by water as described before. The aqueous phase (50 ml.) was made alkaline and repeatedly extracted with ether at 0°C. Total ether extract was evaporated and the residue picked up in dioxane (2 ml.). Sodium hydroxide (20 per cent solution ; 6 ml.) and p-phenylazobezoyl chloride (0·3 gm.) were added to the dioxane solution and the mixture dried under vacuum. The residue was again suspended in water (5 ml.), the suspension filtered through a filter thimble and the residue repeatedly washed with hot water till free of alkali.

Thimble, with its content, was dried at 60-70°C. for 3 hours and then extracted with benzene (100 ml.) for 4 hours in a soxhlet. Benzene extract was reduced to 25 ml. and run on a 50 : 50 alumina : celite column conditioned with 60 : 40 benzene : n-heptane mixture. Elution was also done by the latter solvent mixture. Only two faint yellow bands got separated on the column. These bands were mechanically removed and extracted repeatedly with ethanol. In one case, the amount was negligibly small while in the other case the material was identified to be a mixture of p-phenylazobenzoic acid and its sodium salt. Apparently there was no indication of formation of any benzoyl derivative of the triazine, its isomeric derivative or any of their smaller breakdown fragments.

The above chromatographic separation was also repeated with total material extractable from urine with butanol. In this case also, none of the expected derivatives could be detected. Similarly separation of metabolites by partition chromatography on silicic acid-celite column, as employed by Crounse (1951), also failed to confirm the presence of III (X=Br) or other smaller basic fragments.

As it was not possible to detect any metabolite of M.I.S. 11 by the above techniques, it was argued that either there was little breakdown of this molecule or the metabolites could not be accounted for by the above methods of drug administration and isolation. In order to clarify the position, firstly maximum tolerated dose for M.I.S. 11, when given intravenously, was determined in monkeys. After several experiments, it was observed that a maximum single dose of 10 mg. base/kg. body weight was safe when given intravenously. Secondly, urine of normal monkeys was collected and mixed with known amounts of M.I.S. 11 hydrochloride. This mixture was extracted with butanol and M.I.S. 11 recovered as its picrate quantitatively as described under (*a*) above. Percentage recovery of M.I.S. 11 on this basis was determined several times and results are given in Table I.

TABLE I.

Recovery of M.I.S. 11 picrate from known mixtures of urine and M.I.S. 11.

Serial number.	Urine (in ml.)	M.I.S. 11 hydrochloride added (in terms of mg. base).	Amount of M.I.S. 11 picrate recovered (in mg.)	Per cent recovery of M.I.S. 11 picrate.
1	300	100	70	70·0
2	500	100	75	75·0
3	500	100	67	67·0
4	750	500	352	70·4
5	1,000	750	525	70·0
			Average	70·5

With the above base-line recovery data and considering the fact that there was little evidence to support the presence of other metabolites, quantitative recovery of M.I.S. 11 in the urine of the monkeys, receiving the drug intravenously, was undertaken. Two typical experiments are recorded below :—

(i) A monkey (5 kg.) was administered intravenously M.I.S. 11 hydrochloride in the form of its sterile 0·25 per cent solution at a dosage of 10 mg. base/kg. body weight in the morning. The same dosage was repeated in the evening. Daily intravenous administration of 100 mg. base of the drug was continued for 5 days (total 500 mg. base). Urine was collected over chloroform for 12 days starting from the time of first injection. Total urine (2 litres) was treated with lead acetate and extracted twice with butanol (1000 ml., 500 ml.) for two hours. Butanol extract was worked up as detailed already and finally crude M.I.S. 11 picrate was obtained, from the aqueous phase. On crystallisation it offered 280 mg. of pure M.I.S. 11 picrate m.p. 200°C. Recovery on the basis of blank recovery results (Table I) worked up to 79 per cent.

(ii) Two monkeys were administered intravenously, 100 mg. M.I.S. 11 each in two divided doses (as above) for one day only. Urine from both these monkeys was collected for six days and pooled together. Total urine (1 litre) was worked up as under (i) above and the final yield of M.I.S. 11 picrate was 100 mg. Recovery on the basis of blank results (Table I) worked up to 71 per cent.

DISCUSSION.

Attempts to isolate metabolites 1-p-bromophenyl-2 : 4-diamino-1 : 6-dihydro-6 : 6-dimethyl-1 : 3 : 6-triazine hydrochloride (M.I.S. 11 ; II, X = Br) from the urine of monkeys receiving the drug orally, did not offer any major or minor breakdown product. The presence of M.I.S. 11 alone could be detected. Similarly faeces samples, when analysed according to Crowther and Levi (1953) technique, failed to give any other product excepting the starting material (II, X=Br). In both cases, neither the formation of biguanide derivatives nor isomeric dihydrotriazine (III ; X=Br) as possible breakdown products, was proved. It was, therefore, very doubtful if isomerization of (II) into (III) could take place *in vivo* at all. It seemed probable that M.I.S. 11 was broken down into smaller fragments like, p-bromoaniline, isopropyl-amine, methylamine, ammonia, acetone, etc., because intensive acidic and alkaline hydrolysis of similar compounds had offered such products previously (Crounse, 1951 ; Crowther and Levi, 1953). Benzoylation of materials extracted from urine with p-phenyl-azobenzoyl chloride as well as separation by chromatography indicated that none of these basic fragments were present in any of the extracts in measurable amounts. Even intravenous administration of triazine (II ; X=Br) failed to offer any metabolite in the urine. In fact, about 75 per cent recovery of the injected material from the urine was highly indicative of the fact that this drug was excreted from the system mostly unmetabolised. Failure to account for the remaining 25 per cent of the drug, could be explained on the basis of absorption in the animal system, losses due to perspiration and those during collection of urine. Highly soluble nature of the drug, coupled with its very low toxicity (Sirsi and Bami, 1958), also contributed towards its rapid and extensive elimination from the system unchanged. Robertson (1957) clinically tried the antimalarial activity of proguanil metabolite (II ; X=Cl) and its 3 : 4-dichloro analogue (Crowther and Levi, 1953) and suggested that these compounds were not superior to chloroquin due to either easy destruction or rapid excretion from the system. Studies with M.I.S. 11 have indicated that dihydrotriazines of this class (II) were neither localised in the system (unlike chloroquin, pamaquin) nor destroyed but were capable of rapid elimination from the system without undergoing significant chemical breakdown.

SUMMARY.

1-p-bromophenyl-2 : 4-diamino-1 : 6-dihydro-6 : 6-dimethyl-1 : 3 : 5-triazine hydrochloride (M.I.S. 11) was administered to monkeys both orally (20 to 30 mg. base/kg. body weight) and intravenously (10 mg. base/kg. body weight). Isolation of metabolites from urine and faeces, as well as chromatographic separation of metabolites, revealed that this compound was mostly eliminated from the system unchanged and there was no evidence of its conversion to isomeric triazine (III ; X=Br), biguanides or other smaller fragments. Nearly 75 per cent of the drug could be recovered unmetabolised from the urine after its intravenous administration to monkeys.

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Indian Journal of Malariology, **13**, 4, *December 1959*.

THE SUSCEPTIBILITY OF *CULEX FATIGANS* TO DIFFERENT DENSITIES OF *MF. BANCROFTI*.

BY

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[July 25, 1959.]

THE concept of a critical level of microfilarial density in the blood of the vertebrate host, which is infective to the vector mosquitoes, is an important factor in the transmission of filariasis. This has been studied by many workers in the past (Bahr, 1912; Hu, 1937; Basu and Rao, 1939; Manson-Bahr, 1952; Muirhead Thomson, 1954). The observations have a bearing on programmes for the control of filariasis in which chemotherapeutic agents are used to reduce the reservoir of infection in the community.

An "infective" and a "sub-infective" level of microfilarial density in the host, with reference to the infectivity to the mosquito, were distinguished by some of the earlier workers. Basu and Rao (1939 *loc. cit.*) described a 'minimal', 'optimal' and 'lethal' densities of microfilariae in the blood. The minimum level infective to *C. fatigans* was "12 mf. per 0·2 c.c." (should probably read as 0·02 c.c.). Rosen's studies in Tahiti showed that non-periodic *W. bancrofti* developed successfully in *Aedes polynesiensis* even with microfilarial counts as low as 0·45 per 20 c.mm. Comparative infectivity of different microfilarial densities of periodic *W. bancrofti* in the blood to laboratory-bred *C. fatigans* is reported in this paper.

METHOD AND MATERIAL.

Volunteers positive for *Mf. bancrofti* were selected for these studies in Ernakulam (Kerala State). The microfilariae in this area is known to have a nocturnal periodicity (*Malaria Institute of India*, 1957). Estimation of microfilarial density was made by examination of blood just before applying the mosquitoes for feeding. Some of the volunteers, however, did not have patent parasitaemia during such examinations. The number of microfilariae were enumerated in three smears, each with 20 c.mm. of blood. The average of the three counts represented the microfilarial count at the time of the feed.

Specimens of female *C. fatigans* from the colony, maintained at the laboratory, were conditioned by starving for 24 hours and then fed on the volunteers. Twenty

to thirty such mosquitoes were confined in a bamboo ring covered with bobbinet on either side and applied to the thighs of the volunteers for an hour. The mosquitoes were fed between 20·30 and 21·30 hours. The fully engorged mosquitoes were separated and held in glass chimneys and maintained on glucose.

Routine observations in the laboratory had shown that the extrinsic incubation period was about 14 days. Therefore, observations on the development of the infection in the mosquitoes were restricted to a period of 14 days from the day of feeding. Mortality was noted daily and dead mosquitoes dissected. The live specimens were dissected between the 10th and 14th day of feeding. In positive specimens the number and the stages of development of the larvae found were recorded.

RESULTS.

The microfilarial densities in the volunteers, as determined by the blood examination just before feeding the mosquitoes, ranged from zero to 220 per 20 c.mm of blood. Four hundred and sixty-four mosquitoes were dissected following the infective feeds. The results are presented in Table I and Graph 1.

TABLE I.

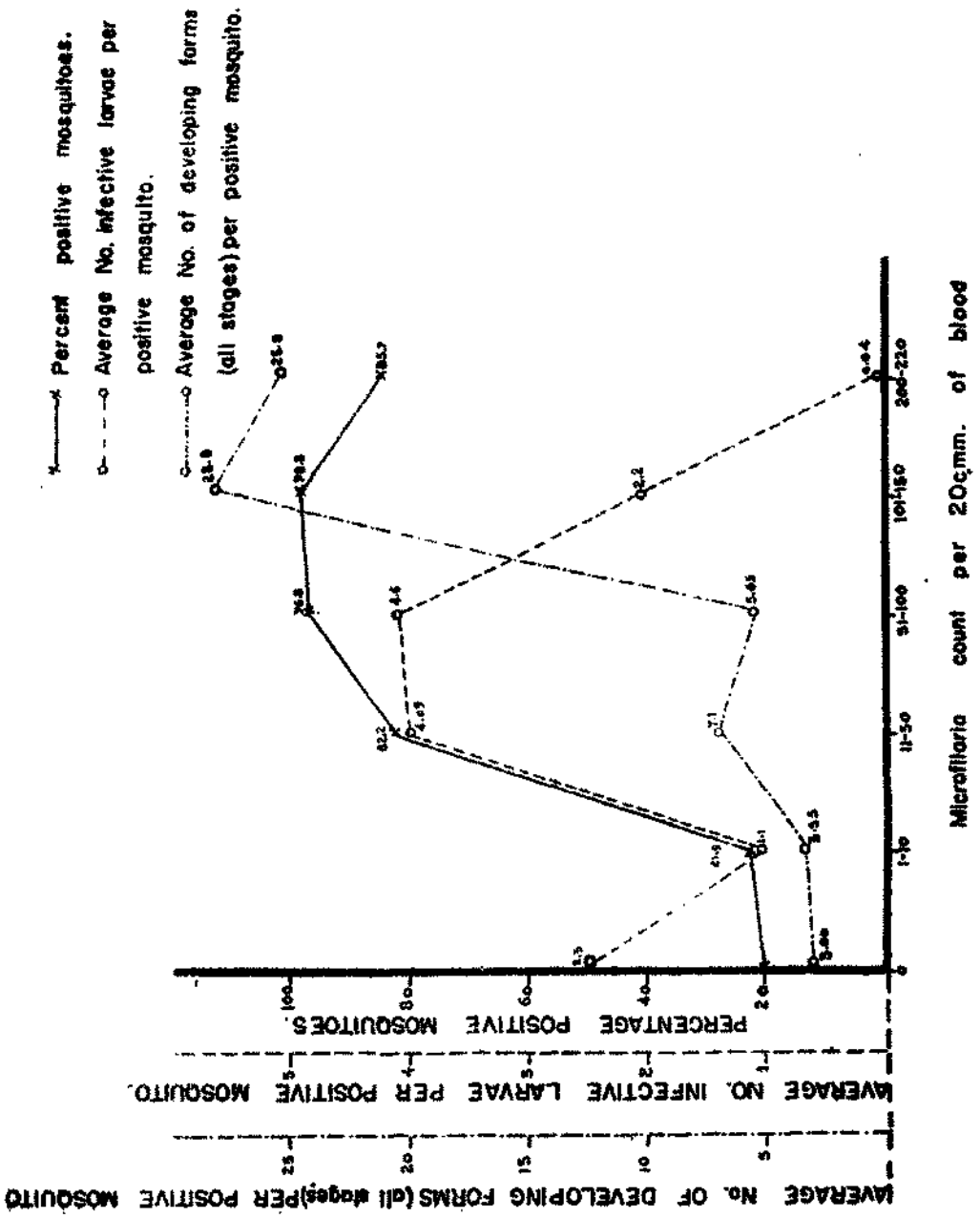
Results of dissection of C. fatigans *fed on volunteers with different microfilarial densities.*

Average Mf. counts per 20 c.mm. of peripheral blood.	Number of mosquitoes dissected.	Percentage of mosquitoes positive.	AVERAGE NUMBER OF LARVAE PER POSITIVE MOSQUITO :	
			All stages.	Infective stage.
0	10	20·0	3·0	2·5
1— 10	82	21·9	3·55	1·1
11— 50	143	82·2	7·1	4·09
51—100	118	96·8	5·65	4·66
101—150	83	98·8	28·8	2·22
210—220	28	85·7	25·8	0·04

DISCUSSION AND CONCLUSION.

It is noteworthy that two out of ten mosquitoes were infected despite the fact that they were fed on volunteers with no patent microfilaraemia at the time of feeding. This could be due to the "ability of mosquitoes to concentrate microfilariae" (Manson, 1883 ; Ashburn and Craig, 1907 and Highby, 1946), which, however, has been questioned by Bahr (1912 *loc. cit.*). Gordon and Lumsdon (1939) made direct observations in the web of a frog infected with *Feleyella dolichoptera*. They noted a difference between the density of microfilariae in the capillaries and that in the pool of blood formed due to the laceration of tissues by the proboscis of the mosquito while feeding. This would appear to be a probable explanation for the finding mentioned above.

GRAPH I.


Percent positive mosquitoes.
Average No. infective larvae per positive mosquito.
Average No. of developing forms (all stages) per positive mosquito.
AVERAGE No. OF DEVELOPING FORMS (all stages) PER POSITIVE MOSQUITO
AVERAGE NO. INFECTIVE LARVAE PER POSITIVE MOSQUITO.
PERCENTAGE POSITIVE MOSQUITOES.
Microfilaria count per 20c.mm. of blood
0
1-10
11-50
51-100
101-150
200-220

The percentage of positive mosquitoes was found to be correlated to the microfilarial count in the donor's blood within certain limits. There appears to be an optimum density of microfilariae favourable for the completion of development in the mosquito (Table I). The average number of infective larvae per positive mosquito showed an increase with counts up to 100 microfilariae per 20 c.mm. With higher densities, the number of infective larvae in the mosquitoes showed a steady fall. It was interesting, however, to note that the total counts of developing forms continued to show an increase with the rise in the microfilarial counts. The overcrowding of the developmental forms in the mosquitoes fed on volunteers with more than 100 microfilariae per 20 c.mm., appeared to be responsible for fewer numbers of larvae reaching the infective stage.

Transmission of filariasis is determined by many factors :—

(i) *In men* :—the microfilariae rate and average infestation ;

(ii) *In the mosquito* :—the infectivity rate, the density of infective larvae per infected mosquito, the vector density, the daily survival and biting rates and the degree of anthropophily.

Of the factors relating to the vector, the first two are intimately associated with the infection in man, and are the common indices used in measuring mosquito transmission. If all the other factors remain constant, the "transmission potential" can be assessed by the formula :

$$\left.\begin{matrix}\text{Transmission} \\ \text{potential}\end{matrix}\right\} = I \times n$$

where 'I' is the infection rate and 'n' the number of infective larvae per positive mosquito (Table II).

TABLE II.

Transmission potential.

Mf. count per 20 c.mm.	Infection rate in mosquito.	Number of infective larvae per positive mosquito.	Transmission potential.
0	20·0	2·5	50·0
1— 10	21·9	1·1	24·1
11— 50	82·2	4·09	337·0
51—100	96·8	4·8	445·3
101—150	98·8	2·2	217·4
200—220	85·7	0·04	3·43

The "transmission potential" would appear to be minimal when microfilarial density is low (below 10 per 20 c.mm.). Densities between 11 and 100 per 20 c.mm. would appear to favour maximum transmission. Densities beyond 100 microfilariae per 20 c.mm. act as a biological brake to mosquito transmission.

The "transmission potential" appears to be mainly governed by and almost directly in proportion to the density of infective forms per positive mosquito and

would, therefore, be dependent on the microfilarial counts in the community. The mosquito infection rate *per se* does not appear to be a true index of transmission, and determination of infective larval counts appears to be important in deciding the quantum of transmission. This has to be borne in mind in comparative studies and in assessing the efficacy of control operations.

SUMMARY.

1. Comparative studies on the effect of different microfilarial densities of *W. bancrofti* in man on the development in mosquitoes (*C. fatigans*) have been reported.

2. It was observed that :

(*a*) mosquitoes showed infective larvae even following feeding on volunteers who did not show circulating microfilariae at the time of feeding and there was no "sub-infective level" of microfilaraemia ;

(*b*) the percentage of infected mosquitoes and the number of infective larvae per positive mosquito increased with mf. counts up to 100 mf. per 20 c.mm. of blood ;

(*c*) the infective larvae count per positive mosquito fell with higher microfilarial counts in the donor which appeared to act as a biological brake to transmission.

3. The importance of recording the counts of infective larvae in the positive mosquitoes for assessment of control programmes has been discussed.

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Indian Journal of Malariology, **13**, *4, December 1959.*

DAY AND NIGHT FILARIA SURVEYS IN SOME PERSONS WITH PERIODIC *W. BANCROFTI* BEFORE AND AFTER DIETHYLCARBAMAZINE THERAPY.

BY

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[July 31, 1959.]

INTRODUCTION.

In India, the only filarial infections recorded in human beings are the nocturnally periodic *W. bancrofti* and *malayi* (Raghavan, 1957). Thus in filarious areas in India the surveys have to be carried out at night, posing a number of practical problems. Many workers have attempted to study the possibility of overcoming them by carrying out day surveys. Korke (1929) in Bihar, India, studied the significance of such surveys and noted that the proportion of positives revealed by day and night surveys was 2:9, thereby emphasising the need for carrying out night surveys in such areas for getting true picture.

Gujral (1957)* observed a reversal of the nocturnal periodicity of *Mf. bancrofti* after treatment with diethylcarbamazine†. Following therapy, there were greater numbers of microfilariae in blood films obtained during the day than at night. In view of the above, it was decided to repeat the work of Korke (1929 *loc. cit.*) and Gujral (1957 *loc. cit.*).

Results of filaria surveys by day and night, prior to and following a five-day course of diethylcarbamazine therapy, as far as possible in the same persons in an area in Uttar Pradesh endemic for *W. bancrofti* transmitted by *Culex fatigans*, are recorded in this paper.

*The observations were made in inmates of the prison at Baraibanki, Uttar Pradesh.

†4 mg./kg. body weight once daily for 5 days.

METHODS AND MATERIAL.

Blood smears from 862 persons, of both sexes and all age-groups, selected at random, were examined during December, 1958 and January, 1959 in Basti, Uttar Pradesh. The smears from each person were obtained both by day and night. The day surveys were carried out between 10 a.m. and 1 p.m. and the night surveys between 9 p.m. and midnight. Each smear consisting of a thick blood film, approximately $1\frac{1}{2} \times \frac{3}{4}$ inches, was prepared with 3 drops of blood (about 20 c.mm.) obtained by pricking the finger tip. The smears were air dried, stained with 0·1 per cent aqueous solution of methylene blue* and examined. The microfilariae were counted in the positive slides.

Thereafter, a five days' course of diethylcarbamazine was administered in a dose 4 mg./kg. of body weight. After completion of the mass therapy, blood smears were obtained during the same time of the day/night as in the first survey. Complete data are available only in respect of 857 persons examined during both the surveys.

RESULTS.

The results are shown in Tables I and II and Graph 1.

TABLE I.

Results of day and night blood surveys of some persons prior to diethylcarbamazine therapy.

Age group in years.	NUMBER OF PERSONS EXAMINED :		POSITIVE BY DAY :		POSITIVE BY NIGHT :	
	Day.	Night.	Number of persons.	Rate, per cent.	Number of persons.	Rate, per cent.
0—5	25	25	0	0·0	3	12·00
6—10	102	102	1	0·9	4	3·92
11—20	307	307	16	5·2	44	14·29
21—30	154	154	7	4·6	23	14·93
31—40	100	100	4	4·0	15	15·00
41—50	80	80	4	5·0	5	7·50
Above 50	86	86	3	3·4	6	6·97
Total	854	854	35	4·2	100	11·7

From Table I, it is seen that some persons were positive in day as well as night in all age-groups except the youngest. The number of people showing microfilariae in their blood in the night is considerably higher as compared to those positive during the day in all age-groups. The overall microfilarial rate by day survey was 4·2 as compared to 11·7 per cent of same persons examined by night.

From Table II it would be noted that the percentage of positives by day and night have been markedly reduced as compared to Table I. In some age-groups,

*Number 1 solution of the J.S.B. stain (Jaswant Singh and Bhattacharji, 1944).

GRAPH 1.

Periodicity of the microfilaria in various age-groups before and after the administration of diethylcarbamazine for five days.

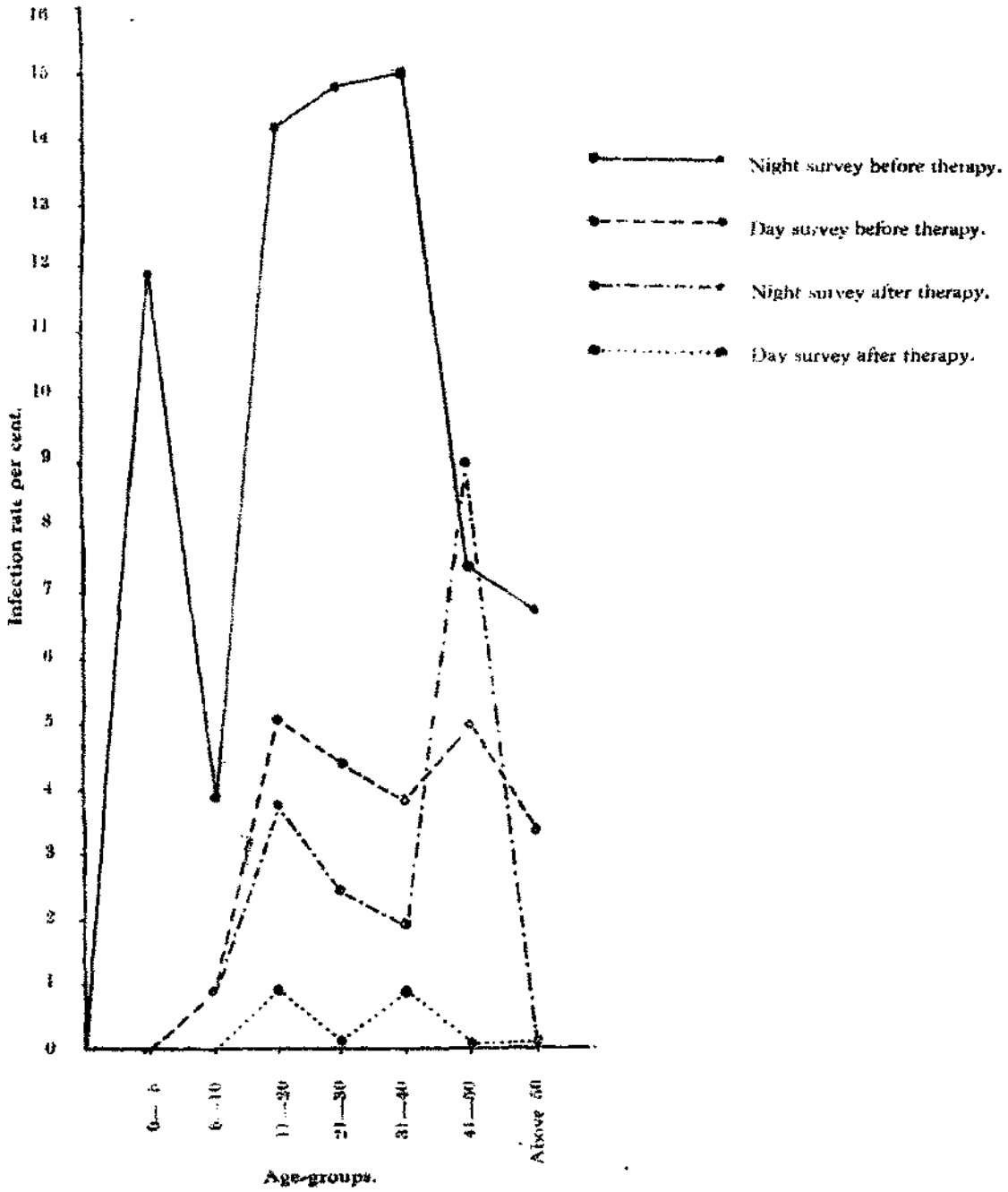

TABLE II.

Results of day and night blood surveys of the same persons as in Table I above, after diethylcarbamazine therapy.

Age group in years.	Number of persons examined:		Positive by day:		Positive by night:	
	Day.	Night.	Number of persons.	Rate, per cent.	Number of persons.	Rate, per cent.
0—5	25	25	0	0·00	0	0·00
6—10	102	102	0	0·00	1	0·98
11—20	307	307	3	0·97	12	3·90
21—30	154	154	0	0·00	4	2·59
31—40	100	100	1	1·00	2	2·00
41—50	80	80	0	0·00	0	0·00
Above 50	86	86	0	0·00	0	0·00
Total	854	854	4	0·46	19	2·22

even by night no positive was found (0-5, 41-50 and over) as compared to 12·00, 7·50 and 6·97 per cent before therapy. In the day surveys, the only age-groups that are positive are 11-20 and 31-40 and even these are 0·97 and 1·00 as compared to 5·2 and 4·0 per cent before therapy. The overall microfilaria rate by day was 0·46 and 2·22 after therapy as compared to 4·2 and 11·7 per cent prior to therapy. Thus the relative proportions of positives by day and night are nearly the same prior to and after therapy. Thus whether with or without therapy, for getting as true a picture as possible of the positives in periodic *W. bancrofti* areas, it is essential to carry out a night survey.

SUMMARY.

1. The ratio of positives by day and night surveys in Basti, Uttar Pradesh, among 862 persons most of whom were examined in common, was 4·2 : 11·7 prior to therapy with diethylcarbamazine. The same after 5 days' therapy in a dose of 4 mg./kg. was 0·46 : 2·22.

2. Diethylcarbamazine caused a number of positive persons to become negative. But from para 1 (above), it would be noted that with or without therapy more positives were revealed by night than the day survey. This emphasises the need for night surveys.

3. There is no evidence to suggest that following therapy there is a change or reversal of periodicity. If this were so, more positives should have been revealed by day than by night surveys.

ACKNOWLEDGMENT.

The authors are grateful to the staff of the Filaria Control Unit, Basti, for their whole-hearted cooperation in the study.

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Indian Journal of Malariology, **13**, 4, December, 1959.

NOTE ON FILARIASIS IN HARDOI TOWN, UTTAR PRADESH.

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INTRODUCTION.

BEFORE the launching of the National Filaria Control Programme in Uttar Pradesh during 1955-56, it was estimated that filariasis was a problem of some 13 districts of the State.

Singh (1958), during his participation in the Filariasis Study Tour, revealed that the disease was prevalent in 22 districts of Uttar Pradesh and about 50 per cent of the population in the State was exposed to the risk of the disease. It was considered that the disease extended in the west up to Lucknow District.

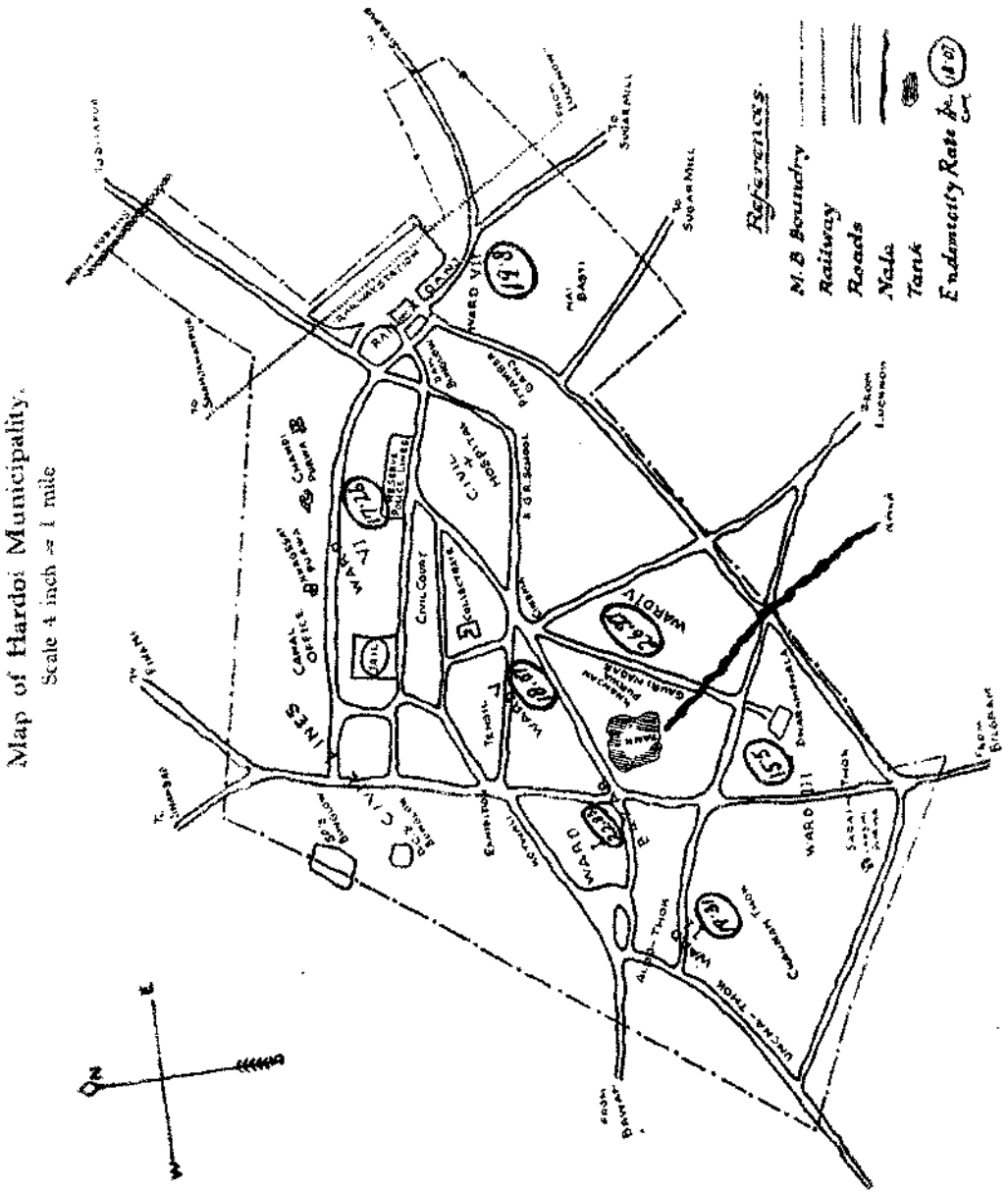
In order to find out if the disease has progressed beyond the district of Lucknow also, the adjoining district of Hardoi was selected for study. The present paper deals with the filaria survey conducted in the township of Hardoi.

DESCRIPTION.

Hardoi town is the headquarter of the Hardoi District which is one of the centrally situated districts of Uttar Pradesh. It is a municipal town having a total population of 29,881 (1951 census) out of which there are 17,085 males and 12,796 females. For administrative purposes, the whole of the town is divided into seven wards (Map 1).

The town has a limited safe water supply. Large number of persons are still using hand pumps and wells as the source of their water supply although a number of stand posts are installed by the local body.

There is no regular drainage system in the town and whatever drains exist, they are of open type (except in a very small area where there are underground drains as well), built without much consideration to the gradient, with the result that water stagnates, forming suitable places for the breeding of the mosquitoes.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M.B Boundry
Railway
Roads
Nala
Tank
Endemicity Rate
Map of Hardoi Municipality.
Scale 4 inch = 1 mile

The disposal of the night soil is by compost pit manuring and the refuse is disposed of by composts and for filling up of the tanks and excavations.

The town has got a modern hospital, an eye hospital and a maternity and child welfare centre. There is a Medical Officer of Health to look after the health activities of the town.

The town is connected with the neighbouring areas by roads and railways. There is a small Developmental and Agricultural Exhibition held in the town annually where persons mainly from the district are attracted.

There is a sugar mill situated just at the border of the town which was established about 15 to 20 years back. Annually, during the crushing season (November to April or May) there is a migration of about 500 to 600 persons (labourers) from the districts of Deoria and Gorakhpur (filarious areas) who reside in the locality for a period of 6 to 7 months. Further a population of about 500 persons has permanently migrated from these districts and has settled down in the town (mainly in Nai Basti, Ward VII).

As regards the climate of the town, the maximum and minimum temperatures are 108·1° F. and 47·2° F., respectively, and the relative humidity varies from 70 to 90 per cent. The average annual rainfall is 50·6 inches.

FILARIASIS—A RETROSPECT.

On enquiries from elderly persons, local medical practitioners and the hospital it is learnt that the disease is recently introduced in the town. About 20 years back there were a couple of cases in the town. It is further learnt that it is for the last 8 to 10 years only that an upward trend in the incidence of filariasis is noted, in the town.

FILARIA SURVEY—PROCEDURE AND TECHNIQUE.

No filaria survey to assess the incidence of this disease was carried out in the past as the area was considered to be non-filarious. The filaria survey under the National Filaria Control Programme, India (Raghavan, 1955) was undertaken due to following reasons :—

(*i*) The incidence of the disease in this area increased so much during the recent years so as to attract the attention of the general public and there was a great demand for some measures against it.

(*ii*) This survey was also undertaken to know if the disease is prevalent in areas on the west of Lucknow and Kanpur as it was considered that filariasis in Uttar Pradesh is prevalent only in the eastern districts of Uttar Pradesh and up to Lucknow and Kanpur only.

The survey was carried out from April 23, 1959 to April 30, 1959 and July 7, 1959 to July 25, 1959 examining as many number of persons as possible belonging to the houses visited and from all the wards of municipality. A door-to-door visit was made between 8 p.m. and midnight to examine as many number of persons as

possible from all the age-groups and from both the sexes in all the wards of the town. The persons were examined for disease manifestations like lymphadenitis, hydrocele, elephantiasis of the limbs and genitalia and chyluria. Along with this, approximately 20 c.mm. of blood was obtained from each individual from finger tip and smear was prepared. These air-dried smears were stained the next morning with J.S.B. stain I (Jaswant Singh and Bhattacharji, 1944), and later on examined for microfilaria. Their number and species in each positive slide were determined.

RESULTS OF SURVEY.

1,846 persons from all age-groups and from both the sexes were examined from all the wards of the town during this period of survey.

The overall infection rate, disease rate and endemicity rate were found to be 10·29 per cent, 10·72 per cent and 19·82 per cent respectively. The average infestation was 13·05 Mf. per 20 c.mm. of blood. The disease manifestations consisted of hydrocele, elephantiasis of the limbs and scrotum and chyluria. None of the nine infants examined was found to harbour the infection, however the youngest child who showed infection was 2¼ years old. The disease was noted in a case of fourteen years of age.

AGE : INFECTION/DISEASE.

The infection and disease prevalence amongst different age-groups of the persons examined are set out in Table I.

TABLE I.

Details of persons of different age-groups with infection and disease.

Age groups (years)	Number of persons examined.	Number of persons with :		Infection rate, per cent.	Disease rate, per cent.	Average infestation per 20 c.mm. of blood.
		Infection.	Disease.			
0 - 1	9	..	..	0·00	0·00	00·00
2 - 5	94	4	..	4·25	0·00	9·0
6 - 10	231	14	..	6·06	0·00	8·5
11 - 20	480	52	28	10·83	5·83	13·3
21 - 30	430	63	58	14·65	13·48	19·2
31 - 40	292	30	53	10·27	18·14	13·9
41 - 50	214	21	40	9·81	18·68	8·2
51 onwards	96	6	19	6·25	19·79	4·9
Total	1,846	190	198	10·29	10·72	13·05

An analysis of Table I shows that the infection rate rises as the age advances till the age-group 11-20 years, after which it tends to remain more or less steady. It further shows that the incidence of filarial disease is negligible in the earlier age-group while it increases as the age advances and steadies itself from the age-groups 21-30 years and above.

TYPE OF DISEASE MANIFESTATIONS.

Table II shows the incidence of various types of disease manifestations as observed in the persons surveyed.

TABLE II.

Type of disease manifestations and their relative prevalence amongst the persons with disease.

Disease manifestations.	Number showing disease manifestations.	Per cent.
Hydrocele and other genital effections.	155	78·28
Right upper limb.	7	3·53
Left upper limb.	8	4·06
Right lower limb.	19	9·59
Left lower limb.	25	12·62
Chyluria.	2	1·01

From Table II, it may be noted that hydrocele and other genital lesions form the bulk of the disease processes.

SEX : INFECTION/DISEASE.

Table III sets out the incidence of filarial infection and disease manifestation as is observed in the two sexes.

TABLE III.

Incidence of infection in males and females.

Sex.	NUMBER OF PERSONS :		Infection rate, per cent.
	Examined.	With infection.	
Male	1,324	145	10·94
Female	521	45	8·63
Total	1,846	190	10·29

It would be noted from Table III that the infection rate differs in the two sexes, being higher in the males than in the females. The same applies equally well to the incidence of disease manifestations as is clear from Table IV. This, in most

TABLE IV.

Incidence of filaria disease in males and females.

Sex.	NUMBER OF PERSONS :		Disease rate, per cent.
	Examined.	With disease.	
Male	1,325	180	13·58
Female	521	18	3·45
Total	1,846	198	10·72

probable case, seems to be due to the fact that sample of the females collected is very much less as compared to the males and also as the women do not readily come forward to report the disease manifestation, particularly genital, in the earlier age-groups.

COMMUNITY : INFECTION/DISEASE.

The incidence of infection and disease as observed in the two communities, Hindus and Muslims, examined is set out in Table V.

TABLE V.

Incidence of infection and disease amongst Hindus and Muslims.

Community.	Number examined.	Infection :		Disease :	
		Number.	Rate per cent	Number.	Rate per cent.
Hindus	1,549	157	10·13	166	10·71
Muslims	297	33	11·08	32	10·82
Total	1,846	190	10·29	198	10·72

From the analysis of Table V, it is evident that the infection and disease rates in the two communities, Hindus and Muslims, are almost the same and do not show any difference.

ENTOMOLOGICAL OBSERVATIONS.

Random adult mosquitoes were collected between 7·00 a.m. and 11·00 a.m. from the different wards of the town during this survey. The mosquitoes collected were later identified and the female mosquitoes were dissected for filarial infection in them. The following species were collected : *A. subpictus. A. annularis, A. pallidus, C. fatigans, Aedes sp., Armegeres Sp.*

Culex fatigans was found to form the major component of the mosquito population.

During this period of survey (from April 23 to April 30 and July 7 to July 25, 1959), 539 mosquitoes (78 anophelines and 461 culicines) were collected. Out of these, 44 anophelines and 336 culicines (279 *Culex fatigans*) were dissected for filarial infection. Only 11 *C. fatigans* were found infected with one or other stages of the developing larvae. Infective forms were also noted in them. The results of dissection are set out in Table VI.

BREEDING PLACES.

The most common type of breeding places for *Culex fatigans*. as noted in the town, are as follows :

1. House drains and small collections of water from the drains.
2. Ablution water near the service types of laterine (cesspools).
3. Water collections from the street drains.
4. Periphery of the tanks (Kutcha) where animals etc. are washed.

TABLE VI.

Results of mosquito dissection for filarial infection.

Species of mosquito dissected.	NUMBER :		HEAD :		THORAX :				ABDOMEN :			
	Dissected.	Positive.	III.	IV.	I.	II.	III.	IV.	I.	II.	III.	IV.
Anophelene	44	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
Culex fatigans	279	11	..	..	..	..	6	1	1	4	..	..
Other culicines	57	*Nil*	..	..	..	..	..	..	..	..	..	..

Due to lack of proper construction of the drains, there are several small water collections on the sides, forming suitable places for breeding of the mosquitoes.

CLIMATE AND ENDEMICITY. (*See* Appendix I).

Appendix I shows the last five years' (1954 to 1958) meteorological data of Hardoi Town as recorded by the Hardoi observatory. The filaria survey revealed that the town is moderately endemic for filariasis (Iyengar, 1938). From the meteorological data, the degree of endemicity noted and the type of disease processes, it would appear that this area fits in as an area with climatic conditions favourable for filarial transmission as an "intermediate" or 4-8 months according to the classification of Acton and Rao (1930).

DISCUSSION.

The reasons which may be attributed to this increase in the incidence of this disease during the recent years, so much as to attract the attention of the general public, may be summarised as follows :—

(*I*) *Migration of population.*—As mentioned earlier, due to the establishment of a sugar mill in the vicinity of the town, there is an annual migration (temporary) of about 500 to 600 persons (labourers) from the districts of Deoria and Gorakhpur (*W. bancrofti* area) during the crushing season for a period of 6 to 7 months (November-April/May) since 1944. Moreover about 100 families (500 persons) have permanently migrated from the districts and have settled down in this area since then.

The infection, disease and endemicity rates, as worked out for these migrants, are set out in Table VII.

TABLE VII.

Infection, disease and endemicity rates in the persons who migrated from the districts of Deoria and Gorakhpur.

Locality.	NUMBER OF PERSONS WITH :				Infection rate, per cent.	Disease rate, per cent.	Endemicity rate, per cent.
	Number of persons examined.	Infection.	Disease.	Both infection and disease.			
Nai Basti	135	19	19	2	14·07	14·07	26·66

Appendix I.

Meteorological Data of Hardoi Town for Five Years (1954--1959).

Months.	YEAR 1954.						YEAR 1955.						YEAR 1956.					
	Temperature :			Humidity :		Rain fall (Inch.).	Temperature :			Humidity :		Rain fall (Inch.).	Temperature :			Humidity :		Rain fall (Inch.).
	Max. °F.	Min. °F.	Mean °F.	08·30 hrs. per cent.	17·30 hrs. per cent.		Max. °F.	Min. °F.	Mean °F.	08·30 hrs. per cent.	17·30 hrs. per cent.		Max. °F.	Min. °F.	Mean °F.	08·30 hrs. per cent.	17·30 hrs. per cent.	
January	69·2	47·2	58·2	86	62	2·73	69·1	48·3	58·7	84	44	1·94	72·2	48·0	60·1	85	56	2·10
February	77·6	56·4	67·0	84	62	2·45	78·1	50·5	64·3	74	40	0·24	75·3	47·5	61·4	67	44	0·00
March	88·6	59·3	74·0	58	33	0·00	93·0	62·3	77·6	59	26	0·01	88·7	61·0	74·8	67	35	0·86
April	102·8	68·6	85·7	33	15	0·00	98·5	65·6	82·0	36	20	0·00	101·3	69·9	85·6	38	24	0·00
May	108·1	79·6	93·9	43	21	0·00	105·5	73·9	89·7	40	27	0·00	104·1	80·6	92·4	50	39	1·81
June	101·5	82·2	91·9	66	47	3·33	101·5	80·1	90·8	70	51	7·14	98·6	80·8	89·7	70	54	2·20
July	92·5	80·5	86·5	83	72	19·80	91·4	79·3	85·4	89	81	17·66	91·8	79·1	85·5	83	72	9·24
August	90·6	79·0	84·8	87	75	20·11	88·0	79·0	83·5	92	83	14·28	90·7	78·6	84·7	85	72	14·19
September	90·5	77·9	84·2	87	80	16·82	90·3	77·3	83·8	86	78	8·36	92·7	77·1	84·9	82	64	5·05
October	87·0	63·2	75·1	77	58	0·00	85·9	69·1	77·5	83	70	10·29	86·1	69·3	77·7	85	73	6·27
November	83·1	52·4	67·7	72	53	0·00	82·8	54·8	68·8	78	55	0·00	81·4	53·2	67·3	82	59	0·00
December	75·9	47·3	61·6	77	57	0·00	74·9	48·9	61·9	84	55	0·00	75·2	48·3	61·8	87	61	0·00

YEAR 1967.

Months	Temperature : Max. °F.	Temperature : Min. °F.	Temperature : Mean °F.	Humidity : 08·30 hrs. per cent.	Humidity : 17·30 hrs. per cent.	Rain fall (Inch.).
January	70·1	49·0	59·6	93	60	3·27
February	75·9	47·3	61·6	79	40	0·00
March	84·9	57·3	71·1	60	39	2·24
April	97·3	68·0	82·7	44	26	0·12
May	105·0	76·4	90·7	46	33	0·00
June	103·6	83·1	93·4	57	41	2·72
July	91·9	80·2	86·1	89	81	11·44
August	92·1	78·9	85·5	89	81	8·43
September	91·0	75·5	83·3	81	71	10·32
October	91·2	65·8	78·5	77	55	0·93
November	84·2	55·4	69·8	72	55	0·00
December	74·4	49·8	62·1	90	67	0·49

YEAR 1968.

Temperature : Max. °F.	Temperature : Min. °F.	Temperature : Mean °F.	Humidity : 08·30 hrs. per cent.	Humidity : 17·30 hrs. per cent.	Rain fall (Inch.).
75·9	48·9	62·4	88	57	0·50
76·6	50·7	63·7	69	41	0·22
91·9	60·6	76·3	56	28	0·07
103·1	72·6	88·0	48	32	0·00
107·4	77·5	92·4	46	29	0·00
107·3	73·7	90·5	55	41	1·88
93·9	80·9	87·4	82	71	5·79
88·8	77·3	83·0	91	82	14·27
90·1	76·2	84·2	86	76	5·45
88·2	68·6	78·6	82	68	3·07
83·8	53·4	68·6	79	56	0·00
75·0	50·5	62·8	91	66	0·31

It is evident from Table VII that the persons migrated from the districts of Deoria and Gorakhpur had higher infection, disease and endemicity rates, thus acting as reservoirs of infection.

(2) Vector.—During this survey it was found that there is a general public complaint that mosquito nuisance has increased many folds during the last few years. This increase in the mosquito population may be due to the water-works having been established about six years back in Hardoi Town without consideration of the drainage system. This has resulted in the collection of water here and there in the absence of proper drainage and thus increasing the total water surface area for breeding of mosquitoes which persists throughout the area.

(3) Congestion and over-crowding.—During the last few years there had been a lot of migration in the town, leading to congestion and overcrowding which ultimately lead to insanitary conditions resulting in profuse breeding of *Culex fatigans* (which is a dirty water breeder) and which is found to be the vector.

(4) Climate.—Optimum conditions of temperature and relative humidity, required for the transmission of the disease, are available for a period of four to eight months per year.

SUMMARY.

Filaria survey was carried out for the first time in the municipal town of Hardoi and about 6 per cent of the population was examined which showed that Hardoi is a moderately endemic area ; endemicity rate being 19·82 per cent.

W. bancrofti was the only infection prevalent. The infection rate was 10·29 per cent. The youngest child who showed infection was 2½ years old. The average infestation was 13·05 Mf. per 20 c.mm. of blood, the maximum being 194 and the minimum 1 Mf. per positive slide.

The overall disease rate was 10·72 per cent consisting of elephantoid limbs, hydrocele, filarial scrotum and chyluria. The youngest age with disease manifestation was 14 years.

Infection was found to be prevalent in all the wards of the town.

Culex fatigans was the only vector for *W. bancrofti*.

Numerous breeding places were noted for *Culex fatigans* which was found to breed in the dirty water collections.

In the males, both infection and disease rates were found higher than in the females. The probable causes for the same have been discussed.

Amongst Hindus and Muslims, the incidence of filarial infection and disease were nearly the same.

The factors like migration of persons from the filarious areas to the town and the establishment of water-works without consideration for the drainage, leading to the spread of the disease, have been discussed.

ACKNOWLEDGEMENT.

The authors are thankful to the municipal authorities and specially to the Municipal Medical Officer of Health, Hardoi, and his staff for their help in the conduct of this survey. They are also thankful to Sri S. Wasi Uddin, Laboratory Assistant, and the staff of the Filaria Survey Unit, Bahraich, Uttar Pradesh for their active part in the field and in the laboratory.

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A SIMPLE MEDIUM FOR LABORATORY REARING OF HOUSEFLY *MUSCA DOMESTICA NEBULO* FABRICIUS, WITH SOME OBSERVATIONS ON ITS BIOLOGY.

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[September 28, 1959.]

INTRODUCTION.

THE common housefly is one of the few widely used insects for experimental work, particularly for testing of insecticides. Attempts to maintain laboratory colonies of the housefly, *Musca domestica**, throughout the year started with the work of Grady (1928) who reared larvae on horse manure and yeast and maintained the adults on a mixture of milk, bread, sugar and yeast.

Richardson (1932), used a larval medium composed of 200 gm. alfalfa, 400 gm. wheat bran, 10 gm. yeast and 16 ml. malt in a litre of water ; and for the adult flies he used milk diluted 50 per cent with water to which a 40 per cent solution of formaldehyde in proportion of 1 to 1,500 may be added to delay the souring of the milk. Among other housefly breeding media are those of Eagleson (1943) who used crimped oats and water for larvae; and a mixture of 500 ml. water, 10 gm. shredded agar, 200 gm. pulp of ripe banana, 150 gm. sugar, 1 litre skimmed milk, 0·5 ml. formalin and 25 gm. gelatin for adults, and Basdon (1947) who modified the Richardson's media for better results. More recently, Deoras (1954) used the following medium for rearing *Musca domestica nebulo*. For larvae he used a mixture of wheat-bran 10 parts, quaker oats 1 part, yeast water (4 tablets of 4 grain each in 200 ml. of water), water 50 ml. ; and for adults a mixture of milk 5 gm., cane sugar 10-15 gm., fresh banana 25 gm., agar 1 gm., formalin 2 drops and water 250 ml.

For mass rearing of flies in the laboratories, the breeding media and methods used by various authors are tedious and expensive. In the Malaria Institute of India, colonies of housefly have been maintained successfully since 1950 using cheaper materials and simpler methods. This paper compares the M.I.I. medium to that used by Deoras (1954), in order to provide an economic and easy-to-handle

* Earlier workers did not mention the sub-species dealt under the specific name.

method for workers in need of large supplies of houseflies of known age for experimental work. Details about some aspects of the biology of the housefly are also included.

MATERIALS AND METHODS.

1. INSECTARY.

The fly colony* is housed in a room 8 feet wide, 10 feet long and 12 feet high. The room is provided with two entrances on opposite sides. Each entrance has a door with glass panes outside which is a wire screen door. Normally one entrance remains closed. There is no control for maintaining constant temperature and humidity. However, during summer ice-slabs are put to lower the temperature and an electric heater is provided during winter months to raise the temperature. The humidity in dry months is provided by spreading water-soaked gunny bags directly under the electric fans. Under these conditions the temperature ranges between 70° F. (winter) to 90° F. (summer) and the relative humidity 65 to 82 per cent.

The work is done with the help of day light that comes through the glass panes of the doors and the skylight.

During the period when sun is bright, light is cut off by closing the wire-screen doors.

2. REARING CAGE (FIG. 1).

Various types of elaborate and expensive cages have been used in rearing adult housefly in the laboratory (West, 1951). However, in the Malaria Institute of India simple one-foot cube wire-frame cages are used for this purpose. The cages are made from six one-foot-square wire-frames (Fig. 1-*a*, *b*) tied together, upon which is fitted a muslin cage provided with a sleeve (Fig. 1-*c*) on one side, and open on the other side (Fig. 1-*d*). The open side is closed by means of a string and the sleeve is also normally closed except for introducing or withdrawing the live flies and removing the dead ones. A petri dish containing cotton pad of 2½ inch diameter and soaked in dilute milk powder is placed at the bottom of the cage to provide the nourishment for adults as well as the medium for oviposition.

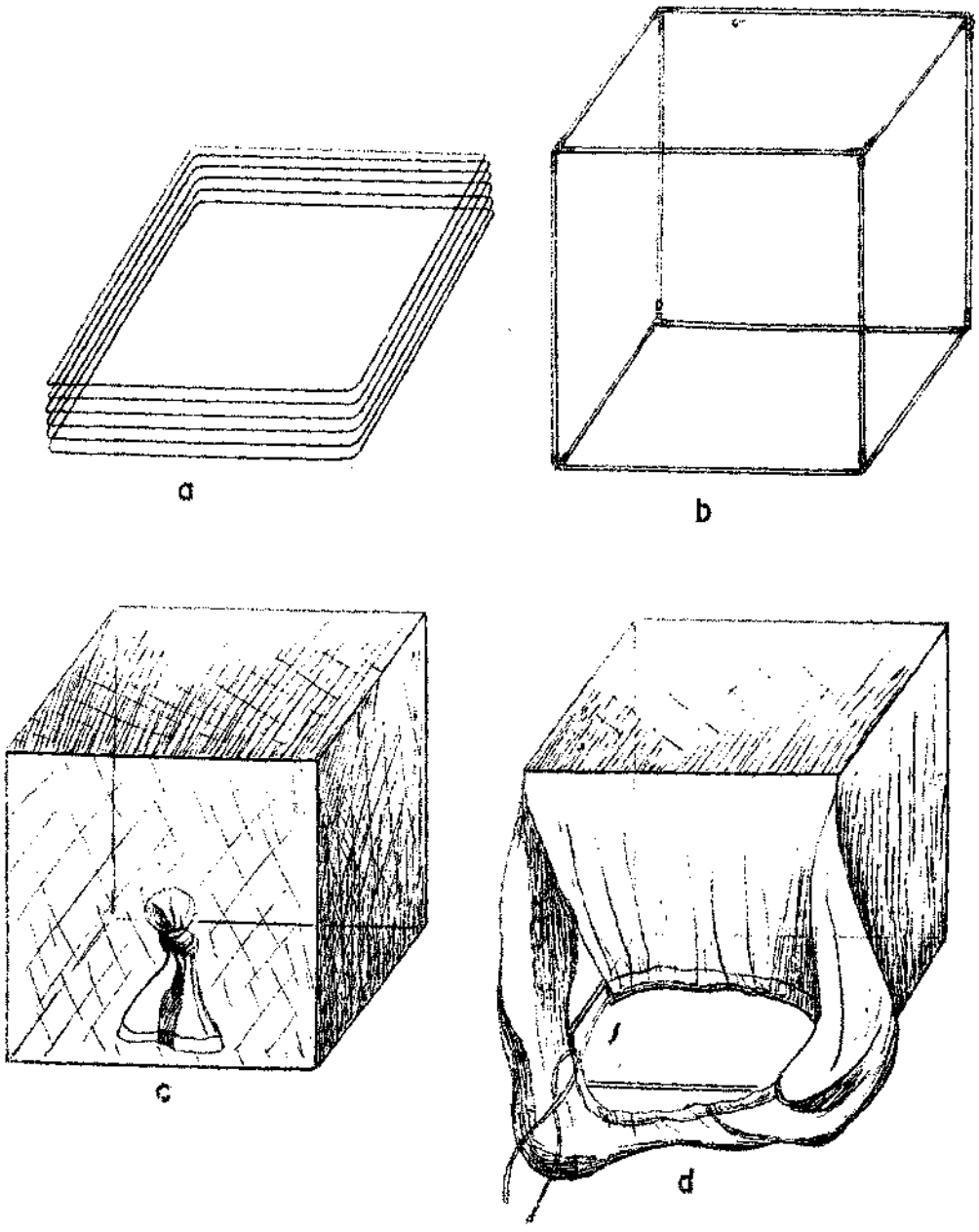
A large number of such cages can be stored conveniently, when not in use. When a large number of flies are needed for experimentation, the number of such cages can be increased conveniently. The cages are cheap to make and can be frequently cleaned.

3. BREEDING POTS.

The larvae are reared in earthen bowls of 8 inch diameter and 2 inch height. The bowl with the maggots is kept inside a one-foot-square wire-frame muslin cage, described above. The bowl is kept on a block of wood to prevent the muslin from becoming moist.

* Colony maintained by the Entomology Section at the Malaria Institute of India (M.I.I.), Delhi.

Fig. No. 1



Housefly rearing cage.

(a) Six wire-frames used for 1 cage ; (b) wire-frame of the cage ;
(c) depicting sleeve in the muslin cage ; (d) the open end with string.

4. FOOD.

For adults, milk powder (Amul powdered whole milk of Milk Producers' Union Ltd., Anand, Bombay) in water in the ratio of 1 : 7 is used. The eggs are also deposited on the same medium as mentioned earlier. The larvae are reared in cottonseed cake (4 oz.) soaked for 24 hours in water (8 oz.) before use.

5. METHOD ADOPTED FOR REARING.

100 to 200 adult flies are introduced in an adult rearing cage. The eggs are laid on the cotton wool pad soaked in adult food. This pad is removed every 24 hours when a fresh pad is introduced into the cage. This method subsequently ensures the age composition of the flies and also prevents souring of milk. In case of freshly emerged flies the eggs are laid 2 to 6 days after emergence. The cotton wool pads with eggs are removed to a glass jar of 4 inch diameter and 8 inch height, and kept in batches of 5 pads in each jar. The eggs hatch within 24 to 72 hours (summer/winter) and the larvae move to the top layers. Usually the larvae that hatch simultaneously are removed along with the cotton wool and put on the larval medium. The cotton wool pads with unhatched eggs are discarded.

For the larvae, 4 oz. of cottonseed cake is soaked overnight in 8 oz. of water in a larval bowl and 400 to 500 maggots are introduced in each such bowl. The medium usually remains soft till the larvae pupate. However, water is sprinkled on the top layer whenever it becomes dry. It takes 4 to 5 days for the maggots to reach the pupal stage in summer and 8 to 10 days in winter. The pupae are found in bottom of the bowl near the periphery*. The adults emerge in 4—5 days during summer and 8—10 days during winter. The life cycle from egg to adult, therefore, is completed within 9—22 days depending on the prevailing temperature.

Four larval pots ensure a regular supply of 1,000 flies per day with additional individuals to replenish the parent colony. This method of rearing is simple, inexpensive and ensures populations of known age for experimentation. The hatching of larvae, pupation, and emergence of majority of adult flies in any single batch is simultaneous.

COMPARISON BETWEEN THE M.I.I. AND THE DEORAS (1954) FLY BREEDING MEDIA.

(i) *Larval media*—Freshly hatched housefly larvae in batches of 50, 100 and 400 were introduced in equal quantities of cotton-seed cake (M.I.I.) and wheat-bran (Deoras, 1954) larval breeding media. The results of some of the experiments carried out are indicated in Table I. It was observed that the duration of developmental period from egg to adult was similar in the two cases. However, the majority of pupation and adult emergence took 24 to 48 hours less in case of cotton-seed than wheat-bran media. From Table I, it can be observed that invariably a higher

* In some of the experiments reported in this paper, larval food was kept in petri-dishes. In such cases, the petri-dishes have to be embedded in soil to allow the larvae to migrate for pupation in the soil. Therefore, it is an advantage to use earthen-bowls to prevent larvae from wandering around in the laboratory. It also does away with the soil and helps in keeping the insectary tidy.

TABLE I.

Per cent adult fly emergence on the M.I.I. and the Deoras' Media.

Serial number.	Number of larvae per replicate.	Number of replicates.	Total larvae.	Amount of food per replicate.	Number of adults: Male.	Female.	Total.	Per cent adult emergence.	Per cent sex ratio: Male.	Female.	Media.
1	50	3	150	1 oz.	57	74	131	87·3	43·5	56·5	M.I.I.
2	50	3	150	1 oz.	30	19	49	32·7	61·2	38·8	Deoras
3	100	6	600	1 oz.	246	259	505	84·3	48·7	51·3	M.I.I.
4	100	6	600	1 oz.	196	194	390	65·0	50·3	49·7	Deoras
5	400	3	1,200	4 oz.	427	504	932	77·7	46·0	54·0	M.I.I
6	400	3	1,200	4 oz.	350	353	703	58·6	49·8	50·2	Deoras

TABLE II.

Average number of eggs laid per female using the M.I.I. and the Deoras' media.

Serial number.	Media used.	Number of females isolated.	Number of repetitions.	Total flies isolated.	Number of flies laying eggs within 24 hours.	Number of flies from which eggs were counted.	Total number of eggs.	Average number of eggs per female.
1	M.I.I.	25	3	75	27	17	1,344	79·0
2	Deoras'	25	3	75	27	13	1,100	78·6

percentage of adults was obtained on the M.I.I. medium as compared to the Deoras' medium.

(*ii*) *Adult media.*—From the fly colonies maintained on the M.I.I. and the Deoras' media, larval/adult batches of 25 females were isolated after mating to study the effect of respective adults on their biotic potential. The flies were confined singly in 3 inch × 1 inch glass tubes containing respective media at the bottom and provided with cotton wool plugs at the open end. The food was covered with moist black cotton cloth to facilitate counting of the eggs. Only those flies that laid eggs within 24 hours, were considered. Cases, where the eggs hatched before counting, were discarded and the average number of eggs were assessed from those tubes where the eggs had not hatched. The results, which are indicated in Table II, demonstrate that the number of eggs laid by females bred out and maintained on the two media is almost equal and there is no particular advantage by using a more expensive adult media and at the same time more difficult to prepare.

(*iii*) *Biotic potential.*—Observations were also made on the frequency and total number of eggs laid by a fly during its life time when reared and maintained on two different media under comparison. Single male and female individuals were paired after emergence in 6 inch × 6 inch wire-frame muslin cage, on the pattern of the regular breeding cage used at the Malaria Institute of India (M.I.I.) and fed on the respective media. Three such pairs were maintained on each medium and the results obtained are given in Table III.

TABLE III.

Average number of eggs laid by a housefly during its life time using the M.I.I. and the Deoras' media.

Serial number.	Media.	Number of days for first oviposition.	Batches of eggs laid.	NUMBER OF EGGS PER FEMALE :		LONGEVITY (AVERAGE IN DAYS) :	
				Total.	Average.	Male.	Female.
1	M.I.I.	4	10	1,743	581	25	29
2	Deoras'	5-7	6	1,113	371	17	27

From the data obtained (Table III), it is evident that flies bred on the M.I.I. medium have higher biotic potential and live longer than those bred on Deoras' medium. The flies in either case laid eggs till their death and did not show any periodicity in oviposition. The frequency of oviposition and the number of eggs per batch became less as the flies grew older in both cases.

(*iv*) *Longevity of adult housefly in the laboratory.*—West (1951), while commenting on the longevity of housefly, mentions, "In the height of summer, it is doubtful if flies ordinarily live longer than two or three weeks. At lower temperatures, however, this may probably be extended to as much as three months. If there is true hibernation of adults, such individuals must, presumably, live even longer." This, of course, largely applies to the housefly under natural conditions. In the laboratory, Deoras and Ranade (1957) found that during hot months flies live

from about 28 to 30 days and in the cold months the flies may live as long as 70 days. This could be true in a laboratory with wide range of temperature.

Experiments conducted during July to October, when the temperature in the laboratory ranged between 75°F. to 90°F., showed that flies survived for 37 days when kept on the Deoras' or the M.I.I. media. Groups of freshly-emerged 50 adult male and female flies each were confined in a breeding cage and kept on two kinds of adult food. The per cent survival rate was recorded after every 24 hours till the last surviving fly died. These experiments were followed through the period mentioned above and the results are incorporated in Table IV and Graph 1. The mortality in the two sexes was about equal for the first two to three days after emergence. Afterwards, the males died much quicker than the females, particularly when Deoras' medium was used. Fifty per cent of the flies died within 10 to 13 days after emergence.

TABLE IV.

Longevity of adult housefly on the Deoras' and the M.I.I. media.

Serial number.	Days after emergence.	Survival rate per cent :			
		Deoras' media.		M.I.I. media.	
		Male.	Female.	Male.	Female.
1	2	94·0	95·0	91·0	96·0
2	3	87·0	88·0	84·0	89·0
3	4	80·0	86·0	78·0	83·0
4	7	65·0	79·0	67·0	65·0
5	9	58·0	70·0	55·0	56·0
6	10	46·0	65·0	49·0	51·0
7	13	32·0	49·0	42·0	47·0
8	17	19·0	30·0	19·0	20·0
9	21	11·0	26·0	10·0	15·0
10	24	6·0	14·0	4·0	7·0
11	27	2·0	7·0	2·0	5·0
12	29	2·0	4·0	1·0	3·0
13	37	0·0	0·0	0·0	0·0

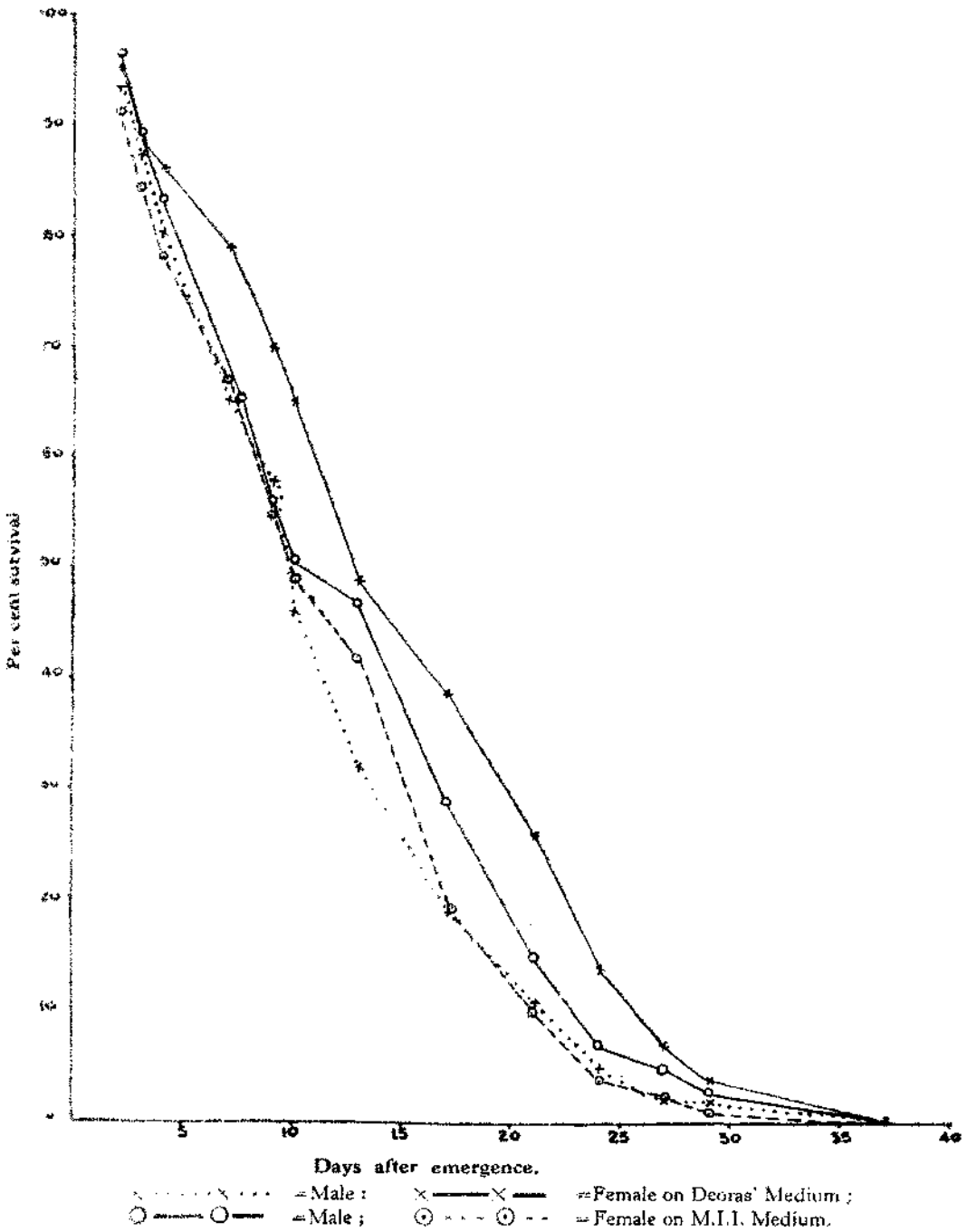
In case of flies maintained on the M.I.I. medium, the survival rate in females was less than those kept on the Deoras' medium. This seems to indicate that flies with higher biotic potential may have a lower survival rate. Further experiments are envisaged to study this aspect. Graph 1 shows that the decline in survival rate follows more or less a linear pattern in both the sexes on either media.

SUMMARY.

A simple medium for the laboratory rearing of housefly, *Musca domestica nebulo*, that has been in use at the Malaria Institute of India since 1950 has been described. The medium consists of cotton-seed cake soaked in water for rearing the larvae and milk powder-water solution for rearing the adults. The method used is simpler than those used by other authors and the materials are cheaper. Four earthen pots, each holding 4 oz. cotton-seed cake soaked in 8 oz. of water in which 400 to 500 maggots are introduced, ensure 1,000 flies per day for experimentation.

GRAPH I.

Per cent survival rate of Musca domestica nebulo *in the laboratory.*



All stages of flies of known age can be obtained by this method. The medium is compared with Deoras' (1948 ; 1954) rearing medium and it has been observed that a higher number of adults were obtained on the M.I.I. medium than on Deoras' (*loc. cit.*) medium. Also, the flies bred on the M.I.I. medium had a higher biotic potential, but a lower survival rate. Further experiments are envisaged to study the relationship between biotic potential and survival rate.

Observations revealed that during July to October the flies lived for 37 days after emergence when equal number of males and females were kept in the breeding cages on two types of media used here. The survival rate declined progressively and showed a straight line relationship.

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Indian Journal of Malariology, **13**, 4, *December 1959*.

A PRELIMINARY NOTE ON COLOUR PREFERENCE FOR OVIPOSITION OF *MUSCA DOMESTICA NEBULO* FAB.

BY

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AND

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[September 28, 1959.]

MANY workers have made observations on the colour response of adult houseflies. Awati (1920) exposed flies to coloured lights provided with tanglefoot and recorded that yellow was the most attractive colour followed by blue, orange, green, red and violet in order of mention.

Somewhat different results were obtained by Waterhouse (1948) who counted flies resting on various coloured removable corners in a Peet-Grady chamber. He found that red was most commonly preferred, with dusky blue a second choice. Yellow and medium-gray surfaces were equally popular as third-choice colours followed by green, light gray, sky blue and white respectively. However, so far, no observations were made on the colour preference for oviposition of the flies. The present note indicates the result of preliminary observations made on this aspect of the bionomics of *Musca domestica nebulo* in the laboratory.

Four to five days old female houseflies in numbers of ten were introduced in 1 × 1 foot wire-frame muslin cages* in which was placed a photographic dish containing cotton wool pads soaked in milk powder-water solution in the ratio of 1 : 7. The cotton wool pads were wrapped in moist cotton cloth of following colours :—

Serial number	*Colour*
1.	Deep red.
2.	Light red.
3.	Blue.
4.	Deep brown.
5.	Light brown.
6.	Yellow.
7.	Black.
8.	Green.
9.	White.

* Described elsewhere by Wattal *et al.* (1959).

The arrangement of different colours in the photographic dishes was made as follows :—

A			B		
1	4	7	1	7	4
2	5	8	3	9	6
3	6	9	5	2	8

C			D		
3	8	6	5	3	8
5	1	4	6	4	9
2	9	7	7	1	2

Three experiments, with 3 replicates each, were made using colours in four permutations (A, B, C, D) as indicated above. The eggs, which were invariably laid in the foldings of the wrapper-coloured-cloth, were counted after 24 hours, after the flies had been introduced in the cages. The results obtained are indicated in Table I and Chart 1.

TABLE I.

Colour preference in oviposition of Musca domestica nebulo.*

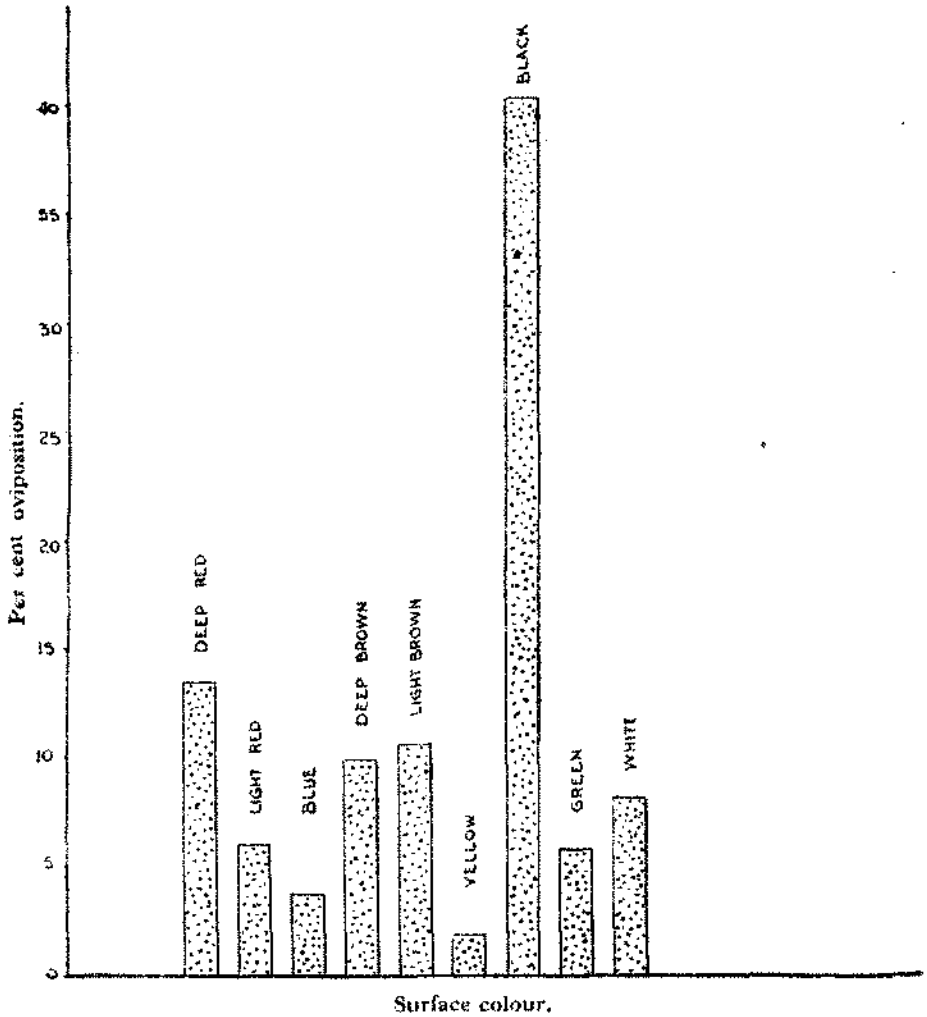
Serial number.	Colours used.	Number of eggs in different permutations.				Total number of eggs.	Per cent oviposition	Order of colour preference
		A.	B.	C.	D.			
1	Deep red	382	296	433	131	1,242	13·6	2
2	Light red	126	63	237	117	543	6·0	6
3	Blue	47	29	17	246	339	3·7	8
4	Deep brown	107	346	297	148	898	9·9	4
5	Light brown	227	266	146	328	967	10·6	3
6	Yellow	41	95	29	..	165	1·8	9
7	Black	1,109	723	832	1,015	3,679	40·4	1
8	Green	..	273	202	61	536	5·8	7
9	White	197	149	140	257	743	8·2	5
	Total	2,236	2,240	2,333	2,303	9,112	100·0	..

*90 female flies each were used for different colour permutations.

Black was the most favourite colour for oviposition in all the four permutations. 40·4 per cent of the total eggs were laid on black surface. This is perhaps to be expected as this colour compares favourably with the colour of breeding sites in nature. There was no marked preference for any other colour. After black, deep red and light and deep brown colours were preferred in order of mention. Yellow proved to be the least attractive colour. It seems that black colour may be used in preference to white for oviposition in laboratories as it will be easier to count eggs or larvae for experimental purposes against this background. Further experiments are being conducted on the probable effect of different colours on the ultimate egg production.

CHART I.

Per cent oviposition of Musca domestica nebulo *on different coloured surfaces in the laboratory.*



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Indian Journal of Malariology, **13**, 4, December 1959.

DISTENTION OF THE ABDOMEN OF *CULEX FATIGANS*, A SIGN OF ACUTE POISONING BY GAMMA BHC*.

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[October 22, 1959.]

INTRODUCTION.

In the course of observations to detect cross-resistance to gamma BHC in a DDT-resistant strain of *C. fatigans*, it was noticed that in some of the specimens exposed to gamma BHC the abdomen was visibly distended. The distention was noted only among the mosquitoes that were knocked down. No record of a similar observation with reference to mosquitoes was traceable in the available literature. However, Brown (1951) has referred to the work of Pasquier (1947) who noticed abdominal distention in desert locusts poisoned by gamma BHC.

It appeared that abdominal distention in mosquitoes may be a readily distinguishable sign of toxicity due to gamma BHC. Systematic studies were, therefore, planned to understand the significance and the nature of this abdominal distention.

THE NATURE OF ABDOMINAL DISTENTION.

Fully and half distended mosquitoes were readily spotted with naked eye. The presence of air bubbles could be made out in the ventral diverticulum through the pleural and intersegmental membranes. The air bubbles could be more easily seen if mosquitoes were fed on coloured solutions before exposure to gamma BHC. In fully bloated mosquitoes the membranes were stretched and the ventral diverticulum was in close apposition to the abdominal wall, thus showing the bubbles easily. The distention of the abdomen was found to vary from slight, medium, to a point of rupture.

The bloated mosquitoes appeared as if they had swallowed air. The proboscis was oriented more or less like that of a mosquito in the act of taking a blood meal. The labium was bent like a bow while the stylets were sticking out (Plate I, Fig. 1 and 2). It appeared possible that the effect of the insecticide resulted in

*The investigation was in part financed by a grant from the Indian Council of Medical Research.

the mosquito swallowing air which filled the fore- and mid-gut if empty, and later the oesophageal diverticulae. A similar sequence of events, resulting in the presence of air in the gut and diverticulae of the adult mosquito at the time of hatching from the pupa, has been described by Marshall and Staley (1932).

It was confirmed by dissection that the distention of abdomen was due mainly to the distention of the enlarged ventral diverticulum which was found extended backwards up to abdominal segment VI. The abdominal contents, like blood in the stomach, or eggs in the ovaries situated above the ventral diverticulum, were pushed backwards and upwards. At the same time, the pleural and intersegmental membranes were stretched. In other words, about two-thirds of the ventral aspect of the abdomen became semi-transparent, and gave a characteristic appearance to the bloated mosquitoes (Plate I, Fig. 1 and 2).

The ventral diverticulum in bloated mosquitoes was found to contain sometimes small air bubbles and at others a single large bubble, elongate, cask-like or rounded in appearance. The normally brisk peristaltic movements of this sac were found to be slow, when distended in paralysed mosquitoes.

Distention was best observed in unfed mosquitoes. In fully gravid females, one or two eggs were found forced out of the genital aperture (noticed in anophelines). Distention was relatively less marked in females containing half digested blood.

The two small dorsal diverticulae also showed a little increase in size. In this condition it was found difficult to pull them out intact from the thorax during dissection.

The fore-gut was found to contain air bubbles of medium size. Sometimes it showed a large bulge, if the mid-gut was already full of blood. When empty, the mid-gut was also seen distended with air. In extreme cases the mid-gut became rounded in shape and difficult to recognise as such but for the presence of the Malpighian tubules.

DEGREES OF DISTENTION.

Preliminary observations were made to determine different degrees of abdominal distention. On the basis of these observations a classification of the condition was arrived at, which is given below and followed throughout the experiments related in this paper.

Normally, the size of the abdomen of a mosquito varied according to its contents. The range is from (*a*) small, containing neither food nor mature ova; (*b*) medium, containing mature ova and no meal or half digested meal and partially mature ova and (*c*) maximum, when freshly engorged with blood. The abdominal distention observed in mosquitoes exposed to gamma BHC was of a higher magnitude (about twice) than the maximum size of the abdomen observable in mosquitoes engorged with blood (Plate I, Fig. 1 and 2). The degree of distention of the abdomen in mosquitoes exposed to BHC was classified as follows :

PLATE I

Fig. 1.

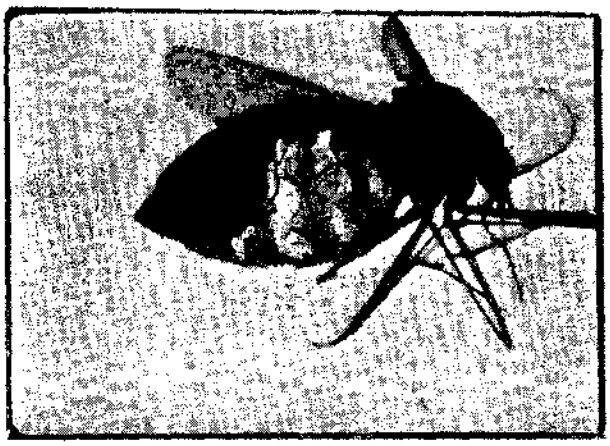

Fig. 2.

Fig. 1. Abdominal distention in blood-fed *C. fatigans* after death from the effects of exposure to gamma BHC. The blood meal is pushed backwards and upwards and the ventral sac has become fully distended with air. The labium is bent as in the act of sucking blood.

Fig. 2. Abdominal distention to a point of rupture in glucose-fed *C. fatigans* after death from the effects of exposure to gamma BHC.

Half distended state comprised mosquitoes with abdominal size (cross-section) increased to about 1½ to 2 times and fully distended state to more than twice the original size.

MATERIALS AND METHODS.

Mosquitoes for the experiments were obtained from the parent colony of normal *C. fatigans* and another colony of the same species resistant to DDT maintained at the South India Branch of the Institute. The degree of resistance to DDT of the latter strain was very high and not measurable by any available method. The adults of this colony could be maintained in continuous contact with DDT. The DDT-resistant mosquitoes, used in the experiments, were of generations 82 and 83.

The mosquitoes were fed either on glucose or blood before exposure to the insecticide. The actual condition of the feed is indicated in the text where relevant.

The filter papers were treated with gamma BHC (lindane) according to the method of Busvine and Nash (1953 ; 1954). The dosage and period of exposure varied as indicated in the text. The observations were made mostly once at the end of the exposure period and again 24 hours after the exposure. Measures were strictly observed to eliminate contamination of the laboratory outfit by insecticides.

RESULTS OF EXPERIMENTS.

It was considered essential to observe the proportion of normal mosquitoes that showed abdominal distention after exposure to lindane. Different dosage levels and periods of exposure were employed. Not less than 25 adult *C. fatigans* (normal) were used for each dosage of exposure to papers treated with 0·025 to 0·2 per cent lindane. Observations for each dosage level were replicated at least 4 times. In this experiment observations were made only 24 hours after exposure to insecticide. Table I below gives the average per cent mosquitoes with distended abdomen of the number exposed to different dosage levels.

TABLE I.

Percentage mortality and incidence of abdominal distention in normal blood-fed C. fatigans *24 hours after exposure to different dosages of lindane. Exposure period one-hour.*

Per cent concentration of lindane.	Per cent mortality.	Percentage of mosquitoes with abdominal distention :		
		Half distended.	Fully distended.	Total distended.
0·025	38	0	0	0
0·05	72	0	0	0
0·1	82	1	4	5
0·2	99	15	27	42

It is seen from Table I that as would be expected, the mortality increased with the dosage. As stated before, the abdominal distention was noticed only in the dead mosquitoes. At the first two levels of dosage, however, none of the dead mosquitoes showed any degree of visible abdominal distention. With higher dosages and higher mortality rates, increasing proportions of the dead mosquitoes showed abdominal distention. The maximum proportion showing distention was with the highest dosage (0·2 per cent) yielding almost 100 per cent mortality.

Even when the mortality was almost 100 per cent, only a proportion of the dead mosquitoes showed a distended abdomen. It, therefore, seemed necessary to investigate whether the incidence of abdominal distention in mosquitoes was related to (1) any particular dose, or (2) the time of exposure. In order that the distention of the abdomen could be readily observed, all the normal *C. fatigans* used for the present as well as the subsequent experiments, were fed only on glucose water. The observations are recorded in Table II.

TABLE II.

Percentage mortality and incidence of abdominal distention in glucose-fed normal C. fatigans *exposed to different concentrations of gamma BHC (lindane) for different periods of exposure.*

Serial number.	Per cent concentration gamma BHC (Lindane).	Exposure period. Hours—Minutes	Per cent mortality 24 hours after exposure.	Percentage of mosquitoes with abdominal distention.	
				Immediately after exposure to lindane.	24 hours after exposure to lindane.
1	0·025	4 — 50	95	0	6
2	0·05	4 — 40	80	0	10
3	0·1	2 — 20	85	20	25
4	0·25	1 — 05	95	50	30
5	0·5	0 — 55	95	85	55
6	1·0	0 — 30	95	70	50
7	2·0	0 — 20	100	75	55

Note: Distinction between half and fully distended abdominal condition was not made.

It is seen from Table II that in all the 7 series, the mortality was high, ranging from 80 to 100 per cent. In general, a larger number of mosquitoes showed abdominal distention at the end of the exposure period than 24 hours after the exposure. The proportion of mosquitoes showing abdominal distention was negligible in series 1 and 2 where the dosage was low but the period of exposure prolonged. As the dosage increased and the period of exposure decreased (Series 3 to 7), the proportion of mosquitoes with abdominal distention increased both at the end of exposure and 24 hours after exposure. It, therefore, seemed that the concentration of the insecticide *per se* causing 100 per cent mortality was not responsible for the manifestation of abdominal distention, but the amount of insecticide absorbed by the insect in unit time. In other words, the higher the dose absorbed in unit time the greater the proportion of mosquitoes that had distended abdomen.

As the distention of the abdomen was manifested in a larger proportion of mosquitoes immediately after exposure than after 24 hours, it appeared that in some mosquitoes at least the distention was reversible before death.

It was obvious from the foregoing that a large proportion of normal *C. fatigans* manifested abdominal distention under specified conditions of exposure to lindane described in Table II. The distention appeared to be a toxic reaction to the insecticide. A logical question was whether the same toxic manifestation to the insecticide would occur in mosquitoes which were resistant to the insecticide. As a strain of mosquitoes specifically resistant to lindane was not readily available, the DDT-resistant strain of *C. fatigans* was used for further experiments. It had already been found to be cross-resistant* to gamma BHC in the absence of any previous exposure to the insecticide.

Adult mosquitoes from the DDT-resistant colony were exposed to papers treated with lindane as described in the previous experiments. In order to obtain some mortality, the period of exposure was increased to 1·5 hours at dosages ranging from 0·025 to 0·2 per cent. The results are presented in Table III.

TABLE III.

Percentage mortality and incidence of abdominal distention in blood-fed DDT-resistant (cross-resistant to gamma BHC) C. fatigans 24 hours after exposure to different concentrations of gamma BHC (lindane). Exposure period 1½ hours.

Per cent concentration of lindane.	Per cent mortality.	Percentage of mosquitoes with abdominal distention :		
		Half distended.	Fully distended.	Total distended.
0·025	0	0	0	0
0·05	0	0	0	0
0·1	16	4	0	4
0·2	36	4	4	8

From the above Table III it would be seen that for the same doses, but with a longer exposure period, the mortality rate was much less in DDT-resistant mosquitoes (cross-resistant to gamma BHC) as compared to normal ones (Table I). Similarly, the percentage of mosquitoes showing abdominal distention, out of the total exposed, was significantly less than that in normal mosquitoes (Table I). It was necessary to determine the results of exposure of resistant mosquitoes to a higher range of concentration. Table IV shows the results of such exposures.

The results (Table IV) showed that exposure to 2·0 per cent concentration of lindane for one hour gave a mortality of 76 per cent. But the percentage incidence of abdominal distention was only 30, a figure lower than 42 per cent in normal mosquitoes at a dose 10 times lower (Table I). The results seemed to confirm that

* MLC of lindane for normal *C. fatigans* was 0·03 per cent×1 (hour).
MLC of lindane for DDT-resistant *C. fatigans* was 0·71 per cent×1 (hour).

TABLE IV.

Percentage mortality and incidence of abdominal distention in blood-fed DDT-resistant (cross-resistant to gamma BHC) C. fatigans 24 hours after exposure to different concentrations of gamma BHC (lindane). Exposure period 1 hour.

Per cent concentration of lindane.	Per cent mortality.	PERCENTAGE OF MOSQUITOES WITH ABDOMINAL DISTENTION.		
		Half distended.	Fully distended	Total distended
0·25	22	6	6	12
0·5	46	14	4	18
1·0	58	16	10	26
2·0	76	10	20	30

only a small proportion of mosquitoes of the resistant strain showed the toxic effects of the insecticide and, therefore, the toxic manifestations did not occur in mosquitoes with a considerable degree of resistance.

The above observations showed that the incidence of the toxic sign of abdominal distention after exposure to lindane was different in normal mosquitoes from that in a strain resistant to DDT with cross-resistance to BHC. It appeared logical to examine the utility of this toxic sign to detect the presence or absence of resistance to BHC in a population of mosquitoes with an unknown susceptibility status to BHC. This could obviously be carried out in the laboratory or field. As an initial step the trial was made with mosquitoes of the two colonies, namely normal and DDT-resistant *C. fatigans*.

Instructions were given to a technician to mark mosquitoes of the two colonies separately with distinctive printers' dye powders, namely, gold and silver. The number of mosquitoes to be marked and the choice of the colour of the dye powder for each of the two strains were left to the technician and were unknown to the authors. The dusted mosquitoes remained undisturbed in separate cages for 24 hours. The usual glucose feed was available to them. This interval ensured that any loose particles of dust would be shaken off the bodies.

The day after the dusting, the two sets of mosquitoes (numbering 104) were released into one cage and exposed in batches to papers treated with 1 per cent lindane for 15 minutes. After the exposure they were released into a clean cage. At the end of 24 hours, 64 mosquitoes were found dead and the remaining 40 were found living and flying normally. Each of the dead mosquitoes was carefully examined for the marking. Twelve were found to have gold dust and the remaining 52 silver dust on them. Among the 12 with gold dust, none was observed with any visible abdominal distention. Out of the 52 with silver dust, however, 8 were found with half distended abdomen and 14 with fully distended abdomen. The remaining 30 with silver dust did not show any abdominal distention. This might mean that the normal strain used for these studies was perhaps not homogeneous for susceptibility. This aspect of the problem, however, requires further

studies. The 40 mosquitoes found alive were killed and examined for their marking. Thirty-eight of them had gold and the remaining 2 silver markings. The data are tabulated for convenience in Table V.

TABLE V.

Percentage mortality and incidence of abdominal distention in a mixed batch of normal and DDT-resistant (cross-resistant to gamma BHC) C. fatigans females exposed to 1 per cent concentration of lindane for 15 minutes.

C. fatigans strain marked with.	Observations 24 hours after exposure.									
	Living.	Dead.	Total.	Per cent mortality.	Number with abdominal distention.			Percentage with abdominal distention.		
					H	F	T	H	F	T
DDT-resistant (Gold powder)	38	12	50	24	0	0	0	0	0	0
Normal (Silver powder)	2	52	54	96	8	14	22	14	26	40

H = Half distended.
F = Fully distended.
T = Total distended.

At the specified dose and exposure period, abdominal distention was seen in 22 out of 52 dead mosquitoes with silver dust and in none of the 12 dead ones with gold dust. It appeared, therefore, that the mosquitoes with gold dust were resistant to lindane judged solely by the absence of the toxic sign of abdominal distention. This was verified by the technician who had marked the mosquitoes.

DISCUSSION AND CONCLUSIONS.

Two clear-cut findings in the course of the series of experiments described were, (1) in a proportion of *C. fatigans* dead due to exposure to gamma BHC the abdomen was markedly distended, and (2) in each one with distended abdomen the proboscis showed the characteristic appearance of that of a mosquito taking a blood meal. As stated earlier, this finding in reference to mosquitoes has not been reported in literature.

The distention of the abdomen was confirmed by dissections to be solely due to the presence of air in the oesophageal diverticulae and gut, chiefly the larger ventral diverticulum. The assumption that it was air that filled the diverticulae seemed justified by the characteristic appearance of the proboscis in mosquitoes with distended abdomen. No specific tests were made to confirm this. Apparently aerophagy had occurred as a result of lindane poisoning. The mechanism of the aerophagy essential to the hatching of the adult from the pupa (Marshall and Staley, 1932 *loc. cit.*) and an identical state as a terminal toxic manifestation in the case of lindane poisoning, are not understood.

Exposure to small doses over prolonged periods did not result in the toxic manifestation of abdominal distention or the characteristic appearance of the proboscis although such exposure ultimately gave rise to 100 per cent mortality. It appeared that the abdominal distention and the characteristic appearance of the proboscis were signs of "acute" and not "chronic" toxicity. This would seem to be confirmed by the evidence that a proportion of mosquitoes which had abdominal distention and the characteristic appearance of proboscis immediately after the period of exposure, recovered from both the signs 24 hours later. It also seemed clear that the toxic manifestations were directly dependent on the dose of insecticide absorbed in unit time. It appeared that the largest dose that effected a 100 per cent knockdown in the shortest exposure period caused the acute toxic symptoms in maximum numbers of exposed susceptible mosquitoes. It is, therefore, suggested to explore the possibility of using this sign of acute toxicity for quick detection of resistant individuals in a mosquito population in the field.

Preliminary trials with a few adult *Anopheles* (males and females) species (*subpictus, vagus, varuna, jamesi, splendidus, pallidus, stephensi**, *fluviatilis**) and *Aedes aegypti** indicated that abdominal distention and the characteristic appearance of the proboscis were signs of acute toxicity in these mosquitoes also, when exposed to BHC.

Under similar conditions of exposure to lindane the signs of acute toxicity occurred in a smaller proportion of BHC-resistant mosquitoes than in normal ones. This was presumably due to the physiological capacity of the resistant mosquitoes to metabolise the insecticide rapidly.

SUMMARY.

Two hitherto undescribed signs of acute toxicity to lindane were noticed in *C. fatigans*. One was distention of the abdomen almost to a point of rupture. The other was the characteristic appearance of the proboscis similar to that of a mosquito taking a blood meal. Both the signs were noticed only in moribund or dead mosquitoes.

Dissection of mosquitoes with the signs of acute toxicity showed that the fore-gut, mid-gut (if empty) and oesophageal diverticulae were filled with air. The appearance suggested aerophagy.

The manifestations of the signs of acute toxicity appeared in the largest proportion of exposed mosquitoes when the dosage was high and the period of exposure short.

Under identical conditions of exposure the signs of acute toxicity were found in a very small proportion of mosquitoes resistant to lindane, as compared to normals. The possibility of exploring this sign of acute toxicity for quick detection of resistant individuals in a mosquito population, has been discussed.

*Laboratory colony strains.

ACKNOWLEDGMENT.

Grateful thanks are due to Dr. B. Ananthaswamy Rao, Director, National Malaria Eradication Programme of India, for his interest and encouragement in the work. Thanks are also due to Mr. S. Cornelius and Mr. P. Raju, Laboratory Assistants at the South India Branch of the Malaria Institute of India.

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Indian Journal of Malariology, **13**, *1*, *December 1959*.

FORCED RETENTION OF EGGS IN *CULEX* (*BARRAUDIUS*) *PUSILLUS* MACQ. (DIPTERA : CULICIDAE.)

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[December 2, 1959.]

INTRODUCTION.

VARIOUS factors may lead to the withholding of eggs in the ovary. This phenomenon may be induced by the absence of an obligate type of oviposition site, low temperature, confinement, crowding, unsuitable relative humidity, application of insecticides, lack of spermatozoa in the spermathecae and other interactory factors. The prolonged forced retention of eggs has been demonstrated among various species of insects belonging to the orders Lepidoptera and Hymenoptera, but very few papers have been published on work concerning mosquitoes. Fielding (1919) reported that fully formed eggs might not be deposited in the absence of water or a moist surface. He believes that the gravid female will retain her eggs until her death or until the conditions become favourable for oviposition. Nicholson (1921), Barber, Komp and Hayne (1924) and Mayne (1926) reported that anopheline mosquitoes, although frequently reported to have taken blood during warm periods in winter, are believed to delay oviposition during cold periods until the temperature is favourable. Senior White *et al.* (1945) suggested that anophelines under certain laboratory conditions may defer oviposition in the absence of more suitable water than that provided by wet blotting paper.

Woke (1955) reported that *Aedes aegypti* Linnaeus retain their eggs in their ovaries indefinitely in the absence of an obligate type of oviposition site. He studied the effects of deferred oviposition on the development and the viability of the eggs. In many cases, however, retardation of oviposition takes place without any certain or known causative condition. Roy and Majumdar (1939) found in *Culex fatigans* Wiedemann that though eggs were formed within three days, and though water was present, their deposition was delayed as long as 13 to 19 days after the blood meal.

The oviposition in *Culex* (*Barraudius*) *pusillus* Macq. occurs normally in the presence of water or a moist surface. Under favourable conditions for oviposition *Culex pusillus* usually produces one or two egg rafts after each blood meal of pigeon blood, the viability of which usually approaches 100 per cent (Shalaby, 1958).

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The individual eggs in each mass are all deposited normally and develop more or less simultaneously.

The study reported in this paper was done with the aim to determine the consequences of prolonged forced retention, on the eggs and on the adults, due to the non-provision of oviposition sites in the laboratory.

METHODS.

A colony of *Culex pusillus* was raised from wild caught larvae and pupae collected in the suburbs of Alexandria. The colony was maintained at an average room temperature of 24°C. and 70 per cent relative humidity. Because of the preference of *C. pusillus* females to pigeon's blood (Shalaby, 1958), pigeons were used to provide the blood meals for the females, whereas cellu-cotton soaked in 10 per cent glucose solution was provided as nourishment for the males.

It was observed that this species laid eggs on any moist surface, such as glucose soaked cellu-cotton, etc., in the absence of water surface. Therefore, some modifications as those adopted by Woke (1955) were used to provide nourishment for the males. The 10 per cent glucose solution was placed in a 100 ml. inverted Erlenmeyer flask with a covering of fine mesh cloth. Occasional and careful blotting was necessary to free it of the surplus moisture. Vibrations and jarring which lead to the formation of moisture on the outside surface of the cloth were avoided.

About 1,500 pupae from the parent colony were distributed equally in five bowls and let to hatch in five large rearing cages, each cage was of 15 inches length, 15 inches width and 15 inches height. Soon after emergence, 10 per cent glucose solution was provided for the nourishment of the adults as described earlier. It was noticed that the emergence of the adults was more or less simultaneous. Five days after emergence the females were let to engorge overnight, and the blood meal (pigeon's blood) was provided only once in every cage. From these cages, immediately after the blood meal was taken and at the end of each six-days interval thereafter, six females were isolated till the last of the females was dead in the original five cages. These groups of females were isolated in cages of $12 \times 10 \times 10$ inches, with wooden framing, wooden top and bottom, glass front and mesh wire screening on the back and sides. In the back is a muslin access sleeve about 30 inches long. The cages were provided with the oviposition dishes. Although *C. pusillus* is known to mate immediately after hatching, three males each, from the parent colony, were introduced in the cages containing the isolated females. This ensured fertilization of such females that might not have been fertilized in the experimental cages. A control cage was maintained similarly, except that necessary conditions for normal oviposition were provided.

The deposited egg rafts were counted, the eggs were measured and their morphological characters noted. Oviposited abnormal eggs were separated from the apparently normal eggs. The percentage hatchability in each egg raft was determined. The emerged larvae were counted and their growth was watched

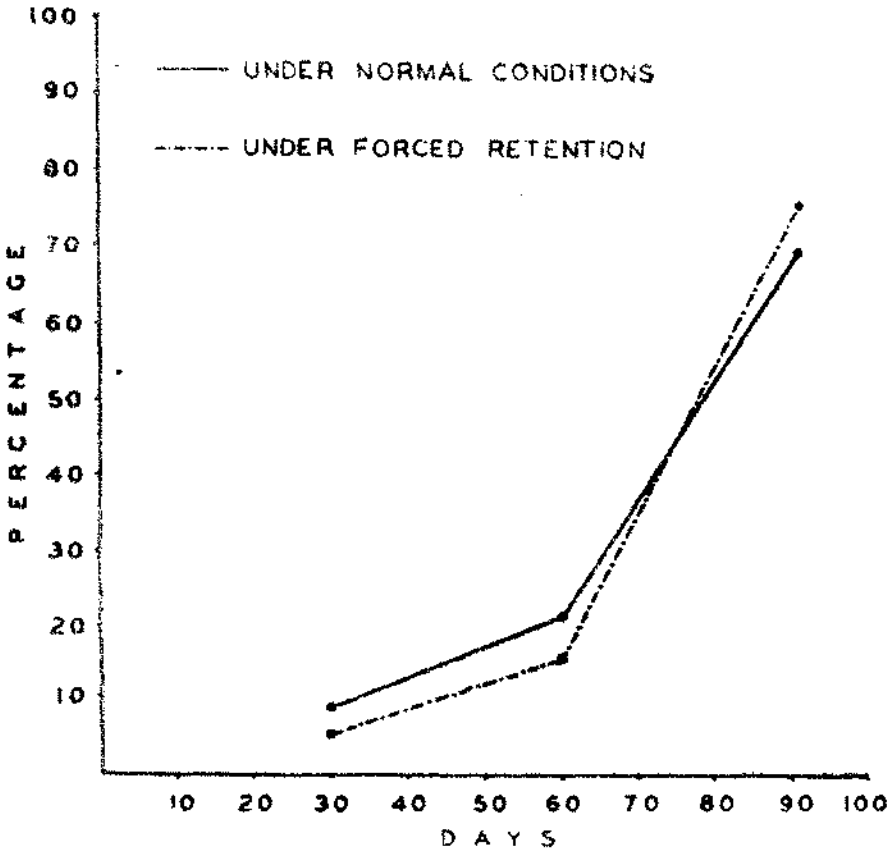
closely through the different instars. The eggs that did not hatch were taken as non-viable.

15 to 20 days after the transfer of the mosquitoes into the cages equipped for oviposition, all the surviving females were chloroformed. Dissection of the ovaries, differential counts, measurements and description of the abnormalities in the undeposited eggs were made. The females that died in the cage were dissected and the undeposited eggs in the ovaries were counted.

In order to determine the consequences of the forced retention of eggs on the mortality rate of females, a group of 150, after having ingested one blood meal, were placed in a cage where they were denied the opportunity to oviposit. The percentage of mortality was estimated at 30, 60 and 90 days after the blood meal. For the purpose of comparison, the percentage of mortality was estimated for a similar group which had recourse to frequent blood meals and provided with favourable conditions for oviposition. The results are indicated in Graph 1.

Graph 1.

Mortality rates of adult Culex pusillus *females which had fed and oviposited under normal conditions, and those which were forced to retain their eggs in the ovaries.*



OBSERVATIONS.

DEPOSITED EGGS.

Normal deposited eggs.—The normal deposited egg is conical in form and evenly tapered distally (Plate 1, *a*). Measurements of 150 eggs, four to seven hours after deposition, gave a range in length of 601—675 microns, with an average of 638 microns. Measurements of 100 eggs, 2 days after deposition, gave a range of 664—754 microns, with an average of 709 microns. Shrunken eggs were observed among the egg masses deposited under normal conditions. 260 shrunken eggs were collected from the total of 2,481 deposited eggs and they showed a range of length of 424—552 microns (mean, 488 microns). The shrunken eggs were found to be viable but their percentage of hatchability was below 40 per cent.

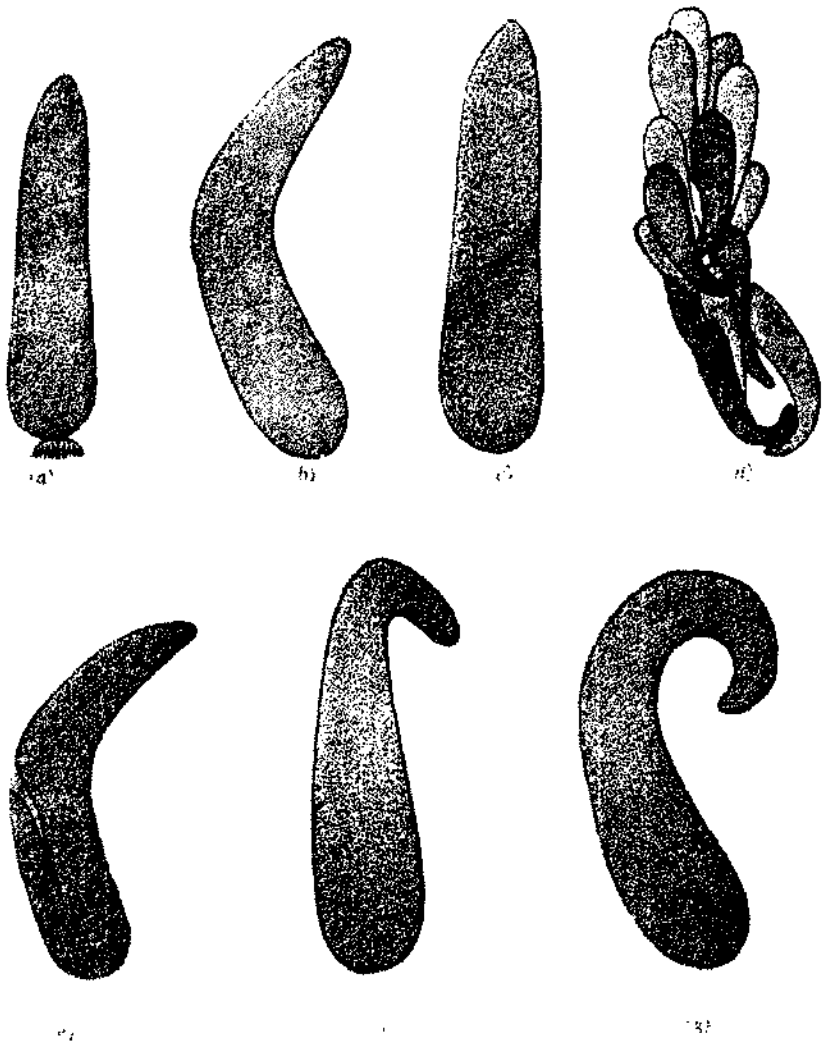
In general the deposited normal eggs are uniform in appearance and regularly arranged side by side in a raft which might be canoe shaped, ribbon shaped or irregular (Shalaby, 1958). Immediately after deposition, the chorion is pale greyish in colour. Few hours later, the colour changes to dark grey, which turns to almost black a short period before hatching. The chorions are filled out and the contents are more or less whitish. Among the normal appearing deposited eggs, however, a small proportion was found to be non-viable.

Forced retention of formed eggs.—During the successive observations of prolonged forced retention, it was of interest to observe that the eggs were retained in the ovaries for a period ranging from one to ten days, in the presence of normal conditions for oviposition (Table I). It is obvious from Table II that the number of deposited eggs decreased and the number of eggs which remained undeposited subsequently increased. The proportion of the total number of eggs which was deposited after providing the suitable conditions for oviposition, is seen to have decreased rapidly after about the 52nd day. Formed eggs that were deposited after retention for extended periods showed visible abnormalities and reduction in viability. Among the apparently normal deposited eggs, the percentage of non-viable eggs increased (Table II).

Abnormal deposited eggs.—Eggs abnormal in appearance were deposited along with the normal eggs after the 12th day of forced retention. It showed a rapid increase in number commencing from the 46th day (Table II). On the 12th day, one egg in a raft of 285 eggs showed an exceedingly abnormal length of 850 microns. All the abnormal eggs deposited were proven to be non-viable. Unlike the normal eggs, some abnormal eggs were shrunken and failed to increase to their average length after deposition. A few were obviously curved (Plate 1, *b*). Many were enlarged and did not gain the characteristic dark colour. Abnormal pigmentation of the chorion varied, as in eggs with more or less black proximal half and a greyish distal half (Plate 1, *c*). Some eggs ranged from pale grey to almost black. In several cases, however, the deposited eggs were not glued together to form the characteristic egg mass of the culicines, instead they were laid at random and scattered on

PLATE 1

Eggs recovered from Culex pipiens fatigans after extended periods of forced retention.



(a) Normal deposited egg.
(b-c) Abnormal deposited egg.
(d) Egg-forms retained in the ovary 76 days after the blood meal.
(e-g) Abnormal undeposited egg.

TABLE I.

Successive groups of mosquitoes provided with the opportunity to oviposit at six-days intervals; and the number of days taken until actual oviposition occurred. (Under ordinary laboratory conditions of 24°C. and 70 per cent R.H.)

Groups.*	Number of days: After blood meal.	Till oviposition.	In oviposition cage before ovipositing.
1st	6	7	1 (20 hours)
2nd	12	14	2
3rd	18	21	3
4th	24	28	4
5th	30	33	3
6th	36	40	4
7th	40	45	5
8th	46	51	5
9th	52	55	3
10th	58	63	5
11th	64	71	7
12th	70	76	6
13th	76	84	8
14th	82	†	†
15th	88	98	10
16th	94	—	—
17th	100	—	—

*Each group consisted of six females except the last group which consisted of three.

†Females escaped from the cage before oviposition.

TABLE II.

Number and condition of eggs recovered from the successive groups of mosquitoes under ordinary laboratory conditions of 24°C. and 70 per cent R.H.

Days after blood meal.	Number of eggs.	Deposited eggs: Normal viable. Number.	Per cent.	Normal non-viable. Number.	Per cent.	Abnormal. Number.	Per cent.	Undeposited eggs: Normal. Number.	Per cent.	Abnormal. Number.	Per cent.
6	464	446	96·00	18	3·10	..	..	..	..	..	..
12	290	277	95·52	7	2·41	1	0·34	5	1·73	..	..
18	173	137	80·00	24	13·90	3	1·15	9	4·95	..	..
24	220	198	86·46	24	10·48	3	1·31	4	1·75	..	..
30	214	146	68·19	58	27·19	8	3·74	2	0·97	..	..
36	248	128	51·61	95	38·30	8	3·22	15	6·05	2	0·82
40	210	83	39·52	110	52·38	6	2·85	10	4·76	1	0·49
46	195	39	20·00	130	66·67	12	6·15	11	5·64	3	0·54
52	192	21	10·94	139	72·30	12	6·25	11	5·73	9	4·69
58	164	8	4·87	81	49·33	21	12·80	25	15·24	29	17·76
64	162	1	0·63	70	43·21	20	12·34	28	17·28	43	26·54
70	204	2	0·99	63	30·88	24	11·78	35	17·15	80	39·20
76	200	..	..	11	5·50	39	19·50	48	24·00	102	51·00
82	*	*	*	*	*	*	*	*	*	*	*
88	186	..	..	2	1·07	6	3·22	12	6·45	166	89·26
94	154	..	..	..	..	..	..	8	5·19	146	94·81
100	148	..	..	..	..	..	..	..	..	148	100·00

*Females escaped from the cage before oviposition.

the water surface. Most of the eggs did not float, but sank to the bottom of the oviposition dish.

Non-viable normal appearing deposited eggs.—Under conditions suitable for oviposition, a small percentage of deposited eggs ranging from one to three per cent had failed to hatch. During the series of observations, a noticeable increase in the non-viability of deposited eggs that appeared normal was recorded (Table II). It reached its maximum 52 days after the blood meal, when 139 out of 172 deposited eggs were proven to be non-viable, though appeared normal. It is of interest to notice that the contents of such non-viable eggs showed a pale greenish tinge instead of whitish, a phenomenon apparently characteristic of abnormal eggs.

Viable deposited eggs.—Under suitable conditions for oviposition, the percentage of viability of deposited eggs reached about 98 per cent. An obvious gradual decline was noticed with the increase in the period of forced retention of eggs. The decline was most obvious after the 30th day, when the percentage of viable deposited eggs decreased to 68·19 per cent. After the 30th day the decline in per cent viable eggs was regular and significant till the 76th day, when all the deposited eggs were non-viable (Table II). It was observed that the vitality of the larvae, emerging from eggs laid on and after the 36th day of forced retention, was declining and a large number died within a few hours after hatching.

'Incomplete hatching' of normal appearing deposited eggs.—During the series of observations, it was noted that some of the normal appearing eggs failed to hatch properly. The embryo was capable of breaking the chorion in order to emerge half-way only. The head, thorax and a part of the abdomen of the newly emerging first instar larvae were capable to wriggle themselves out of the chorion, while the rest of the abdomen remained in the egg unable to free itself. Such larvae died within five to ten minutes after the chorion was broken. Death was caused probably due to drowning. This phenomenon occurred in the larvae emerging from eggs retained for 36, 46, 64 and 70 days after the blood meal. The percentages of such eggs to the total number of deposited eggs was 4·76, 2·24, 0·97 and 2·35, respectively.

UNDEPOSITED EGGS.

It was observed that no eggs were retained in the ovaries of the females six days after the blood meal, after providing the suitable conditions for oviposition. 12 days after the blood meal, five normal appearing eggs were retained in the ovaries (Table II). The proportion of the total number of formed eggs which were retained in the ovaries after providing suitable conditions, increased rapidly after the 30th day. On and after the 94th day, all the formed eggs were retained in the ovary (Table II). Because of extended periods of retention in the ovarioles, an increasing number of eggs acquired abnormal features and only 5·19 per cent were normal.

Abnormal undeposited eggs.—The abnormalities noticed were in size, shape and colour in addition to the chorion malformations. The observation of the ovary of

a female that had carried its eggs for 76 days, showed alteration in shape and colour in addition to obvious disarrangement of the eggs (Plate I, *d*). Eggs with overly large size which reached up to 910 microns in length were found in the ovary of a female that had carried its eggs for 94 days after the blood meal. Abnormalities as regards to colour, ranged from pale yellowish, greyish, light and dark browns and black. Some eggs were as black as 24 hours old deposited eggs. The chorion malformation occurred from the 46th day but were especially numerous in the females that carried their eggs 70 days after the blood meal and longer. This varied from somewhat collapsed chorions to chorions which were ruptured or split (Plate I, *e*). Abnormalities as regards to shape, ranged from slightly curved, distally bent (Plate. I, *f*) or looped (Plate I, *g*).

Effect of forced retention on adult mortality.—Among 150 females which had freely taken blood meals and had been allowed to deposit their eggs, eight per cent died at 30 days, 22 per cent at 60 days and 68·4 per cent at 90 days. Among the same number which were denied the opportunity to lay their eggs after one blood meal, six per cent died at 30 days, 16 per cent at 60 days and 78·66 at 90 days (Graph I).

DISCUSSION.

The causative conditions of withholding of eggs among various arthropods are unknown in some circumstances. The retention of eggs in the ovaries of insects could be attributed to the inhibitory effect of the brain, as to deposit in places sure to hatch the eggs and keep the species flourishing. It was reported by Wigglesworth (1950) that the females of *Tipula* (*Diptera*), after living for some days without egg-laying owing to unsuitable conditions, can be brought by decapitation to immediate oviposition. Woke (1955) reported that four *Aedes aegypti* which were decapitated 30 days after each had taken a blood meal and had been in the meantime without opportunity to oviposit, immediately deposited 10 to 20 normal eggs each in a dry petri dish. DeCoursey and Webester (1952) in their experiments with *Aedes sollicitans* (Walker), reported that under conditions in which oviposition would not normally occur, the females may be forced to oviposit after treatment with insecticides.

Cases of lengthened egg-laying periods in various arthropods were observed to be due to confinement alone and to crowding (Woke, 1955). It is of interest to notice that in *Culex pusillus* the females in which retention of eggs was enforced did not oviposit immediately after being given the opportunity to do so (Table I). Six days after the blood meal, oviposition occurred about 20 hours after being provided with the suitable conditions for oviposition. The lapse between the date of release into the oviposition cages and the actual date of oviposition had increased considerably and more or less gradually with the increased periods of forced retention, until it reached 10 days in those which were forced to carry their eggs 88 days after the blood meal (Table I), when only eight eggs were deposited (Table II).

Failure to deposit all the formed eggs after extended periods of retention may be attributed in part to mechanical inability to pass many of the enlarged and the malformed eggs.

The variation of the total of the viable and the non-viable eggs of all the deposited eggs which appear normal and those which were retained in the ovaries, reflects in part the varied quantities of blood that may be taken by the individual mosquitoes in addition to individual differences. It is also believed that age and physical conditions influence the rate of deposition of formed eggs.

The reduced viability of deposited eggs noted during the successive observations under the conditions of these experiments could result from a failure in the mechanism of fertilization. This could, perhaps, arise from damage to the spermathecae due to long retention, which might have caused occlusion or injury to the spermathecal duct. Damage to the micropylar apparatus, substantial death to the sperms, failure of the females to retain the sperms or to liberate them at the proper time, or inability of the sperms to penetrate the eggs could also be causative factors in the non-viability of deposited eggs.

It is noticeable that the percentages of the mortality in the females which had freely taken blood meals and had been put under conditions favourable for oviposition, and the females which were allowed one blood meal and forced to retain their eggs, were more or less similar. Woke (1955) reported similar results in *Aedes aegypti*. However, Flanders (1942) working on the Hymenopterous parasite *Encyrtus fuliginosus* Compere reported that the parasites that were allowed to oviposit lived three times as long as those that were not permitted to oviposit.

The vitality of larvae that emerged from eggs laid after 18 to 30 days of forced retention, was markedly reduced, and a large number of these died within a few hours after hatching. In addition they showed the phenomenon of 'incomplete hatching'. This would indicate that the vitality had declined already in the formed eggs before deposition.

From the observations reported in this paper, it becomes evident that any designed plan for the control of breeding sites, particularly by proper sanitation, needs to be a thorough one, as otherwise species that can retain viable eggs for over two months can upset the whole control programme. Such behaviour of *C. pusillus* should be studied with other culicines and anophelines in addition to other subjects such as, oviposition, feeding and mating habits along with the longevity of each species before launching control programmes in any given area. This information, beside being of academic interest, is of importance in control procedures.

SUMMARY.

Forced retention of eggs in the mosquito *Culex (Barraudius) pusillus* Macq. by non-provision of oviposition sites was carried out.

Instantaneous oviposition was not encountered by providing the favourable conditions for egg laying. A gradual delay of one to ten days occurred before the successive groups of mosquitoes had actually oviposited.

A gradual decrease in the deposited eggs and a consequent increase in the formed undeposited eggs occurred. 94 days after the blood meal, the formed eggs were all retained in the ovaries.

A decline in the viability of deposited eggs and the vitality of emerged larvae due to lengthened periods of retention had taken place. Cases of 'incomplete hatching' among deposited eggs were noticed. Non-viability of deposited eggs reached 100 per cent on and after the 76th day of forced retention.

Abnormalities occurred in the deposited and formed undeposited eggs. These ranged from variations in size, alteration in shape, discolouration of fluid content and malformation of chorion. A gradual increase in the proportion of abnormal eggs was noticed in eggs deposited by the successive groups of mosquitoes. Abnormalities were more prominent in eggs retained in the ovaries. On and after the 88th day of forced retention, the formed eggs exhibited more than 90 per cent abnormality. 100 days after the blood meal, the formed eggs exhibited 100 per cent abnormality.

Effect of prolonged forced retention on the mortality of females, in comparison with those which had the opportunity to feed and oviposit freely, did not show a noticeable difference.

ACKNOWLEDGMENT.

Sincere appreciation is expressed to Professor M. M. Ramadan, Head of the Department of Zoology, Faculty of Science, University of Alexandria, for his kindness in extending all facilities while carrying out these investigations.

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Indian Journal of Malariology, **13**, 4, *December 1959*.

Review

Malariology, with special reference to Malaya. By A.A. Snadhosham. University of Malaya Press, Singapore. (1959). *Price 35s.*

PROFESSOR SNADHOSHAM is to be congratulated on his admirable review in his book on the extensive work in the field of malariology by workers in Malaya. The text has the lucidity which is only acquired through long years of teaching of the subject. As a teaching manual, the book will be welcomed by the students of malariology in Malaya.

The chapters on malaria parasite and notes on Malayan vectors have been presented excellently. 'These merozoites do not invade liver cells any longer' (p. 50) creates doubts in the reader's mind as to whether the merozoites, released from blood schizonts, do so at any stage. Accepted rapid staining method for both thick and thin films, such as J.S.B., has not been mentioned and in this context the statement 'for at a pH below 7·0 stippling is seldom seen even with intense staining' (p. 54) cannot be supported.

Certain considerations on epidemiology have been included. Great stress has been laid on classical species sanitation in consideration of the local vector habits. The importance of infant parasite index in the assessment of transmission has not been mentioned.

The current concept of eradication of malaria by the use of residual insecticides, aided by antimalarials, now undertaken by various Nations, has not been introduced to the reader. Some of the statements appearing in the text such as 'elimination of mosquitoes as a means of eradicating malaria' (p.15), 'eradication cannot be hoped for by house spraying alone (p. 259) compared to the highly successful antilarval measures for the protection of towns and villages' read somewhat back-dated. While discussing antimalarials, no mention has been made of primaquin which is known to be less toxic than plasmoquine.

The get-up of the book is good ; the plates, diagrams, printing and binding are praise-worthy.

P. C. B.

Indian Journal of Malariology, **13**, *4*, *December 1969*.

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