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Indian Journal of Malariology, **14**, *1, March 1960.*

STUDIES ON METABOLIC DEGRADATION OF PAMAQUIN (PLASMOQUIN) IN MONKEYS.

BY

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AND

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[November 17, 1959.]

PAMAQUIN (plasmoquin), [8-(4'-diethylamino-1' methyl-butylamino)-6-methoxy quinoline ; Chart 1, Fig. I], a member of 8-aminoquinoline series, was the first synthetic antimalarial to be discovered. Although a poor blood schizonticide, it proved to be of considerable value in radical cure of malaria (Wiselogle, 1946 ; Schmidt and Coatney, 1955). However, its wide-spread use had to be restricted because it was saddled with a major handicap on account of its toxicity. Attempts to improve upon the activity of pamaquin led to the discovery of several closely related 8-aminoquinolines, viz., pentaquin, isopentaquin and primaquin. These drugs showed higher chemotherapeutic indices, but dose for dose they were perhaps as toxic as pamaquin (Schmidt and Coatney, 1955). It has been observed that though pamaquin was markedly plasmodicidal *in vivo*, it was relatively inactive when added to cultures of *P. falciparum in vitro* (Berliner quoted by Josephson, Taylor *et al.*, 1951). Gieman (1948) made essentially the same observation for the related 8-aminoquinolines, pentaquin and isopentaquin, using culture of *P. knowlesi in vitro*. It was further confirmed that, although pamaquin when added to *in vitro* cultures of *P. gallinaceum* was inactive, concentrates from blood, tissues and droppings of the chicken treated with pamaquin, possessed antimalarial activity *in vitro* which could not be accounted for on the basis of pamaquin present in these concentrates (Josephson, Taylor *et al.*, 1951). This suggested that anti-plasmodial and possibly toxic methemoglobin forming properties of pamaquin were perhaps due to metabolite (s) which was formed in the system. It had also been observed by Zubrod, Kennedy and Shanon (1948) that pamaquin was extensively metabolised in the vertebrate body and extremely little of the drug was recovered as such in the excretory products of treated patients. Brodie and Udenfriend (1950) and Hughes and Schmidt (1950) undertook studies on metabolic intermediates of pamaquin and related 8-aminoquinolines, but none of these could be chemically characterised.

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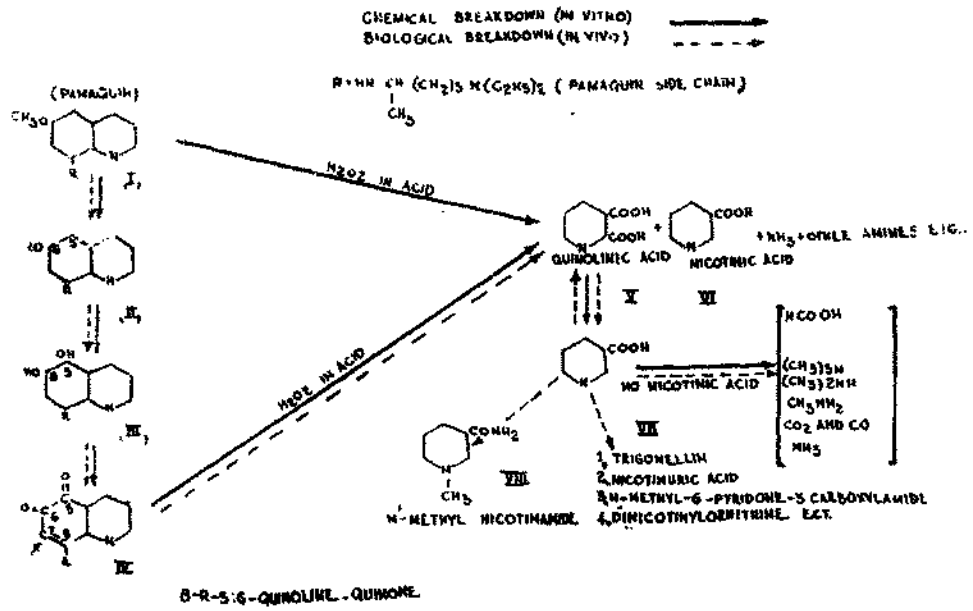
CHART I.

Metabolic Degradation of Pamaquin.



Josephson, Greenburg *et al.* (1951) and Greenberg *et al.* (1951) later on confirmed that one of the degradation products of pamaquin, following a single oral dose to chickens, was 8-(4'-diethylamino-1'-methylbutyl-amino)-5 : 6-quinoline-quinone (Chart 1, Fig. IV). No other metabolite of pamaquin was, however, isolated in these studies. Studies have been undertaken in these laboratories for the last several years with a view to confirm the above results in monkeys, if possible, and to search for other metabolites of pamaquin in order to further elucidate the mechanism of pamaquin degradation *in vivo*. Such knowledge was not only expected to help to rationalise the clinical use of pamaquin and related drugs but could also offer some clue for the synthesis of newer derivatives which would have the essential curative properties but little toxic side-effects. Preliminary work has already been reported by Bami (1955) and the detailed findings are recorded in this paper.

EXPERIMENTAL AND RESULTS.

In order to study the metabolic degradation of pamaquin *in vivo*, pamaquin citrate (a soluble salt) was administered orally to monkeys at varying doses. A dose of 10 mg. base/kg. body-weight proved to be toxic and even fatal while 5 mg./kg. body-weight was adopted successfully for the purpose. Urine was collected over dilute hydrochloric acid and preserved in the refrigerator. Different solvents like ethylene-dichloride, butanol and benzene : butanol mixture were used for extraction of the metabolites at different pH. These extracts were either concentrated under vacuum at low temperature or directly chromatographed on

cellulose column. Nature of the metabolites was studied and some typical experiments are recorded as follows :—

(A) EXTRACTION WITH BUTANOL AND BENZENE : BUTANOL AT pH 7·0 AND 5·0.

Urine (200 ml.) was adjusted to pH 7·0 with solid sodium acetate and extracted with butanol (100 ml.) by shaking mechanically for an hour. The extract was run on a cellulose column conditioned with butanol saturated with water at pH 7·0. Very little adsorption took place on the column and the extract was recovered as such. Use of 50 : 50 benzene butanol, as a solvent in the above procedure, offered identical results. Similarly, extraction at pH 5·0 was also unsuccessful.

(B) EXTRACTION WITH BUTANOL AT pH 2·0

Preserved urine (2,000 ml.) was filtered, adjusted to pH 2·0 and extracted twice with butanol (500 ml.). Total butanol extract was reduced to about 200 ml. under reduced pressure at room temperature. This extract was suitably diluted with benzene to obtain a 30 : 70 benzene : butanol mixture.

In order to prepare a chromatographic column, cellulose powder (washed with 0·1 N HCl) was made into slurry with 0·1 N HCl saturated with butanol. It was poured into a column (40×2·5 cm.) and packed with suction applied at the lower end. The packed column was successively washed with 0 : 100, 10 : 90, 20 : 80 and 30 : 70 mixtures of benzene : butanol saturated with 0·1 N HCl. The above 30 : 70 benzene butanol extract was run on the column using suction. Brown material adsorbed at the top was eluted with 30 : 70 benzene : butanol saturated with 0·1 N HCl. A diffused pinkish band was ultimately obtained on the column while majority of the metabolites under these conditions were not adsorbed on the column. The pinkish band fraction and the unadsorbed extract were treated with large excess of petroleum ether and the metabolites obtained in a small aqueous phase in each case.

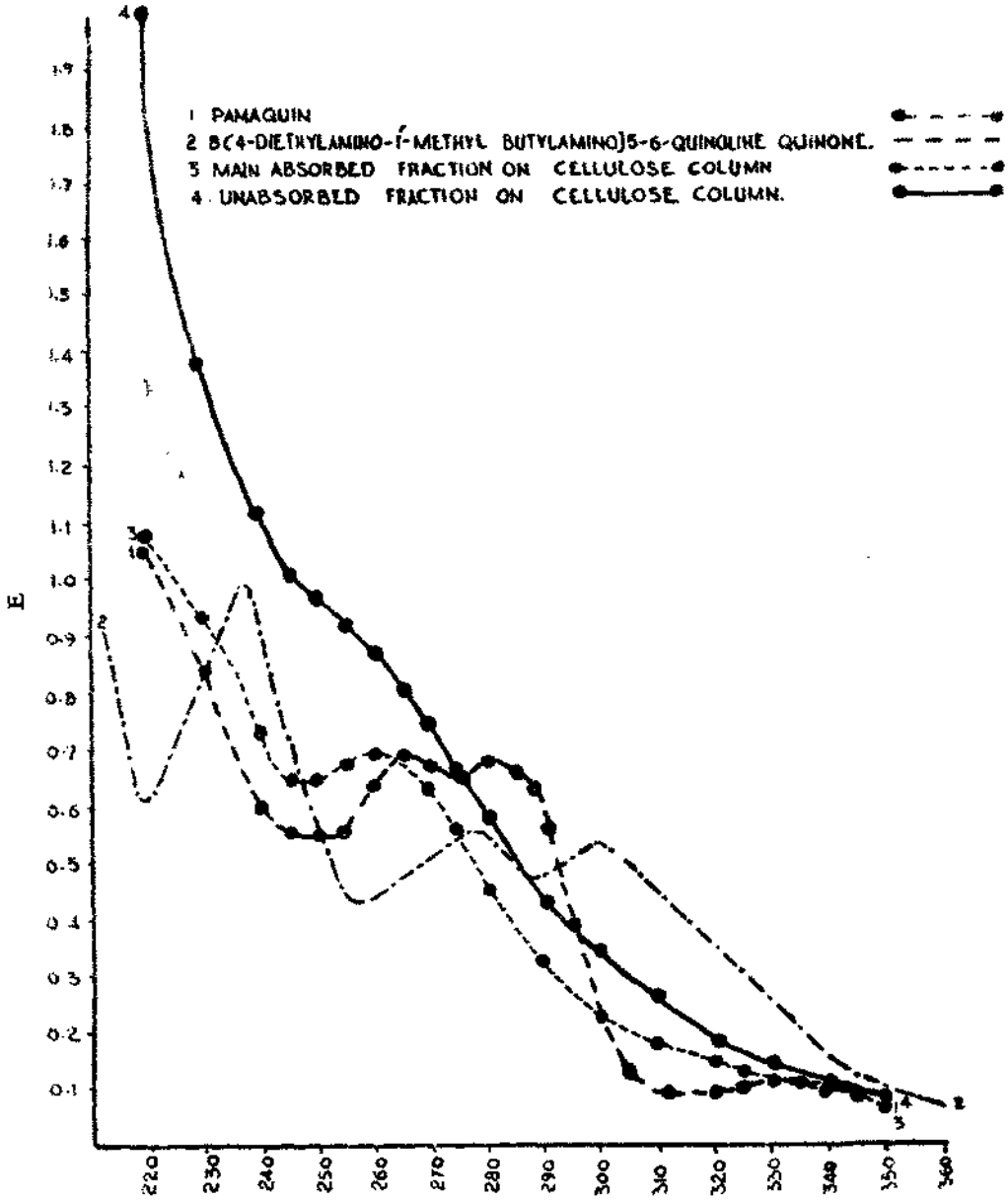
Ultraviolet absorption spectra of pinkish cut, as well as of unadsorbed material, was determined on a model DU Beckman spectro-photometer and compared with that of pamaquin and its known 5 : 6-quinoline : quinone metabolite (Graph 1). The unadsorbed material appeared to be similar to acid insoluble fraction isolated by Brodie and Udenfriend (1950) so far as their UV absorption spectra were concerned. The main adsorbed fraction showed only one peak when compared to two in case of UV spectra of pamaquin and its metabolite. Attempts to prepare a quinoxaline derivative, benzoyl derivative, picrate or a 2 : 4-dinitrophenyl-hydrazine derivative of these two fractions were unsuccessful.

(C) EXTRACTION WITH 30 : 70 BENZENE : BUTANOL AT pH 6·5 TO 7·0.

Urine (1000 ml.) preserved in acid was filtered and adjusted to pH 7·0 with solid sodium acetate. It was directly extracted with 30 : 70 benzene : butanol mixture (500 ml.) for an hour. The organic solvent phase was separated and run

GRAPH 1.

Ultraviolet absorption curves for pamaquin and its metabolites.



Wave Length in $m\mu$.

on a cellulose column (40 cm. × 2·5 cm.) conditioned as described under (B). The unadsorbed material was collected separately. A broad brown band was formed at the top which was eluted with 30 : 70 benzene : butanol saturated with 0·1 N hydrochloric acid. It got separated into three small diffused bands, yellow with greenish tinge (I), brown (II) and a larger pinkish (III). Each collection was diluted with excess of petroleum ether (40-60°C.), and an aqueous extract containing the metabolites was separated. Similarly, the unadsorbed material (IV) was also diluted with petroleum ether and the aqueous layer collected. These aqueous extracts (I to IV) were examined in detail as follows :—

(*i*) Aqueous extracts I to IV, when screened against *P. gallinaceum* in young chicks, were found to be inactive. Total material extracted by ethylene-dichloride from the urine similarly, was also found to be inactive.

(*ii*) The larger pinkish cut (III) was analysed for the presence of unchanged pamaquin, if any, by Brodie *et al.* method (1947). It was found to contain none.

(*iii*) Pinkish cut (III) was concentrated under reduced pressure and with the residue, several spot tests were conducted in order to determine the presence of various functional groups. The tests were positive for primary aliphatic amine and carboxyl group but negative for phenol, carbonyl, alcoholic and secondary aliphatic amino groups.

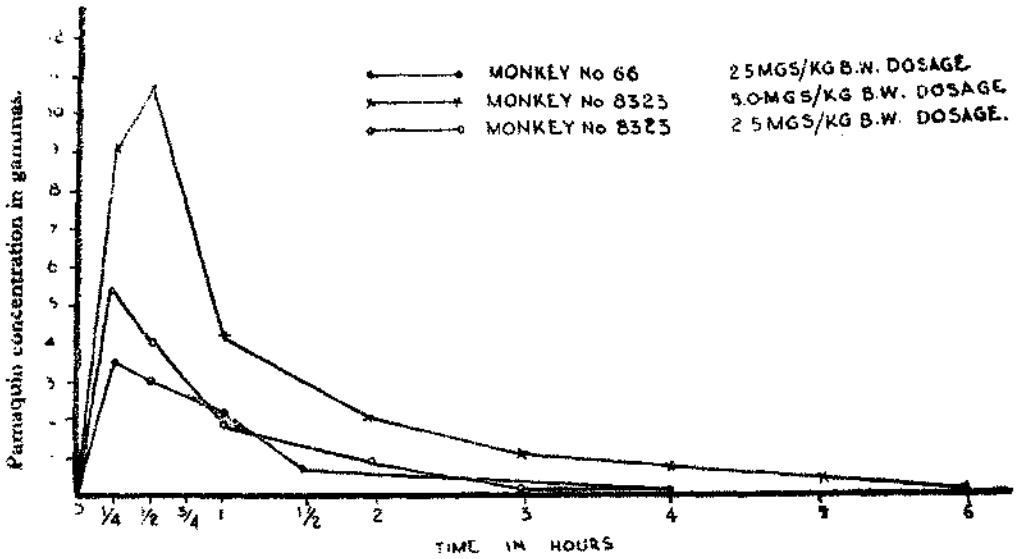
(*iv*) Attempts to prepare quinoxaline derivatives, benzoyl derivative, p-phenylazobenzoyl derivative, picrate and 2 : 4 dinitrophenyl-hydrazine derivative have also been unsuccessful in all cases.

(D) Intravenous administration of pamaquin.

Considering that oral administration of pamaquin citrate might lead to improper absorption of the drug and some of the metabolites might not be present in the urine in sufficient quantities, pamaquin citrate was injected intravenously. A dosage of 2·5 mg. base/kg. body-weight was found to be well tolerated by monkeys for the purpose.

Estimation of pamaquin in blood was undertaken in the first instance in order to determine the time of disappearance of the drug from the blood. A single dose of pamaquin 2·5 mg. base/kg. body-weight was injected intravenously to a monkey and blood samples were drawn at different intervals of time. Pamaquin in centrifuged plasma was estimated by Brodie *et al.* method (1947) and the results are presented in Graph 2. It would appear from Graph 2 that pamaquin as such was not present in the blood after 3 to 4 hours of its intravenous administration. This did not exclude the possibility of its conversion into such metabolites which were not detectable by Brodie *et al.* method of analysis (1947). However, it was possible that bulk of metabolites might be available in the urine or blood within a reasonable time. Consequently, urine of treated monkeys was studied by solvent extraction at different pH and chromatography as described before. Excepting very slight contamination with unmetabolised pamaquin, physical and chemical characteristics of the fractions isolated were similar to those obtained with oral feeding. None of

GRAPH 2.



the fractions isolated, showed any antimalarial activity when screened against *P. gallinaceum* in young chicks.

(E) CHEMICAL BREAKDOWN OF PAMAQUIN.

Schonhofer (1942) had indicated the possibility of biological oxidation of pamaquin *in vivo* which was also considered responsible for its therapeutic activity. Studies on metabolic degradation of pamaquin by Josephson, Greenberg *et al.* (1951) also supported the above hypothesis. It was, therefore, thought worthwhile to study the chemical breakdown of pamaquin molecule using mild chemical oxidising agents. Oxidation of substituted quinolines, to nicotinic acid and quinolinic acid, has been achieved by several workers (Stix and Bulgatsch, 1932; Hogerwerff and Vandorp, 1879, 1883; Miller, 1891; Guha and Muller, 1944), using a variety of oxidising agents like dilute hydrogen peroxide, potassium permanganate, dilute nitric acid and catalytic and electrolytic oxidation. However, in the present studies, mildest of the oxidising agents, viz., dilute hydrogen peroxide and dilute sulphuric acid were employed as follows:

Pamaquin citrate (9·3 g.), hydrogen peroxide (3 per cent; 510 ml.), sulphuric acid (25 per cent; 11 ml.) and copper sulphate (15·4 g. dissolved in 38 ml. water) were heated together at 60°C. for 12 hours. Again hydrogen peroxide (3 per cent; 50 ml.) and sulphuric acid (25 per cent; 11 ml.) were added and the mixture was neutralised to pH 4, heated at 90°C. for 30 minutes and kept over night. Supernatant layer was decanted off and on basification, gave strong ammonical smell. Precipitated copper salt thus separated was suspended in water (50 ml.). Hydrogen

sulphide was passed into it at 60°C., the black precipitate filtered and the clear filterate kept over-night. A crystalline product was obtained which was purified by re-crystallisation from glacial acetic acid. White amorphous crystals m.p. 190°C., yield 0·24 g. (mixed melting point with an authentic sample of quinolinic acid, 190°C.). Mother liquor obtained after filtering crude quinolinic acid was treated with excess of ethanol and filtered. Filterate gave a strong positive reaction for nicotinic acid and on basification gave indication of presence of amines.

The above experiment lent indirect support to the assumption that if pamaquin was extensively oxidised in the system, products like quinolinic acid, nicotinic acid, their further derivatives and simpler breakdown products like ammonia, methylamine, dimethylamine, trimethylamine, formic acid etc., (Chart I) could be its normal breakdown products. Most of these oxidation products were, however, normal biological constituents of the urine as well. Conversion of only a very small dose of pamaquin into these highly soluble products could, therefore, get masked and escaped detection unless their excretion was very much above the normal. It was suggested, therefore, that estimation of nicotinic acid in the urine and blood both before and after intravenous administration of pamaquin might throw more light on these aspects.

(F) Estimation of nicotinic acid in urine.

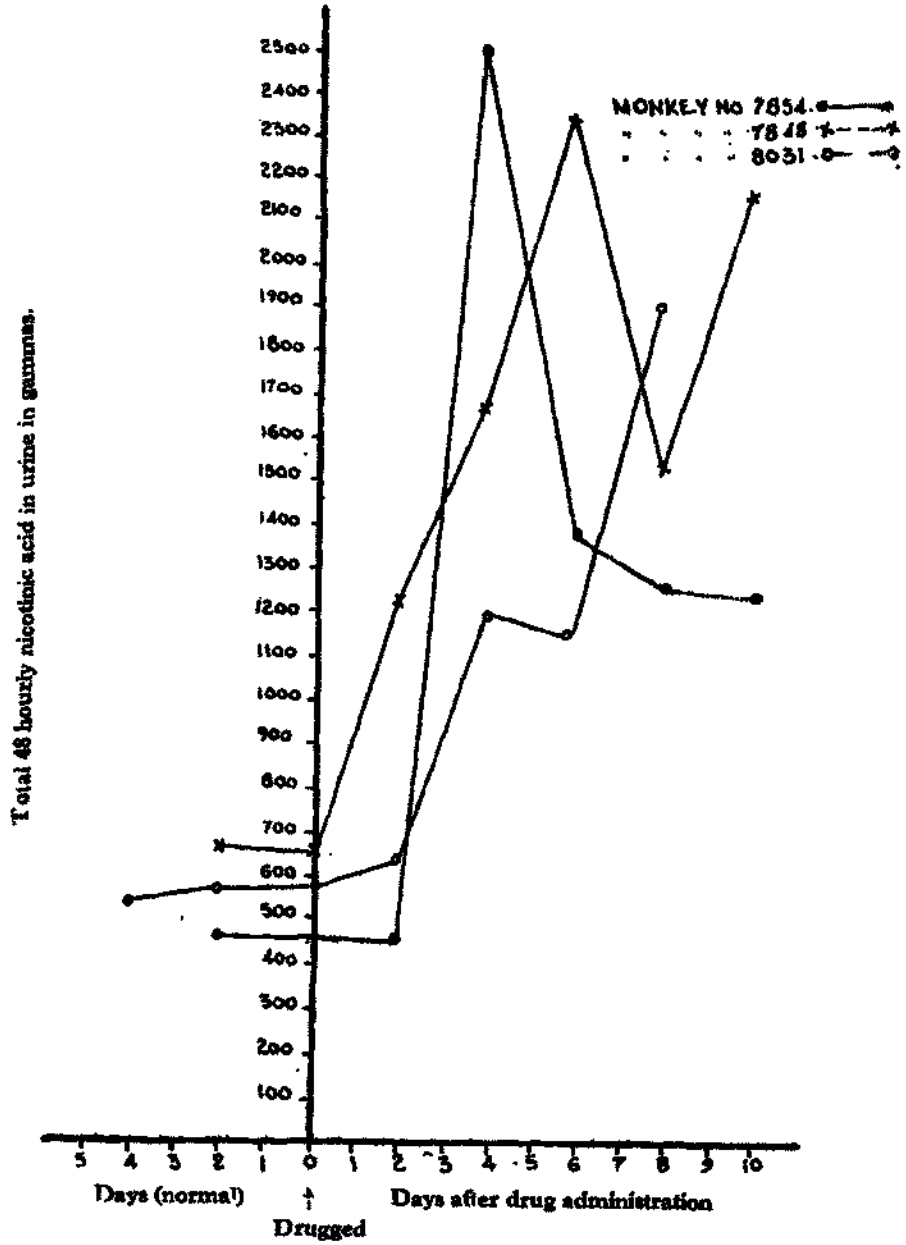
Original method of Harris and Raymond (1939) for nicotinic acid estimation was adopted after confirming its specificity. For this purpose, pamaquin citrate solution was heated with alkali as required by the method and tested for Konig's reaction (1904) which was found to be negative. Urine of normal monkeys was collected at 48 hourly intervals for several days. Pamaquin citrate solution was administered intravenously at 2·5 mg. base/kg. body-weight in a single dose and 48 hourly urine collections made subsequently for several days. Nicotinic acid was estimated in these samples on the day of collection as follows :—

Urine (50 ml.) was centrifuged and hydrolysed with sodium hydroxide on a boiling water bath for forty-five minutes. The mixture was cooled and re-centrifuged. The clear liquid was adjusted to pH 6 and diluted to 100 ml. Of this solution, 50 ml. was used for estimation of nicotinic acid using cyanogen bromide and p-aminoacetophenone (Konig's reaction, 1904). The level of nicotinic acid was found to be same both before and 48 hours after the drug administration. However, considering the possibility of localisation of pamaquin in the tissues and/or extensive oxidative processes which the drug molecule has to undergo in the system, urine samples collected at 48 hourly intervals for ten days after the drug administration were also similarly analysed. The findings showing increase in nicotinic acid excretion have been represented in Graph 3.

Theoretically oxidation of 25·0 mg. of pamaquin should give rise to 10·2 mg. of nicotinic acid. Hence, the total excretion of nicotinic acid over the normal should not normally exceed this theoretical limit, presuming all was converted into nicotinic acid only. In order to verify the trend in increase of nicotinic acid

GRAPH 3.

Nicotinic acid in monkey urine after intravenous administration of pamaquin (3·5 mg. per kg. body-weight).

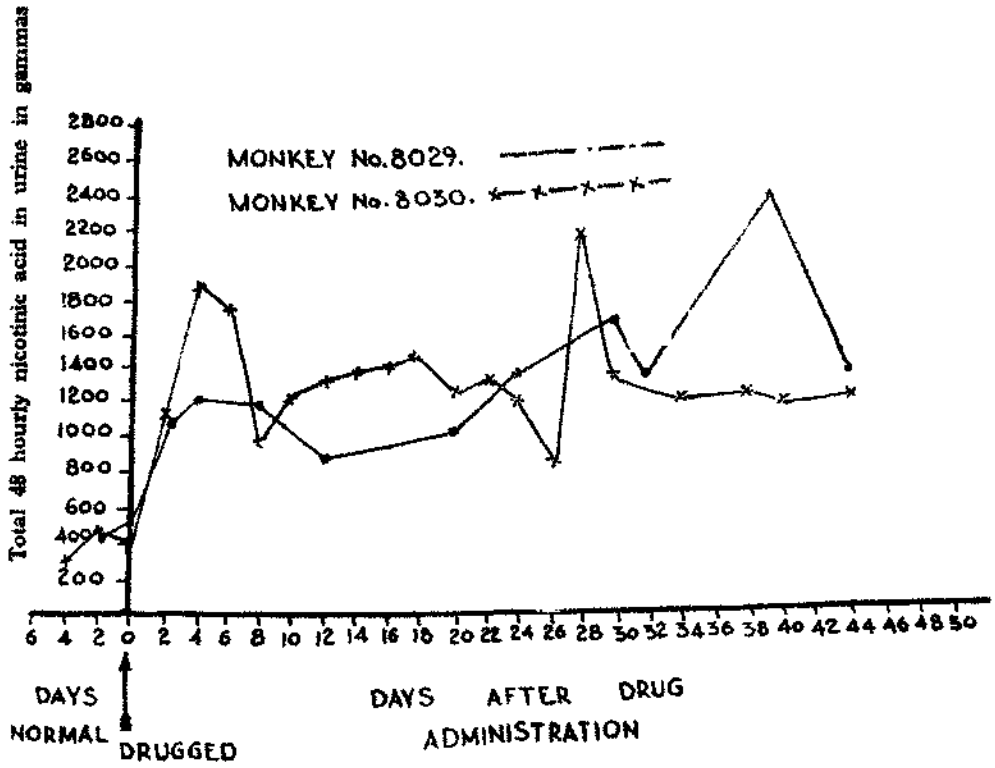


excretion (Graph 3), estimation of nicotinic acid was continued for several weeks subsequent to the pamaquin administration. Estimations were carried out for 44 days (Graph 4) and the total excretion of nicotinic acid in the urine above the

normal value approximately determined. It was observed that total excretion of nicotinic acid had been above the theoretical value and the concentration of nicotinic acid in the urine did not seem to fall back to the normal at least up to 44 days. The increase in nicotinic acid excretion, therefore, could not be explained on the basis of pamaquin degradation alone.

GRAPH 4.

Excretion of nicotinic acid in monkey urine after intravenous administration of pamaquin (2·5 mg. base/kg. body-weight).



(G) ESTIMATION OF NICOTINIC ACID IN BLOOD AND OTHER PATHOLOGICAL INVESTIGATIONS.

Nicotinic acid was estimated in the blood by Kodicek's method (1940) both before and after intravenous pamaquin administration to a monkey at 2·5 mg. base/kg. body-weight dosage. The values varied from 4 to 6 gamma/ml. of nicotinic acid during 44 days period of observation.

Considering the possibility that intravenous administration of pamaquin might also upset the normal spleen, kidney or liver functions; sugar, albumen and bile pigments were determined in the urine both before and after drug administration for the entire period. These tests were negative in all cases. Similarly, test

for bile in serum was also negative. Apparently, there was little evidence of direct damage to these organs due to pamaquin administration, although possibility of other pathological damage, such as triggering of increased nicotinic acid production, could not be ruled out.

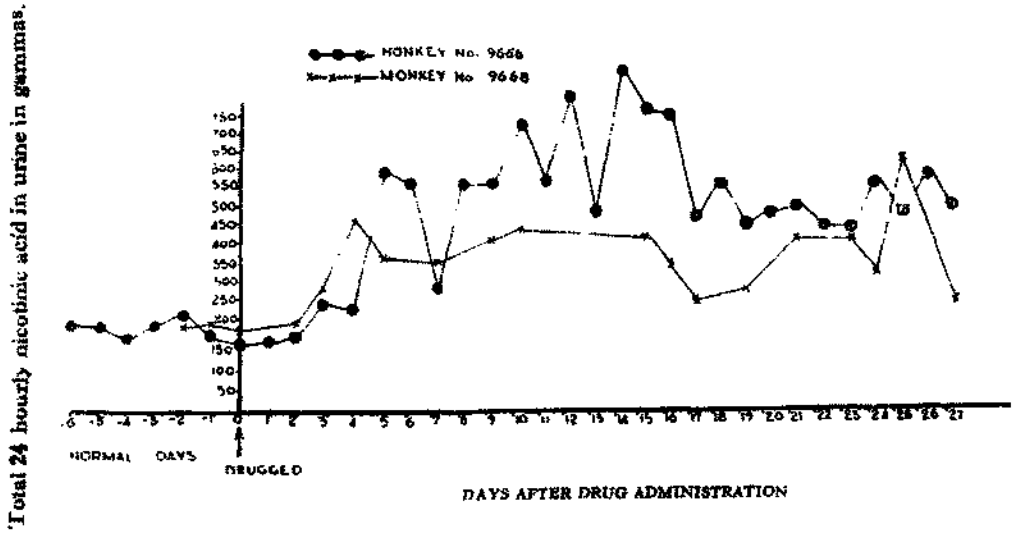
(H) ESTIMATION OF NICOTINIC ACID BY MODIFIED KODICEK'S METHOD.

Before final consideration of the above results for nicotinic estimation, it was suggested that in the above analytical procedure (F), urine was not decolourised and as such might lead to less reliable results due to its highly coloured nature. Consequently, estimation of nicotinic acid in the urine was repeated using modified method by Wang and Kodicek (1943). According to this technique, urine (collected at 24 hourly intervals) after hydrolysis with sodium hydroxide, was cooled and adjusted to pH 2-3 with concentrated hydrochloric acid. It was extracted with half the volume of iso-butanol and the aqueous phase evaporated on a water bath for 15 minutes. The solution was diluted with 15 ml. of distilled water, cooled, acidified and then decolourised with potassium permanganate till the pinkish colour persisted for 30 seconds. It was brought to pH 6·5-6·8 with alkali carefully and made up to 50 ml. This decolourised solution was centrifuged and analysed as described under the previous original method (Harris and Raymond, 1939).

In these experiments also, it has been confirmed that there was an increase in nicotinic acid excretion in urine subsequent to intravenous administration of pamaquin (Graph 5). However, it was evident that total increase in nicotinic acid

GRAPH 5.

Excretion of nicotinic acid in monkey urine after intravenous administration of pamaquin (2·5 mg. base/kg. body-weight).



excretion was not more than that theoretically expected from the amount of pamaquin administered. Previous findings to the contrary, using original method

of Harris and Raymond (1939), could be explained on the basis of highly coloured nature of the urine subsequent to pamaquin administration.

DISCUSSION.

Present studies had been undertaken to elucidate the mechanism of pamaquin degradation and to search for a lead to synthesise more potent and less toxic 8-amino-quinoline derivatives. Some progress has been made towards the first objective only. Previous general findings regarding extensive degradation of pamaquin in the animal system (Craig *et al.*, 1948, Hughes and Schmidt, 1950; Brodie and Udenfriend, 1950 and Bami, 1955) have been confirmed. It has also been observed that there was only transient plasma drug level in such cases. Metabolites, obtained through oxidative degradation of pamaquin (Chart 1), were generally water and alkali soluble but insoluble in ethylenedichloride. Detection of a preliminary oxidation product of pamaquin, viz., 5:6-quinoline-quinone derivative (Chart I, Fig. IV) has not been possible in the case of monkey urine both after oral and intravenous administration of pamaquin. Most probably it was due to extensive oxidation of the quinoline molecule in the system prior to its excretion in the urine. Similarly, it has not been possible to isolate any other similar quinoline : quinone derivatives such as one with the side chain removed (Chart I ; Fig. IV without-R). Presumably cleavage of the side chain and breaking up of the quinone ring took place simultaneously at this stage.

Mild chemical oxidation of pamaquin, though not directly related to *in vivo* studies, has supported the possibility of pamaquin being ultimately oxidised in the system to products like nicotinic acid, quinolinic acid and other smaller fragments (Chart I). The route of this oxidation might also be via the 5:6-quinoline : quinone (Chart I ; Fig. IV). This is supported by the fact that oxidative irradiation of pamaquin with ultra-violet light has led to the formation of this compound (Elderfield and Werble, 1950). Similarly, Drake and Pratt (1950) have found that 6-hydroxy analogue of pentaquin could be oxidised to 5:6-dihydroxy-pentaquin with oxygen in alkaline solution at room temperature. Although, Brodie and Udenfriend (1950) postulated the formation of 5-hydroxy-pamaquin and pamaquin-quinonimine *in vivo*, the current evidence (Drake and Pratt, 1950 ; Hughes and Schmidt, 1950 ; Smith, 1956), has indicated that de-alkylation at 6-position was the first step towards degradation, and not the formation of a 5-hydroxy derivative. Consequently, only one oxidative pathway has been shown in Chart I. Although, in case of chloroquin, it appeared that dialkylamino-alkylamino side chain was attacked first (Titus *et al.*, 1948), metabolic degradation studies with radio-active labelled pentaquin by Elderfield and Smith (1953) and Smith (1956) have shown that side-chain was more resistant to cleavage initially. Subsequently, however, it was detached and ruptured with deamination into smaller fragments. These findings also supported the present scheme (Chart I) wherein pamaquin was first converted into 5:6-quinoline : quinone, then shed its side chain with simultaneous opening of the ring and ultimately got converted into nicotinic acid, its metabolites and other smaller fragments.

Studies on nicotinic acid metabolism *in vivo*, in man and rats, indicated that administration of tryptophan lead to the formation of kynurenic acid, 3-hydroxy-anthranilic acid, xanthurenic acid (4 : 8-dihydroxy quinoline carboxylic acid) and quinolinic acid as intermediates prior to the formation of nicotinic acid and its derivatives (Reddi and Kodicek, 1953 ; Sarett and Goldsmith, 1947, 1949, 1950 ; Perlzweg *et al.*, 1947 ; Henderson, 1949*a*; Sarett, 1959*a*, 1959*b*; Heiderlberger *et al.*, 1949). Similarly dosing with quinolinic acid, lead to increase in the excretion of nicotinic acid and tertiary nicotinyl compounds (Reddi and Kodicek, 1953, Henderson, 1949*a*, 1949*b*; Krehl *et al.*, 1950). Although, tryptophan is a major precursor in nicotinic acid synthesis in the animal system, apparently xanthurenic acid and quinolinic acid also serve as intermediates in secondary metabolic pathways. Increased excretion of nicotinic acid, when viewed with the back-ground of existing data on chemical oxidation of pamaquin and normal nicotinic acid metabolism, would also support the hypothesis that via the 5 : 6-quinoline : quinone, pamaquin could be oxidised to quinolinic acid and then to nicotinic acid. Intermediates in pamaquin oxidation would thus serve as precursors of nicotinic acid and cause increase in its excretion in healthy animals. However, it has been observed that relative rise in nicotinic acid concentration in urine started two days after the drug administration. During this period, probably the drug under-went a series of oxidative changes and several of the intermediate products leading to nicotinic acid formation could also be considered as its precursors. It was also possible that apart from pamaquin metabolites serving as nicotinic acid precursors, pamaquin metabolism might also bring about some abnormal changes in the animal system whereby nicotinic acid production and excretion remained at a high level for a considerable period. However, it may be pointed out that preliminary investigations to prove any direct damage to the system have not given positive results. Considering the complicated nature of nicotinic acid metabolism itself, results dealing with its excretion have to be viewed with the back-ground of other supporting data on the metabolic and chemical degradation of pamaquin. As the maximum tolerated dose of pamaquin was very small, detailed chemical investigations have been greatly handicaped. But even in the absence of a direct evidence in support of the present scheme of pamaquin metabolism (Chart 1), there was considerable indirect evidence that this route of oxidation was quite probable in case of pamaquin and perhaps also applicable to allied 8-aminoquinoline antimalarials.

SUMMARY.

Metabolic degradation of pamaquin [8-(4′-diethylamino-1′-methylbutyl amino)-6-methoxy-quinoline] has been studied in monkeys. Studies on the matabolites separated from the urine, mild chemical oxidation of pamaquin, and increased excretion of nicotinic acid in urine, subsequent to intravenous administration of pamaquin, have suggested the possibility that pamaquin was rapidly and extensively oxidised into simpler water-soluble materials, such as quinolinic acid, nicotinic acid and their further break-down products. Based upon present and previous data, a scheme for pamaquin degradation *in vivo* has been suggested (Chart 1).

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A NOTE ON THE DISTENTION OF ABDOMEN OF *CULEX FATIGANS*, WHEN EXPOSED TO DIELDRIN.

BY

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[November 18, 1959.]

INTRODUCTION.

Ramakrishnan and Mohan (1959) noted, among the paralysed and dead female *C. fatigans* exposed to lindane, distention of abdomen almost to the point of rupture and the characteristic appearance of proboscis similar to that of a mosquito taking blood meal. Under identical conditions of exposure, these signs of acute toxicity of lindane were found in a smaller number of individuals of the BHC-resistant strain than the normal strain of *C. fatigans*. Studies were undertaken to see whether similar signs of acute toxicity also occurred in two strains of *C. fatigans* when exposed to dieldrin. The results obtained are recorded in the present communication.

MATERIAL AND METHODS.

Filter papers were impregnated with dieldrin solution in white oil according to the method of Busvine and Nash (1953 : 1954). The insecticidal concentrations ranged from 0·25 to 4 per cent both for the normal and resistant *C. fatigans* (resistant to DDT and cross-resistant to dieldrin and gamma BHC). At least 100 mosquitoes of each of the two strains of *C. fatigans* were exposed to each dosage. The resistant mosquitoes used in the experiments were of generations 82 and 83.

THE NATURE OF ABDOMINAL DISTENTION.

The characteristic appearance of the bloated mosquitoes, caused by the action of dieldrin, was similar to that produced by gamma BHC. Observations as regards aerophagy by bloated mosquitoes, after exposure to dieldrin, were the same as after exposure to gamma BHC. The sign of abdominal distention was found among paralysed or dead mosquitoes after exposure to dieldrin as in the case of gamma BHC.

RESULTS OF EXPERIMENTS.

In the first series of experiments with normal *C. fatigans*, the period of exposure was two hours. The results are recorded in Table I. It is seen that with two hours' exposure period, the abdominal distention among dead mosquitoes appeared at concentrations ranging from 1 to 4 per cent of the insecticide. At these concentrations, the incidence of abdominal distention

remained the same, namely, 8 per cent. The difference in the percentage mortality at the same concentrations was also slight or negligible, which suggested heterogeneity of the strain.

In the second series of experiments, the period of exposure was increased to three hours. This resulted in increase in the incidence of abdominal distention but not in mortality (Table I).

TABLE I.

Percentage mortality and incidence of abdominal distention in blood-fed C. fatigans *of the normal and D.D.T.-resistant (cross-resistant to dieldrin) strains exposed to different concentrations of dieldrin (technical grade), using laboratory-impregnated filter papers. Exposure period is indicated separately for each strain. Observations were recorded 24 hours after release from exposure.*

Strain of *C. fatigans.*	Exposure period in hours.	PER CENT CONCENTRATION :									
		0·25		0·5		1·0		2·0		4·0	
		Per cent.		Per cent.		Per cent.		Per cent.		Per cent.	
		Mortality.	Abdominal distention.	Mortality.	Abdominal distention.	Mortality.	Abdominal distention.	Mortality.	Abdominal distention.	Mortality.	Abdominal distention.
Normal	2	5	0	34	0	61	8	66	8	70	8
Normal	3	..	..	48	5	68	12	70	30	79	30
D.D.T.-resistant (cross-resistant to dieldrin)	24	..	..	37	0	47	3	56	0	77	12

Note :—1. Specimens with fully or half distended abdomen combined.

2. Specimens showing abdominal distention were all dead in both the normal and D.D.T.-resistant strains.

3. MLC of dieldrin for normal *C. fatigans* : 0·76 per cent × 1 (hour of exposure).
MLC of dieldrin for DDT-resistant *C. fatigans* : 1·26 per cent × 24 (hours of exposure).

Only one experiment was carried out with resistant *C. fatigans* (resistant to DDT and cross-resistant to dieldrin and gamma BHC). The period of exposure was extended to 24 hours in order to obtain comparable mortality. The highest incidence of abdominal distention was 12 per cent associated with 77 per cent mortality at 4 per cent concentration of the insecticide (Table I). Resistant *C. fatigans* showed a lower incidence of the toxic sign of dieldrin poisoning than normal ones, despite the prolonged exposure.

To confirm the above results further tests were carried out in which specimens of the normal and resistant strains of *C. fatigans* were marked separately with gold dust and silver dust. They were left overnight in the same cages with glucose water. The number of mosquitoes of each strain, marked with either gold or silver dust, was known only to the technicians.

The marked mosquitoes of each strain were then mixed together. From this mixed batch, mosquitoes were removed and exposed to 1·6 per cent dieldrin (using the standard World Health Organization adult kit) for 50 minutes. After exposure, the mosquitoes were released into a clean cage. The usual feed of glucose was available. Observations were recorded 24 hours after exposure. The results are shown in Table II.

TABLE II.

Percentage mortality and incidence of abdominal distention in a mixed batch of normal and D.D.T.-resistant (cross-resistant to dieldrin) strains of C. fatigans *females exposed to 1·6 per cent dieldrin (using the standard World Health Organization adult kit) for 50 minutes. The mosquitoes of both the strains had the opportunity to feed on glucose water.*

C. fatigans strain marked with :	Living.	Dead.	Total.	Per cent mortality.	Number with abdominal distention.			Per cent abdominal distention.		
					H	F	T	H	F	T
D.D.T-resistan (Gold dust)	19	8	27	30	1	0	1	4	0	4
Normal (Silver dust)	1	76	77	99	9	10	19	12	13	25

H = Half distended.
F = Fully distended.
T = Total distended.

The percentage incidence of abdominal distention was significantly higher in specimens marked with silver dust than in specimens marked with gold dust. The mosquitoes with gold dust were, therefore, diagnosed as belonging to the DDT-resistant (cross-resistant to dieldrin) strain and those with silver dust to the normal strain. This was verified. A low abdominal distention rate of 4 per cent in the *C. fatigans* resistant strain, as compared to 25 per cent in the normal strain, confirmed the above findings. Further work with homogeneous susceptible and and dieldrin resistant strains may throw some light on the association of abdominal distention rates and susceptibility of the strains.

SUMMARY AND CONCLUSIONS.

C. fatigans exposed to dieldrin showed the two toxic signs reported earlier with reference to gamma BHC, *i.e.*, distention of the abdomen and the characteristic appearance of the proboscis as in the act of biting.

Under identical conditions of exposure, the two toxic signs were manifest in a smaller proportion of *C. fatigans* resistant to D.D.T. with cross-resistance to dieldrin than in normal *C. fatigans*.

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A NOTE ON HIGH DDT TOLERANCE IN RAT FLEAS COLLECTED FROM GUNDLUPET TOWN OF MYSORE STATE*

BY

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[March 31, 1960]

CERTAIN villages in Gundlupet Taluk of Mysore State are known to have been endemic for plague. To control this disease, a few selected villages were sprayed for the first time with gamma BHC in 1951 and with DDT in 1952. Gundlupet Town itself came under spraying operations after the adoption of National Malaria Control Programme by the State authorities in 1953. The town was sprayed with 112 mg. of DDT (75 per cent water wettable powder) per square foot once a year from 1954 to 1957, inclusive. As the reduction in the flea index was less marked in 1953 as compared to previous years, gamma BHC was used in 1958 and 1959.

The information outlined above led to the suspicion of resistance in rat fleas to DDT and possibly to gamma BHC in certain treated localities of Gundlupet Taluk. It was, therefore, decided to test the susceptibility of fleas in the area and Gundlupet Town was selected for the purpose. For comparison, fleas from Mettupalaiyam Town in Madras State were used. This town was sprayed for the first time with DDT four months prior to the carrying out of susceptibility tests on local rat fleas. Rats for obtaining fleas were, however, actually trapped from the central part of the town, which was left unsprayed.

For the collection of fleas, rats were trapped from human dwellings and killed by strangulation. As the fleas left the dead bodies of rats, they were gently picked up by means of a sucking tube and transferred into clean tubes. They were tested for their susceptibility soon after removal from rats according to the method of Busvine and Nash (1953 : 1954) with slight modifications. Test tubes (3 inch × 1 inch) were lined on the inside including the cork with filter papers taken from the World Health Organization adult mosquito kit. Each filter paper was cut into two halves and each half was used to prepare one tube. The number of fleas enclosed in each tube was usually small, the maximum being 13 in one instance. During exposure, the tubes were kept in an upright position with cork end as the bottom. After exposure, the fleas were released into glass jars. The bottom of the jars was previously covered with a perforated circular piece of cardboard over which a small quantity of saw dust was evenly spread. Appropriate controls were observed for each test. Mortality counts were made 24 hours after the exposure period.

*The investigation was in part financed by a grant from the Indian Council of Medical Research.

RESULTS.

Results of susceptibility tests with rat fleas from Gundlupet (sprayed town) and from Mettupalaiyam (comparison town) are shown in Table I.

TABLE I.

Results of susceptibility tests on rat fleas from Gundlupet (sprayed town) and from Mettupalaiyam† (comparison town).*

Locality.	Exposure period in hours.	Number of fleas tested.	24-HOUR MORTALITY (PER CENT). Per cent concentration DDT.			Remarks.
			0·25	2	4	
Gundlupet (sprayed town)	1	23	0	..	..	There was no mortality in control fleas from Gundlupet. Tests with some mortality in control fleas from Mettupalaiyam are excluded. Mortalities are based both on *Xenopsylla cheopis* and *Xenopsylla astia*.
	8	10	0	..	..	
	1	9	..	33	..	
	1	27	..	..	70	
Mettupalaiyam (comparison town)	1	119	86	..	..	

*Tests were carried out during March, 1960.

†Tests were carried out during December, 1959 and January, 1960.

It will be seen that exposure of fleas from Mettupalaiyam (comparison town) to 0·25 per cent DDT filter papers for one hour gave an average mortality of 86 per cent. In contrast, mortality in fleas from Gundlupet (sprayed town) at the same dosage was nil, even when the exposure poriod was increased to 8 hours. Exposure to increased concentrations of 2 and 4 per cent DDT for one hour resulted in 33 and 70 per cent mortality respectively. The data, although of a preliminary nature, provide positive evidence of the development of a fairly high tolerance to DDT in rat fleas indigenous to Gundlupet Town.

Indentification of rat fleas from Gundlupet and Mettupalaiyam could be done only after mounting the specimens by the usual method. In Gundlupet *Xenopsylla cheopis* was the dominant species while in Mettupalaiyam *Xenopsylla astia* was predominant.

Under field conditions, very few instances of insecticide resistance have been recorded in fleas of the Genus *Xenopsylla*. Saenz-Vera (1953) included *X. cheopis* among the fleas that could not be controlled by DDT applications in Ecuador. The first confirmed case of high DDT resistance in *X. cheopis* has recently been reported in parts of Poona District of Bombay State by Patel, Bhatia and Deobhankar (1960). In the present study, rat fleas obtained from Gundlupet were found to show fairly high tolerance to DDT.

SUMMARY.

Susceptibility tests on rat fleas collected from Gundlupet Town of Mysore State have shown fairly high tolerance to DDT.

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OBSERVATIONS ON THE SEASONAL PREVALENCE OF ADULT ANOPHELINES NEAR DHANBAD.

BY

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[November 21, 1959.]

INTRODUCTION.

VERY little has been published regarding the seasonal prevalence of anophelines in the south-eastern part of Bihar State. The only work on the anopheline fauna of this area has been that of Rao (1941 : 1944), discussing the epidemiology of malaria in the Jharia Mining Settlement. Published records on the seasonal prevalence of anophelines, of other areas of India, are those of Russell and Rao (1941) dealing with the prevalence of twelve species of anophelines in south-eastern Madras ; Brooke Worth (1953) and Bhombore *et al.* (1954) dealing with twenty-three anopheline species of the western hill-tracts of Mysore State ; and Bhatia *et al.* (1958) recording the observations on ten species of anophelines over a period of about five years around Delhi.

During the course of regular malaria control work with D.D.T. residual spraying that has been in progress in the coalfield area of Bihar since 1947, a considerable amount of information has been recorded regarding the prevalence of anopheline adults to gauge the effect of the control measures.

The present paper puts on record the entomological findings with regard to the prevalence of adult anophelines made from July, 1953 to April, 1958 in Dhokra —a village about four miles from Dhanbad. Of the seventeen* species of anophelines recorded for Bihar State (Puri, 1955), only *A. aconitus*, *A. karwari*, *A. maculatus*, *A. minimus*, *A. phillipinensis*, and *A. ramsayi* were not recorded during the present

*There are 24 Anopheles species known to occur in Bihar according to the unpublished records of Malaria Institute of India—*Editor*.

investigation. The other eleven species, viz., *A. annularis, A. barbirostris, A. culicifacies, A. fluviatilis, A. hyrcanus, A. pallidus, A. stephensi, A. subpictus, A. tessellatus, A. vagus,* and *A. varuna,* as also *A. splendidus* (which was not included for Bihar by Puri, 1955), were recorded during the present study. Altogether twelve species were recorded. Of the fourteen species recorded by Rao (1941 : 1944) for the Jharia Mining Settlement, three species, viz., *A. jeyporensis, A. jamesi* and *A. umbrosus,* were not obtained during the present investigations*.

METHODS.

Dhokra, a totally unsprayed village situated about four miles south-east of Dhanbad, was selected for making the routine observations on the anopheline densities in man-made shelters. Indoor collections from three fixed catching stations, comprising a cattle-shed, a human-dwelling and a mixed-dwelling, were made regularly thrice a week. During the early part of the observations (from July, 1953 to February, 1955) collections were made only from a cattle-shed and a human-dwelling. However, from March, 1955 onwards, collections were commenced from a mixed-dwelling also. All collections were made by suction-tube and flash-light by trained insect-collectors, between the hours 06·00 and 08·30. Half an hour was devoted for each station. The collections were brought to the laboratory of the Malaria Institute of the Coal Mines Welfare Organisation where identification of species and recording of the daily collections were made.

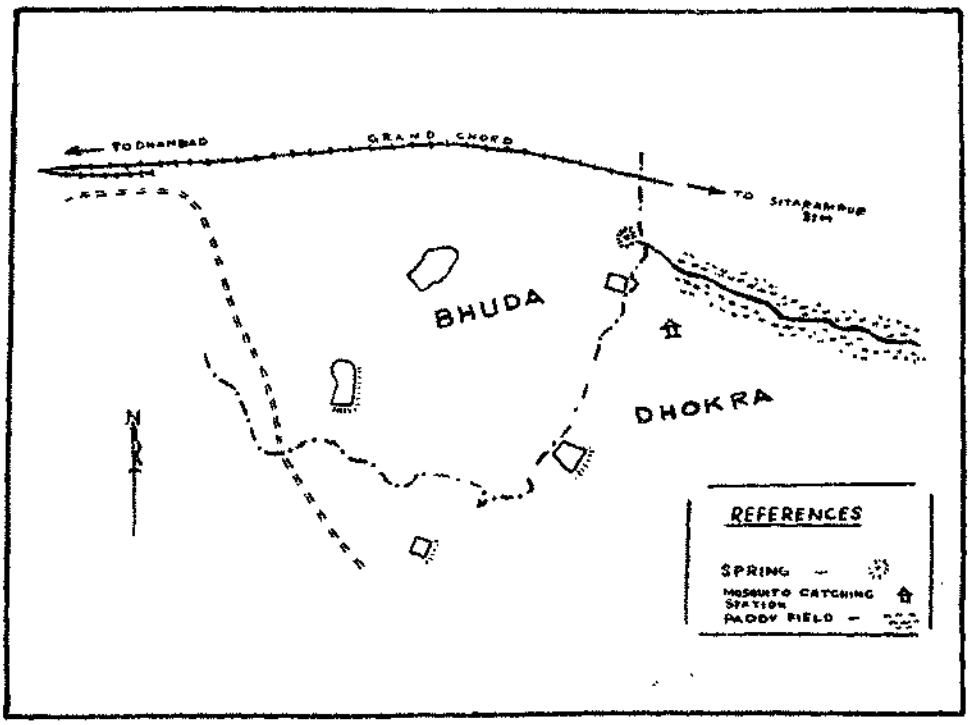
AREA OF STUDY.

Dhokra village is situated about three miles from Dhanbad station in the south-eastern direction (Map I). It is a big village spread out over an area of about four square miles. The area covered by the village is undulating but there is a general slope downwards from the north-west to the south-east. The northern boundary of the village is low-lying. The village is surrounded by rice-fields which are more numerous on the northern low-lying area. There are a large number of ponds of various sizes scattered all over the village and a number of wells as well. On the north-eastern corner of the village bordering Bhuda, an adjoining village, there are a few perennial springs from which water flows out continuously. Since the lie of the land is towards the south-eastern direction, the water from the springs flows in that direction. During and soon after the monsoon months, the spring develops into a small stream flowing rapidly and irrigating the fields adjoining it. The whole area north of the village thus becomes very much water-logged. During the winter months the flow of water from the stream becomes very much reduced and numerous pools and puddles are formed constituting ideal breeding grounds for anophelines. During the peak summer months most of the wells and ponds of

*The other species not recorded in the paper but known to occur in Bihar are *A. aitkeni, A. majidi, A. moghulensis,* and *A. theobaldi.* —*Editor.*

MAP 1.

Dhokra and the location of the catching stations.



the village dry up and then the spring forms the chief source of water supply for the village. There are a large number of *lantana* bushes and numerous other shrubs and trees between the fields and the human dwellings.

The houses are scattered in groups, all over the village and there are numerous open spaces and gaps between the different groups of houses. The majority of houses are built of mud walls and thatched roofing. A few houses, however, have brick walls and tiled roofing. In most houses, the cattle-sheds also form part of the house. In certain houses the cattle-sheds are removed to one side with a court-yard separating it from the part occupied by men. In the case of mixed-dwellings, there is a common roof for both animals and men but an incomplete low wall separates the two parts.

METEOROLOGICAL DATA.

The meteorological data for Dhanbad for the period July, 1953 to April, 1958 have been taken into consideration for the present observations. These readings recorded by the Meteorological Department were obtained from the Regional

Meteorological Centre, Calcutta. It has not been possible to get the meteorological data for a period of five months from March, 1956 to July, 1956, since the observatory at Dhanbad was not functioning during those months. Table I shows the monthly averages of the maximum, minimum, and mean temperatures, relative-humidity at 08·30 hours and 17·30 hours and the mean, and total rainfall for each month from July, 1953 to February, 1956 and from August, 1956 to April, 1958.

From the available meteorological data, it is found that January is the coldest month of the year with an average minimum temperature of 54·9°F. and May is the hottest month of the year with an average maximum temperature of 105·6°F. May, 1957, with an average maximum temperature of 108·5°F., was the hottest month and January, 1954, with an average minimum temperature of 51·9 degrees F., was the coldest month for the period under observation. The average mean relative-humidity for the entire period ranges from 83 per cent in August to 24·3 per cent in April. The lowest monthly relative-humidity recorded during the entire period was 10 per cent in April, 1954 at 17·30 hours. The corresponding highest figure was 87 per cent in August, 1955 at 08·30 hours. Thus it is seen that the range of fluctuation of relative-humidity is very high. The average mean relative-humidity for April, May and June was less than 30 per cent, whereas the average mean relative-humidity for August, September and October was above 80 per cent.

The rainfall figures indicate that the average total rainfall for the period January, 1953 to April, 1958 was 47·2 inches of which 44·09 inches was recorded during June to October. The year 1953 was the wettest year during the period under study, with an annual rainfall of 70·15 inches.

It will, therefore, be seen that March, April and May are hot and dry ; July, August and September are warm and humid with the maximum amount of rainfall, while November, December and January are cold and dry months. The months of February, June and October are transitional months between these clear-cut periods.

OBSERVATIONS.

During the entire period of 58 months from July, 1953 to April, 1958, 33054 adult anophelines, comprising 12 Anopheles sp., were collected in 826½ hours. Of the twelve species, *A. fluviatilis* and *A. stephensi* have been incriminated as the primary and secondary vectors respectively, and *A. annularis* as the sole carrier in the south-eastern corner of the Settlement. *A. pallidus* and *A. culicifacies* are considered to play relatively unimportant rôle in malaria transmission.

Table II presents the data of anopheline adult collection month-wise and species-wise for the entire period under observation. Table III indicates the total consolidated adult collection for each species month-wise and Table IV shows the monthly per man-hour density of all anophelines and the number of hours spent during each month.

TABLE II.

Total catch of anophelines near Dhanbad (July, 1953 to April, 1958).

Months.	Species: *A. annularis.*	*A. barbirostris.*	*A. culicifacies.*	*A. fluviatilis.*	*A. hyrcanus.*	*A. pallidus.*	*A. splendidus.*	*A. stephensi.*	*A. subpictus.*	*A. tessellatus.*	*A. vagus.*	*A. varuna.*	Total.
1953													
July	158	..	342	48	..	40	36	..	147	..	..	..	780
Aug.	208	..	450	3	1	107	43	..	458	..	..	..	1,270
Sep.	138	..	254	16	..	46	2	..	763	..	..	..	1,219
Oct.	222	..	60	151	2	110	16	..	535	..	..	..	1,096
Nov.	197	2	2	260	13	106	..	..	488	..	..	..	1,068
Dec.	167	..	42	538	14	117	28	..	309	..	15	..	1,230
1954													
Jan.	156	..	275	528	..	111	10	..	65	..	..	..	1,145
Feb.	193	..	332	447	..	132	17	..	65	..	5	..	1,191
March	164	..	143	64	..	56	5	..	21	..	1	..	454
April	119	..	34	5	..	22	..	..	27	..	..	..	207
May	90	..	23	..	..	18	1	..	72	..	..	..	204
June	30	..	53	..	..	7	..	..	150	10	10	..	260
July	63	..	376	..	..	33	..	..	179	..	31	..	682
Aug.	57	..	371	..	..	115	..	..	242	..	33	..	818
Sep.	59	..	276	9	..	161	..	..	186	..	24	..	715
Oct.	33	1	112	98	..	146	2	..	68	..	4	15	479
Nov.	26	..	19	338	..	146	6	..	38	..	1	65	639
Dec.	76	..	23	448	..	142	15	..	49	..	..	52	805
1955													
Jan.	172	..	51	580	..	72	21	..	34	..	1	10	941
Feb.	247	..	117	523	..	28	20	..	41	..	..	..	976
March	235	..	78	202	..	5	5	..	34	..	..	..	559
April	67	..	9	4	..	12	..	..	141	..	3	..	236

TABLE II. (*Contd.*)

Months.	Species:												Total.
	A. annularis.	*A. barbirostris.*	*A. culicifacies.*	*A. fluviatilis.*	*A. hyrcanus.*	*A. pallidus.*	*A. splendidus.*	*A. stephensi.*	*A. subpictus.*	*A. tessellatus.*	*A. vagus.*	*A. varuna.*	
1955 (contd.)													
May	1	..	..	..	..	..	..	..	116	..	10	..	127
June	9	..	9	..	..	..	..	..	176	..	12	..	206
July	19	..	98	..	..	7	..	..	383	..	13	..	520
Aug.	20	..	218	..	..	25	..	..	598	..	70	..	931
Sep.	13	..	119	..	..	110	..	..	494	..	42	..	778
Oct.	61	..	27	46	2	196	2	..	280	..	19	..	633
Nov.	128	..	1	226	1	350	24	..	134	..	4	..	868
Dec.	80	..	3	257	..	224	6	..	47	..	2	5	624
1956													
Jan.	65	..	9	376	..	276	4	..	18	..	..	4	752
Feb.	180	..	39	135	1	211	7	..	7	..	..	..	580
March	214	..	11	63	..	139	15	..	16	..	..	..	458
April	46	..	4	4	..	31	..	..	257	..	28	..	370
May	20	..	2	..	..	17	..	..	235	..	7	..	281
June	4	..	33	..	..	10	..	..	240	..	4	..	291
July	4	..	106	..	..	12	..	..	358	..	15	..	495
Aug.	8	..	109	..	..	42	..	..	333	..	27	..	519
Sep.	10	..	107	..	..	31	..	..	210	..	12	..	370
Oct.	35	..	2	29	..	82	1	..	52	..	1	..	202
Nov.	110	1	4	307	10	198	11	..	83	..	3	7	734
Dec.	149	..	..	348	4	106	29	..	76	..	1	3	721
1957													
Jan.	218	..	..	349	1	34	61	..	33	1	1	1	699
Feb.	397	..	..	285	..	43	76	..	16	..	..	..	817
March	525	..	4	90	..	17	36	..	10	..	..	..	682

TABLE II. (*Contd.*)

Months.	Species: *A. annularis.*	*A. barbirostris.*	*A. culicifacies.*	*A. fluviatilis.*	*A. hyrcanus.*	*A. pallidus.*	*A. splendidus.*	*A. stephensi.*	*A. subpictus.*	*A. tessellatus.*	*A. vagus.*	*A. varuna.*	Total.
1957 (*contd.*)													
April	234	..	1	8	1	6	7	..	14	..	..	..	271
May	59	..	3	3	..	9	2	..	10	..	1	..	87
June	31	..	1	2	..	5	..	..	47	..	..	..	86
July	16	..	9	3	..	3	..	..	139	..	2	..	172
Aug.	14	..	13	9	..	8	..	..	298	..	4	..	346
Sep.	16	..	1	3	..	24	..	..	91	..	..	..	135
Oct.	34	..	..	34	..	116	..	..	34	..	..	..	218
Nov.	232	..	..	180	..	157	..	..	41	..	..	..	610
Dec.	180	..	..	172	..	52	1	..	34	..	..	..	439
1958													
Jan.	234	..	..	203	..	21	11	..	47	..	..	..	516
Feb.	220	..	..	68	..	7	12	..	11	..	..	..	318
March	130	..	1	19	..	2	2	1	16	..	..	..	171
April	18	..	1	9	..	1	..	1	29	..	..	..	59

(*b*) *Monthly per man-hour density of anophelines near Dhanbad (July, 1953 to April, 1958).*

Months.	Species: *A. annularis.*	*A. barbirostris.*	*A. culicifacies.*	*A. fluviatilis.*	*A. hyrcanus.*	*A. pallidus.*	*A. splendidus.*	*A. stephensi.*	*A. subpictus.*	*A. tessellatus.*	*A. vagus.*	*A. varuna.*	Total.
1953													
July	17·5	..	38·0	5·3	..	5·4	4·0	..	16·3	..	..	..	86·5
Aug.	13·9	..	30·0	0·2	0·07	7·1	2·9	..	30·5	..	..	..	84·6

TABLE II (*Contd.*)

Months.	Species :												Total.
	A. annularis.	*A. barbirostris.*	*A. culicifacies.*	*A. fluviatilis.*	*A. hyrcanus.*	*A. pallidus.*	*A. splendidus.*	*A. stephensi.*	*A. subpictus.*	*A. tessellatus.*	*A. vagus.*	*A. varuna.*	
1953 (*contd.*)													
Sep.	6·0	..	11·0	0·7	..	2·0	0·09	..	33·2	..	0·3	..	53·3
Oct.	10·6	2·9	2·9	7·2	0·1	5·2	0·8	..	25·5	..	..	..	55·2
Nov.	9·4	0·1	0·1	12·4	0·62	5·05	..	..	23·2	..	..	..	50·9
Dec.	7·6	..	1·9	24·5	0·64	5·3	1·3	..	14·0	..	0·7	..	55·9
1954													
Jan.	8·2	..	14·5	27·8	..	5·8	0·5	..	3·4	..	..	..	60·2
Feb.	12·1	..	20·8	27·9	..	8·3	1·1	..	4·0	..	..	..	74·2
March	14·9	..	13·0	5·8	..	5·1	0·5	..	1·9	..	0·1	..	41·3
April	10·8	..	3·1	0·5	..	2·0	..	2·5	..	..	..	..	18·9
May	6·9	..	1·8	..	..	1·4	0·1	..	5·5	..	..	..	15·7
June	2·3	..	4·1	..	..	0·5	..	..	11·5	..	0·8	..	19·2
July	4·5	..	26·9	..	..	2·4	..	..	12·8	..	2·2	..	48·8
Aug.	4·7	..	30·9	..	..	9·6	..	..	20·2	..	2·7	..	68·1
Sep.	4·9	..	23·0	0·7	..	13·4	..	..	15·5	..	2·0	..	59·5
Oct.	3·6	0·1	12·4	10·9	..	16·2	0·2	..	7·6	..	0·4	1·7	53·1
Nov.	2·2	..	1·6	28·2	..	12·2	0·5	..	3·2	..	0·1	5·4	53·4
Dec.	7·6	..	2·3	44·8	..	14·2	1·5	..	4·0	..	..	5·2	80·5
1955													
Jan.	17·2	..	5·1	58·0	..	7·2	2·1	..	3·4	..	0·1	1·0	94·1
Feb.	22·5	..	10·6	47·5	..	2·5	1·8	..	3·7	..	..	..	88·6
March	13·4	..	4·5	11·5	..	0·2	0·3	..	1·9	..	..	..	31·9
April	3·7	..	0·5	0·2	..	0·7	..	..	7·8	..	0·2	..	13·1
May	0·1	..	..	..	..	..	..	..	9·7	..	0·8	..	10·6
June	0·5	..	0·5	..	..	..	..	..	8·0	..	0·6	..	10·6
July	1·0	..	5·0	..	..	0·4	..	..	19·6	..	0·7	..	26·7

TABLE II (*Contd.*)

Months.	Species :												Total.
	A. annularis.	*A. barbirostris.*	*A. culicifacies.*	*A. fluviatilis.*	*A. hyrcanus.*	*A. pallidus.*	*A. splendidus.*	*A. stephensi.*	*A. subpictus.*	*A. tessellatus.*	*A. vagus.*	*A. varuna.*	
1955 (*contd.*)													
Aug.	1·1	..	12·1	..	..	1·4	..	..	33·2	..	3·9	..	51·7
Sep.	0·7	..	6·1	..	..	5·6	..	..	25·3	..	2·1	..	39·8
Oct.	3·4	..	1·5	2·6	0·1	10·9	0·1	..	15·6	..	1·1	..	35·3
Nov.	7·1	..	0·06	12·5	0·06	19·4	1·3	..	7·4	..	0·2	..	48·0
Dec.	5·9	..	0·2	19·0	..	16·6	0·4	..	3·5	..	0·15	0·4	46·2
1956													
Jan.	3·9	..	0·5	22·8	..	16·7	0·2	..	1·1	..	..	0·4	45·6
Feb.	15·0	..	3·2	11·2	0·1	17·6	0·6	..	0·6	..	..	..	48·3
March	20·4	..	1·0	6·0	..	13·2	1·4	..	1·5	..	..	..	43·5
April	3·1	..	0·3	0·3	..	2·1	..	..	17·1	..	1·9	..	24·8
May	1·2	..	0·1	..	..	1·0	..	..	14·2	..	0·2	..	16·7
June	0·3	..	2·2	..	..	0·7	..	..	16·0	..	0·2	..	19·4
July	0·2	..	5·9	..	..	0·7	..	..	19·9	..	0·8	..	27·5
Aug.	0·6	..	8·1	..	..	3·1	..	..	24·8	..	2·0	..	38·6
Sep.	0·9	..	10·2	..	..	3·0	..	..	20·0	..	1·1	..	35·2
Oct.	5·8	..	0·3	4·8	..	13·7	0·2	..	8·7	..	0·2	..	33·7
Nov.	6·7	0·06	0·2	18·6	0·6	12·0	0·6	..	5·0	..	0·2	0·4	44·4
Dec.	9·9	..	..	23·2	0·3	7·1	1·9	..	5·1	..	0·07	0·5	48·1
1957													
Jan.	12·1	..	..	19·4	0·06	1·9	3·4	..	1·8	0·06	0·06	0·06	38·8
Feb.	29·4	..	..	21·1	..	3·2	5·6	..	1·2	..	..	..	60·5
March	29·2	..	0·2	5·0	..	0·9	2·0	..	0·6	..	..	..	37·9
April	12·0	..	0·05	0·4	0·05	0·3	0·4	..	0·7	..	..	..	13·9
May	3·3	..	0·2	0·2	..	0·5	0·1	..	0·6	..	0·06	..	4·9
June	2·9	..	0·1	0·2	..	0·5	..	..	4·5	..	..	..	8·2

TABLE II (*Contd.*)

Month.	Species :												Total.
	A. annularis.	*A. barbirostris.*	*A. culicifacies.*	*A. fluviatilis.*	*A. hyrcanus.*	*A. pallidus.*	*A. splendidus.*	*A. stephensi.*	*A. subpictus.*	*A. tessellatus.*	*A. vagus.*	*A. varuna.*	
1957 (*contd.*)													
July	1·3	..	0·8	0·3	..	0·3	..	..	11·6	..	0·2	..	14·5
Aug.	1·3	..	1·2	0·9	..	0·8	..	..	28·4	..	0·4	..	33·0
Sep.	1·8	..	0·1	0·3	..	2·7	..	..	10·1	..	..	..	15·0
Oct.	3·2	..	..	3·2	..	11·0	..	..	3·2	..	..	..	20·6
Nov.	17·2	..	..	13·3	..	11·6	..	..	3·0	..	..	..	45·1
Dec.	17·1	..	..	16·4	..	4·0	0·1	..	3·2	..	..	..	41·7
1958													
Jan.	22·3	..	..	19·3	..	2·0	1·0	..	4·5	..	..	..	49·1
Feb.	24·4	..	..	7·6	..	0·8	1·3	..	1·2	..	..	..	35·3
March	10·8	..	0·1	1·6	..	0·2	0·2	0·1	1·3	..	..	..	14·3
April	2·0	..	0·1	1·0	..	0·1	..	0·1	3·2	..	..	..	6·5

TABLE III.

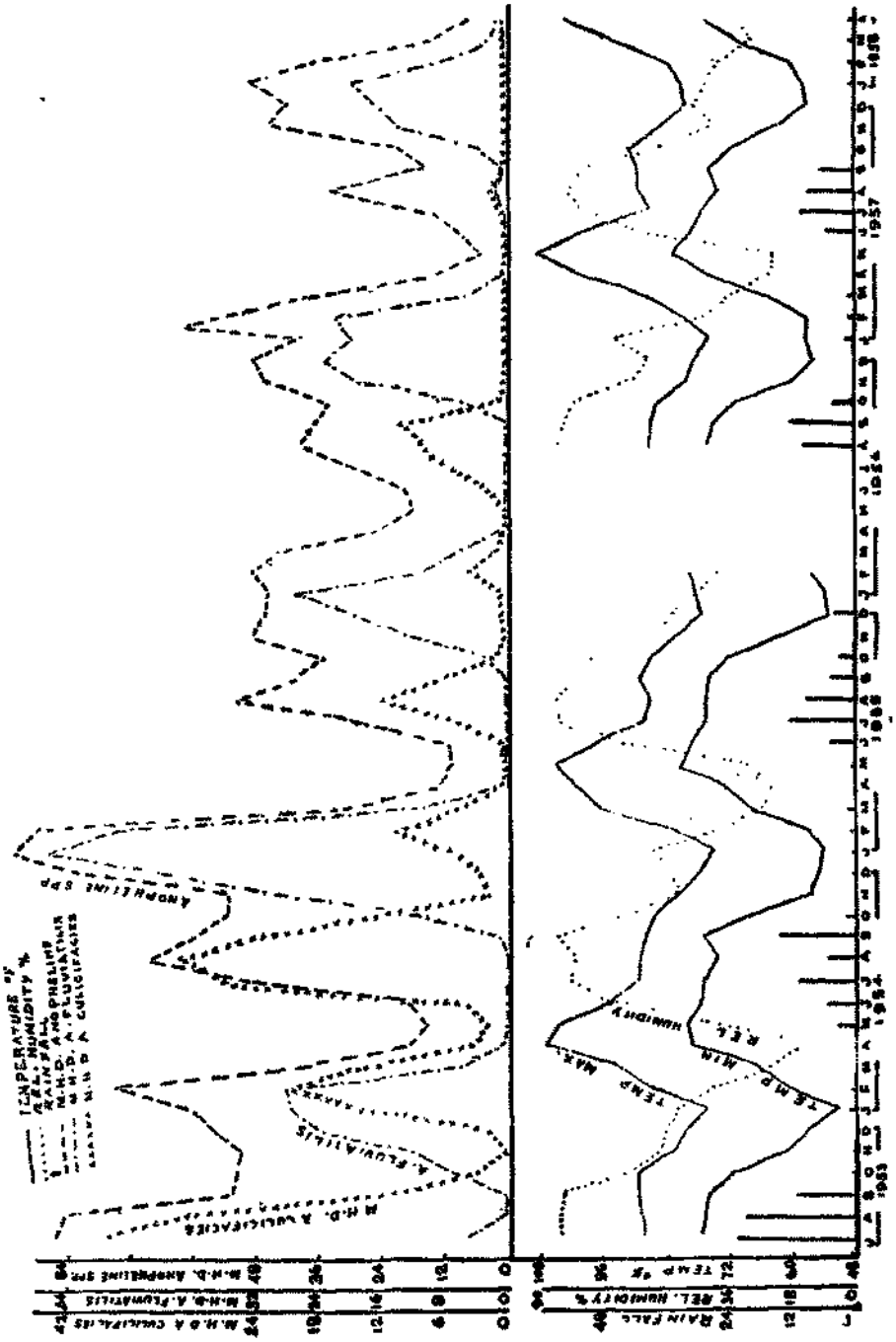
Total adult mosquito collections listed by species and months during the period July, 1953 to April, 1958 near Dhanbad.

Serial number.	Species.	Adult mosquito collections:												Total.
		Jan.	Feb.	March	April	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	
1	*A. annularis*	845	1,237	1,268	484	170	74	260	307	236	385	693	652	6,611
2	*A. barbirostris*	..	..	..	..	..	..	..	..	..	1	3	..	4
3	*A. culicifacies*	335	485	237	49	28	96	931	1,161	757	201	26	68	4,374
4	*A. fluviatilis*	2,036	1,458	438	30	3	2	51	12	28	358	1,311	1,763	7,490
5	*A. hyrcanus*	1	1	..	1	..	..	..	1	..	4	24	18	50
6	*A. pallidus*	514	421	219	72	44	22	104	297	372	650	957	641	4,313
7	*A. splendidus*	107	132	63	7	3	..	36	43	2	21	1	79	534
8	*A. stephensi*	..	..	1	1	..	..	..	..	..	..	..	..	2
9	*A. subpictus*	197	140	97	468	433	613	1,206	1,929	1,744	969	784	515	9,095
10	*A. tessellatus*	1	..	..	..	..	..	..	..	..	..	..	..	1
11	*A. vagus*	2	5	1	31	18	26	81	134	85	24	8	18	413
12	*A. varuna*	15	..	..	..	..	..	..	..	..	15	72	65	167
	Total	4,053	3,879	2,324	1,143	699	833	2,649	3,884	3,224	2,628	3,919	3,819	33,064

TABLE IV.

Per man-hour density of adult mosquito species and total time spent in collection near Dhanbad (July, 1953 to April, 1958).

Serial number.	Species.	MEAN DENSITY PER MAN-HOUR :												Total.
		Jan.	Feb.	March	April	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	
1	*A. annularis*	11·4	20·1	18·4	6·7	2·9	1·3	3·6	4·5	3·2	6·0	8·6	9·2	7·2
2	*A. barbirostris*	..	..	..	..	..	..	..	..	..	0·02	0·4	..	0·005
3	*A. culicifacies.*	4·5	7·9	3·4	0·7	0·5	1·7	12·8	16·8	10·2	3·1	0·3	0·9	5·3
4	*A. fluviatilis*	27·5	23·7	6·3	0·4	0·05	0·03	0·7	0·2	0·4	5·6	16·2	24·8	9·1
5	*A. hyrcanus*	0·01	0·02	..	0·01	..	..	..	0·01	..	0·06	0·3	0·3	0·06
6	*A. pallidus*	6·9	6·8	3·2	1·0	0·7	0·4	1·4	4·3	5·0	10·1	11·8	9·0	5·2
7	*A. splendidus*	1·4	2·1	0·9	0·1	0·05	..	0·5	0·6	0·03	0·3	0·5	1·1	0·6
8	*A. subpictus*	2·7	2·3	1·4	6·5	7·3	10·6	16·6	28·0	23·6	15·0	9·7	7·3	11·0
9	*A. tessellatus*	0·01	..	..	..	..	..	..	..	..	..	..	..	0·001
10	*A. vagus*	0·03	0·08	0·01	0·4	0·3	0·4	0·8	1·9	1·1	0·4	0·1	0·3	0·5
11	*A. varuna*	0·02	..	..	..	..	..	..	..	..	0·2	0·9	0·9	0·2
Time spent (Hours.)		74·0	61·5	69·0	72·5	59·5	58·0	72·5	69·0	74·0	64·5	81·0	71·0	826·5
Per man-hour density of all anophelines.		54·6	63·0	33·6	15·8	11·8	14·4	36·4	56·3	43·5	40·7	48·8	53·8	..



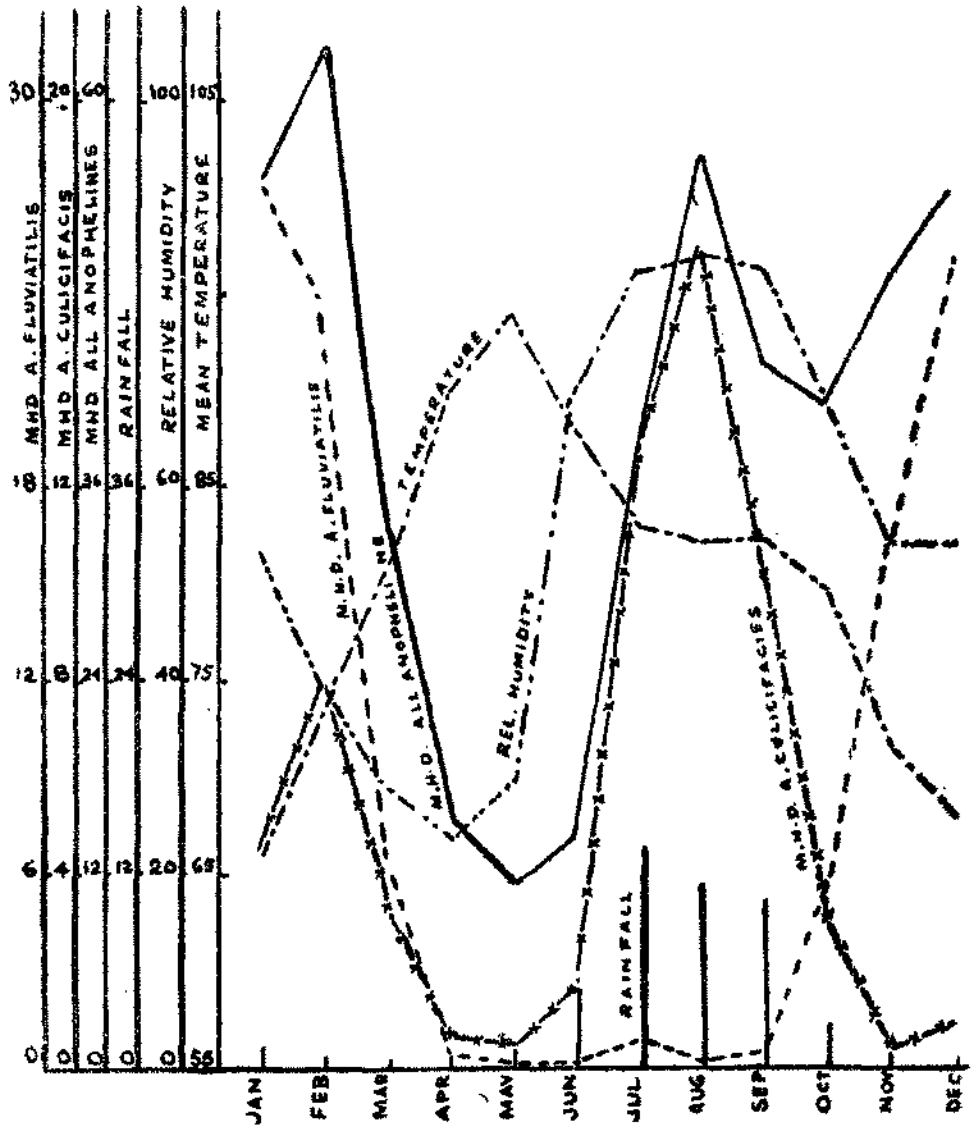
GRAPH 1.

Meteorological data and per man-hour density of (i) Anopheline spp., (ii) A. fluviatilis and (iii) A. culicifacies. (July, 1953 to April, 1958).

M.H.D.=Man-hour density.

GRAPH 2.

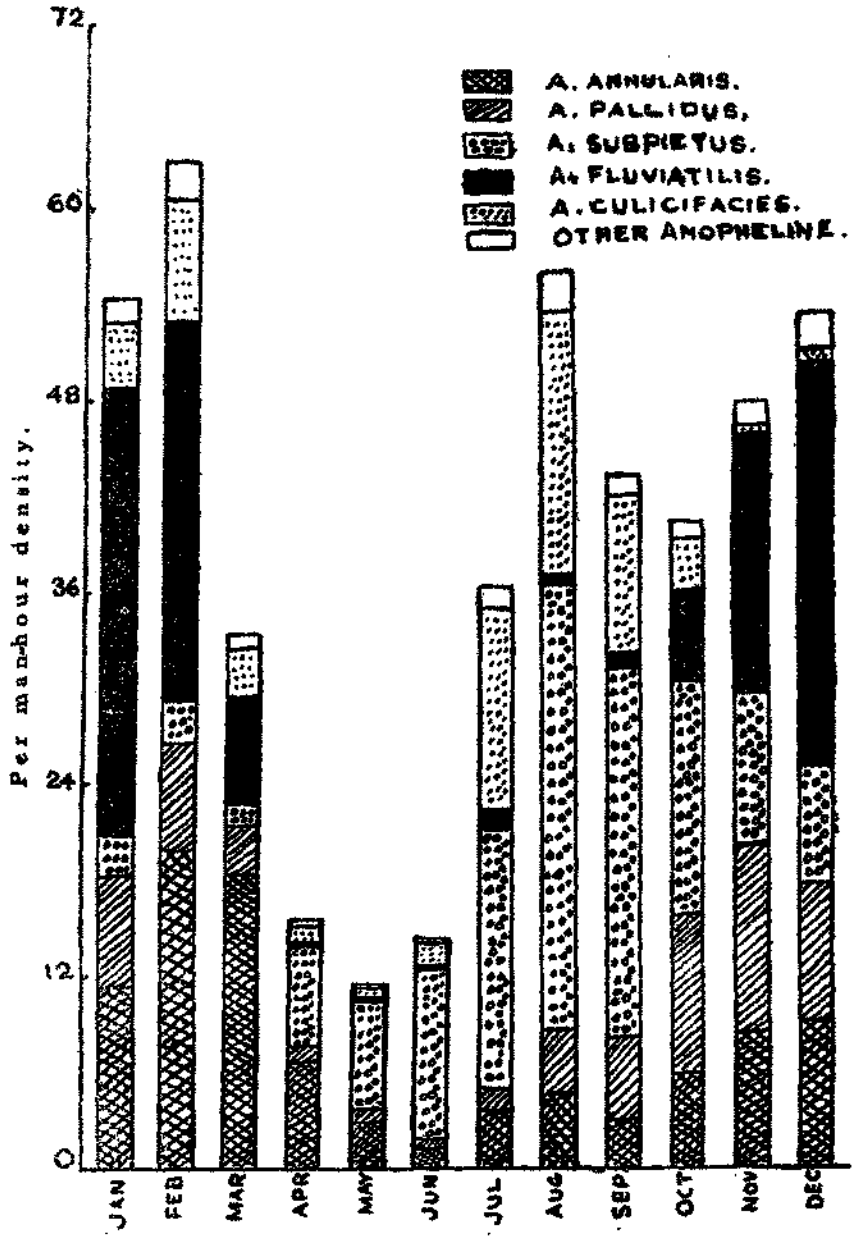
Meteorological data (monthly means) from July, 1958 to April, 1958 and seasonal prevalence of Anopheles spp., A. culicifacies and A. fluviatilis near Dhanbad.



M.H.D.=Man-hour density.

GRAPH 3.

Relative monthly prevalence of the different species of anophelines near Dhanbad (July, 1953 to April, 1958).



SEASONAL FLUCTUATION IN DENSITY OF ALL ANOPHELINE SPECIES.

From the data in Table II and Graph 1, it is found that the monthly per man-hour density of all anophelines has fluctuated from 4·8 in May, 1957 to 94·1 in January, 1955. From Graphs 1, 2 and 3 it will be found that there are two peaks of maximum anopheline abundance every year. One of these occurs in February (63·1 per man-hour) and the other in August (56·3 per man-hour). The anopheline density reaches its lowest point in the month of May, with an average per man-hour density of 11·7. If the monthly mean per man-hour density is plotted along with the monthly mean temperature, relative-humidity and rainfall (Graph 2), it is seen that as the temperature increases and the relative-humidity goes down from March to May, the anopheline density decreases to the lowest level in the year in May, and starts rising as the average mean temperature falls down to about 80 degrees F., with the relative-humidity rising, consequent to the advent of the rainy season from June onwards. During the warm humid months of July and August, the anopheline density increases and in September it is maintained at a fairly high level, though there is a fall from that in August. From October to January, the relative-humidity and temperature decrease considerably, but the anopheline density remains at a high level. In spite of the decreasing relative-humidity, the density gradually rises from November onwards to reach the peak density in February. Probably this is due to the low prevailing temperatures which may be more favourable than those in other months. Bhatia *et al.* (1958), in their observations near Delhi, have found that at low mean monthly temperatures (December to January) and low percentage relative-humidity (May), the anopheline density is either low or is on a decline. But during the present investigation in Dhanbad area, the anopheline density is actually on the increase when both the temperature and relative-humidity are on the decline from October to January. The cause underlying this is better revealed by studying the relative densities of the different species of anophelines. Table III shows that out of the twelve species recorded, five species of anophelines viz., *A. annularis*, *A. culicifacies*, *A. fluviatilis*, *A. pallidus* and *A. subpictus*, form the bulk (96·5 per cent) of the catch and are the predominant species in numbers of the total collection of each month. In the case of *A. fluviatilis* and *A. annularis* (Table IV), the density starts increasing from October onwards and reaches the peak in January and February respectively. *A. pallidus* also shows an increase in density in October and November while from December onwards it falls gradually. It is, therefore, clear that it is because of the abundance of *A. fluviatilis* and *A. annularis*, and *A. pallidus* to some extent, that there is a winter peak in spite of the decreasing relative-humidity and temperature. These three species occur mainly during the winter months, either early, mid or late. In the Delhi area (Bhatia *et al.*, 1958), since *A. fluviatilis* is very rare and *A. pallidus* also does not seem to occur in abundance, the winter months show a decline with *A. annularis* alone forming the bulk of the anopheline catch during these months. The August peak in Dhanbad area is formed by the abundance of *A. culicifacies* and *A. subpictus*

which reach their peak densities during this month, and is augmented to a small extent by *A. annularis* and *A. pallidus* which maintain a fair density. All species show a steep decline in May, when the temperature is at its highest and the relative-humidity is very low, except *A. subpictus* and *A. vagus* which not only maintain their numbers at a good level, but are on the increase from April onwards. Bhatia *et al.* (1958), in their observations near Delhi, state that enough rainfall, high humidity, and temperature between 75 to 95 degrees F., are ideal for high density of anophelines around Delhi, but such a statement cannot be made with regard to the Dhanbad area as the composition of the anopheline fauna is slightly different from the Delhi area, though the climatological factors are almost similar. The maximum anopheline density is reached in February (63·1 per man-hour) when the mean temperature is around 74 degrees F., the mean relative-humidity around 40 per cent and the rainfall is meagre.

Graph 1 shows that the man-hour density of all anophelines as well as that of *A. fluviatilis* and *A. culicifacies* has declined considerably from 1955 onwards. *A. culicifacies* especially seems to have almost disappeared from 1956 onwards. This may, perhaps, be due to the fact that spraying operations have been extended considerably covering most of the villages around Dhokra since the advent of the National Malaria Control Programme.

The seasonal fluctuations in density and the preferential day-time indoor resting places of each species are considered individually below.

Anopheles annularis *van der Walp.*

This species was the third dominant anopheline and formed 20·0 per cent (Table V) of the total collections for the entire period. It was prevalent throughout the year (Graph 4) but the per man-hour densities fluctuated from 1·3 in June to 20·1 in February. Bhatia *et al.* (1958) have reported that the high density for this species is recorded during the months of February, March and April in the Delhi area. The findings at Dhanbad also point to a similar occurrence, the highest densities being in February and March with 20·1 and 18·4 per man-hour respectively. However, in this area, the density drops down considerably in April and this may perhaps be due to the fact that in Dhanbad the summer starts earlier, with higher temperature than in Delhi. The present study confirms the findings of Bhatia *et al.* (1958) that this species does not appear to be a cold-weather species as reported by Senior White (1930) but rather tends to be a mosquito of the spring season, and that heavy rainfall, irrespective of temperature, leads to low densities of this species. Brooke Worth (1953) and Bhombore *et al.* (1954) state that *A. annularis* shows a marked seasonal prevalence in the Western Hill-tracts of Mysore, with the peak of its adult population cycle falling earlier in the year, occupying the late winter months preceding the hot weather of April and May.

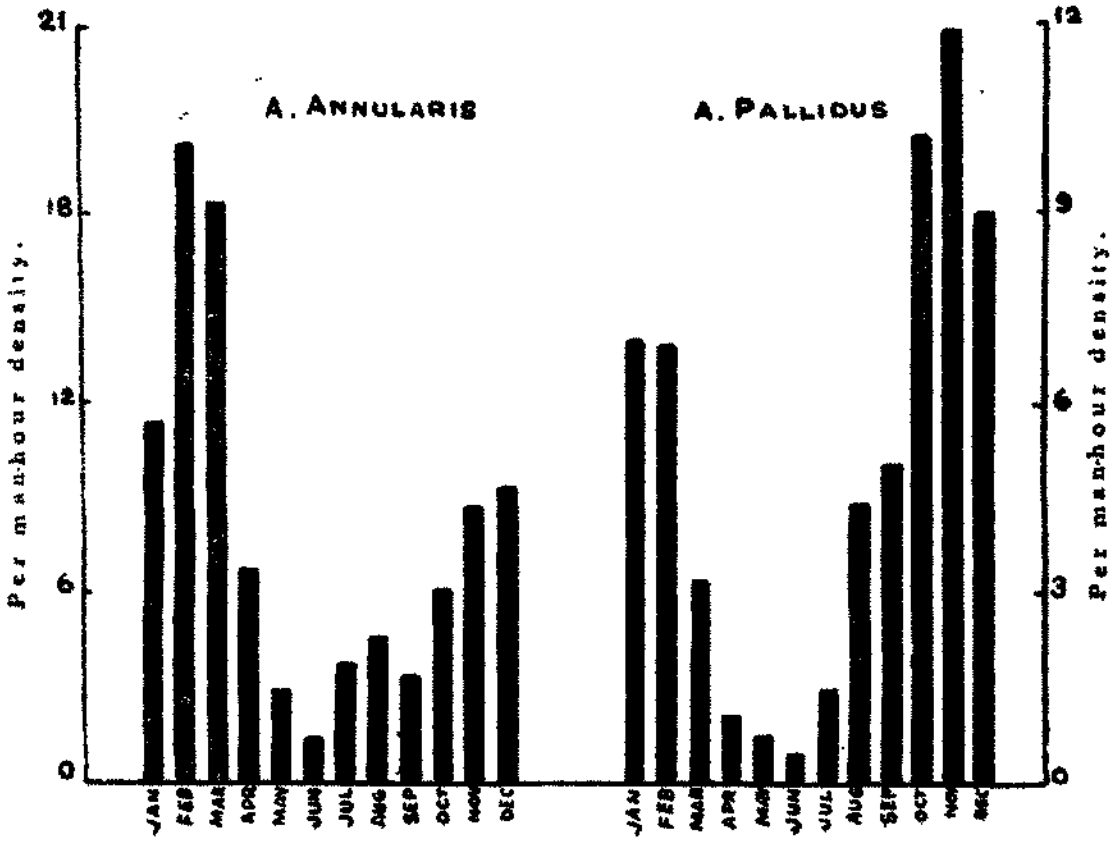
TABLE V.

Percentage of abundance of the different species of anophelines near Dhanbad. (July, 1953 to April, 1958).

Serial number.	Species.	Total collected.	Per man-hour density.	Percentage of abundance.
1	*A. subpictus*	9,095	11·0	27·5
2	*A. fluviatilis*	7,490	9·1	22·7
3	*A. annularis*	6,611	7·9	20·0
4	*A. culicifacies*	4,374	5·3	13·2
5	*A. pallidus*	4,313	5·2	13·1
6	*A. splendidus*	534	0·6	2·5
7	*A. vagus*	412	0·5	
8	*A. varuna*	167	0·2	
9	*A. hyrcanus*	50	0·06	
10	*A. barbirostris*	4	0·005	
11	*A. stephensi*	2	0·002	
12	*A. tessellatus*	1	0·001	

GRAPH 4

Monthly incidence of A. annularis *and* A. pallidus *near Dhanbad (July, 1953 to April, 1958).*



Unlike the observations of Bhatia *et al.* (1958) the present study confirms the findings of Christophers (1933), Senior White *et al.* (1943) and Rao (1944), in that this species prefers to rest in cattle-sheds. The ratio of per man-hour density in cattle-shed, human-dwelling and mixed-dwelling was found to be 15·1, 2·0, 5·9 respectively (Graph 8 and Table VI).

Rao (1944) has incriminated this species as the sole carrier in the south-eastern corner of the Jharia Mining Settlement with a gut-infection rate of 0·5 per cent and gland-infection rate of 0·1 per cent.

TABLE VI.

Resting preferences of adult anophelines near Dhanbad (July, 1953 to April, 1958).

Serial number.	Type of shelter:	Human dwelling:			Mixed dwelling:			Cattle shed:		
	Time spent.	323 hours.			180·5 hours.			323 hours.		
	Species.	Total catch.	Per man-hour.	Per-centage.	Total catch.	Per man-hour.	Per-centage.	Total catch.	Per man-hour.	Per-centage.
1	*A. annularis*	660	2·0	8·7	1,064	5·9	25·65	4,887	15·1	65·05
2	*A. barbirostris*	..	..	..	..	..	..	4	0·01	..
3	*A. culicifacies*	131	0·4	2·9	68	0·4	2·9	4,175	12·9	94·2
4	*A. fluviatilis*	291	0·9	3·6	676	3·7	14·1	6,523	20·2	82·3
5	*A. hyrcanus*	..	..	..	4	0·02	16·7	46	0·1	83·3
6	*A. pallidus*	402	1·2	7·9	755	4·2	27·6	3,156	9·8	64·5
7	*A. splendidus*	41	0·1	5·9	59	0·3	17·6	434	1·3	76·5
8	*A. stephensi*	..	..	..	1	0·0006	..	1	0·003	
9	*A. subpictus*	1,058	3·3	10·4	1,401	7·8	24·7	6,636	20·5	64·9
10	*A. tessellatus*	..	..	..	1	0·006	..	..	..	..
11	*A. vagus*	25	0·08	5·8	61	0·3	21·7	327	1·0	72·5
12	*A. varuna*	1	0·003	0·6	2	0·01	2·0	164	0·5	97·4

*Percentages not worked out as the numbers collected are meagre.

Anopheles barbirostris *van der Walp.*

This species was very rare during the present investigation and altogether only 4 specimens were caught from cattle-sheds in the months of October/November.

Anopheles culicifacies *Giles.*

This species is reputedly a species of the plains (Bhatia and Krishnan, 1957). The vectorial capacity and its importance in the transmission of malaria in the different parts of India have been a point of dispute. Christophers (1933) is of the opinion that this species could be regarded as an important vector wherever it is found. Senior White (1937 : 1938) has stated that *A. culicifacies* plays no part in the transmission of malaria in the Jeypore Hills and East Central India even though found in large numbers. Russell and Rao (1940), discussing the role of *A. culicifacies* as a carrier of malaria in the south-eastern part of Madras, feel that Senior White's (1937 : 1938) findings are not convincing, in view of the fact that it is a

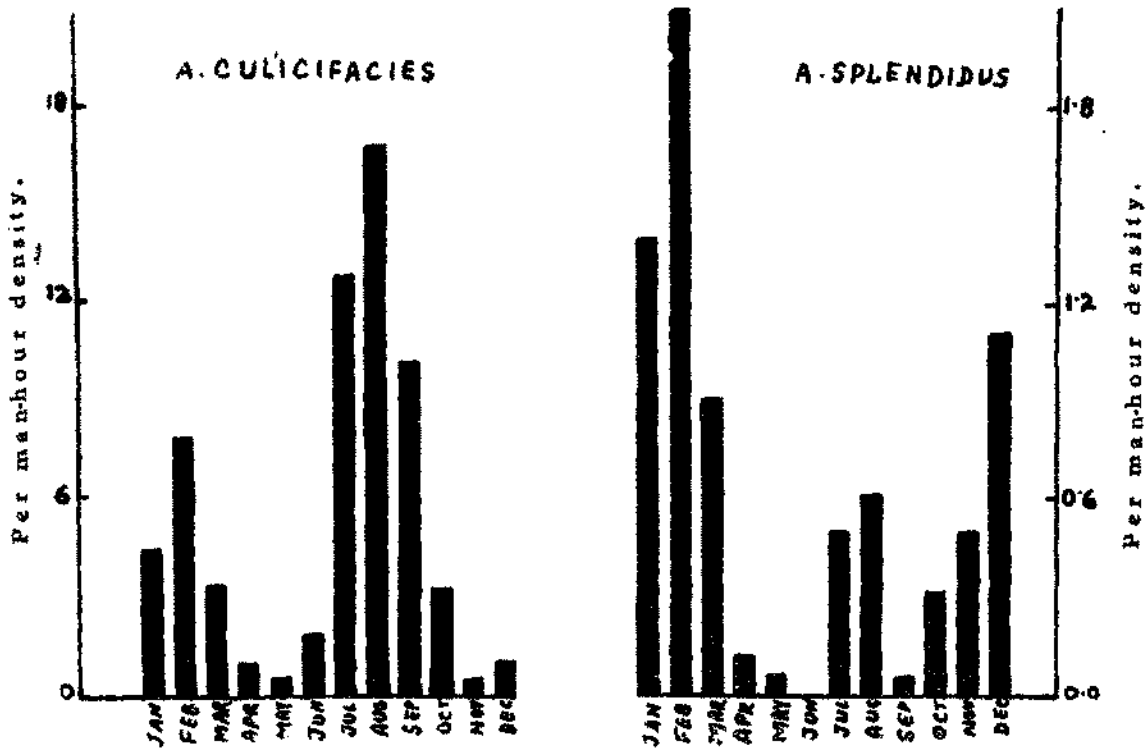
weak carrier which is capable of doing great harm when in sufficient numbers and that there is a need for many more dissections of this species in North Central and North-East India. They also say that the possibility of two or more biotypes of *A. culicifacies* in India is not ruled out. But so far no evidence has come forth regarding the presence of two or more biotypes of this species. However, the records so far show that the vectorial capacity of *A. culicifacies* is limited to Peninsular India and north-west India (Bhatia and Krishnan, 1957). Senior White (1943) has recorded that on the Ranchi plateau there is a moderate endemicity caused by *A. culicifacies*.

The role of this species in the transmission of malaria in the Jharia Mining Settlement has not been decided conclusively. Rao (1944), after dissecting 1,509 specimens over a period of five years, has recorded gut-infection rate of 0·6 per cent and gland-infection rate of 0·1 per cent and has concluded that with *A. fluviatilis* and *A. stephensi* showing higher infection rates, *A. culicifacies* can be considered as relatively unimportant. But viewed against the light of Russell's work at Tanjore (Russell and Rao, 1940) where 13,156 *A. culicifacies* were dissected and the infection rate was 0·073 per cent for gut-infection and 0·061 per cent for gland-infection, but still this was the chief carrier maintaining an average endemic malaria spleen-index greater than 40 per cent., Rao's (Rao, 1944) figures of 0·6 per cent and 0·1 per cent gut and gland infection rates from a dissection of 1509 adults should be considered to be of considerable importance in the transmission of malaria in the Jharia Mining Settlement area. Rao (unpublished report) has recorded a sporozoite infection rate of 0·045 per cent in August, 1950 alone, after dissecting 2,211 *A. culicifacies* in the north-western corner of the Settlement during an experimental study. Rao (1944) has also mentioned that eight gland-positive *culicifacies* were recorded in 1943. Considering the above points, it is felt that the role of *A. culicifacies*, as a vector of considerable importance in the coal-field area, has been under-estimated.

Afridi and Puri (1940), reviewing the work of Senior White and Das (1938) in the hilly-tracts of Singhbhum, have stated that the adults of *A. culicifacies* were present from March to September, the period of maximum prevalence being from June to September with a smaller rise in April. A record of similar prevalence has been made by Senior White and Adhikari (1934) near Singhbhum, where adults were encountered in large numbers from August to October. The present findings (Table IV and Graph 5) show that the species is abundant from July to September with a peak of 16·8 per man-hour in August and again a smaller rise in February (7·9 per man-hour) and not in April as in the Singhbum Hills. From March onwards there is a rapid decline and in April and May the average densities are 0·7 and 0·5 per man-hour respectively. Periods of comparatively low densities are April to June, November and December. The species is at its highest density during the monsoon months but with the termination of the monsoon in September, the density falls down rapidly and it is not possible to say exactly what factors influence the rise and fall of the species. Bhatia *et al.* (1958) have stated that the most important controlling factor seems to be the formation of fresh rain-water

GRAPH 5.

Monthly incidence of A. culicifacies *and* A. splendidus *near Dhanbad (July, 1953 to April, 1958).*



pools devoid of amorphous matter during July, August and September, in the Delhi area. It is not definitely known if the same factors, as reported by Bhatia *et al.* (1958), influence the prevalence of this species in the Dhanbad area also.

A. culicifacies is a predominantly domestic species preferring human-dwellings and cattle-sheds for its day-time resting places (Bhatia and Krishnan, 1957). Senior White (1937), Senior White and Das (1938), Afridi, Jaswant Singh and Harwant Singh (1939) have all reported of catches being higher in cattle-sheds than in human-dwellings. Timbers (1935) for Bengal and Bhatia *et al.* (1958) for the Delhi area have recorded larger numbers in human-dwellings than in cattle-sheds. Rao (1944) has recorded a ratio of 5 : 1 for the density in cattle-sheds and human-dwelling in the Jharia Mining Settlement. The present study reveals that *A. culicifacies* is predominantly found in cattle-sheds (Graph 8), the per man-hour density for cattle-shed, human-dwelling and mixed-dwelling being 12·9, 0·4, and 0·4 respectively. (Table VI).

Anopheles fluviatilis *James*.

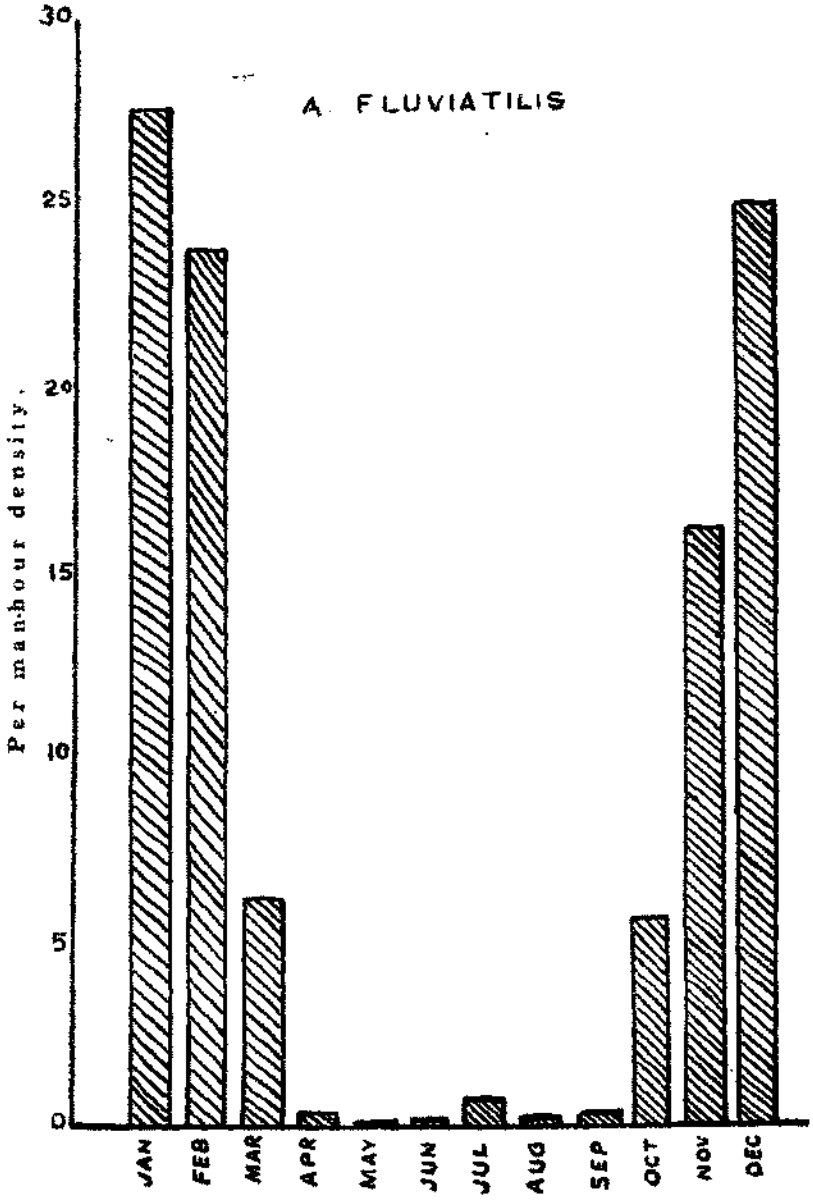
This species has been incriminated as the primary vector in the Jharia Mining Settlement by Rao (1941 : 1944), with sporozoite infection rates of 2·0 per cent and 1·8 per cent for the respective years. It has also been incriminated as the vector in the Hazaribagh ranges (Senior White, 1943) and the Singhbhum Hills (Senior White and Das, 1938), both of which are adjacent to the Dhanbad district. So far, all the records (Measham and Chowdhury, 1934 in the Annamalais ; Senior White and Das, 1938 in the Singhbhum Hills ; Senior White, 1938 in the Jeypore Hills ; Covell and Harbagwan, 1939 in Wynaad ; Russell and Jacob, 1942 in the Nilgiris ; Jaswant Singh and Jacob, 1944 in North Kanara ; Viswanathan and Ramachandra Rao, 1946 in North Kanara ; Senior White, 1943 in the Hazaribagh ranges ; Ramakrishnan *et al.*, 1948 in South Kanara ; and Issaris *et al.* 1953 in the Tarai region of U. P.) show that *A. fluviatilis* is primarily a vector in hilly and foot-hill regions. However, Rao (1944) incriminated this species as a vector of primary importance in the Jharia Coal-field, which is a plain area.

In the area under study, *A. fluviatilis* was the second dominant species forming 22·7 per cent of the total catch (Table V). Table IV and Graph 6 show that this species has a strictly seasonal distribution, being abundant only during the winter months (November to February), with the mean peak density of 27·5 per man-hour in January. Senior White (1943) reported that *A. fluviatilis* exists in fair numbers during September to March and is maximal in December in the Ranchi Plateau. The seasonal distribution of the species in Dhanbad, therefore, compares favourably with the findings of Senior White in the Ranchi Plateau. The present findings indicate that this species prefers temperatures between 55°F. and 75°F. and a mean relative-humidity between 50 and 60 per cent.

Brooke Worth and Sitaraman (1952) have stated that there is enough evidence to believe that two geographic races of *A. fluviatilis* are present in Peninsular India though no morphologic evidence supporting the belief is as yet available. They visualize the two races with the following habits: (*i*) anthropophilic, out-door resting, hill-inhabiting, strongly vectorial and low in density, (*ii*) zoophilic, in-door resting, plains-inhabiting, weakly vectorial and high in density. Issaris *et al.* (1953) have stated that in the Tarai region of U.P., there is a mixture of both anthropophilic and zoophilic races, if it is assumed that there are two such races existing. Rao (1944) has stated that adults of *A. fluviatilis* have been found almost exclusively in human-dwellings in the Jharia Mining Area. During the present study, however, this species was considerably abundant in cattlesheds than in human-dwellings or mixed-dwellings, the percentage of abundance for the three types of shelters being 82·3, 3·6 and 14·1 respectively (Table VI). It is difficult to arrive at a conclusion regarding the habits of a species merely from the densities in the different types of catching stations, especially so in the case of *A. fluviatilis* whose habits have been controversial (Brooke Worth and Sitaraman, 1952). Thompson (1951) has stated that the feeding habits of mosquitoes are influenced by a variety of factors such as, type of construction of houses and cattle-sheds and also by the relative proportion

GRAPH 6.

Monthly incidence of A. fluviatilis *near Dhanbad (July, 1953 to April, 1958).*



of man and animals. The type of construction of houses and cattle-sheds in this area are all similar and hence Rao's (Rao 1944) findings can be compared with the present findings. Regarding the second factor, viz., proportion of man and animals, there are no correct figures available and as such no conclusion can be arrived at. During the course of the present investigation some specimens were sent to the Malaria Institute of India for precipitin tests, from November, 1957 to February, 1958. Table VII shows the data of the precipitin tests carried out by the Malaria Institute. The number of specimens examined from human-dwellings are very meagre, but since the density of *A. fluviatilis* in cattle-sheds formed more than 80 per cent of the total (Table VI), the figures for the cattle-shed collection can be taken to give a fair idea about the feeding preferences of this species. The percentage with human blood resting in cattle-shed, was 28·9 while in mixed-dwellings it was 50 per cent. The percentage with animal blood in cattle-shed was 71·1. The bulk of the catch is from cattle-sheds and precipitin test indicates that the species has a preference for animal blood, though a small percentage also rests there after feeding on man. Senior White (1947) in East Central India and Issaris *et al.* (1953) in the U.P. Tarai region have reported the presence of *A. fluviatilis* in out-door shelters, having a high anthropophilic index. As no out-door collections were conducted during the present study, it is not definitely known whether this species is zoophilic or anthropophilic or a mixture as in the Tarai region (Issaris *et al.*, 1953).

TABLE VII.

Results of precipitin tests on A. fluviatilis *from Dhokra (November, 1957 to February, 1958).*

Type of shelter.	Total positive.	Man :		Cow :		Goat :	
		Number positive.	Per cent.	Number positive.	Per cent.	Number positive.	Per cent.
Human dwelling	2	1	50·0	1	50·0	..	..
Mixed dwelling	16	8	50·0	8	50·0	..	..
Cattle-shed	45	13	28·9	27	60·0	5	11·1
Total	63	22	34·9	36	57·1	5	8·0

As regards the vectorial capacity of this species in the Dhanbad area, it appears that it is weakly vectorial. The dissection records of the Jharia Malaria Laboratory (unpublished) for the Jharia Coal-field area from 1949 to 1958 inclusive, show that 22,021 *A. fluviatilis* (including the collections from the present study) have been dissected and not a single case of gut or gland infection has been recorded. From the data so far discussed, it appears that *A. fluviatilis* of this area (Dhanbad plains) is prevalent in high densities, prefers cattle-sheds as day-time in-door resting places, and does not seem to be vectorial, which are contrary to the findings of Rao (1944). However, further studies on the behaviour of this species in this area are warranted to find out conclusively whether the species has changed its habits during the course of the past fifteen years or that one geographic race (assuming there are two races) has given place to another.

Anopheles hyrcanus *var* nigerrimus *Giles*.

This species is generally considered to be wild and not commonly found resting indoors (Christophers, 1933). Bhatia *et al.* (1958), from their observations around Delhi, have reported that the species may be present throughout the year in relatively low densities. During the present study only 50 specimens of this species were encountered out of which 42 were collected during November and December (Table III). Bhombore *et al.* (1954) have reported that this species attains peak abundance in October and November in the Western Hill-tracts of Mysore. The present study also reveals that this species has a definite preference for cattle-sheds as day-time resting places over human or mixed dwellings. 87·5 per cent of the catch was from cattle-sheds, 12·5 per cent from mixed-dwellings and none from human dwellings (Table VI).

Anopheles pallidus *Theobald*.

Although a gut-infection rate of 0·8 per cent and a gland infection rate of 0·1 per cent has been recorded for this species, it has been considered to be relatively un-important in transmission of malaria (Rao, 1944). During the study, *A. pallidus* was the fifth dominant species in number, forming 13·1 per cent of the total collections during the entire period of investigation (Table V). Bhatia *et al.* (1958) have found the species to abound in large numbers in late monsoon and early winter months in the Delhi area. The present study at Dhanbad also points to a similar prevalence (Table IV and Graph 4), the species starting to build up its density from August onwards and reaching the peak of 11·8 per man-hour in November. The density gradually falls down in December and the early months of the year. From April to June the density is very low, not exceeding 1·4 per man-hour. Bhombore *et al.* (1954) also report of highest prevalence of adults from September to November, i.e., after the monsoon and a minor peak in April-May, in the Western Hill-tracts of Mysore. Brooke Worth (1953), commenting on this minor rise in April-May, states that the adults are able to survive in hot dry periods with a measure of considerable efficiency. The mean temperature in April and May for the Mysore area (Bhombore *et al.*, 1954) ranges from 75°F. to 80°F., and the mean relative humidity from 65 to 80 per cent. The mean temperature in Dhanbad during April and May ranges from 90°F. to 95°F., and mean relative humidity between 20 and 30 per cent. The conditions in Dhanbad, therefore, appear to be too hot and too dry for the species to survive in large numbers during these months. Bhatia *et al.* (1958) are of the opinion that low temperatures, combined with numerous breeding places augmented by monsoon, determine the relative abundance of this species. The same opinion seems to hold good in Dhanbad area also for this species as evidenced by the present findings. Russell and Rao (1941) observed that the adults of this species seem to prefer to rest in out-door places in south-east Madras. Rao (1944) has stated that adults of *A. pallidus* are found in about equal numbers in human dwellings and cattle-sheds in the Jharia Mining Settlement. Bhombore *et al.* (1954) have been able to record high densities from in-door resting places in the low rainfall area of the Western

Hill-tracts of Mysore. Bhatia *et al.* (1958) have also reported that the species is an indoor-rester in the areas surveyed around Delhi. In the absence of out-door collections, but the comparatively abundant indoor densities, it appears that this species can be considered as an indoor-rester in the Dhanbad area also. As regards the percentage of abundance in the different types of shelters, Christophers (1933) states that *A. pallidus* is commonly taken in houses and cattle-sheds, but with special attraction to stables and cow-sheds. Table VI and Graph 8 show that during the present study *A. pallidus* was found to prefer cattle-sheds, the percentage of abundance for cattle-shed, human-dwelling and mixed-dwelling being 64·5, 7·9 and 27·6 respectively.

Anopheles splendidus *Koidsumi*.

Puri (1955) does not list this species for Bihar State. Rao (1941 : 1944), however, has recorded this species in the Jharia Mining Settlement. Christophers (1933) states that adults are "usually found in small numbers in houses, out-houses, cow-sheds etc." Bhombore *et al.* (1954) have confirmed this statement during their investigation in the Hill-tracts of Mysore. During the present study, this species was encountered in fairly good numbers and ranked sixth in abundance (Table V). It was present during eleven months of the year (Table III) and only in June it was not encountered. Graph 5 shows that maximal densities are reached during the mid and late winter months (December to February). There is a small rise in July-August. During the other months the density is very low. Bhombore *et al.* (1954) have, however, recorded the maximal densities during the hottest season of the year in the Mysore Hill-tracts. Senior White and Das (1938) have recorded *A. splendidus* exclusively in cattle-sheds in the Singhbhum Hills during their check catches over a period of sixteen months. In the area under consideration, the species was found in the ratio of 1 : 2·5 : 10·5 for human-dwelling, mixed dwelling and cattle-shed respectively (Table VI).

Anopheles stephensi *Liston*.

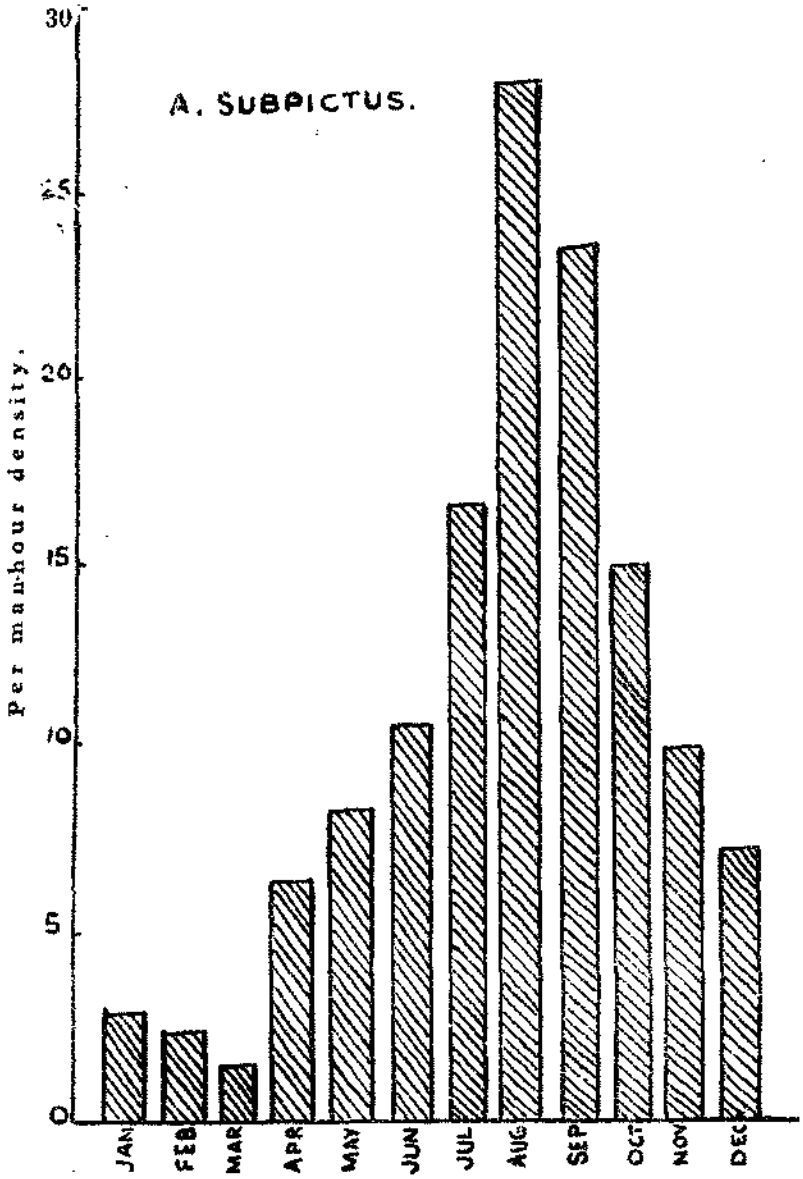
This species has been incriminated as a vector of secondary importance in the Jharia Mining Settlement by Rao (1944). In numbers it ranked next to *A. annularis* and *A. subpictus* during the earlier investigations (Rao, 1944). However, during the present study which was confined to Dhokra village alone, only two specimens were collected during the entire period of 58 months (Table III). This species was very rare and, therefore, it is not possible to comment on its prevalence.

Anopheles subpictus *Grassi*.

A. subpictus and *A. vagus* are two of the commonest anophelines in India (Puri, 1955). This species was most abundant during the present investigations and formed 27·5 per cent of the total collection for the entire period. In Delhi area, which is climatologically comparable with Dhanbad, Bhatia *et al.* (1958) report that the species is not found throughout the year but shows a definite seasonal trend. In the Dhanbad area it is recorded throughout the year, the

GRAPH 7.

Monthly incidence of A. subpictus *near Dhanbad (July, 1953 to April, 1958).*



average per man-hour density ranging from 1·4 in March to 28·0 in August, with a definite seasonal abundance during the monsoon months (Table IV). The findings of Bhatia *et al.* (1958) that this species shows a typical rise during monsoon, is confirmed in this area (Graph 7), being abundant during the months of July, August and September. The density decreases considerably during the winter months, but again rises from mid-summer (April) onwards. Bhombore *et al.* (1954) state that this species shows a late winter and early spring rise in the Mysore Hill-tracts. Bhatia *et al.* (1958) find that this species has a very low density during January to April. As such, this species seems to show varied types of seasonal distribution in different areas. The rise during the peak summer months in Dhanbad area is, however, strange in that the species can withstand very high temperatures combined with very low humidities. This species and *A. vagus* are the only two among the twelve species recorded (Table IV), that show a definite rise from April onwards, when the other species are all on the decline during the summer months.

Bhatia *et al.* (1958) state that *A. subpictus*, along with *A. annularis* and *A. culicifacies*, seems to have a preference for human and mixed dwellings over cattle-sheds, in the Delhi area. The present study indicates that in the Dhanbad area this species definitely prefers cattle-sheds to human dwellings or mixed-dwellings, the percentage of abundance being 64·9, 10·4 and 24·7 for the three types of shelters, respectively, (Table VI and Graph 8).

Anopheles tessellatus *Theobald*.

Christophers (1933) describes this species as a more or less domestic species. Puri (1955) states that the distribution of this species in India is mainly Eastern and Southern. During the present study, this was a rare species, only one speciman being collected for the entire period (Table III). Rao (1944) has also recorded only two specimens during a period of five years. Nothing is known about the prevalence of this species in this area except that it is very rarely encountered in indoor shelters.

Anopheles vagus *Donitz*.

As has already been mentioned, *A. subpictus* and *A. vagus* are the two commonest anophelines of India (Puri 1955). This species has been recorded in very large numbers in the Jharia Mining Settlement by Rao (1944). In the Dhanbad area it ranked seventh in abundance. Like *A. subpictus* it was found throughout the year with the peak density in August and again a rise from April onwards. The seasonal prevalence of this species is exactly similar to that of *A. subpictus*. Brooke Worth (1953) states that the habits of *A. vagus* are almost similar to that of *A. subpictus*. This species also prefers cattle-sheds to human-dwellings or mixed-dwellings in the Dhanbad area, the percentage of abundance being 72·5, 5·8 and 21·7 for the three types of shelters respectively (Table VI).

Anopheles varuna *Iyengar.*

Brooke Worth (1953) states that this species is rather puzzling because of its apparently discontinuous prevalence in the hill-tracts of Mysore. During the present investigation also, *A. varuna* showed an erratic distribution, being found in abundance in 1954 and in small densities in 1955 and 1956 (Table II). During the other years it was completely absent in indoor shelters. During the three years they were collected, the species showed a definite preference for winter months, being found only during October and January. The reason for this discontinuous distribution during the same year as well as during different years is not known so far, unless further detailed studies are undertaken. Senior White and Venkat Rao (1943) have found in Vizagapatam that *A. varuna* has special and somewhat peculiar resting habits which involve not houses or ordinary cattle-sheds or outdoor shelters, but a special type of cow-shed situated far away from houses. It is not known if such a special habitat is preferred in this area also. However, *A. varuna* showed a distinct preference for cattle-sheds over human-dwellings and mixed-dwellings in this area. The 167 *varuna* collected during the entire period were distributed as follows :—

Cattle-shed 164, human-dwelling 1, and mixed-dwelling 2, (Table VI).

ADULT RESTING PLACES.

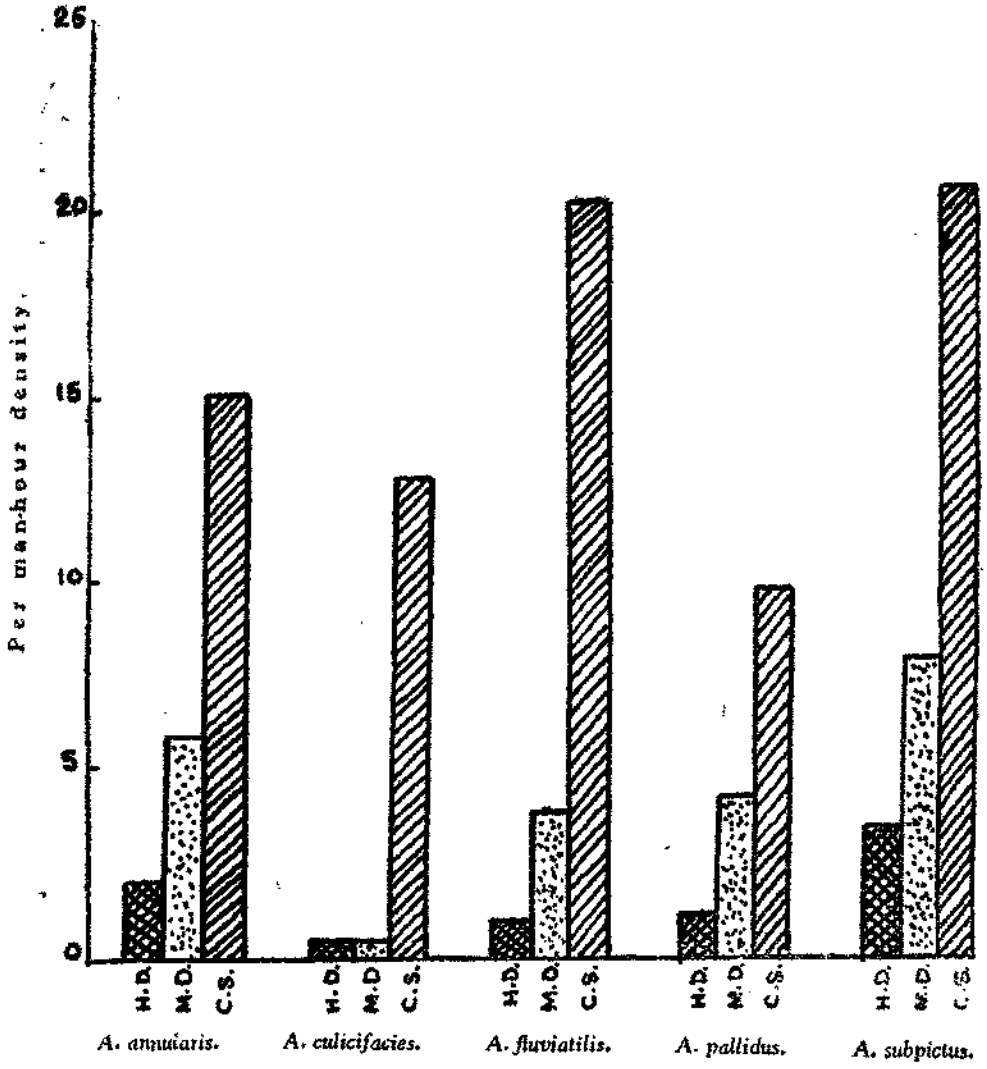
Table VI shows the total per man hour density and percentage of collection for each species from the three types of indoor shelters, viz., human-dwelling, mixed-dwelling and cattle-shed. Graph 8 shows the relative abundance (per man-hour density) of the five dominant species, viz., *A. annularis, A. culicifacies, A. fluviatilis, A. pallidus* and *A. subpictus* in the three types of shelters. The preferences of shelters are discussed under each species. On the whole it is found that in the Dhanbad area, all the anophelines show a marked preference for cattle-sheds over human-dwellings or mixed-dwellings. The reason for this preference is not quite apparent. Bhatia *et al.* (1958) find that in the Delhi area, *A. annularis, A. culicifacies,* and *A. subpictus* seem to prefer human and mixed-dwellings than cattle-sheds. Rao (1944) states that *A. fluviatilis* is almost exclusively found in human-dwellings, *A. stephensi* and *A. pallidus* in about equal numbers in human-dwellings and cattle-sheds, whilst *A. annularis* and *A. culicifacies* appear to prefer cattle-sheds as the day-time resting places, in the Jharia Mining Settlement. It is not definitely known why *A. fluviatilis* and *A. pallidus* have shown a definite change in the preference of day-time resting place in this area when compared to the findings of Rao (1944).

SUMMARY.

1. Entomological field data pertaining to the habits of adult anophelines over a period of about six years (July, 1953 to April, 1958) in Dhokra, an unsprayed village near Dhanbad, is discussed with reference to the seasonal prevalence and the day-time indoor-resting habits of the different species.

GRAPH 8.

Relative abundance in the different indoor resting places of A. annularis, A. culicifacies, A. fluviatilis, A. pallidus *and* A. subpictus *near Dhanbad (July, 1953 to April, 1958)*



H.D. = Human dwellings.
M.D. = Mixed dwellings.
C.S. = Cattle-sheds.

2. Tweleve species of anophelines were recorded and the seasonal preference of seven species, viz., *A. annularis, A. culicifacies, A. fluviatilis, A. pallidus, A. splendidus, A. subpictus* and *A. vagus* which were available in considerable numbers, is discussed with reference to the climatological conditions.

3. It was found that five species of anophelines, viz., *A. annularis, A. culicifacies, A. fluviatilis, A. pallidus* and *A. subpictus* formed over 95 per cent of the catch and the presence or absence of these species affected the total anopheline density in any month. The average per man-hour density for all anophelines showed two peaks of abundance, one in February and the other in August. The month of February had the highest density. It was found that these five species could be divided into two groups, (*i*) *A. annularis, A. fluviatilis* and *A. pallidus* which were abundant during the winter and early spring months giving rise to the February peak and (*ii*) *A. culicifacies* and *A. subpictus* which were typical monsoon species accounting for the peak in August.

4. *A. annularis* was the third dominant species in numbers and was found to be present throughout the year but predominantly abundant during the late winter and early spring months.

5. *A. culicifacies* was the fourth dominant species and showed two peaks of abundance, one during the monsoon (July to September) and a smaller rise in February.

6. *A. fluviatilis* (incriminated as a vector of primary importance in 1944) was second in abundance and appeared to have changed its resting and feeding habits, and probably vectorial capacity also is replaced by a different race (assuming that two races are present). It was found to be purely a winter species prevalent in high densities from November to December.

7. *A. pallidus*, the fifth in abundance, was also found throughout the year like *A. annularis* but the peak densities were attained during early winter.

8. *A. splendidus*, the sixth in abundance, was found in large numbers only during late winter, like *A. annularis*, but disappeared completely in June.

9. *A. subpictus*, the most dominant species, showed a typical monsoon peak and also a rise during the hottest months of the year.

10. *A. vagus*, though not in large numbers, was prevalent throughout the year and showed a seasonal distribution exactly similar to that of *A. subpictus*.

11. *A. barbirostris, A. hyrcanus, A. tessellatus, A. stephenei* and *A. varuna* were too few to indicate any definite seasonal distribution. *A. varuna*, however, showed a discontinuous distribution.

12. The day-time resting habits of the different species are also discussed. It was found that all the species preferred cattle-sheds to human dwellings and mixed-dwellings as the day-time resting places.

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RESULTS OBTAINED IN THE SECOND YEAR OF PILOT STUDY OF MALARIA SURVEILLANCE IN MYSORE STATE.

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INTRODUCTION.

THE results of pilot study carried out from June to October, 1956, on the setting up of surveillance organisation in North Kanara District, have already been communicated by Patel *et al.* (1957). The first year's results of pilot study (from October, 1956 to September, 1957) in selected areas of Mysore State, including North Kanara District, have also been communicated by Shama Sastry and Narayana Iyengar (1959).

The observations continued during the second year of the programme (October, 1957 to September, 1958) are presented here. However, the entomological data are presented from 1954 onwards. The data are presented areawise on the same basis as in the previous communications referred to.

THE *FLUVIATILIS* AREA.

In erstwhile Mysore State, areas selected for maintenance work are of two types. The first area, consisting of Shikaripur, Sorab, Sagar, Hosanagar and Thirthahalli Taluks in Shimoga District, Sakalespur Taluk of Hassan District and Koppa and Sringeri Taluks of Chickmagalur, was under complete interruption of spraying. The results of the second year of the pilot study are described in Part I of the present series. The second area, consisting of Mudigere Taluk in Chikmagalur District and Belur Taluk in Hassan District, was under one round of spraying (October—November in the former and May—June in the latter), till the year 1959. The results of the pilot study during the second year are described in Part II.

The area of past *A. culicifacies* malaria, was also under one round of spraying (April—May, 1959); the study and its results in the second year form Part III.

The observations in North Kanara District, which is the past *A. fluviatilis* area, now under complete interruption of spray, are reported in Part IV.

In Parts V and VI results in Coorg District and the District Malaria Scheme area, Puttur, in South Kanara District, respectively, are presented.

PART I.

A. *FLUVIATILIS* AREA OF ERSTWHILE MYSORE STATE WHERE SPRAYING WAS SUSPENDED.

This area comprises 8 Taluks, as already referred to, with a total population of 4,03,971 (1951 census). All these 8 Taluks are covered by a net-work of Primary Health Units and Modified Health Units. Each Primary Health Unit is provided with 2 Health Inspectors and each Modified Health Unit with 1 Health Inspector. Active surveillance work is being carried out by the Health Inspectors of the Health Units. Surveillance workers, specially appointed for the purpose, supplement the active surveillance work in areas not provided with Health Units, and in Municipal Towns, and areas with Health Units in difficult terrain and with defective communications. Eighteen surveillance workers have been allotted to the work in this area in addition to 118 sanctioned posts of Health Inspectors. Surveillance work is supervised by the Assistant Medical Officer of Health at the Primary Health Unit level and by the Medical Officer of Health, who is incharge of the Secondary Centre, at the Taluk* level. The population of this area is covered for surveillance in a period of 10 days during each fortnight. The average population covered on each day by a Junior Health Inspector or a surveillance worker during the year was 313 as against 297 during the previous year. No vehicle was provided for surveillance work. The population to be surveillanced by each worker is less than the targeted figure because the Health Inspectors of the Health Units have to attend to other public health activities, and general sanitation, etc., in addition to the surveillance work.

Table I furnishes the details of active malaria surveillance work from October, 1957 to September, 1958. The population surveillanced during each month ranged from 0·34 to 0·39 million as against 0·29 to 0·38 million during the previous year. This fluctuation, as pointed out by Shama Sastri and Narayana Iyengar (1959 *loc. cit.*) is due to (*i*) vacancies among the sanctioned posts having been not filled in, (*ii*) there being not enough substitutes to be posted when permanent staff proceed on leave or deputed to other urgent health works such as epidemic control or fair sanitation duty, and (*iii*) frequent resignations of surveillance workers after brief periods of service. Out of the sanctioned posts of 118 Junior Health Inspectors and 18 surveillance workers, the number working in any single month varied from 83 to 93 and 13 to 18 respectively. Provision was made for leave reserves in the Health Units but the posts could not be filled in due to dearth of suitably qualified persons. The situation is under critical examination.

FEVER MORBIDITY.

A total of 10,322 fever cases were investigated during the year under report, as against a total of 10,889 fever cases investigated during the previous year. The average number of fever cases per 1,000 population during the first fortnight ranged

* Taluk means a Revenue Division of the District.

TABLE I.

Details of active malaria surveillance work during the period October, 1957 to September, 1958.

A. *fluviatilis* area where spraying has been interrupted since October, 1956 (In five Taluks of Shimoga, 2 Taluks of Chickmagalur and 1 Taluk of Hassan Districts.)

(Population : 0·4 million)

Month and year.	Population surveillanced.	Number of surveillance workers:		Particulars:					
		Health Inspectors.	Malaria surveillance workers.	Cases investigated.	Blood smears collected.	Results of blood smears.		Anti-malaria pills issued.	Number of cases investigated per 1,000 population.
						Positive.	Negative.		
Oct. 1957	3,87,996	93	15	1,061	1,017	..	1,017	3,311	2·73
Nov. 1957	3,82,595	91	15	1,239	1,151	..	1,151	3,946	3·23
Dec. 1957	3,91,518	85	17	984	923	..	923	3,265½	2·50
Jan. 1958	3,92,045	83	17	919	876	..	876	3,068	2·33
Feb. 1958	3,99,764	86	18	1,076	1,016	..	1,016	3,563	2·69
Mar. 1958	3,97,723	84	18	760	709	..	709	2,402½	1·91
Apr. 1958	3,87,434	83	18	698	638	..	638	2,238	1·71
May 1958	3,99,933	87	18	793	748	..	748	2,457½	1·98
Jun. 1958	3,68,973	89	16	737	672	..	672	2,334½	1·99
Jul. 1958	3,44,649	86	16	589	509	..	509	1,961	1·70
Aug. 1958	3,88,691	83	14	653	606	..	606	2,154	1·66
Sep. 1958	3,85,454	86	13	803	724	3	721	2,521	2·07
Totals	46,26,775	1,036	195	10,322	9,589	3	9,586	33,222	2·23

from 0·05 to 1·3 and during the second fortnight from 0·06 to 0·16. The monthly average number of fever cases per 1,000 population ranged from 1·66 to 3·23 as against the range of 0·97 to 3·59 during the previous year. There was a slight rise in the incidence of fever cases from October to February, 1958.

Out of 10,322 fever cases investigated, a total of 8,829 cases were analysed age and sex wise (Table II). The number of fever cases in the age-group 0-4 years formed 1/7th of the total as against 1/5th during the previous year.

BLOOD SMEAR EXAMINATION.

Blood smears were examined after staining with J.S.B. stain. A smear was declared negative only after examining at least 100 fields of thick smear in 3 to

TABLE II.

Analysis of fever cases investigated—Age and sex wise.

Age-group. (years).	Total number of fever cases.	Male.	Female.	Percentage of total cases.	Percentage of population under each age-group.
0—4	1,285	691	594	14·6	13·5
5—14	1,872	1,002	870	21·2	24·8
15 and above	5,672	3,451	2,221	64·2	61·6
Grand Total	8,829	5,144	3,685	..	..

5 minutes. The smears were examined at the laboratories attached to the Bureau of Malariology, Bangalore; Malaria Investigation Centre, Mandya; Malaria Field Station, Sakalespur; and at the District Health Offices and Secondary Centres.

9,589 blood smears from fever cases were examined during the year. Three smears were found to be positive for *P. vivax*. Of these three cases encountered in Koppa and Sringeri Taluks of Chikmagalur District, 2 belonged to South Kanara District, a major portion of which has hypoendemic malaria and therefore was included under spray programme only with effect from 1959. It has not been possible to fully investigate the third *P. vivax* positive case. All these 3 cases were encountered during the month of September, 1958, which is a non-transmission period in this area. Apart from these three positive cases, one case of *P. falciparum*, infection* encountered casually, was reported to have been imported into the area from a village in Honnali Taluk, which continues to be under spraying since 1953.

Incidentally, during the half-yearly spleen and parasite survey conducted in April, 1958, which continues to be a routine feature of Health Unit activities, two child blood smears were found positive for *P. vivax*†. Both of them were children of the estate workers who have been living in the estate for several years. These children were born in their native places (Sidlaghatta in South Kanara District) and gave definite history of movement once a year. Detailed investigations reveal that there is no autochthonous transmission and these two cases appear to be relapse cases. Particulars of parasite positive cases are detailed in Appendix I. As against 4 parasite positive cases encountered in fever surveillance during the previous year, only 3 cases were encountered during the year under report.

DRUG DISTRIBUTION.

A total of 33,222 Avlochlor and Resochin tablets were distributed to the fever cases during the period (Table I).

ENTOMOLOGICAL DATA.

Appendix II shows the collection of all anophelines and vectors from October, 1954 to September, 1958. In Sagar area, the per man-hour density of all

* This case was not encountered during fever surveillance.

† These two cases were not encountered during fever surveillance.

anophelines is being built up gradually from 0·38 and 0·42 in 1954-55 and 1955-56, respectively, (period prior to interruption of spraying), to 5·03 in 1956-57 and 5·32 in 1957-58. The vector density during the two years immediately prior to interruption was 0·0. Both *A. fluviatilis* and *A. culicifacies* have started appearing and continued in low density in all types of shelters. *A. fluviatilis* and *A. culicifacies* first appeared in January, 1958 and December, 1957, respectively, the last round of spraying in the area being in May-June, 1956.

In Shikaripur area, the per man-hour density for all anophelines was 5·3 during 1957-58. *A. culicifacies* density was 0·019 and 0·029 in human dwellings (including mixed dwellings) and cattle-sheds respectively. The per-man-hour density for *A. fluviatilis* was 0·001 and 0·019 in human dwellings (including mixed dwellings) and cattle-sheds respectively. The total anopheline density is being built up gradually from 1·15 in 1954-55 and 0·69 in 1955-56 (prior to interruption) to 2·47 in 1956-57 and 5·3 in 1957-58. The per man-hour density of *A. culicifacies* was 0·0 in 1954-55, 0·0007 in 1955-56, 0·0055 in 1956-57 and 0·019 in 1957-58, in human dwellings and mixed dwellings (combined). In cattle-sheds it was 0·004 in 1954-55, 0·004 in 1955-56, 0·02 in 1956-57 and 0·029 in 1957-58. For *A. fluviatilis* the per man-hour density was 0·0 in 1954-55, 0·0014 in 1955-56, 0·008 in 1956-57 and 0·001 in 1957-58 in human dwellings and mixed dwellings (combined). In cattle-sheds the density for *A. fluviatilis* was 0·009 in 1954-55, 0·000 in 1955-56 (prior to interruption), 0·008 in 1956-57 and 0·019 in 1957-58.

In this area the vector species, though not completely absent during the period prior to interruption of spraying, have increased in density since interruption. They are collected in all types of shelters.

*Sakalespur area**.—There was a marked rise in the total anopheline density in all the areas from the year 1955 to 1958 and particularly so in the low rainfall area from 2·1 and 0·9 in 1955 and 1956 respectively to 3·35 in 1957 and 16·55 in 1958. During the previous year (1956-57) the highest density was recorded in the intermediate rainfall area. In the high and the low rainfall areas, *A. culicifacies* was absent all through 1955 and 1956 calendar years. It appeared during 1957 in both these rainfall areas; during 1958 there was a slight drop in the *A. culicifacies* density in all the three rainfall areas, from what it was during the previous year (1956-57). *A. fluviatilis*, absent in intermediate rainfall areas but present in the other areas during 1955-56, reappeared in 1957 and 1958 in low densities, particularly in cattle-sheds.

PART II.

A. FLUVIATILIS AREA OF ERSTWHILE MYSORE STATE (REDUCTION OF FREQUENCY OF SPRAYING TO ONE ROUND).

Mudigere Taluk of Chikmagalur District and Belur Taluk of Hassan District were selected for reduction in the number of spraying rounds. One round of

* This area can be divided into three zones depending upon annual rainfall :—
(1) High Rainfall area—more than 200 inches average annual rainfall.
(2) Intermediate Rainfall area—about 85 inches average annual rainfall.
(3) Low Rainfall area—about 45 inches average annual rainfall.

spraying with 112·0 mg. DDT per sq.ft. was carried out in October-November in Mudigere Taluk and in May-June in Belur Taluk. These Taluks are also covered by a net-work of Primary and Secondary Health Units. 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. The total population of the two taluks is 0·12 million (1951 census) and the average population surveillanced per day by each worker was 405 as against 300 during the previous year. The pattern of surveillance has been similar to one described under Part I. Details of malaria surveillance work done from October, 1957 to September, 1958 are depicted in Table III.

TABLE III.

Details of active surveillance work during the period October, 1957 to September, 1958.

A. fluviatilis area where frequency of spraying was reduced to one round—Belur and Mudigere Taluks ; (Population : 0·12 million.)

Month and year.	Population surveillanced.	Number of surveillance workers :		Particulars :					
		Health Inspectors.	Malaria Surveillance workers	Cases investigated.	Blood smears collected.	Result of blood smears.		Anti-malaria pills issued.	Number of cases investigated per 1,000 population.
						Positive.	Negative.		
Oct. 1957	1,01,127	30	..	146	138	..	138	381½	1·439
Nov. 1957	1,06,837	32	..	145	140	..	140	382	1·34
Dec. 1957	1,19,507	33	..	176	148	..	148	588	1·47
Jan. 1958	1,09,592	25	4	181	166	..	166	632	1·64
Feb. 1958	1,03,128	24	4	205	172	..	172	707½	1·91
Mar. 1958	1,02,007	24	3	135	112	..	112	458½	1·31
Apr. 1958	1,25,935	27	4	127	107	..	107	423½	1·0
May 1958	1,26,165	20	5	207	143	..	143	713½	1·63
Jun. 1958	1,22,045	21	5	193	148	..	148	642	1·52
Jul. 1958	1,26,286	22	5	194	148	..	148	646	1·51
Aug. 1958	1,25,709	23	5	222	156	..	156	735½	1·75
Sep. 1958	1,09,012	19	5	214	152	..	152	690	1·96
Totals	13,77,650	300	40	2,145	1,730	..	1,730	7,000	1·55
Average	1,14,804								

FEVER MORBIDITY.

A total of 2,145 fever cases were investigated during the year covering a population of 0·101 to 0·126 million during different rounds. The average number of fever cases per 1000 population during the year works out to 1·00 to 1·96 (Table III) as against the average of 0·018 to 1·52 during the previous year.

Analysis of 1,904 fever cases, is depicted in Table IV. Details in respect of age and sex of 241 fever cases were not readily available.

TABLE IV.

Analysis of fever cases age and sex wise—Belur and Mudigere Taluks.

Age-group. (Years).	Total number of fever cases.	Male.	Female.	Percentage of total cases.	Percentage of population under each group (1951 census).
0—4	209	96	113	10·9	13·5
5—14	346	207	139	18·2	24·8
15 and above	1,349	838	511	70·9	61·7
Total	1,904	1,141	763	..	..

The percentage of fever cases in the age-group 0-4 years formed about 1/9th of the total cases investigated (Table IV) as against 1/5th during the previous year.

BLOOD SMEAR EXAMINATION.

1,670 blood smears from fever cases were examined and all found negative for malaria parasite. No parasite positive cases were reported during the previous year also.

DRUG DISTRIBUTION.

A total of 7,000 tablets of Resochin and Avlochlor were distributed to fever cases (Table III).

ENTOMOLOGICAL DATA.

Appendix II shows the collections of all anophelines and vectors from October, 1954 to September, 1958. There is a rise in the all anopheline density from 0·94 during 1954-55 to 4·8 during 1956-57 and 6·14 in 1957-58. While *A. fluviatilis* was absent in human dwellings and mixed dwellings during 1954-55, it has appeared in low density (0·0009 per man-hour) during 1956-57 and has recorded a slight rise to 0·007 during the year under report. While the man-hour density of *A. fluviatilis* in cattle-sheds increased from 0·003 in 1954-55 to 0·031 during 1956-57, it has recorded a further rise from 0·031 to 0·237 during the year under report. *A. culicifacies*, which appeared in low density during the first year of pilot study (1956-57), has recorded a rise in density from 0·006 (1956-57) to 0·008 in human dwellings and mixed dwellings (combined and from 0·12 (1956-57) to 0·323 in cattle-sheds during the year under report.

PART III.

PAST *A. CULICIFACIES* AREA OF ERSTWHILE MYSORE STATE (REDUCTION OF FREQUENCY OF SPRAYING TO ONE ROUND).

In Krishnarajnagar Taluk of Mysore District, reduction to one round of spraying was introduced during the year 1956. Spraying is being carried out in this area at 112 mg. per sq. ft. of D.D.T. 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 7 Primary Health Units with a Secondary Centre, and only one Senior Health Inspector is provided for each Primary Health Unit. The pattern of surveillance in this area was confined to one visit to all the villages during the month. No transport was provided. For a total population of 0·107 million, in addition to 7 Health Inspectors, 2 Malaria Surveillance Workers were posted during April, 1957. The average population covered per day by a Senior Health Inspector or a surveillance worker comes to about 1,200. However, the daily performance per worker varied from unit to unit, depending on density of population and terrain. The details of work done are presented in Table V.

FEVER MORBIDITY.

A total of 641 fever cases were investigated during the year. The average number of fever cases per 1000 population varied from 0·22 to 1·23 during the year under report as against the average varying from 0·19 to 0·91 during the previous year. There was a rise in fever rate investigated during October to February in contrast to the rise recorded during May to September during the preceding year.

Analysis of 566 fever cases, is depicted in Table VI. Data in respect of 75 cases was not readily available.

The percentage of fever cases in the age-group 0-4 years formed about 1/6th of the total cases as against 1/5th during the previous year. In the *A. fluviatilis area* where spraying was interrupted or partially interrupted, the percentage of fever cases in the age-group 0-4 years formed about 1/7th and 1/6th respectively of the total cases investigated.

BLOOD SMEAR EXAMINATION.

603 blood smears from fever cases were found negative for malaria parasite.

DRUG DISTRIBUTION.

A total of 2,060½ tablets of Resochin and Avlochlor were distributed to the cases investigated.

ENTOMOLOGICAL DATA.

A. culicifacies appeared in low densities during 1956-57 and during 1957-58. There was a decline from 0·006 (1956-57) to 0·004 (1957-58) in human dwellings and mixed dwellings (combined). In cattle-sheds it declined from 0·034 in 1956-57 to 0·004 during 1957-58 (Appendix II).

TABLE V.

Details of active surveillance work during the period October, 1957 to September, 1958 in Krishnarajnagar Taluk, Mysore District.

A. culicifacies area where frequency of spraying has been reduced to one round since June, 1956—Spraying interrupted since October, 1958. (Population : 0·1 million.)

Month and year.	Population surveillanced.	Number of surveillance workers :		Particulars :					
		Health Inspectors.	Malaria surveillance workers.	Cases investigated.	Blood smears collected.	Result of Blood smears.		Anti-malaria pills issued.	Number of cases investigated per 1,000 population.
						Positive.	Negative.		
Oct. 1957	72,342	7	..	75	75	..	75	243	1·03
Nov. 1957	1,10,418	8	1	43	42	..	42	127	0·39
Dec. 1957	96,558	8	..	82	68	..	68	286	0·84
Jan. 1958	79,258	8	1	72	68	..	68	194½	0·90
Feb. 1958	74,166	6	2	92	74	..	74	310	1·23
Mar. 1958	1,12,539	4	3	25	25	..	25	80	0·22
Apr. 1958	1,04,260	4	3	59	57	..	57	182	0·44
May 1958	95,860	5	2	61	59	..	59	188	0·63
Jun. 1958	81,658	4	2	31	31	..	31	105	0·37
Jul. 1958	1,04,713	6	2	38	36	..	36	129	0·35
Aug. 1958	56,950	3	2	35	33	..	33	117	0·61
Sep. 1958	59,782	3	2	28	27	..	27	90	0·54
Totals	10,39,510	66	20	641	603	..	603	2,060½	0·67

TABLE VI.

Analysis of fever cases—age and sex-wise.

Age-group. (years)	Total number of fever cases.	Male.	Female.	Percentage of total cases.	Percentage of population under each group.
0—4	96	58	38	17·0	13·5
5—14	123	72	51	21·7	24·8
15 and above	347	187	160	61·3	61·7
Total	566	317	249	..	..

PART IV.

MALARIA SURVEILLANCE IN NORTH KANARA DISTRICT.

After completion of spraying for the tenth year in succession, D.D.T. spraying was interrupted in North Kanara District from June, 1956. An organisation was simultaneously started to study the after-effects of interruption of spraying. From August, 1956, a comprehensive surveillance programme was taken up in the villages of North Kanara District. Surveillance work was to be restricted to the malaria transmission season. It was contemplated to work 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 from December to June next. Work turned out in the rice-tracts up to October, 1956, has already been described by Patel *et al.* (1957 *loc. cit.*) and the works turned out in the garden tracts and rice-tracts from December, 1956 up to November, 1957 have been presented by Shama Sastry and Narayana Iengar (1959 *loc. cit.*).

(A) MALARIA SURVEILLANCE IN GARDEN TRACTS.

The garden tracts comprise a population of 0·27 million in Sirsi, Yellapur, Supa and Siddapur Taluks of North Kanara East Division and Karwar, Ankola, Kumta, Honnavar and Bhatkalpeta of Kanara West Division. The malaria surveillance work, which was carried out in the garden tracts from December, 1956 to May, 1957 during the previous year, was resumed during December, 1957 and concluded in May, 1958. Like the previous year, the entire population in the garden tracts excluding that under-spraying, and that of certain towns viz , Ankola, Kumta, Gokarna, Honnavar and Bhatkal, was surveillanced. In addition to the available staff, the malaria staff from adjacent districts was also deputed. Boats were hired for the purpose of malaria surveillance in riverain villages in Honnavar and Bhatkal taluks. Particulars in regard to the number of villages surveillanced and total population allotted to each team are contained in Table VII.

In addition to the staff detailed in Table VII, 4 Insect Collectors were posted to each division for routine fortnightly mosquito collection work, each working in 10 villages.

As usual, D.D.T. spraying with 112·0 mg. dose per sq. ft. was carried out once in the hitherto unsprayed hypo-endemic 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 0·092 million so as to create a "Cordon Sanitaire". The details of malaria surveillance work from December, 1957 to May, 1958 are presented in Table VIII.

FEVER MORBIDITY.

A total of 3,393 fever cases were investigated in 5,14,340 houses visited. The number of fever cases per 1,000 population was 1·31 as against the average of 1·26 during the previous year. Analysis of 3,385 fever cases, is depicted in Table IX. Details in respect of 8 cases were not readily available.

TABLE VII.

Number of villages surveillanced, and total population allotted to each team.

Serial number.	Taluk.	Number of malaria surveillance workers allotted.	Number of villages.	Population.
1	Sirsi	3	146	28,661
2	Siddapur	4	195	43,271
3	Yellapur	3	125	18,548
4	Supa	1	59	6,429
Total for Kanara East Division		11	525	95,909
5	Karwar	3	34	49,758
6	Ankola	4	58	41,636
7	Kumta	3	71	31,038
8	Honnavar	3	53	30,486
9	Bhatkal	3	58	30,243
Total for Kanara West Division		16	274	1,89,161

TABLE VIII.

Details of active malaria surveillance work carried out in garden tracts of North Kanara district for the period December, 1957 to May, 1958.

Total population under surveillance in garden tracts = 0·27 million.

Serial number.	Month and year.	Number of surveillance workers.	Number of village visits.	Number of house visits.	Number of fever cases investigated.	Number of fever cases per 200 houses (per 1000 population).	Number of blood smears taken.	Result of blood smears examined.	Number of anti-malaria tablets distributed.
1	Dec. 1957	20	916	54,653	583	2·13	577	1 Pos.	2,193
2	Jan. 1958	26	1,069	79,855	632	1·58	631	2 Pos.	1,828½
3	Feb. 1958	27	1,113	88,681	566	1·27	565	Neg.	1,751½
4	Mar. 1958	26	1,140	89,508	587	1·31	584	2 Pos.	1,819½
5	Apr. 1958	27	1,139	1,02,608	467	0·91	467	Neg.	1,335½
6	May 1958	27	1,336	99,035	558	1·12	556	1 Pos.	1,629½
Total		153	6,973	5,14,340	3,393	1·31	3,380	6 Pos.	10,557½

The number of fever cases in the age-group 0-4 years formed 17·9 per cent of the total cases (Table IX) as against 18·25 per cent during the preceding year. The incidence of fever cases among females was slightly less than males.

TABLE IX.

Age and sex-wise analysis of fever cases (Garden tract).

Age-group. (years)	Male.	Female.	Total number of fever cases.	Percentage of total.	Percentage of population under each age-group.
0— 4	309	297	606	17·9	13·5
5—14	493	409	902	26·6	24·8
15 and above	1,074	803	1,877	55·5	61·7
Total	1,875	1,509	3,385		

BLOOD SMEAR EXAMINATION.

Out of the total number of 3,385 blood smears examined, 6 were positive, 4 for *P. vivax*, 1 for *P. falciparum* and 1 for mixed infection (*P. vivax* plus *P. falciparum* plus *P. malaria*). Appendix I furnishes details of investigation conducted in respect of positive cases. Five of the six positive cases were treated with 8-aminoquinolines and the sixth case could not be treated as the patient returned to Hyderabad State. Out of the six positive cases encountered, 3 would appear to have been imported, one from Hyderabad, one from Bombay and the third from Kerala State.

DRUG DISTRIBUTION.

A total of 10,557½ tablets of Avlochlor and Resochin were distributed to fever cases (Table VIII).

ENTOMOLOGICAL DATA.

Entomological collections were analysed from representative villages in both rice tracts and garden tracts of the district. The entomological data for the calendar year 1955 and onwards up to May, 1958 are presented in Appendix II-A. One specimen of *A. fluviatilis* in human dwelling (mixed dwelling) and 22 *A. fluviatilis* in cattle-sheds were collected during the period from December, 1957 to May, 1958, yielding a density of 0·0003 and 0·021, respectively, per man-hour.

(B) MALARIA SURVEILLANCE IN RICE TRACTS.

The rice tract of North Kanara district covers the Taluks of Sisri (part), Mundagod, Halliyal, and Yellapur (2 villages) of Kanara East Division comprising a total population of 0·08 million. After the conclusion of surveillance work in the garden tract at the end of May, 1958, surveillance work in the rice tract was resumed from June, 1958 with 2 Malaria Supervisors of Kanara East Division and 8 Havildars* who are trained in blood smear collection and malaria case finding work. The rural

* Havildar is a superior field worker who supervises the work of spraying squads.

population of 0·05 million was assigned to 7 Havildars and the urban population of 0·02 million and 2 villages with a population of 1,000 were entrustsd to the two Malaria Supervisors. On Havildar was kept as reserve to work as a substitute in case of necessity. Since, from a review of the work done previously, it was seen that the staff were not able to visit the population twice during the month, the pattern of surveillance work was changed as follows :—

(*a*) One visit to each one of the houses in the area during the month.

(*b*) Transport was not provided to the surveillance worker. The Havildars resided in the centrally situated Head-quarter villages and covered the villages on foot or bicycle.

(*c*) The staff posted for surveillance work belonged to malaria organisation.

Details in regard to the number of villages, population and surveillance workers in each Taluk are given in Table X.

TABLE X.

Number of villages surveillanced, and total population allotted to each team.

Serial number.	Taluk.	Number of Malaria surveillance workers allotted.	Number of villages allotted.	Population alloted.
1	Sirsi	3	99	29,011
2	Mundagod	2	68	15,871
3	Halliyal	3	79	36,002
4	Yellapur	1 (Part-time)	2	1,006
	Total:	9	248	81,890

Table XI furnishes the details of malaria surveillance work carried out from June to November, 1958.

FEVER MORBIDITY.

A total of 1,591 fever cases were investigated in 97,588 house visits. The number of fever cases per 1,000 population (per 200 houses) was 3·26 as against the average of 2·9 during the previous year.

Analysis of the 1,591 fever cases investigated is depicted in Table XII. It is evident from Table XII that the number of fever cases in the age-group 0—4 years formed 1/7th of the total and the incidence of fever was comparatively lower among females.

BLOOD SMEAR EXAMINATION.

None of the 1,591 blood smears examined was found positive as against 3 positives among 1,527 blood smears examined during the previous year.

DRUG DISTRIBUTION.

A total of 4,701½ anti-malaria tablets (Resochin and Avlochlor) were distributed to the fever cases investigated.

TABLE XI.

Details of active malaria surveillance work carried out in rice tracts of North Kanara district for the period June, 1958 to November, 1958.

A. fluviatilis area—Spraying interrupted since June, 1956.

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

Serial number.	Month and year.	Number of surveillance workers.	Number of house visits.	Population surveillanced.	Number of fever cases investigated.	Average number of fever cases per 200 houses (per 1000 population).	Number of blood smears taken.	Results of blood smears examined.	Number of anti-malaria tablets issued.
1	Jun. 1958	9	16,401	82,006	200	2·4	200	Neg.	552
2	Jul. 1958	9	16,279	81,393	238	2·9	238	Neg.	628½
3	Aug. 1958	9	16,395	81,976	297	3·62	297	Neg.	870½
4	Sep. 1958	9	15,915	79,575	288	3·61	288	Neg.	918½
5	Oct. 1958	9	16,299	81,494	298	3·6	298	Neg.	899
6	Nov. 1958	10	16,299	81,494	270	3·3	270	Neg.	833
	Total	55	97,588	4,87,938	1,591	3·26	1,591	Neg.	4,701½

TABLE XII.

Analysis of fever cases investigated—age and sex-wise (Rice tracts).

Age-group. (Years)	Male.	Female.	Total number of fever cases investigated.	Percentage of total.	Percentage of population under each age-group.
0—4	121	108	229	14·4	13·5
5—14	291	199	490	30·8	24·8
15 and above	564	308	872	54·8	61·7
Total	976	615	1,591		

ENTOMOLOGICAL DATA (Appendix II-A).

During the period June to September, 1958, six *A. fluviatilis* were collected in human dwellings and mixed dwellings, and 27 *A. fluviatilis* in cattle-sheds. (Appendix II-A). Precipitin tests have not been done so far, and arrangements are being made to have analysis of blood meals carried out in future.

PART V.

MALARIA SURVEILLANCE WORK IN COORG DISTRICT.

(*A. fluviatilis* area—Spraying interrupted—Population 0·2 millions).

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

health work has been integrated with malaria work. There are 8 sanctioned posts of Health Inspectors and 8 posts of Health Sub-Inspectors each of whom is assigned a population of 10,000 on an average and is required to visit the assigned population once during the month in 20 work days. A population of 30,000 concentrated around the dispensaries and hospitals, is excluded from malaria surveillance work. Eight additional surveillance workers were appointed during July, 1958 and they started working from August, 1958. Table XIV furnishes the details of malaria surveillance work from October, 1957 to September, 1958.

FEVER MORBIDITY.

A total of 2,606 fever cases were investigated during the period. The average number of fever cases per 1,000 population varied from 1·1 to 3·08, during the different months. The incidence of fever cases was maximum during the month of September, 1958.

The number of fever cases in the age-group 0-4 years, formed about 1/8th of the total and the incidence of fever is slightly lower among females than males as in the case of other areas (Table XIII).

TABLE XIII.

Analysis of fever cases investigated—age and sex-wise.

Age-group. (Years)	Male.	Female.	Total fever cases investigated.	Percentage of total.	Percentage of population under each age-group.
0—4	172	158	330	13·09	13·5
5—14	347	246	593	23·52	24·8
15 and above	953	645	1,598	63·39	61·7
Total	1,471	1,049	2,521	Analysis of 85 cases is not available	

DRUG DISTRIBUTION.

A total of 7,776 anti-malaria tablets (Resochin and Avlochlor) were distributed to fever cases investigated.

BLOOD SMEAR EXAMINATION.

Out of 2,070 blood smears examined, 11 were found positive, 4 for *P. falciparum*, 3 for *P. vivax*, 1 for mixed infection with *P. vivax* and *P. falciparum* and 3 for rings only.

Apart from these, 3 blood smears were found positive, two for *P. vivax* and 1 for *P. falciparum* among 12 child blood smears collected at random. The details of blood smears are furnished in Appendix I. All these positive cases were found in Yerwars (nomadic tribal people, without a permanent abode) who had come to live in the villages and forest labour camps.

ENTOMOLOGICAL DATA (Appendix II-B).

Collection of *A. fluviatilis* were negligible during the year 1958-59. It appeared for the first time one in August, 1958 and one in September, 1958. In special collections made in September, 1958, by the Malaria Investigation Centre, 2 *A. fluviatilis* were collected in 3½ hours.

TABLE XIV.

Details of active surveillance work during the period October, 1957 to September, 1958.

Coorg District—*A. fluviatilis* area—Spraying interrupted since June, 1957.

Population : 0·2 million.

Month and year.	Population surveillanced.	Number of surveillance workers :		Particulars :						Remarks.
		Health Inspectors.	Malaria Surveillance workers	Cases investigated.	Blood smears collected.	Result of blood smears. Positive.	Result of blood smears. Negative.	Anti-malaria pills issued.	Number of cases investigated per 1,000 population.	
Oct. 1957	74,587	11	..	83	70	..	70	224½	1·1	
Nov. 1957	86,386	13	..	143	117	..	117	444	1·65	
Dec. 1957	98,984	13	..	230	186	..	186	659	2·31	
Jan. 1958	1,00,591	13	..	282	190	..	190	735	2·79	
Feb. 1958	95,867	14	..	255	164	..	164	665	2·59	
Mar. 1958	60,505	11	..	113	113	..	113	377	1·83	
Apr. 1958	83,263	14	..	187	128	..	128	425½	2·24	
May 1958	1,04,758	14	..	220	220	2	218	657	2·05	1 *P. vivax*, 1 *P. falciparum*.
Jun. 1958	1,05,196	14	..	172	160	..	160	558	1·63	
Jul. 1958	1,17,061	14	..	207	195	4	191	695½	1·72	1 *P. falciparum*, 2 *P. vivax*, 1 mixed (*vivax*+*falciparum*)
Aug. 1958	1,11,534	14	3	235	199	2	197	752	2·16	1 *P. falciparum*, 1 ring only (species?)
Sep. 1958	1,55,385	13	7	479	328	3+3*	325	1,583½	3·08	2 *P. falciparum*, 2 *P. vivax*, 2 rings only.
Totals	11,94,117	158	10	2,606	2,070	11+3	2,059	7,776	2·18	

*Of these 3 cases, 1 *P. falciparum* and 2 *P. vivax* were from random smears—afebrile cases.

PART VI.

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

The South Kanara District Malaria Scheme area, Puttur, comprises a population of 0·23 million living in the foot-hill areas and adjacent plains on the western slopes of the western ghats. This is *A. fluviatilis* and *A. culicifacies* area with transmission season extending from December to May.

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 a whole time Malaria Health Officer. Each one of the Field Assistants was assigned a population of 10,000. He visited each house once a month. No transport was provided. Out of the 26 Field Assistants, 23 were detailed for surveillance work and 3 for entomological work. The work of 4 Field Assistants was supervised by one Health Inspector and the entomological work of Field Assistants was supervised by Assistant Entomologist by actual random test collections in the villages. The blood smears were examined at the laboratory attached to the Health Office, Puttur, and test-checks made at the Central Laboratory, Bangalore. The details of malaria surveillance work in Puttur area are contained in Table XV.

FEVER MORBIDITY.

A total of 5,157 fever cases were investigated. The average number of cases per 1,000 population ranged from 1·22 to 3·17 during the different months. The incidence of fever cases was maximum during the month of October, 1957.

Analysis of the fever cases investigated, is depicted in Table XVI. Analysis of 762 cases was not available.

The number of fever cases in the age-group 0—4 years, formed about 1/11th of the total and incidence of fever was lower among females than males.

BLOOD SMEAR EXAMINATION.

Out of 4,993 blood smears examined, only one was found positive for *P. vivax*. The positive case had an enlarged spleen (4th to 5th size) and gave history of fever for several years. Subsequent examinations, at repeated short intervals up to 16 weeks, were negative.

DRUG DISTRIBUTION.

A total of 15,908½ anti-malaria tablets (Resochin and Avlochlor) were distributed to the cases investigated.

ENTOMOLOGICAL DATA.

These are presented in Appendix II-B. No specimen of *A. fluviatilis* was encountered during the year. Ten specimens of *A. culicifacies* in human dwellings

(mixed dwellings) and 25 *A. culicifacies* in cattle-sheds were collected. The per man-hour density for the different types of shelters worked out to 0·02 and 0·11.

TABLE XV.

Details of active surveillance work during the period October, 1957 to September, 1958.

South Kanara District—District Malaria Scheme Area :—*A. fluviatilis* and *A. culicifacies* area where spraying has been interrupted since June, 1957.

(Population : 0·23 millions.)

Month and year.	Population surveillanced.	Number of surveillance workers:		Particulars:					
		Health Inspectors.	Malaria Surveillance workers.	Cases investigated.	Blood smears collected.	Results of Blood smears: Positive.	Results of Blood smears: Negative.	Anti-malaria pills issued.	Number of cases investigated per 1,000 population.
Oct. 1957	2,33,702	..	23	757	743	1*	742	2,836	3·17
Nov. 1957	2,14,946	..	22	485	481	..	481	1,249½	2·25
Dec. 1957	2,24,343	..	23	478	468	..	468	1,227½	2·12
Jan. 1958	2,24,343	..	23	353	345	..	345	1,022½	1·56
Feb. 1958	2,21,456	..	23	348	282	..	282	1,031	1·52
Mar. 1958	2,24,343	..	23	293	293	..	293	871	1·29
Apr. 1958	2,24,343	..	23	375	372	..	372	980	1·22
May 1958	2,17,715	..	23	393	378	..	378	1,212	1·78
Jun. 1958	2,24,343	..	23	402	380	..	380	1,313	2·46
Jul. 1958	2,24,343	..	23	418	407	..	407	1,417½	1·55
Aug. 1958	2,24,343	..	23	440	433	..	433	1,453½	1·95
Sep. 1958	2,24,343	..	23	415	411	..	411	1,295	1·84
Totals	26,82,562	..	275	5,157	4,993	1*	4,992	15,908½	1·92

* *P. vivax.*

TABLE XVI.

Analysis of fever cases investigated—age and sex-wise.

Age-group. (Years)	Male.	Female.	Total fever cases investigated.	Percentage of total.	Percentage of population under each age-group (1951 census).
0—4	200	178	378	8·6	13·5
5—14	891	514	1,405	31·9	24·8
15 and above	1,748	864	2,612	59·5	61·7
Total	2,839	1,556	4,395		

DISCUSSION AND CONCLUSION.

Active malaria surveillance was continued in several areas of the Mysore State on the same pattern as described by Shama Sastry and Narayana Iyengar (1959 *loc. cit.*). In rice tracts of North Kanara District, however, the pattern of surveillance has been changed over to one visit during the month since June, 1958. The previous house visits which were restricted to malaria transmission season (June-November in the rice tracts and December-May in the garden tracts) were converted to one of monthly visits throughout the year.

In 8 taluks, namely, Thirthahalli, Hosanagar, Sagar, Sorab and Shikaripur (Shimoga District), Koppa and Sringeri (Chickmagalur District) and Sakalespur (Hassan District), where spraying has been completely withdrawn, surveillance work was carried out through fortnightly visits to every house in the area. General public health work and malaria surveillance work are integrated in this area.

In Mudigere and Belur Taluks of Chickmagalur and Hassan Districts, respectively, where spraying rounds were reduced to one round in the year, fortnightly visits were paid by the staff, and the public health work and malaria surveillance work are integrated in this area.

In Krishnarajnagar Taluk of Mysore District where one round of spraying is done annually and in Coorg District where spraying has been suspended, general public health work and malaria surveillance work are integrated and house visits paid once a month.

In the District Malaria Scheme area of South Kanara District, where the surveillance staff belonged exclusively to malaria organisation, house visits were paid once a month.

In the garden tracts of North Kanara District, fortnightly visits were paid to the houses by the staff (both of general public health and malaria) deputed for the purposes.

In the rice tracts of North Kanara District, some modifications were made in the pattern of surveillance, as has been described under Part IV; the important changes being that the staff belonged to malaria organisation purely; no transport facility was given to them for surveillance work and monthly house visits were paid instead of fortnightly visits during the earlier periods.

FEVER MORBIDITY.

In the *A. fluviatilis* area where spraying has been completely interrupted, there is a slight decline in the average fever incidence. The average per 1,000 population ranges from 1·66 to 3·23 as against 0·97 to 3·59 during the preceding year.

The epidemiological surveillance data from the different areas is summarised in Appendix III. It is observed that the performance of the surveillance staff has improved over the previous years. A certain proportion of the population still remained uncovered during some of the visits. The reasons for such deficiencies appear to be mainly administrative and are being attended to.

There has been an increase in the number of fever cases recorded, both absolute and per 1,000 population surveillanced, indicating a better efficiency in the performance of the teams.

POSITIVE BLOOD SMEARS.

The positive blood smears encountered in the several areas were investigated and the findings are depicted in Table XVII.

TABLE XVII.

Area.	Number of positive blood smears : During the year under report.	During the previous year.
Part I.—*A. fluviatilis* area where spraying is completely withdrawn	*3 *P. vivax*+1 *P. falciparum*=6. 2 imported+3 with history of movement to unsprayed areas of South Kanara District.	†4 *P. vivax*+2 *P. falciparum* = 6.
Part II.—*A. fluviatilis* area where spraying rounds are reduced to one round in the year.	Nil	Nil
Part III.—*A. culicifacies* area where spraying rounds are reduced to one round.	Nil	Nil
Part IV.—(*a*) North Kanara Garden tract.	4 *P. vivax*+1 *P. falciparum*+1 (triple)=6 (3 imported).	2 *P. vivax*+1 *P. falciparum*=3.
(*b*) North Kanara Rice tract	Nil	2 *P. vivax*+1 (*P. vivax*+*P. falciparum*) =3. Imported.
Part V.—Coorg District	5 *P. vivax*+5 *P. falciparum*+1 (*P. vivax*+*P. falciparum*)+3 rings (species ?) =14.	Nil. (Only 3 months of surveillance work)
Part VI.—District Malaria Scheme, Puttur	1 *P. vivax* Chronic case with 4 to 5 size spleen encountered in October, 1957, with history of relapses for several years.	Surveillance was commenced just a month prior to the year under report.

*Only 3 positives were encountered during fever surveillance.
†Only 4 were encountered during the fever surveillance.

It is seen from the above data (Table XVII) that inspite of increased vigilance under surveillance programme, the number of positives encountered during the year under report has not increased over that of the previous year except in Coorg District where 14 positives have occurred in a localised area. The investigation of these cases will be communicated elsewhere separately. In all the other areas, positive cases tend to dwindle gradually.

In Coorg District, so far as is known, the positive cases are confined to Srimangala and Balale Divisions and they are occurring among Yerwars (tribal population) and other forest labour population who have a nomadic life. It is, therefore, likely that these positives have contracted infection during their stay in several abodes, temporarily erected, and had escaped spraying. As their stay amidst a population, where spraying has been interrupted, would be dangerous and as it is not possible to protect these wandering people effectively, strict watch over their movement into the area of surveillance has been kept. Further, mass blood surveys among Yerwars are being carried out. The positive cases are treated with 8 aminoquinolines.

The analysis of fever cases, age and sex wise, shows that the percentage of fever cases, as compared to the population in different age-groups, is more in the age-group 0—4 in the areas described under Parts I, III and IV. The percentage of fever cases is more in the age-group 5—14 in the District Malaria Scheme area, Puttur, (Part VI) and the percentage of fever cases is more in the age-group "15 and above" in the areas described under Parts II and V. The incidence of fever cases is less among females in all the areas.

A total of 81,226½ anti-malaria tablets (4 amino-quinolines) were distributed in a population of 14,12,019 as against 84,721 tablets estimated at the rate of 60,000 tablets per million population.

SUMMARY.

The surveillance programme already described was continued during the second year (October, 1957 to September, 1958). 25,855 fever cases were encountered in all the areas out of which 21 cases were found microscopically positive. In addition, 6 parasite positive cases were encountered in the study area during other surveys. Epidemiological investigation of these cases showed that 8 were imported, 4 were sporadic, and in respect of one full investigation could not be done. The remaining 14 cases were among a particular section of the population (Yerwars—tribal population) living in the interior forest areas confined to south-eastern portion of Coorg District. The investigation of these cases will be communicated elsewhere.

The observations of pilot study are being continued. The organisation of surveillance has been reviewed and the lacunae has been discussed. The attempts to overcome the lacunae have been mentioned.

ACKNOWLEDGEMENT.

The author wishes to express his thanks to all the Officers and Officials in the respective Districts who have actively participated in the pilot studies and to Sri K. Sitaramiah, Malaria Supervisor, Bureau of Malariology, for his assistance. The valuable criticisms offered by the Director, National Malaria Eradication Programme, Delhi, are gratefully acknowledged.

Thanks are also due to the Director of Public Health, Mysore State, for permitting to publish this paper.

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Indian Journal of Malariology, **14**, *1*, *March 1960*.

PYRALIS PICTALIS CURT. (PYRALIDAE : LEPIDOPTERA) LARVAE AS PREDATORS OF EGGS OF BED BUG, *CIMEX HEMIPTERUS* FAB. (CIMICIDAE : HEMIPTERA).

BY

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AND

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[February 25, 1960.]

INTRODUCTION.

DESPITE the large scale use of residual insecticides for interruption of malaria transmission under the National Malaria Eradication Programme in India, the existence of large populations of bed bugs is being reported from certain parts of the country. In fact, the impression would appear to be that the populations of bed bugs, which were considerably reduced following the use of residual insecticides under the said programme, have now increased over the populations of this insect prevalent in the pre-D.D.T. era. Such impressions are particularly common in the humid and warm districts of Bombay and Orissa States. Rao and Halgeri (1956) studied bed bugs in Kolaba District of Bombay State and listed the following among the possible causes for the presence of bed bug populations in that area :—

(*a*) Development of resistance to D.D.T. ;

(*b*) Change in behaviour of bed bugs resulting in the bugs not coming in adequate contact with treated surfaces ;

(*c*) Upsetting of the environmental factors resulting in the removal of the factors adverse to bed bugs, such as for instance, the destruction of parasites and predators of bed bugs.

The authors dealt with the first factor listed above and experimentally proved that bed bugs in Poona City (Bombay State) had changed in their susceptibility to D.D.T. between 1952 and 1955. While in 1952 they obatined 50 per cent mortality in bed bugs with two minutes' exposure to a 5 per cent D.D.T. dust in Talcum, in 1955 even a ten minutes' exposure to the same concentration of D.D.T. did not result in any mortality in the bed bugs from areas sprayed regularly during the period (1952-1955). They further reported that bed bugs from Chiplun Town, 80 miles away from Poona, which was not sprayed, showed a mortality of 66·6 per cent when exposed for ten minutes to 5 per cent D.D.T. dust.

Although the first hypothesis put forth by Rao and Halgeri (1956 *loc. cit.*) stands proven, both this and their second hypothesis of a change of behaviour alone cannot account for the impression that bed bug populations in some of the sprayed

areas have increased over the populations noticed in the pre-D.D.T. era. The logical inference, therefore, appears to be, either

(i) the lowered susceptibility to D.D.T. has increased the biotic potential of the insect, or

(ii) the application of insecticide has upset the balance of nature (host, parasite, predator ratio).

There is no information on the first factor and this paper deals with observations made on the second factor.

OBSERVATIONS.

Two colonies of bed bug *Cimex hemipterus* Fab. have been maintained at the Malaria Institute of India since 1958. During 1959, with the onset of rainy season which resulted in an increase in atmospheric humidity, it was observed that the colonies, instead of thriving, were decreasing in numbers. A thorough search of the colony cages revealed the presence of lepidopterous larvae in the tunnels of the small cages (to be described separately) as well as in the crevices of the big colony cage. Some of the larvae were reared into moths which were later found to be *Pyralis pictalis* Curt. (Pyralidae : Lepidoptera)*.

The adult moth [Plate I, fig. (*a*)] has the wing length of $\frac{1}{2}$ inch or slightly longer. The base and the apex of the fore-wing are deep-brown while the middle area is light-brown. There are two wavy white lines on either side of the middle light area. The hind wing has similar pattern except for the apical area which has both light and deep-brown scales as shown in the figure.

The larvae [Plate I, fig. (*b*)] were found in silken tubes which they spin around them and were $\frac{3}{4}$ inch in length when full-grown. They are grey in colour except the head and the twelfth segment which are deep brown.

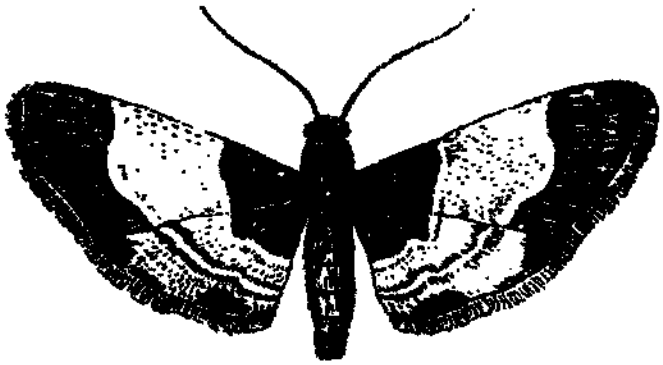
Three of the larvae of the moth were removed from the silken tubes and isolated in a specimen tube (3 × 1 inch) provided with a filter paper base at the closed end and a cotton wool plug in the open end. Three live specimens, each of the first and the second nymphal stages of the bed bugs, were introduced into the specimen tube. The larvae did not eat or injure any of the nymphs provided and died within 48 hours. Subsequently two of the larvae were isolated in the manner mentioned above and confined with 16 eggs of the bed bugs. The larvae were observed to eat the eggs readily. The observations were repeated twice using eggs of bed bugs and the pyralid larvae were seen to eat the same readily.

DISCUSSION.

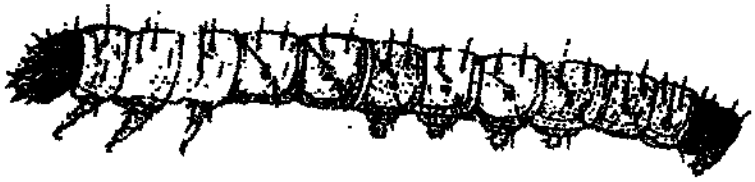
Three species of the genus *Pyralis*, viz., *P. farinalis* Linn; *P. pictalis* Curt. and *P. manihotalis* Guen. are common household pests of a variety of food articles in India. Amongst these *P. farinalis*, the meal snout moth, is well known and is cosmopolitan in distribution. These insects abound in damp places and the larvae

*The identification of the moth was made in the Division of Entomology, Indian Agricultural Research Institute, New Delhi.

PLATE I.



(a)



(b)

Perulis pectinis. (a) adult ; (b) full grown larva.

are known to attack all sorts of grain, particularly when slightly out of condition; potatoes, seeds of mustard, coriander, sago, cumin, haystraw and other roughage, bran, oat meal and flours (Cotton, 1947; Pruthi and Mohan Singh, 1948). None of these species have so far been observed to be predacious on other insects. However, in India the larvae of another pyralid, *Hypsopygia mauritialis*, have been known to feed on the larvae and pupae of the wasp *Polistes hebraeus* (Lefroy and Howlett, 1909).

In rural India the grain and other vegetables are stored in the living room itself or in its immediate vicinity. The fodder for cattle is also stored nearby or in the cattle-sheds that are not far from human dwellings. Therefore, these moths as well as others of the family pyralidae (*Ephestia cautella*, and *Plodia interpunctella*) which are known pests of stored grain and other food articles, are always present in or near the human habitations. It seems likely, in view of the observations reported here, that the larvae of these moths devour a large number of the eggs of bed bugs when the same are available. However, due to residual sprays the moths, which are highly susceptible to contact insecticides, apparently have been considerably reduced in density and any check that was being maintained on the growth of bed bug populations has thereby been eliminated. This phenomenon, in conjunction with the development of resistance in bed bugs to insecticides, seems to account for the large populations of bed bugs now a days noticeable in areas sprayed with the residual insecticides.

As a result of the observations recorded and the comments made earlier in this note, it is proposed to (*a*) study the change, if any, in the biotic potential of the bed bug with the development of resistance to insecticides, and (*b*) try to colonise the different species of pyralid group of grain moths in order to determine the most efficient natural predator of the bed bugs.

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INDIAN COUNCIL OF MEDICAL RESEARCH

Colonel Amir Chand Trust Fund Prizes for Medical Research

Lieut.-Col. Amir Chand, lately Principal of Lady Hardinge Medical College, New Delhi, donated Rs. 50,000.00 to the Indian Council of Medical Research for creating a prize Fund. From the interest earned by this sum, prizes are awarded for the best published research work in Medical Sciences. The Governing Body of the Council has constituted a Trust, called the 'Colonel Amir Chand Trust' for the administration and management of the Fund.

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

It has been decided to award *four prizes in 1960* each of Rs. 300.00 *to graduates of not more than 40 years of age on 1st January, 1960, for the best* research papers in medical sciences published by them during 1959. These prizes will be known as 'Shakuntala Amir Chand Prizes'.

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

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

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

THE AWARD of the prizes will be announced at the meetings of the Council's Advisory Committees in November/December, 1960.

CANDIDATES for award of prizes are required to submit 10 REPRINTS of their papers published during 1959. These should be sent to the DIRECTOR, INDIAN COUNCIL OF MEDICAL RESEARCH P.O. BOX 494, NEW DELHI, so as to reach him NOT LATER THAN 1st SEPTEMBER, 1960. The PAPERS should be accompanied by a short biographical sketch and two copies of passport size photographs of the candidate/candidates concerned.

Indian Journal of Malariology, **14**, 2, *June 1960*.

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

II. THE RÔLE OF WATER HYACINTH (*EICHHORNIA SPECIOSA* KUNTH) AS AN IMPORTANT HOST PLANT IN THE LIFE CYCLE OF *MANSONIA UNIFORMIS* (THEOBALD)

WITH NOTES ON THE DIFFERENTIATION OF THE LATE EMBRYONIC AND NEWLY HATCHED STAGES OF *MANSONIA UNIFORMIS* (THEOBALD) AND *MANSONIA ANNULIFERA* (THEOBALD).

BY

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[*Entomologist (Filariasis), U.S. Technical Co-operation Mission to India.*]

[February 18, 1960.]

In a previous study (Burton, 1959) it was established that water hyacinth (*Eichhornia speciosa* Kunth) was important as a host plant for attachment of larvae and pupae of *Mansonioides* (Fig. 26, 32, and 33), but the full epidemiological significance of this plant had not then been completely determined. After ascertaining in detail the behaviour of *Mansonia uniformis* larvae, investigations were carried out in all available water hyacinth tanks in Ernakulam, Kerala, where many adults of *M. uniformis* had been caught, in order to determine as completely as possible the rôle of water hyacinth as a host plant and its relationship to *Pistia stratiotes* Linn. (water lettuce) and to other tank plants in the bionomics of *M. uniformis*.

The extensive water hyacinth plots in the Karakkamury area, Ward Number 3, of Central Ernakulam are typical of an ecological and epidemiological situation involving *Mansonia uniformis* (Fig. 1), which is a vector of the human filarial parasite, *Wuchereria malayi*. More or less similar situations are scattered between Cochin and Shertallay, Kerala. The following ecological situations are apparent in the Karakkamury area :

1. *Eichhornia* alone, either in a tank or as a separate group with no other plant nearby (Fig. 1 and 5).
2. *Eichhornia* stands with no other interspersed plant, but with *Pistia stratiotes* adjacent (Fig. 4).
3. *Eichhornia* with interspersed *Lemna polyrrhiza* (Fig. 17).
4. *Pistia* tank with small groups of *Eichhornia* here and there (Fig. 3).
5. *Pistia* tank with no *Eichhornia* or *Lemna* (Fig. 6).

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

The discovery of tremendous numbers of *M. uniformis* larvae in the Karakkamury area led to a detailed investigation of the water hyacinth stands in October and November, 1959, the months during which very large numbers of *M. uniformis* adults appear in Ernakulam. It was known by then that *M. uniformis* larvae had certain habits of behaviour, i.e., very tenacious attachments among the mud-encrusted roots of water hyacinth (Fig. 32 and 33), and resting or swimming for long periods of time in the bottom mud (Fig. 22 and 23). Using the previously ascertained technique of great dilution of the black, muddy water, or else examining a little water at a time, necessitating considerable time and patience, thousands of *M. uniformis* larvae were found. Considerable washing of the mud-covered roots revealed the tightly attached larvae and pupae. Even after very thorough swirling of the roots in water some larvae remained tightly attached, particularly those which were soon due to change into pupae. It is important to note that water hyacinth usually has its roots embedded in the bottom mud, whereas *Pistia* is primarily a floating plant.

Where water hyacinth is concerned, the egg-laying preferences of *M. uniformis* appear to be in accordance with the age and height of the plants, as well as the presence of other plants such as *Pistia* and *Lemna* (in this case *L. polyrrhiza* Linn.). After searching for egg masses on thousands of *Pistia* and *Lemna* leaves adjacent to water hyacinth, the following conclusions were drawn :

1. Where all the water hyacinth is tall so that no leaves touch the water (Fig. 2), *M. uniformis* lays its eggs preferably on nearby *Pistia* (Fig. 17, 18 and 19), or on *Lemna* if there is no *Pistia* present (Fig. 15, 16 and 17). When the eggs hatch, whether on *Pistia* or *Lemna*, the larvae instinctively swim over to the water hyacinth roots nearby for attachment, by preference, but some attachments also take place to roots of *Pistia* (Fig. 24).

2. Where water hyacinth plants are small, so that the bottom leaves rest on or in the water (Fig. 7 and 8), *M. uniformis* lays its eggs on such leaves if there is no *Pistia* nearby (Fig. 9 to 14). Even where there is *Pistia* nearby, some of the water hyacinth leaves touching the water will often show egg masses.

3. Where low water hyacinth plants are associated with *Lemna* only, without *Pistia* or any other plant being present, *M. uniformis* appears to prefer laying its eggs on low water hyacinth leaves lying in the water, but egg masses will also be found on *Lemna*. Perhaps this has something to do with the denser texture or size of the water hyacinth leaves, or the better support which they provide for resting of the female during egg laying.

4. Where tall water hyacinth plants are isolated, with no other plant present, and no leaves touching the water, in some cases larvae and pupae of

PLATE I.

FIG. 1. Large water hyacinth stands in Karakkamoola area of Central Ernakulam, Cochin, Kerala State, South India.

FIG. 2. Tall water hyacinth (*Eichhornia crassipes*) plants adjacent to water lettuce (*Pistia stratiotes*) plants.

PLATE II.

FIG. 3. *Pistia* tank having scattered groups of water hyacinth.

FIG. 4. *Eichhornia* plants surrounded by *Pistia* plants.

PLATE III.

FIG. 5. Water hyacinth tank (*Eichhornia speciosa*).

FIG. 6. Water lettuce tank (*Pistia stratiotes*).

PLATE IV.

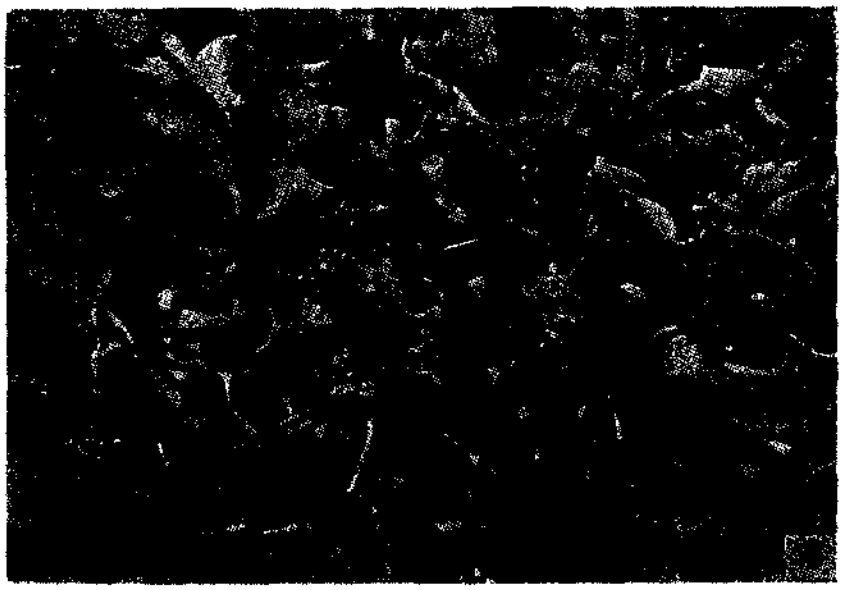


FIG. 7. Low water hyacinth plants in foreground, tall plants in background

Fig. 8. Low water hyacinth plants with leaves touching the water, conducive to egg mass laying by *M. uniformis*.

Plate V.

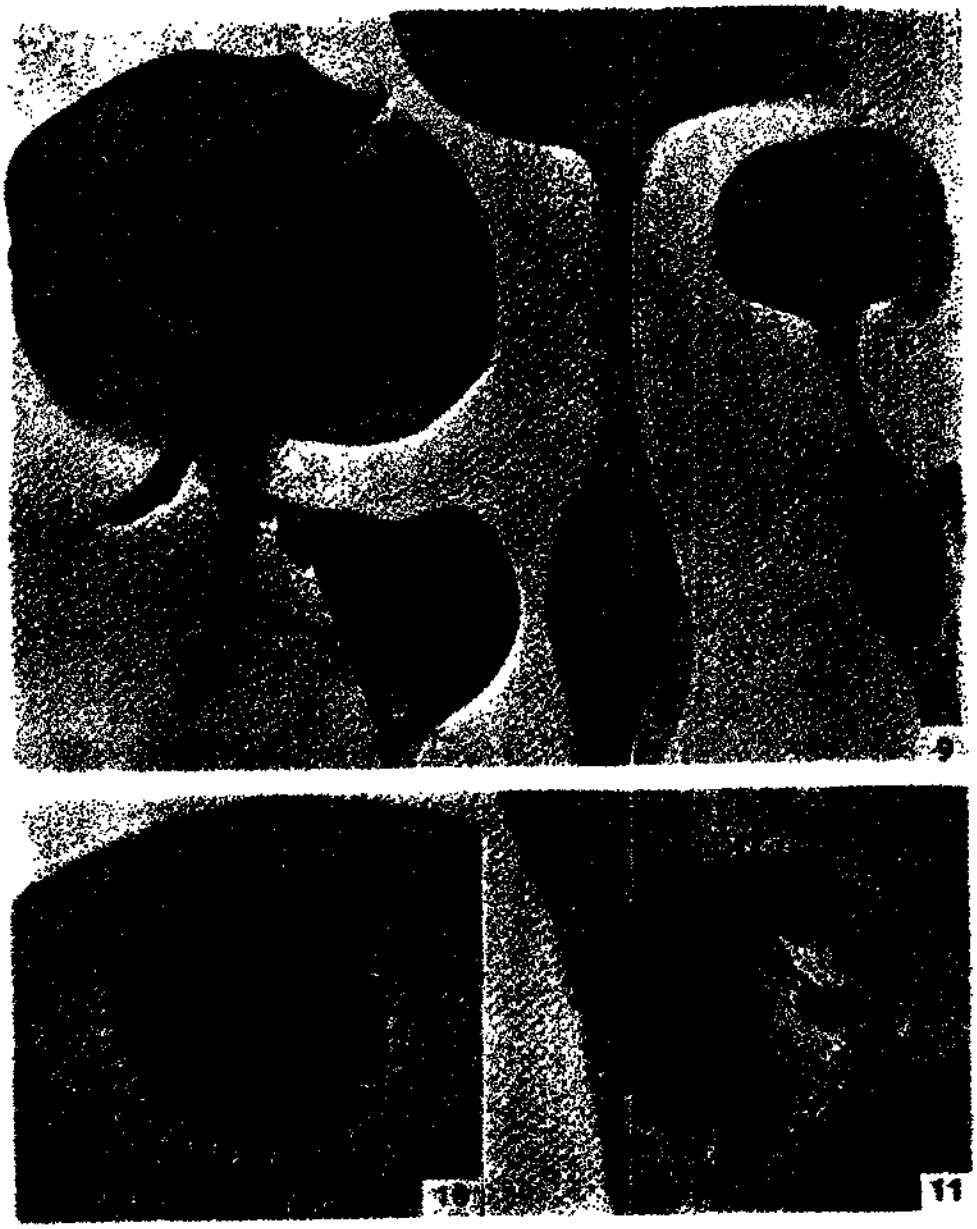
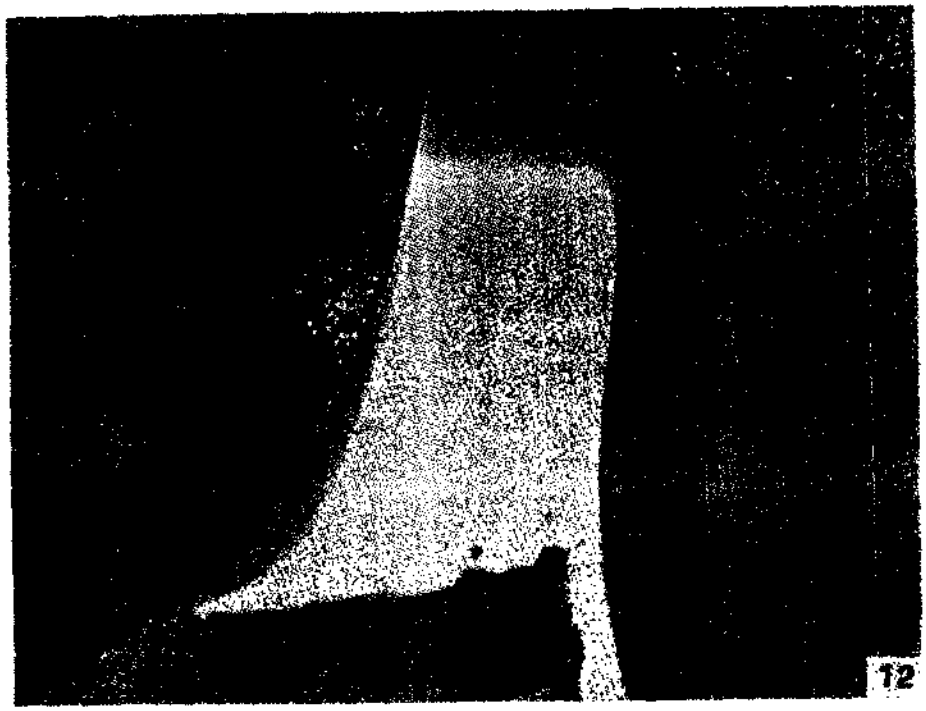


Fig. 9. [illegible]

Fig. 10. Magnified *M. uniformis* egg mass on water lily [illegible]

Fig. 11. Magnified egg mass of *M. uniformis* [illegible]

PLATE VI.



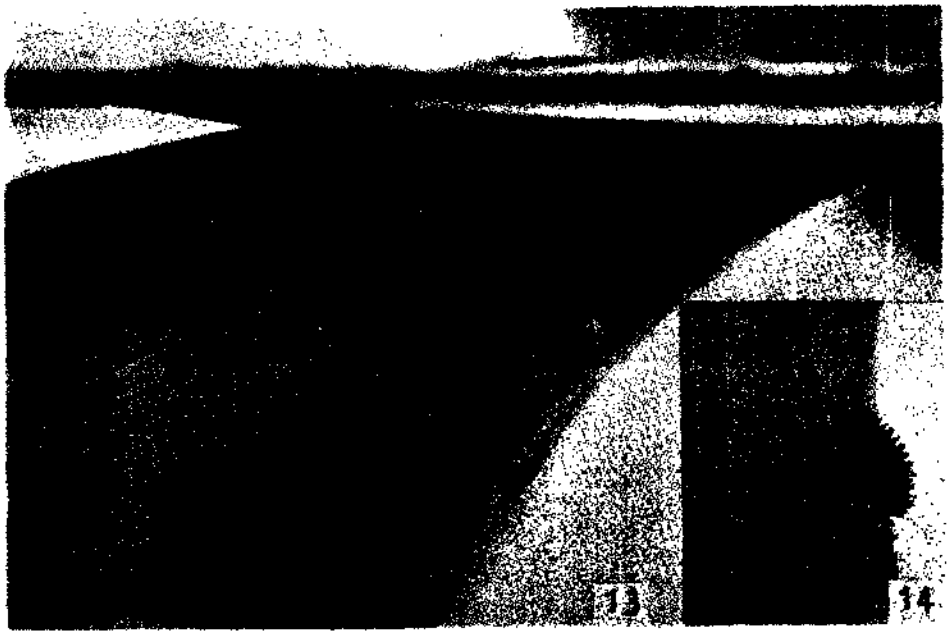


FIG. 12. Close up of *M. uniformis* egg masses on water hyacinth leaves.

FIG. 13. Egg mass of *M. uniformis* laid on undersurface of water hyacinth leaf just below water surface.

FIG. 14. Lateral aspect of *M. uniformis* egg mass.

PLATE VII

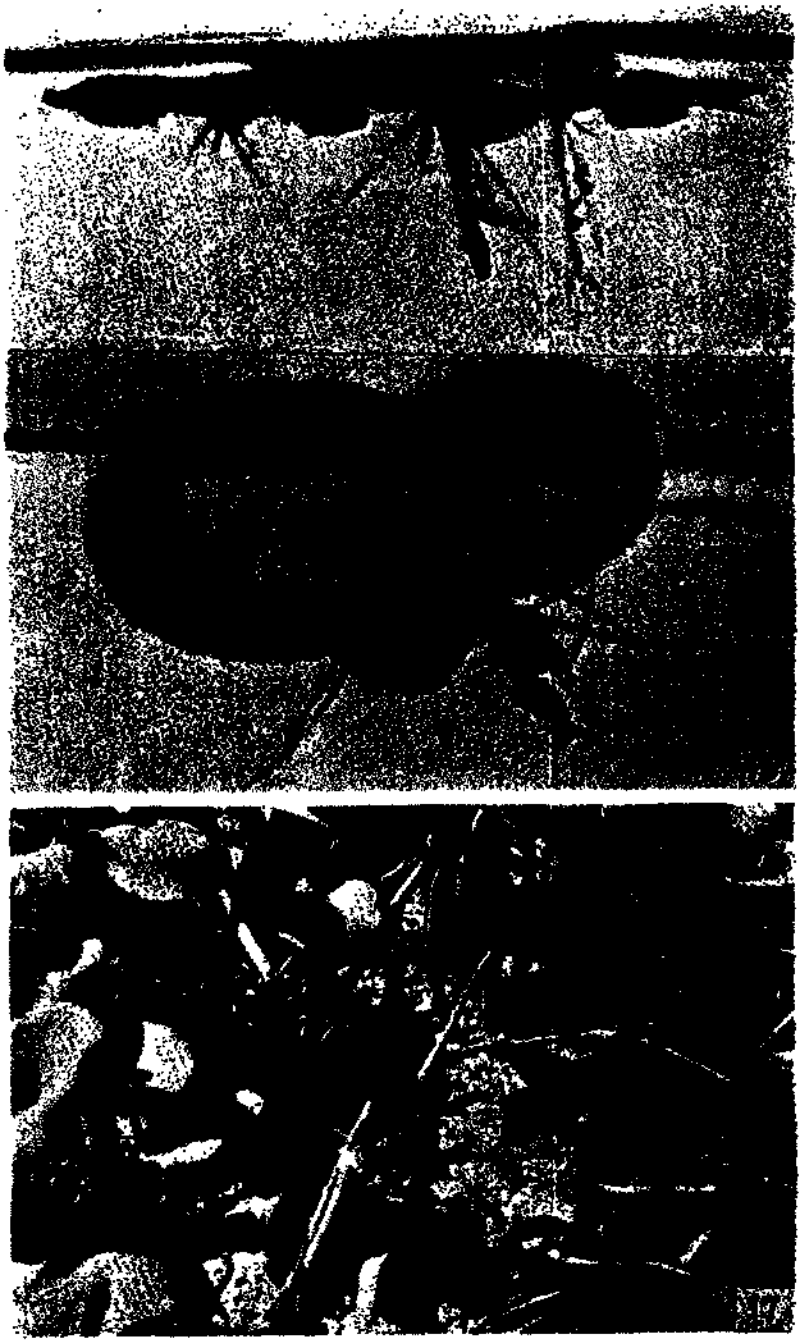


FIG. 15. *M. uniformis* egg masses laid on *Lemna polyrrhiza* blades.
FIG. 16. Two irregular egg masses of *M. uniformis* on *Lemna polyrrhiza*.
FIG. 17. Association of *Lemna polyrrhiza* with *Eichhornia speciosa*.

PLATE VIII.

FIG. 18. Four *M. uniformis* egg masses on *Pistia* leaves.

FIG. 19. *M. uniformis* egg masses on *Pistia* leaves. Note at left one mass on the upper surface of the leaf and another mass on the under surface of the same leaf.

PLATE IX.

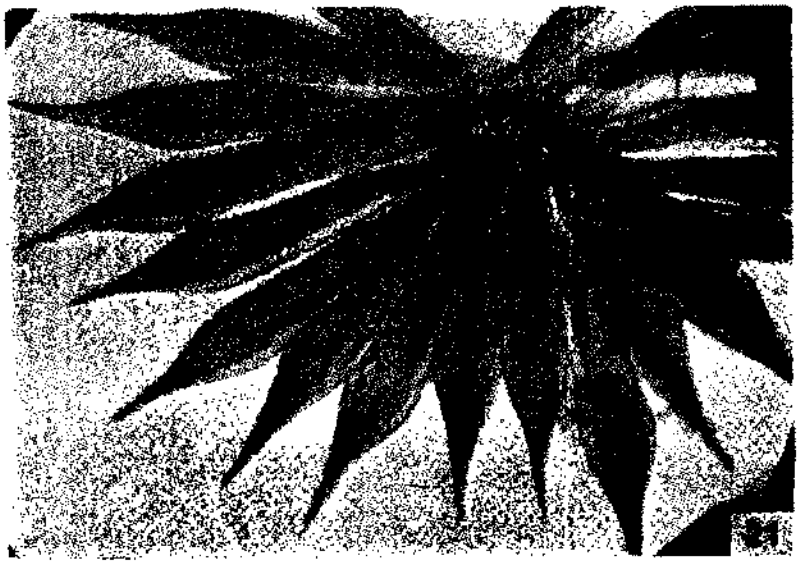


FIG. 20. Oval sunken *M. uniformis* egg mass on [illegible] surface of adult.
FIG. 21. Close-up of *M. uniformis* egg mass showing embryos inside the eggs.

Plate V.

Fig. 22. [illegible] in the [illegible] mud or water hyacinth [illegible]

Fig. 23. [illegible] of *M. uniformis* larvae in mud.

M. uniformis have been found attached to the roots. Searching among the water hyacinth plants next to the bank disclosed no egg masses on the stalks. In such cases it may be that the egg masses have been laid on grasses along the bank (which occurs frequently in nature), or perhaps on some flattened, floating debris such as dead, deciduous leaves or bark. Due to the internal inaccessibility of such water hyacinth stands, searches could not be made deeper within them.

5. Where *Pistia* tanks are near the water hyacinth stands but are completely separated (Fig. 6), and there is no *Eichhornia* or *Lemna* in the tank, only a small number of *M. uniformis* egg masses have been found. In this type of situation it appears as if *Mansonia annulifera* predominates, this being borne out repeatedly by tank examinations in Palluruthy in the Cochin area. Very few *annulifera* larvae and pupae were found on water hyacinth during the peak of *M. uniformis* production in October and November; however, the following February, with considerable reduction in *uniformis*, *M. annulifera* was found in larger numbers on *Eichhornia*.

While no exhaustive comparisons were conducted on the shapes of *M. uniformis* and *M. annulifera* egg masses, it was noticed that a large proportion, of *M. uniformis* egg masses are not round, but rather are rectangular, pentagonal, hexagonal, rhomboidal, oval, or irregular (Fig. 11, 12, 13, 16, and 20). On the other hand, egg masses of *M. annulifera* tend to be rounder. Identifications of egg masses were made by hatching out, or if this did not occur, the presence of nothing but *M. uniformis* larvae and pupae in the place of examination, correlated with practically all *M. uniformis* adults in nearby catching stations. It is also possible to identify mature egg masses having well-advanced embryos, and in many cases even egg masses which have hatched out for the most part, using certain differentiating characteristics to be described below.

Even without specific identification of each and every egg mass the general picture of species domination can be ascertained. For instance, in Palluruthy, near Cochin, there is one area where *M. uniformis* is seldom found ; what *M. uniformis* there is, comes from some water hyacinth tanks nearby. In this area practically every adult, larva and pupa is that of *M. annulifera*, and collected egg masses hatch out into *M. annulifera* larvae. In such cases it is impractical and unnecessary to check every single egg mass for species, in view of the evidence supporting the species identification. Likewise, in the Karakkamury area, the species found in October and November on water hyacinth was overwhelmingly that of *M. uniformis*, although very small numbers of *M. annulifera* were found on both *Pistia* and *Eichhornia* during that period. As noted above, by the following February, the number of larvae, pupae, and adults of *M. uniformis* had decreased considerably, and the proportion of *M. annulifera* on water hyacinth had increased. When the proportions of the two species are similar, it is best to check more carefully by hatching out of egg masses, if accurate epidemiological data are to be determined.

Newly hatched first-instar larvae of *M. annulifera* and *M. uniformis* from the Cochin area show certain specific differentiating characteristics which have been quite constant. Since there are two such characteristics, one can be checked against the other. First involved are the paired dorso-lateral hairs which are attached on about the same level as the long hairs of the anal segment, about $\frac{1}{5}$ to $\frac{1}{4}$ of the way back from the end of the anal segment. In *M. uniformis* this hair is quite stout, being wide almost from its origin, as wide as or wider than the basal tubercle, and at least as wide as the bases of the long terminal hairs of the anal segment. This hair does not really begin to taper down until its distal fourth (Fig. 36). In the early first-instar of *M. uniformis* it averages about 104 microns in length. The same hair in *M. annulifera* (Fig. 34 and 35) is finer, narrower than the basal tubercle, with more parallel sides which begin to taper off considerably at about the middle, resulting in a fine termination. It is never as wide as the basal portion of the long hairs of the anal segment. Its average length is 82 microns in the early first-instar of *M. annulifera*.

The second identifying character in the first-instar is the transparent "sabre" or long spine at the tip of the antenna. Three elements are present at the tip : a long, clearly marked sabre, a shorter spine, and a long, somewhat flattened, transparent sabre which is cleft along part of its length so as to produce a fine, accessory spine lying parallel to the rest of the sabre. The location of the fork at the proximal end of the cleft with respect to the entire length of the sabre, and the position of the basal segmentation, are the important features to be ascertained. In *M. uniformis* the basal portion of the sabre is segmented at about $\frac{1}{3}$ of the way along the entire sabre (Fig. 37 and 38) ; also, the fork of the sabre is about in the middle of its length from the proximal end, including the segmented basal portion. The average length of the entire transparent sabre in the *M. uniformis* first-instar larva is 120 microns. Usually the sides of the sabre show up in parts as darker lines, outlining the location of the forked portion. In *M. annulifera* the forking occurs earlier, being located at about $\frac{1}{4}$ of the way from the proximal end, thus making the accessory, parallel spine appear longer ; also the segmentation occurs closer to the base, being located at $\frac{1}{7}$ to $\frac{1}{8}$ of the way along the sabre. The line of segmentation appears as a very fine, almost invisible transverse demarkation, visible clearly when the lighting is right. The indentations at the sides of the sabre where the segmentation occurs are, however, very clearly marked. The average length of the sabre, including the basal segment, is 112 microns in *M. annulifera*.

Since both the sabres of the antennae and the dorso-lateral hairs of the anal segment are present at hatching, it is evident that they must also be present in the pre-hatching egg, at which time the organism is regarded as a mature embryo. Examination of such mature eggs under a compound microscope shows the moving, segmented, mature embryo, having two very evident, black eye spots, and the black, chitinous tube making up the terminal portion of the siphon (Fig. 41). If a cover glass is placed over the egg mass which is in a drop of water, and pressure is exerted very slightly, the opercula will come off, or the shell will crack open,

PLATE XI

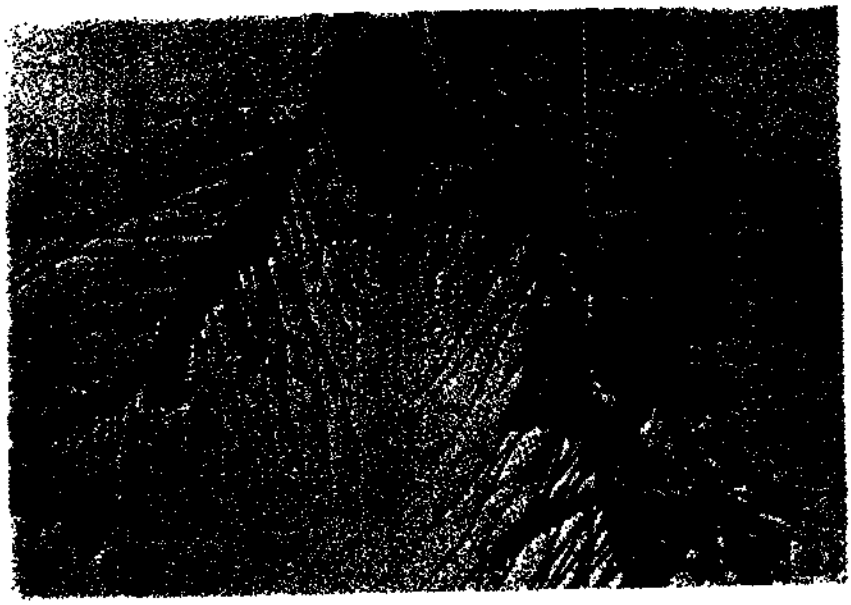


FIG. 24. *M. uniformis* fourth-instar larvae attached to main roots.

FIG. 25. Close-up showing 2 - 3 branched hairs on anal segment of *M. uniformis* larva and tuft hair on siphon.

PLATE XII.

FIG. 26. *M. uniformis* pupae attached to main roots of water hyacinth.
FIG. 27. Close-up of *M. uniformis* pupa attached at base of a main root.

PLATE XIII.

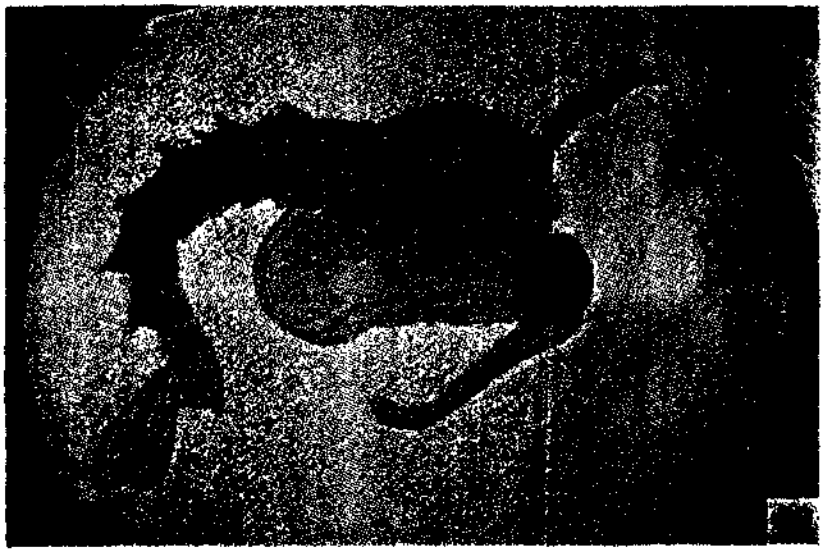


FIG. 28. *M. uniformis* larvae attached to *Pistia stratiotes* roots. This may occur in the absence of water hyacinth.

FIG. 29. Cleared *M. uniformis* pupa attached to main root of water hyacinth.

PLATE XIV.

FIG. 30. Siphon of *M. uniformis* larva hooked into main root of water hyacinth.

FIG. 31. Close-up showing *M. uniformis* pupal respiratory trumpets embeded in water hyacinth main root. Note that stout, parallel trumpets diverge at tips to provide hook-like attachment.

PLATE XV.

FIG. 32. *M. uniformis* larvæ and pupæ attached to mud covered roots of water hyacinth.

FIG. 33. Close-up of *M. uniformis* pupa and larvæ attached to mud covered water hyacinth roots.

PLATE XVI

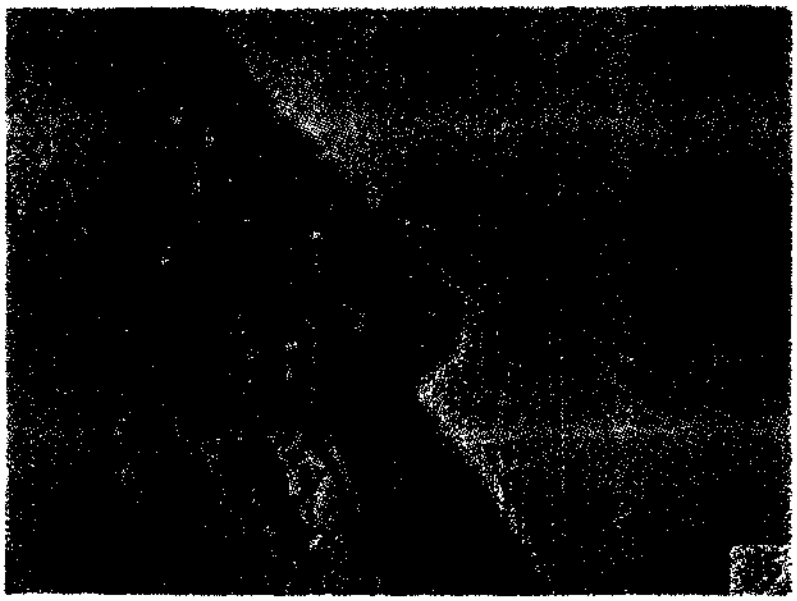


FIG. 34. Stout, dorso-lateral hair on anal segment of newly hatched *M. amnigena* larva.

FIG. 35. Closer view of hair in Fig. 34. Note that hair is narrower than basal tubercle, and tapers rapidly after the middle.

FIG. 36. Stout, dorso-lateral hair on anal segment of newly hatched *M. uniformis* larva. Note that hair is at least as wide as basal tubercle, and tapers only in the last fourth. Magnification same as in Fig. 35.

PLATE XVII.

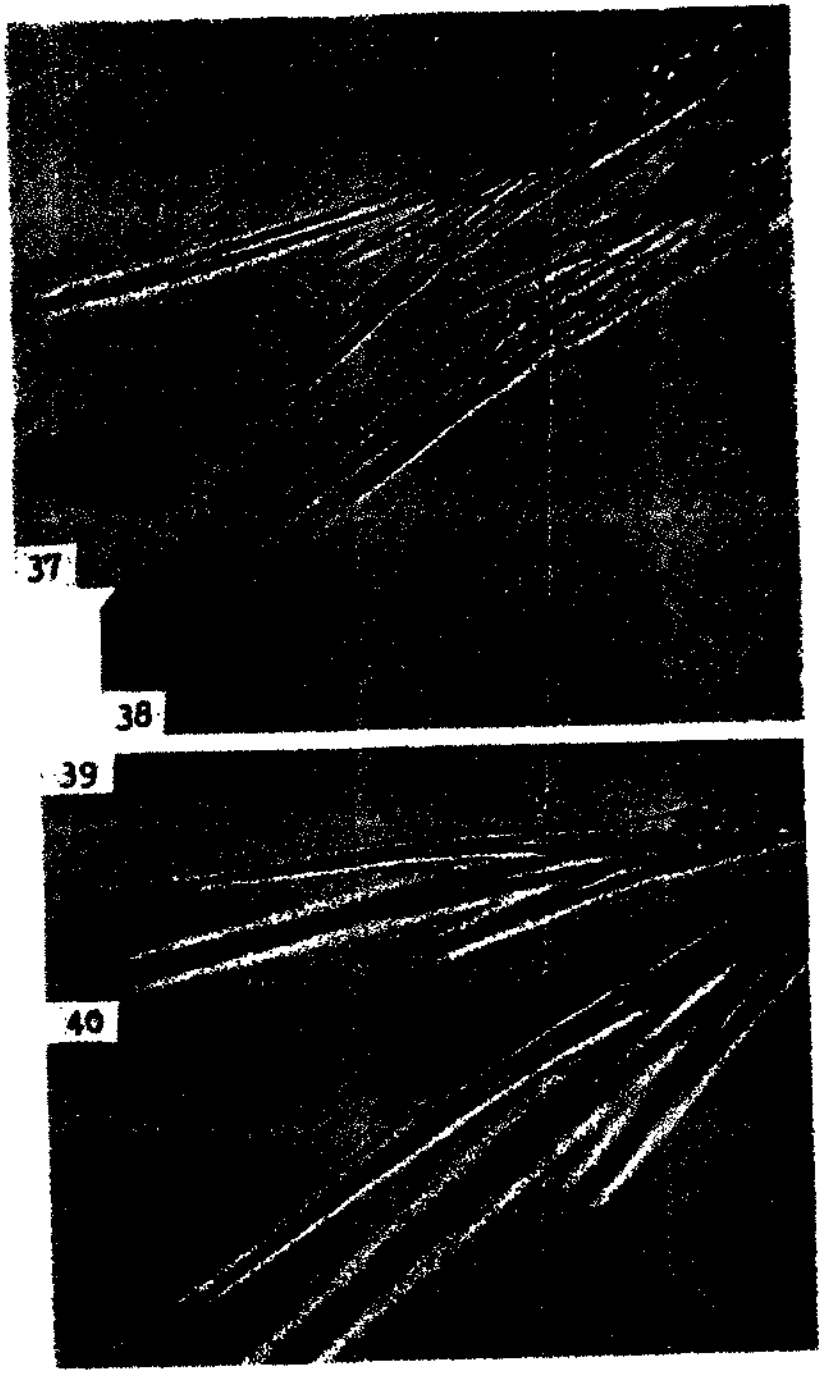


FIG. 37. Greatly magnified view of forked, transparent sabre at tip of antenna of newly hatched *M. uniformis* first-instar larva. Note that forking takes place at about middle and segmentation occurs about 1/3 from the proximal end.

FIG. 38. Closer view of forked sabre shown in Fig. 37.

FIG. 39. Greatly magnified view of forked, transparent sabre at tip of antenna of newly hatched *M. annulifera* first-instar larva. Note that forking takes place about 1/4 of the distance from the proximal end, and segmentation occurs 1/7 to 1/8 of the length of the sabre.

FIG. 40. Closer view of forked sabre shown in Fig. 39.

...ose of E. speciosa *Kunth in the life cycle of* M. uniformis.

PLATE XVIII.

FIG. 41. Mature, pre hatching *Mansonioides* embryos within eggs.

FIG. 42. Hatched *Mansonioides* egg mass with two unhatched mature eggs and one dead, well developed embryo (arrow).

FIG. 43. *M. uniformis* mature embryos extracted for examination by pressure on egg shell with lower glass.

PLATE XIX.

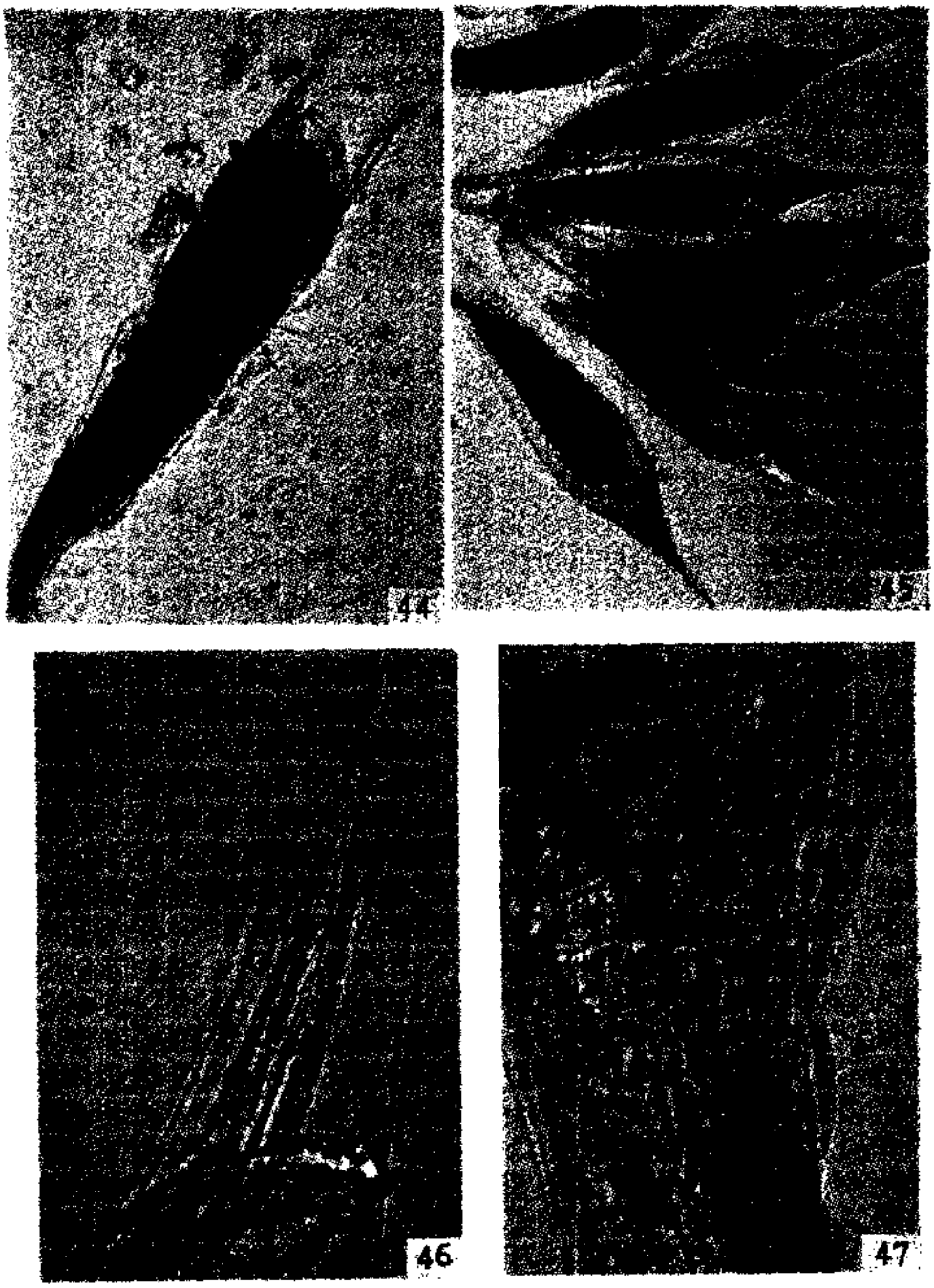


FIG. 44. Partially developed but identifiable Mansonioides embryos.

FIG. 45. Partially developed *Mansonioides* embryos within eggs.

FIG. 46. Close-up of partially developed *M. uniformis* embryo identified by position of segmentation in transparent sabre. (arrow).

FIG. 47. Partially developed *M. annulifera* embryo identified by two dorso-lateral hairs on developing anal segment.

revealing the fully formed first-instar larva, which can then be identified by the characteristics which have been noted above (Fig. 43).

In most hatched egg masses collected in the field, there are generally several unhatched eggs, and even dead larvae which could not swim out from the shell for some reason ; thus making it possible to identify the species by the same means (Fig. 42). Sometimes, when the embryo is only partially developed (Fig. 44 and 45), there is evidence of the condition of the sabre of the antenna, or of the dorso-lateral hair of the anal segment, if enough embryos are examined after being expressed from the shells (Fig. 46 and 47). Immature as such specimens may be, they can still be identified as to species, but it has been found that not all specimens of such an egg mass will have the identifying characters at this stage.

The identification of *M. uniformis* versus *M. annulifera* pupae and fourth-instar larvae is relatively simple. In *M. uniformis* the four ventral hairs of the anal segment are 2 to 3 branched (Fig. 25), as compared to simple hairs in *M. annulifera* larvae. Also, in *M. uniformis* the lateral hairs of the siphon are practically always bifid, whereas in *M. annulifera* they are trifid. *M. uniformis* pupae are larger and stouter than those of *M. annulifera* (Fig. 27), and the respiratory trumpets are likewise much shorter and stouter in *M. uniformis* pupae (Fig. 29 and 31). *Mansonia indiana* and *M. longipalpis* are very rare in the Cochin area of Kerala.

An experienced technician or field collector can separate *M. annulifera* larvae and pupae from those of *M. uniformis* fairly accurately with the naked eye. Larvae of *M. uniformis* are stouter and often darker in appearance, whereas those of *M. annulifera* are more slender and more transparent. When these two types of larvae are together in a vessel, it is seen that those of *M. uniformis* tend to remain near or on the bottom, wriggling very sinuously and rapidly, usually flat against the bottom, whereas larvae of *M. annulifera* have a somewhat less sinuous movement, and move throughout the water area, resting often at the surface.

In the second-instar there is considerably more variation in the nature of the hairs of the ventral portion of the anal segment. In *M. uniformis* a single bifid hair alone may be seen, with gradual development of the other three hairs, which may appear single or bifid ; or else by the end of the second-instar only the initial bifid hair may be present. The bifid lateral hair on the siphon may or may not be present by the end of the second-instar. In *M. annulifera* there is at first only a single, fine hair on the ventral portion of the anal segment, and gradually the other three make their appearance, being spaced about equally apart. In many cases the lateral hair of the siphon is still single, not becoming trifid until the third or fourth-instar. It is often difficult to tell where the second-instar ends and the third begins. In the case of the first to second-instar, there is some guide in that the serrated plate of the siphon tip is formed just before the end of the first-instar after a reorganization of the cylindrically arranged, modified plates of the early and middle first-instar stage.

The above discussion on the identification of *M. uniformis* versus *M. annulifera* has been included because in an area where both vectors occur simultaneously it is necessary to differentiate between them as accurately as possible, in order to ascertain correct epidemiological data. Because of differences in strains these same species may show varied characteristics in other areas or countries, as often occurs.

SUMMARY.

Water hyacinth (*Eichhornia speciosa* Kunth) has considerable epidemiological importance in the life cycle of *Mansonia uniformis* (Theobald) in the Cochin area of Kerala State, South India, being the preferred host plant for attachment of the larvae and pupae, especially during months of greatest density of the vector. When water hyacinth is associated with *Pistia stratiotes* (water lettuce), the egg masses of *M. uniformis* are preferably laid on *Pistia* leaves ; after hatching the larvae prefer to attach to the nearby mud-covered water hyacinth roots, this being associated with the *M. uniformis* larva's usual habit of remaining in the mud for many hours. Attachments to *Pistia* main roots do occur, but definite preference is shown for the roots of water hyacinth, possibly because *Pistia* roots are generally far from the mud in which the *M. uniformis* larvae prefer to remain.

M. uniformis egg masses are laid often on young water hyacinth leaves which lie in or on the water, and secondarily on the under surface of *Lemna* or duckweed fronds if this plant also happens to be present. In the absence of *Pistia* or low water hyacinth leaves, the eggs are laid on *Lemna* alone. In the absence of *Pistia*, *Lemna* or low water hyacinth plants, where tall water hyacinth plants show attached larvae and pupae, it is probable that the egg masses have been attached to nearby grasses, or possibly on floating dead leaves or flattened debris. Since all the aquatic stages of *M. uniformis* occur on water hyacinth, this plant should be regarded of primary epidemiological importance wherever *M. uniformis* occurs.

Late embryos and first-instar larvae of *M. uniformis* show a stout dorso-lateral hair on the anal segment, and a centrally-located fork on the transparent sabre at the tip of the antenna. In *M. annulifera* the dorso-lateral hair is much finer, and the fork of the transparent sabre occurs more proximally. By rupturing the egg shells of pre-hatching eggs, these characters can also be seen in the late embryo, and sometimes earlier. Forty-seven figures illustrate attachments of the aquatic stages of *M. uniformis* to *Eichhornia*, *Pistia*, and *Lemna*, and identifying characters of late embryonic and first-instar larvae of *M. uniformis* and *M. annulifera*.

REFERENCE.

BURTON, G. J. (1959) ... ... Studies on the bionomics of mosquito vectors which transmit filariasis in India. I. Attachment of *Mansonia annulifera* and *Mansonia uniformis* larvae to host plants occurring in *Pistia* tanks in Kerala, South India. *Ind. J. Mal.* 13, 2/3, pp. 75-115.

Indian Journal of Malariology, **14**, 2, *June 1960*.

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

III. FURTHER STUDIES ON ATTACHMENT OF *MANSONIA UNIFORMIS* LARVAE TO HOST PLANTS OCCURRING IN *PISTIA* TANKS IN KERALA, SOUTH INDIA.

BY

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[February 15, 1960.]

In a previous study (Burton, 1959) descriptions were given of attachments of *Mansonia annulifera* (Theobald) and *Mansonia uniformis* (Theobald) to eleven species of plants occurring in *Pistia* tanks in Kerala State, South India. These plants were *Pistia stratiotes, Eichhornia speciosa, Bacopa monnieri, Commelina salicifolia, Trapa bispinosa, Marsilea quadrifoliata, Limnophila gratioloides, Azolla pinnata, Lemna minor, Nelumbo nucifera,* and a species of *Nymphaea*. It was decided to continue this study and gradually to cover every plant in *Pistia* and water hyacinth tanks, in order to throw additional light on the behaviour of the aquatic stages of vectors of filariasis in India. Of course, from the epidemiological standpoint, those plants which have *Mansonioides* egg masses laid on them, in addition to attachments by larvae and pupae, would be the most important plants in considerations of the life cycles of the vectors ; however, it is interesting and informative to note that there are certain alternate plants which have secondary or tertiary importance in so far as attachment is concerned, but which may be important enough to sustain the vector until the emergence of the adult.

At the time of the present study, egg masses, larvae, and pupae of *Mansonia uniformis* were far more prevalent than those of *M. annulifera*, hence the actions of the former are recorded here. As in the previous study, in order to see and photograph the actions of the larvae and pupae, the plants were taken from the water and immediately placed into a clear, rectangular glass jar having several hundred *M. uniformis* larvae. Attachments took place generally within one minute, often instantly in large numbers (Fig. 1 and 20), and records were made of their behaviour.

As before, photographs were taken with an Edixa (German) 35 mm. single-lens reflex camera having a Takumar (Japanese) f.2.2, 55 mm. lens, using close-up rings and a bellowscope. The cross sections of roots were cut with the naked eye, using a thin, double-edged razor blade after the root had been fixed and hardened in 4 per cent formalin. The microscope photographs were made by

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

attaching the camera minus lens above the microscope with an adapter, and using the lenses of the microscope to replace the camera lens.

This study presents additional observations as to attachments to *Pistia stratiotes*, and in addition covers three grasses (*Brachiaria eruciformis*, *Leersia hexandra*, and *Pseudechinolaena polystachya*), one stonewort (*Chara wallichii*), one leafy aquatic herb (*Colocasia antiquorum*), one submerged, rootless herb (*Ceratophyllum demersum*), a minute, floating flowering plant (*Wolffia arrhiza*), and *Salvinia auriculata* which in some other countries has considerable epidemiological importance in filariasis. First-instar attachments to *Lemna polyrrhiza* roots are also described.

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

In the previous study it had been seen that *M. annulifera* larvae and pupae undertook attachments which at that time appeared to be unusual, particularly the habit of attaching directly to leaves which lie horizontal in the water. Gaseous exchange in this case takes place within the aerenchyma of the leaf, as considerable oxygen is available after being formed by the green chloroplasts during photosynthesis.

The nature of a single unit of *Pistia stratiotes* and the normal means of attachment of *M. uniformis* larvae and pupae are shown clearly in Figure 1. Most *M. annulifera* larvae tend to hang upside down while attached to *Pistia roots*, but here *M. uniformis* larvae are attached both right side up and upside down. It was repeatedly observed in the present study that attachments to leaves is a *normal*, and not an unusual form of behaviour, and provides an alternate method of performing gaseous exchange. This is shown clearly in Fig. 2, 4, and 5. In Figure 2 note that the larvae are attached to the portion of the *Pistia* leaf which normally lies beneath the water, and need not necessarily be horizontal. These larvae remain attached for several days, before detaching in order to pupate.

Another frequent place of attachment of *M. uniformis* larvae is directly to the sympodial mass from which the main roots originate, and where the leaves come together (Fig. 3). Note here the single pupa attached at the base of a main root.

It has long been known that in *Pistia* the attachments take place to the main roots rather than to the rootlets. After studying cross sections of rootlets and comparing them with sections of main roots, it was thought that early larval stages might be able to attach to the rootlets. This was borne out after detailed watching of the early stages, as seen in Figure 8, where two first-instar larvae are attached to rootlets, and remained so for several days. The first-instar larvae also can attach to main roots, but after the formation of the serrated plate prior to the first ecdysis, the larvae prefer to attach to the main roots. Since the early and middle first-instar larva does not possess a serrated plate, but rather a hooked, cylindrical, chitinized siphon tip, the latter is merely pushed into the root or rootlet with a few jabs after which the relatively large hooks catch hold.

PLATE I.

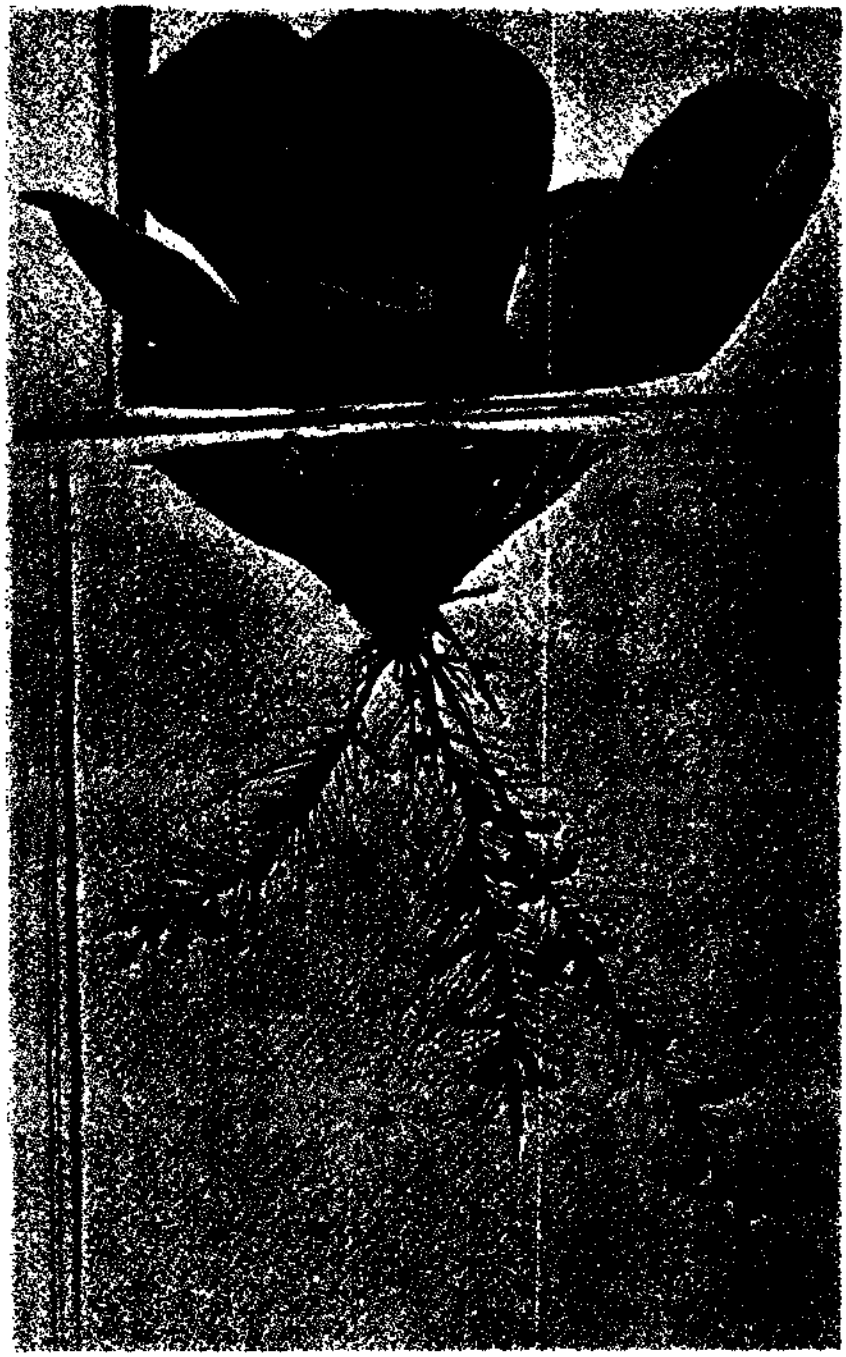


FIG. 1. *Mansonia uniformis* larvae and pupae attached to main roots and leaves of *Pistia stratiotes* water lettuce.

PLATE II.

FIG. 2. Close-up of *M. uniformis* larvae attached to leaf of *Pistia stratiotes*.
FIG. 3. *M. uniformis* larvae and pupae attached near origins of main roots.

PLATE III.

FIG. 4. Close-up of *M. uniformis* pupa attached to leaf of *Pistia stratiotes*.

FIG. 5. Close-up of *M. uniformis* larva with siphon tip embedded in *Pistia* leaf

PLATE IV.

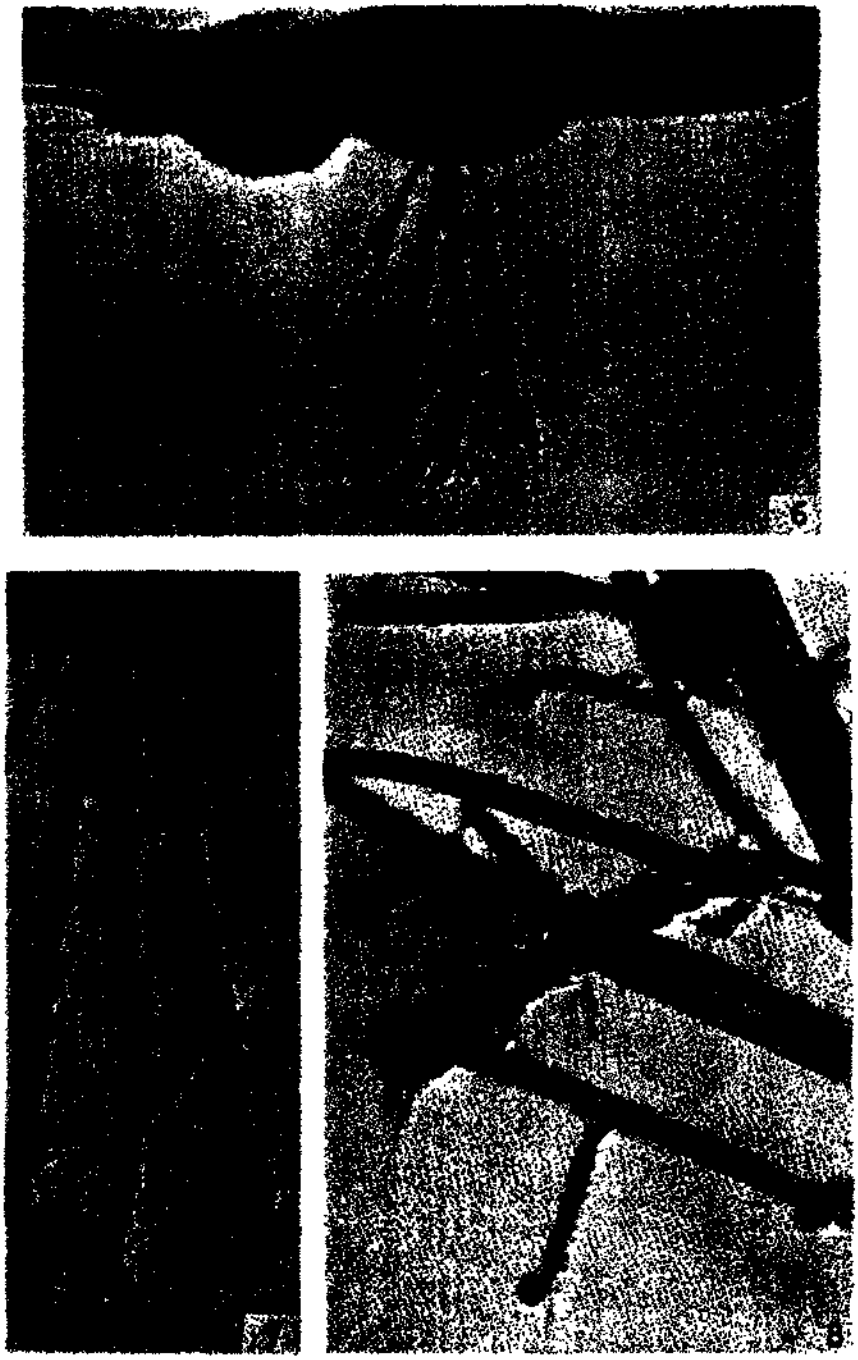


FIG. 6. First-instar larva of *M. uniformis* attached to rootlet of *Lemna polyrrhiza.*

FIG. 7. Close-up of *M. uniformis* first-instar larva attached to rootlet of *Lemna polyrrhiza.*

FIG. 8. Two first instar larvae of *M. uniformis* attached to rootlet of *Pistia stratiotes.*

PLATE V.

FIG. 9. *Colocasia antiquorum* growing in *Pistia* tank.
FIG. 10. Complete plant of *Colocasia antiquorum*.

PLATE VI.

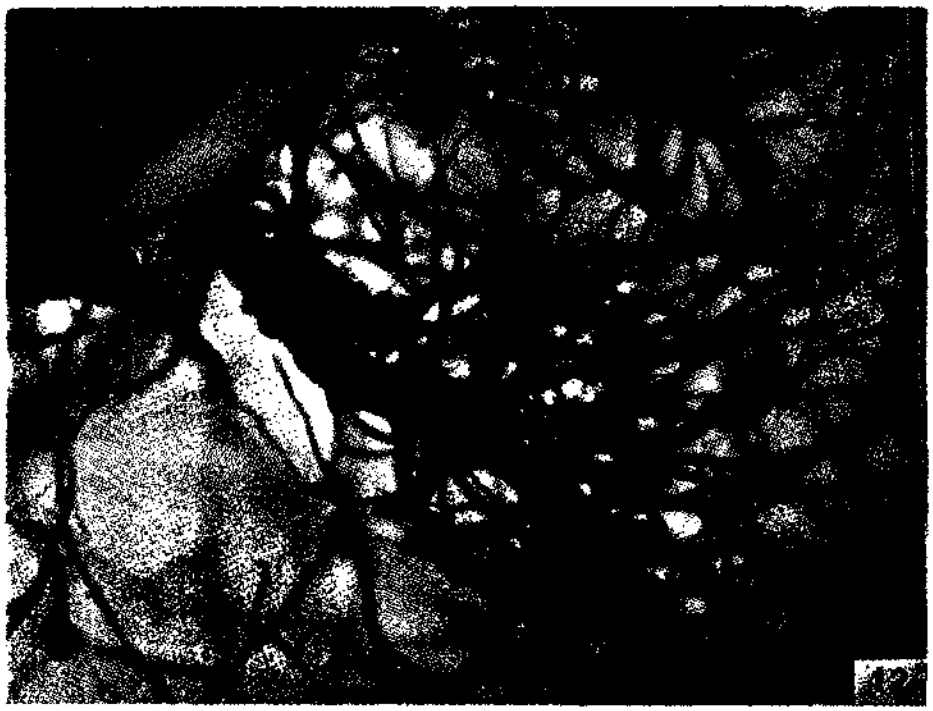


FIG. 11. *M. uniformis* larvae attached to roots of *Colocasia antiquorum* (Natural size).

FIG. 12. Close-up showing many *M. uniformis* larvae attached to dark roots and rootlets of *Colocasia antiquorum*.

PLATE VII.

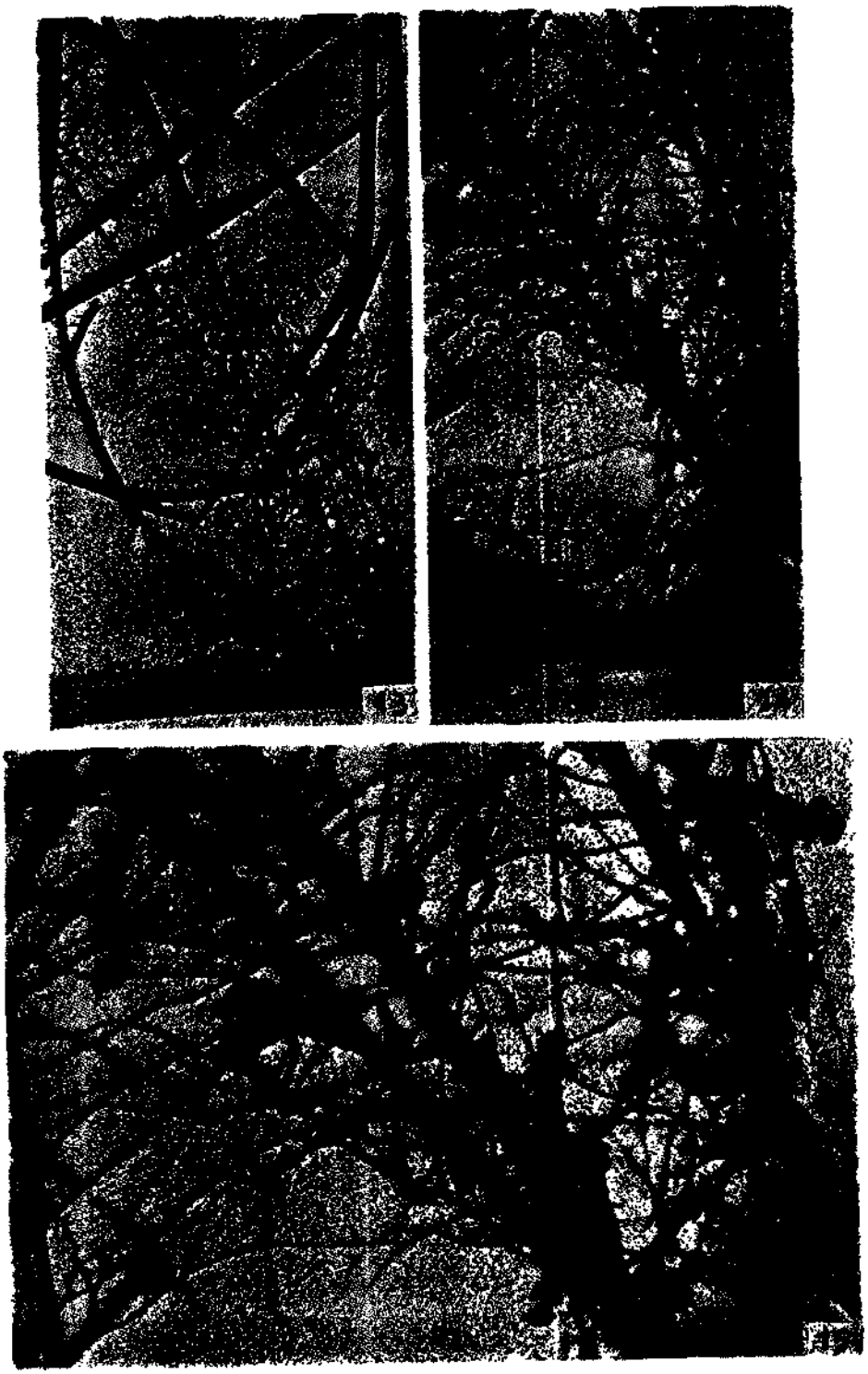


FIG. 13. *Leersia hexandra*, showing nature of roots and leaves.

FIG. 14. *M. uniformis* larvae attached to roots of *Leersia hexandra*.

FIG. 15. Close-up showing *M. uniformis* larvae attached to roots of *Leersia hexandra*.

PLATE VIII.

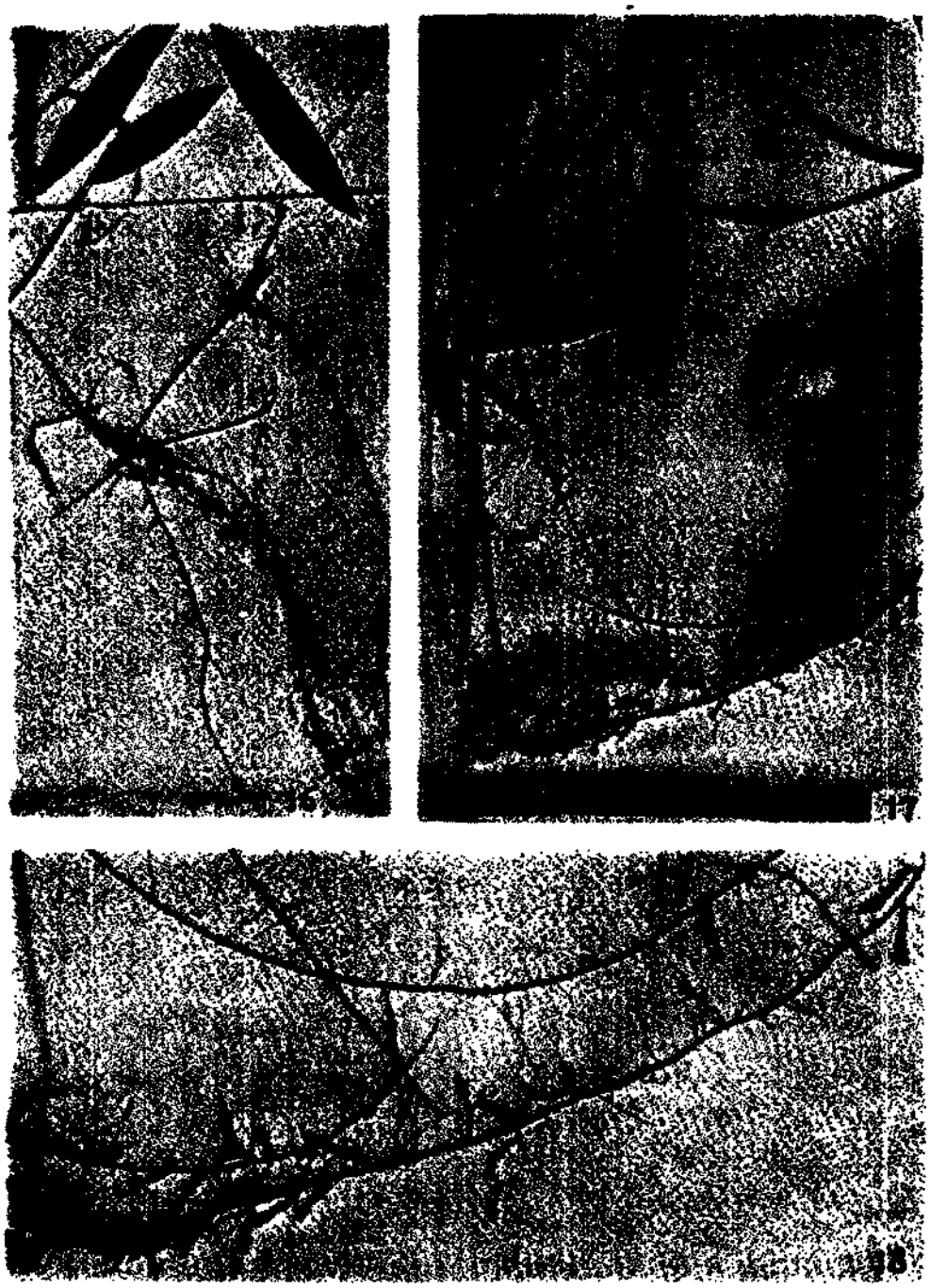


FIG. 16 *Brachiaria eruciformis*, showing *M. uniformis* attached to main roots and rootlets.

FIG. 17. *Pseudechinolaena polystachya*, with *M. uniformis* larvae attached to main roots and rootlets.

FIG. 18. Closer view of *M. uniformis* larvae on roots of *Pseudechinolaena polystachya*.

PLATE IX.

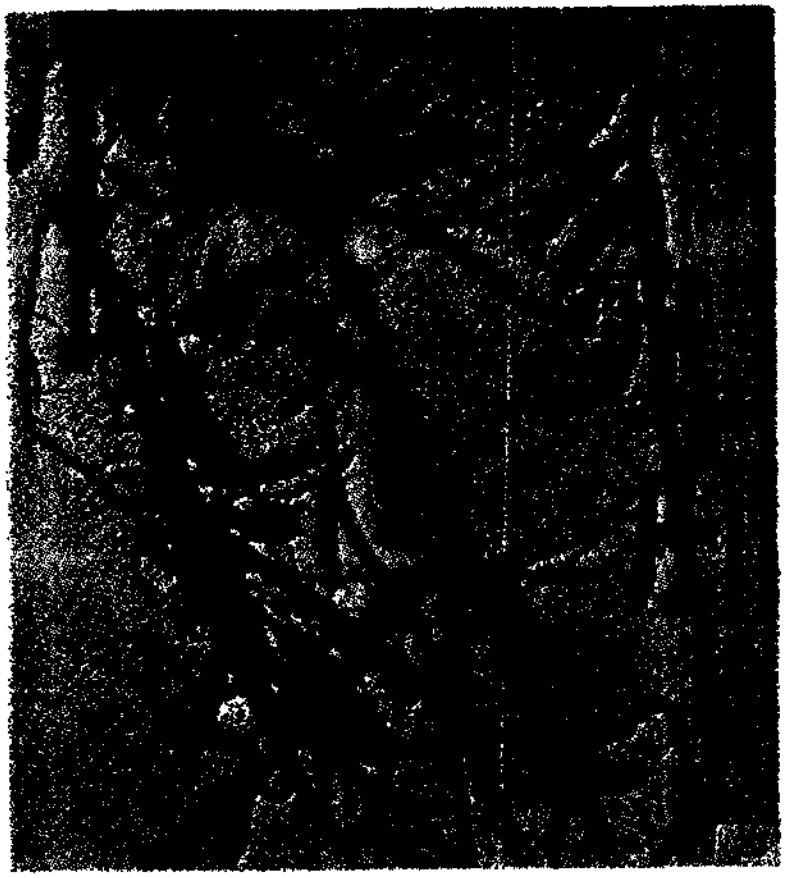


FIG. 19. Details of attachment of *M. uniformis* larvae to roots and rootlets of *Pennisetum polystachya*.

FIG. 20. Close up showing *M. uniformis* larvae attached to roots and rootlets of *Brachiaria eruciformis*.

PLATE X.

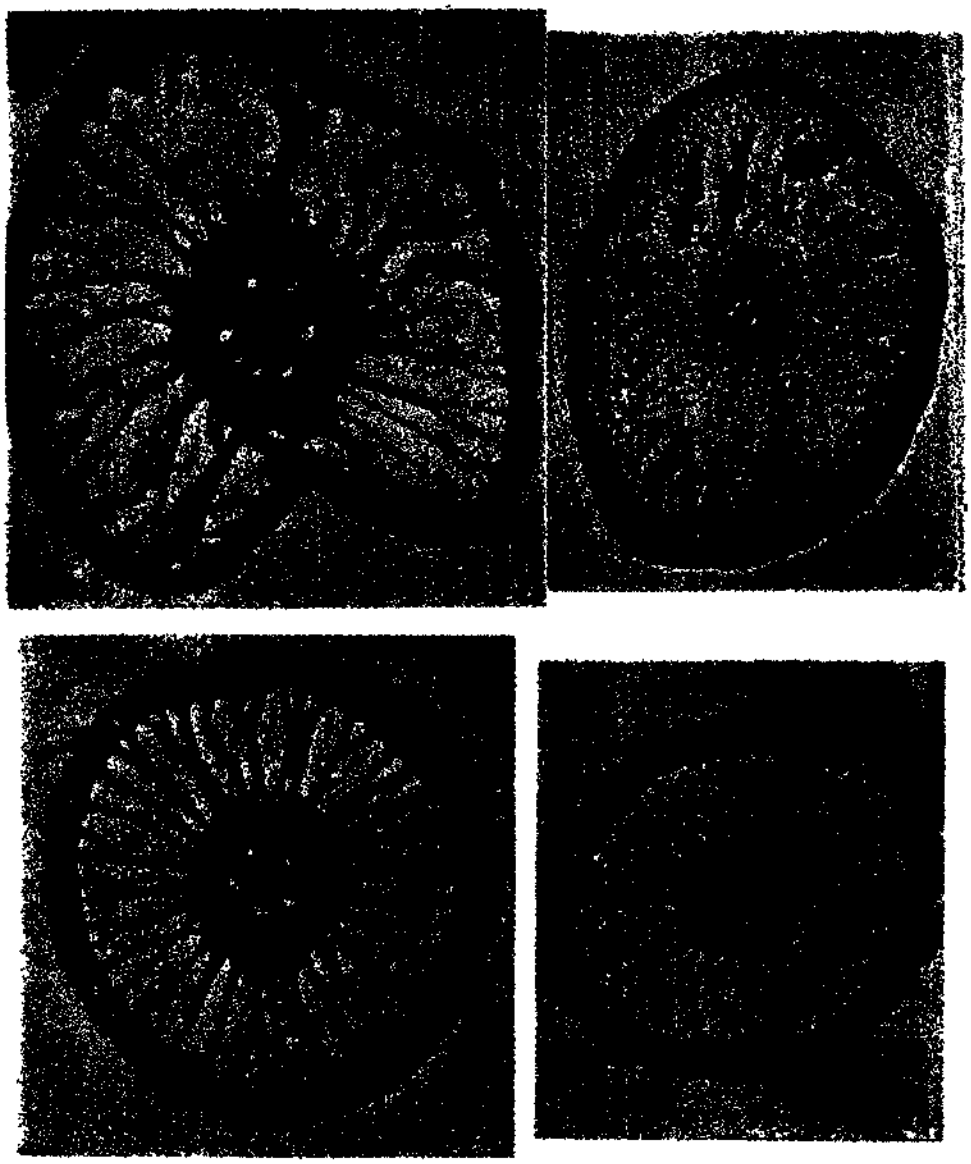


FIG. 21. Cross section through root of *Brachiaria eruciformis*.
FIG. 22. Cross section through root of *Colocasia antiquorum*.
FIG. 23. Cross section through root of *Pseudechinolaena polystachya*.
FIG. 24. Cross section through root of *Leersia hexandra*.

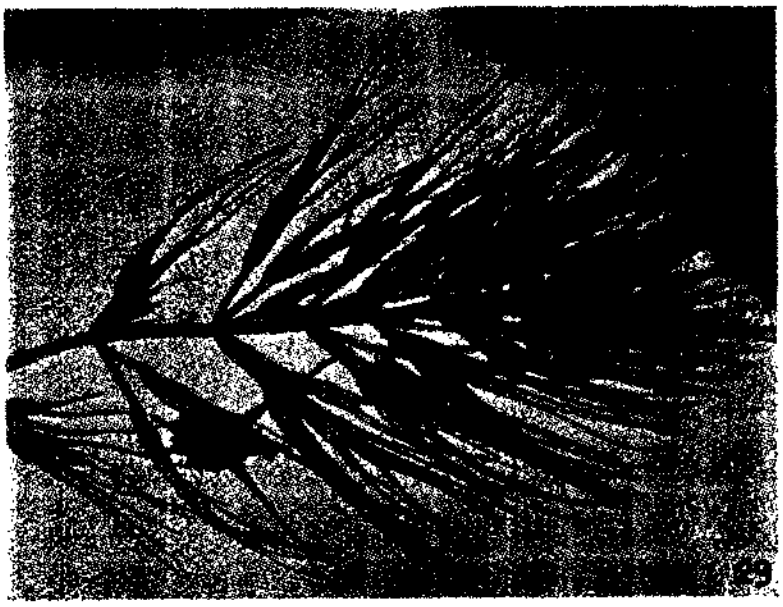
Fig. 25. Nature of the stem-mat of *Chara wallichii*, showing main stem and sheath of branches. 3 sp.

Fig. 26. Two larvae of *M. uniformis* with siphons inserted into a node and a branch of *Chara wallichii*.

Fig. 27. Close-up of *M. uniformis* larvae attached to *Chara wallichii*.

Fig. 28. Close-up showing rows of dissociated chl-capsules giving stringy appearance to branch of *Chara wallichii*.

PLATE XII.



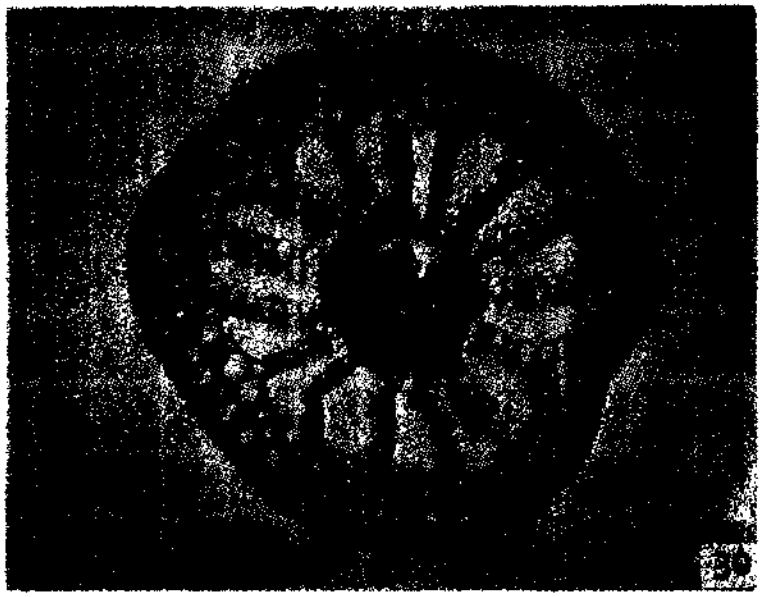


FIG. 29. Branches and fruit of *Ceratophyllum demersum.*

FIG. 30. Cross section through main stem of *Ceratophyllum demersum.*

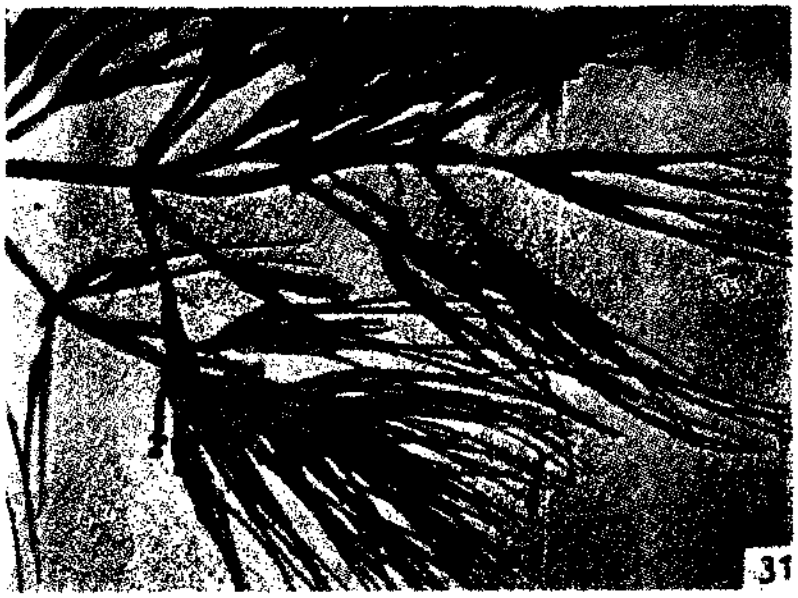
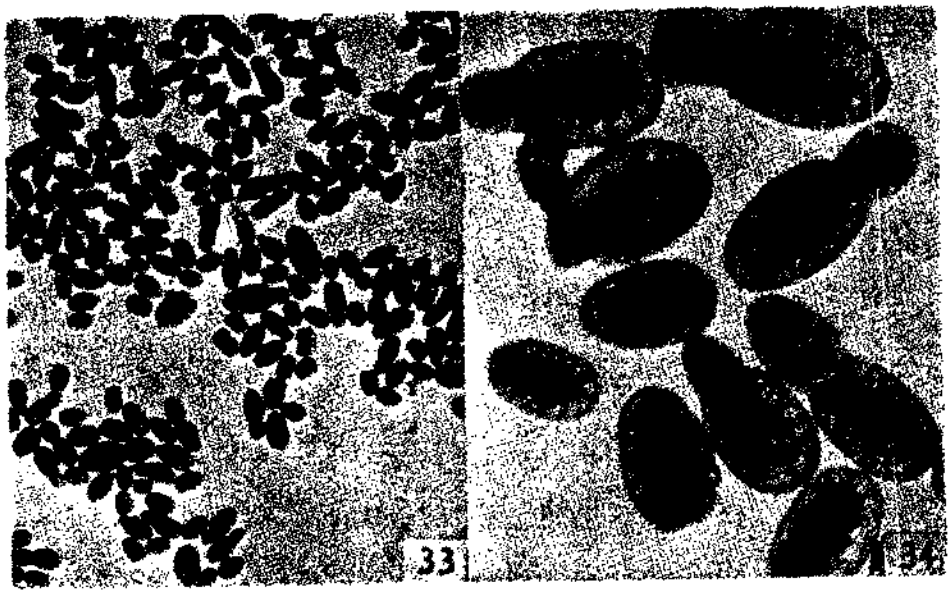


FIG. 31. *M. uniformis* larvæ attached to stem, stipes, and branches of *Ceratophyllum demersum*.

FIG. 32. Close up showing *M. uniformis* larvæ attached to node of *Ceratophyllum demersum*.



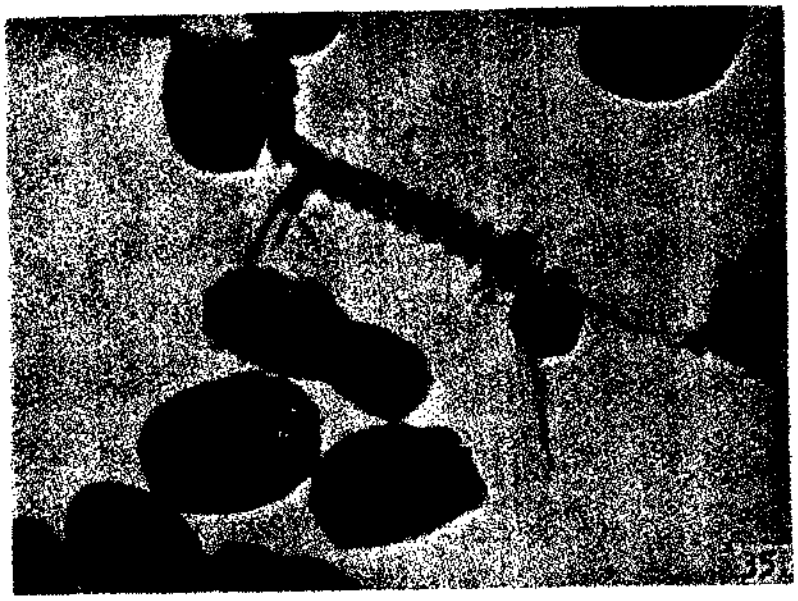


FIG. 33. Grass like aspect of tiny fronds of *Wolffia arrhiza*
FIG. 34 Close-up of *Wolffia arrhiza* showing formation of daughter fronds
FIG. 35 Close-up showing first instar *M. uniformis* larva attached to a frond of *Wolffia arrhiza*

PLATE XV

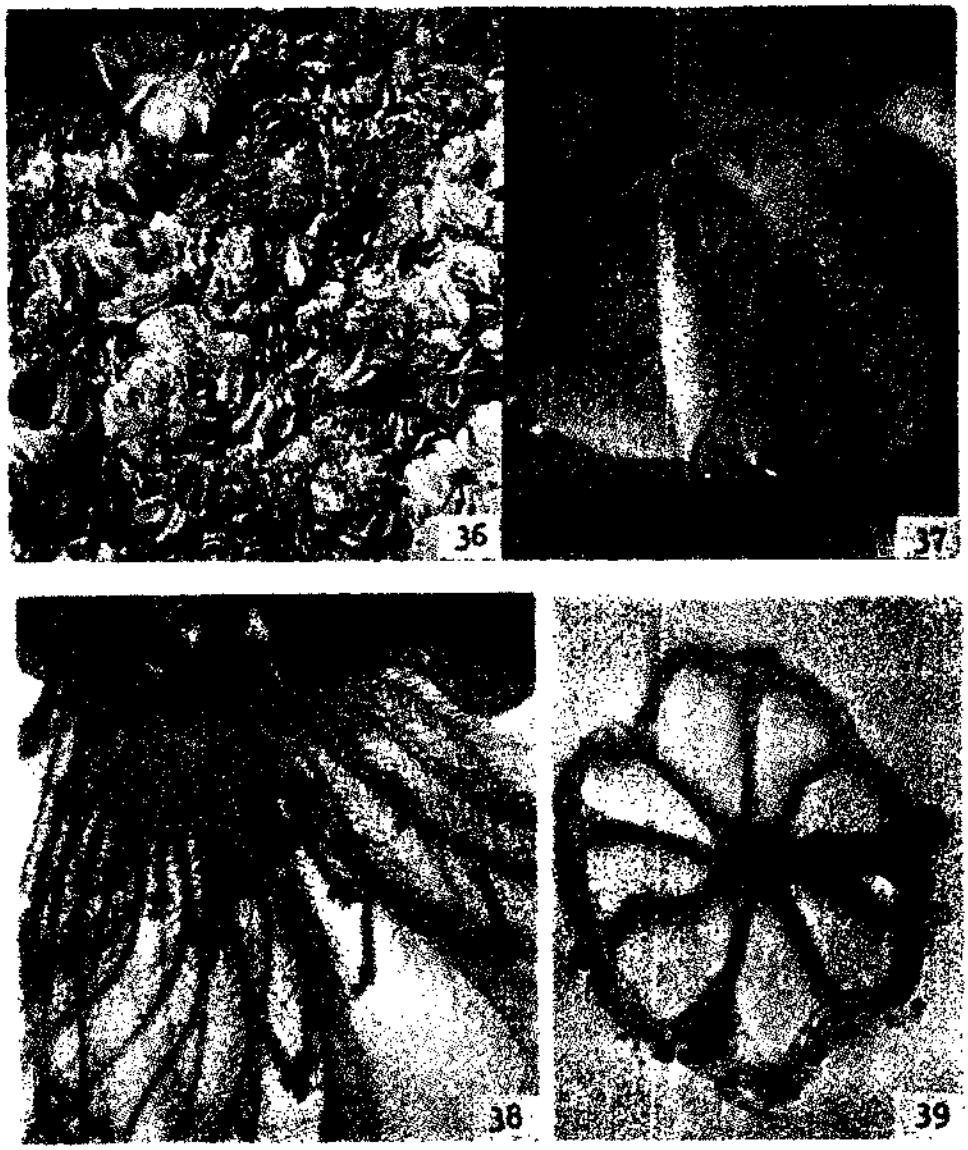


FIG. 36. Massed, tightly-packed *Salvinia auriculata* plants.

FIG. 37. Close-up of papillose *Salvinia auriculata* leaves.

FIG. 38. Close-up of *M. uniformis* larvae attached to root-like modified leaves of *Salvinia auriculata*.

FIG. 39. Cross section of root-like structure of *Salvinia auriculata* showing 9 large air chambers and single peripheral layer of cells.

PLATE XVI.

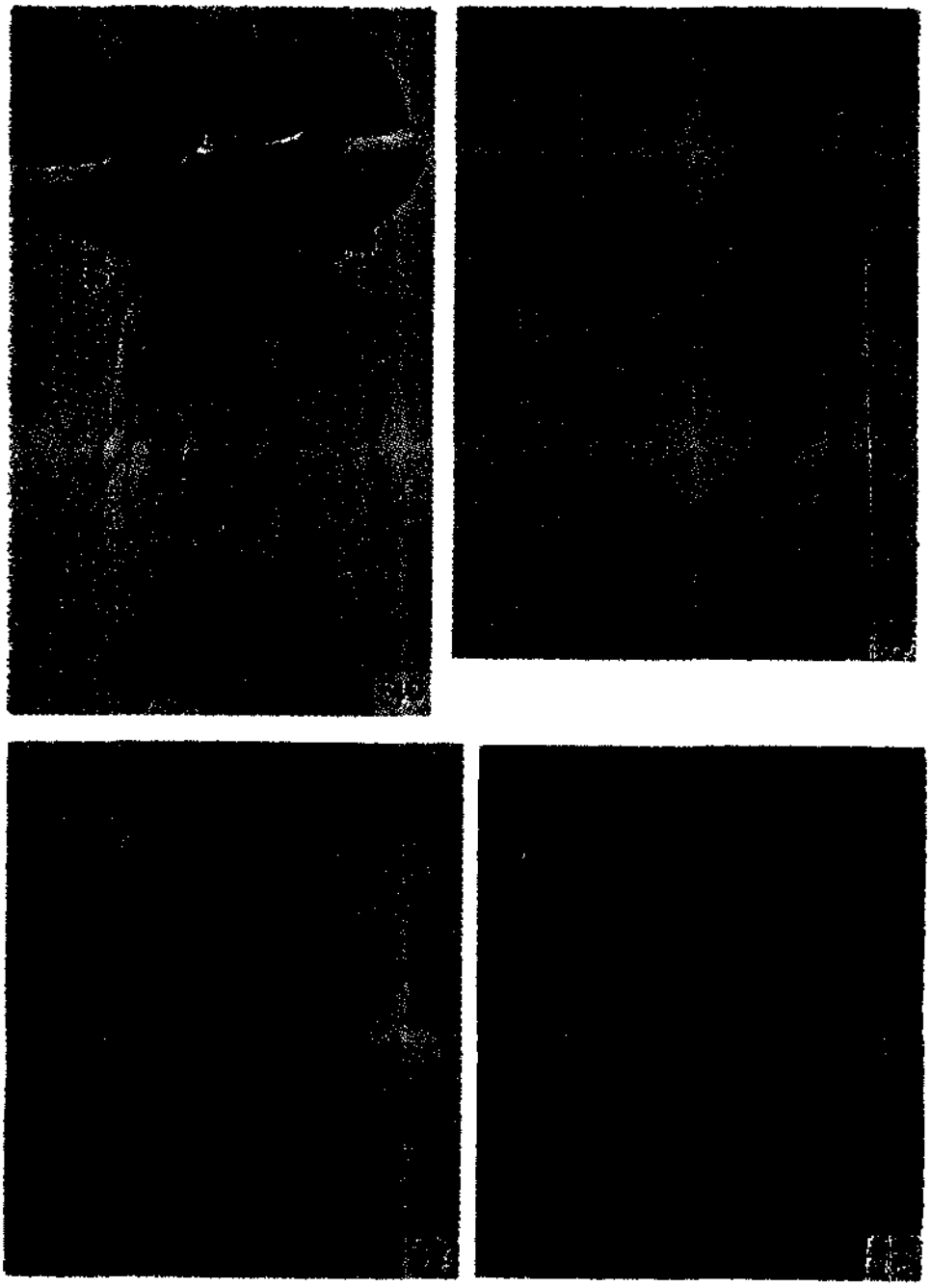


FIG. 40. Complete unit of *Salvinia auriculata* showing aquatic, subaerial, and root-like leaves with *M. uniformis* attached larvae.

FIG. 41. Close-up of *M. uniformis* larvae attached to root-like structures of *Salvinia auriculata.*

FIG. 42. *M. uniformis* larvae attached to leaf of *Salvinia auriculata*, as a normal and frequent means of effecting gaseous exchange.

FIG. 43 *M. annulifera* egg masses laid on *Salvinia auriculata* leaves.

2. *LEMNA POLYRRHIZA* LINN., DUCKWEED (FAMILY LEMNACEAE).

In the previous study, attachments of *M. annulifera* larvae to blades of *Lemna minor* were described. *L. minor* is a small, floating plant having only a single, fine rootlet to which no attachments were observed. In the case of *Lemna polyrrhiza*, however, there is a tuft of coarse rootlets arising from each frond, the latter being ⅛ to ¼ inch in diameter. First-instar larvae of *M. uniformis* were observed to remain attached to these rootlets for many hours (Fig. 6 and 7). In Figure 6 an egg mass of *M. uniformis* is also seen attached to the under surface of the frond.

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

Colocasia antiquorum is a tall, coarse herb with undulate-margined, arrowhead-shaped leaves on long petioles attached to a tuberous rhizome (Fig. 10). Previously it had been reported that relatively few attachments of *M. annulifera* larvae had been noted. In the present study, however, it was found that *M. uniformis* larvae attached in large numbers to the coarse roots which are 1/16 inch in diameter. The plants are shown growing in a *Pistia* tank in Figure 9. Figures 11 and 12 show the attachments of the larvae both to main and secondary roots. They remained tenaciously attached in the same place for several days. A cross section of a *Colocasia* root (Fig. 22) revealed a wide, soft cortex and epidermis, and about 30 main air chambers separated by delicate walls. Attachments generally did not take place on the white roots which are up to ⅛ inch in diameter, or to their stout, lateral rootlets.

4. *LEERSIA HEXANDRA* SW., BAREET GRASS (FAMILY GRAMINEAE).

This is a tall, floating, perennial grass having 3 to 8 flat, tapering linear blades which are ⅛ to ¼ inch wide and 6 to 12 inches long, and sheathed at the base. The erect, slender branches, which are 2 to 4 feet high, originate from stems rooted in the mud, there being a number of long main roots and numerous rootlets (Fig. 13). The panicles are 2 to 4 inches long, somewhat alternately branched, and the spikelets are 1/10 to ⅛ inch long. *M. uniformis* larvae attached readily and very tenaciously to the main roots, and a few attached themselves to secondary roots (Fig. 14). A cross section of a *Leersia* root (Fig. 24) shows about 25 main air chambers and a number of secondary air chambers, separated by thin walls of cells. In practically every case the attachment took place at or near the origin of a rootlet (Fig. 15) as has been previously reported for *Pistia* and *Eichhornia*, the place of penetration evidently being guided by a pair of sensory hairs attached near the base of the serrated plate.

5. *PSEUDECHINOLAENA POLYSTACHYA* (H. B. K.) STAPF, A GRASS (FAMILY GRAMINEAE).

This is a slender, perennial grass with flat, flaccid blades 2 to 3½ inches long and a few spikelike racemes in contracted terminal panicles which are about ½ to

$\frac{3}{8}$ inch long, the spikelets being $\frac{1}{8}$ inch long. The culms or aerial stems are one to two feet high. The roots are very long and slender, purplish proximally, with short, lateral, secondary rootlets. The nodes are $1\frac{1}{2}$ to 2 inches apart. *M. uniformis* larvae attached instantly not only to main roots, but also to rootlets (Fig. 17 and 18). A cross section of a root of this plant (Fig. 23) shows 28 main air chambers and a number of secondary smaller chambers. As seen in Figure 19, here also the attachment is made at or near the origin of a rootlet.

6. *BRACHIARIA ERUCIFORMIS* (J. E. SM.) GRISEBACH, A GRASS (FAMILY GRAMINEAE).

This is a slender, annual grass having lanceolate blades $\frac{3}{8}$ to $\frac{1}{2}$ inch wide and up to 3 inches in length. Its inflorescence is 2 to 3 inches long, and the spikelets are solitary and 1·8 to 2·5 mm. long. The loosely-tufted culms are erect or ascending, being one to two feet high. Large numbers of *M. uniformis* larvae attached instantly to the main roots, and many also attached to the rootlets (Fig. 16 and 20). A cross-section of a main root (Fig. 21) shows once again the numerous air chambers within a grass root, 25 in this case, and the usual secondary air chambers. The epidermal and cortical layers appear to be narrower than those of the two preceding grasses, possibly accounting for the large number of ready attachments because of ease of penetrability.

7. *CHARA WALLICHII* BRAUN, A STONEWORT (FAMILY CHAROPHYCEAE).

This is an erect plant having a cylindrical main axis and whorls of 5 branches arising at the nodes (Fig. 25), anchored by means of rhizoids. It grows to a height of up to one metre. Under the microscope the plant appears to be striated, and careful examination reveals many small, discoidal chloroplasts lying in parallel, longitudinal, spirally twisted rows. These oxygen-rich chloroplasts are embedded in the layer of cytoplasm which lies between the corticated cell wall and the large central vacuole. This cytoplasm revolves continuously and longitudinally, down one side and up the other. At the nodes there is no vacuole, but only a layer of cells forming a diaphragm between elongated cells. No air-containing chambers were present, yet *M. uniformis* larvae attached themselves to the nodes and branches, embedding the siphon into the layer of chloroplasts from which they can apparently obtain oxygen. The attachments were therefore shallow, as seen in Figure 26, this being magnified in Figure 27. In some cases the larvae remained attached in this manner for at least 8 hours.

8. *CERATOPHYLLUM DEMERSUM* LINN., HORNWORT (FAMILY CERATOPHYLLACEAE).

This perennial, submerged, rootless, aquatic plant with a weak stem, can float due to the many oxygen bubbles which are formed at the base of the leaves about $\frac{1}{8}$ inch from the main stem. These leaves are dichotomously cleft several times, terminating in filiform, toothed, fine prolongations which are needle-like. There

are 7 to 8 branches around the stem at a node. The fruit, an achene, is small, hard, dark-brown, compressed, and leathery, with two long thorn-like spines $\frac{3}{8}$ to $\frac{1}{2}$ inch long attached at one end, and a third shorter spine, the persistent style, 1/16 inch long, at the other. It is attached at intervals at the node, along with the regular branches. The plant can grow to eight feet in length. The leaves are one inch in length (Fig. 29).

Contrary to what one would assume, very few attachments were made by *M. uniformis* larvae to the dichotomous portion containing oxygen bubbles. Instead, attachments were noted to the main stem, branches, and at the nodes (Fig. 31 and 32), the larvae remaining in place for many hours. A cross-section through the main stem shows a *Pistia*-like structure (Fig. 30), there being 15 clear-cut air chambers inside a wide cortical area. More attachments were made by M. *uniformis* when the *Ceratophyllum* leaves were closer to the bottom of the vessel. When *M. annulifera* larvae were introduced, instead, they effected more attachments to the floating portions than did *M. uniformis*.

9. *WOLFFIA ARRHIZA* WIMM, (FAMILY LEMNACEAE).

This is the simplest and smallest of the flowering plants. The tiny grain-like fronds, about $\frac{1}{4}$ mm. to one mm. long, are rootless, subglobose or ovate, and cover the surface of the water as a thick bright green film, appearing to be a thick scum (Fig. 33). Solitary, sessile daughter fronds originate from a cleft or funnel-shaped opening at the base, and soon become detached (Fig. 34). The flowers are central and burst through the upper surface of the frond.

When the cushiony nature of these fronds was seen, a similarity was noted to that of *Azolla* and it was suspected that first-instar *Mansonioides* larvae would be able to penetrate into the oxygen-filled interior. This supposition was borne out when first-instar *M. uniformis* larvae were seen to attach to the fronds, remaining so attached for hours (Fig. 35). In this figure the entire terminal, black cylindrical portion of the siphon is seen to be inserted into the frond.

10. *SALVINIA AURICULATA* AUBLET, A WATER FERN, (FAMILY SALVINIACEAE).

The floating stem of this annual or perennial aquatic herb gives off short-petioled or sessile leaves or fronds mostly in close pairs with the papillose surfaces of each pair facing each other. The leaves, which measure about one inch in either direction, are rounded-triangular, and vertical or suberect, some floating horizontally, but most being subaerial with the proximal portions under the water surface. When the two leaves of a pair are removed as a unit, they can be opened and spread out like the wings of a butterfly. Their upper surface is very papillose (Fig. 37), the papillae increasing in size to several millimetres in the centre of the leaf, where they are very thin and more separated. The under side is also soft, with very fine parallel veinlets having finer connecting veinlets. The interior of the leaf is quite spongy with loose, air-containing tissue. The tightly

packed leaves are so numerous as to form a complete mat across the water's surface in the same manner as *Pistia* (Fig. 36). This plant was found in a round fountain-tank at Maharaja's College in Ernakulam, Kerala, and has been reported to occur in Shertallay, Kerala, during the rainy season.

The most unusual feature of this plant is what is ordinarily taken to be roots. *Salvinia* actually has no roots. Botanically the so-called "roots", resembling those of *Pistia* or *Eichhornia* except for the fine, delicate "rootlets", are actually modified leaves which are arranged in brown fascicles, each of which is attached to a short, vertical stem that has branched downwards from the floating, horizontal stem (Fig. 40). The cross-section of the root-like structures (Fig. 39) somewhat resembles the other cross-sections described herein in that there are large air chambers (9 in the figure shown), and central conducting tissue, but only one layer of cells peripherally. This single layer accounts for the fact that *Mansonia uniformis* larvae, which on other plants are very tenaciously attached, do not have as firm a grasp on the root-like structures, as there is no bulk of tissue into which the terminal hooks can be sunk deeply. This is the reason why after attaching to the root-like structures for a while, both *M. uniformis* and *M. annulifera* leave their positions (Fig. 40) and swim up to the floating, suberect leaves or the floating, horizontal leaves, and attach either to the under surface or to the papillose upper surface of the leaves (Fig. 38 and 42). Wherever they are attached the terminal black, valvular portion of the siphon is sunk deeply into the tissues (Fig. 41) in order to maintain a firm hold in the absence of solid tissues for embedding the hooks; however, once attached, whether to modified leaves or to the regular leaves (Fig. 38), the larvae and pupae remain for very long periods of time effecting gaseous exchange, especially when attached to the floating leaves.

In Ceylon this plant covers acres of water surface area, said to have been introduced into that country in order to hide the waterways by total green coverage, thus confusing enemy pilots during World War II. Since *Mansonioides* readily lays its egg masses on *Salvinia* in Ceylon, this plant is of considerable epidemiological importance there. It is intended to seek out *Salvinia* in Kerala wherever it occurs, especially during the monsoon season, and check for the prevalence of *Mansonioides* egg masses. Two other species, *S. natans* Hoffm. and *S. cucullata* Roxb, are said to occur throughout India.

No *Mansonioides* egg masses were found on the *Salvinia auriculata* plants in the fountain-tank in Ernakulam, probably because the closest *Mansonioides* breeding place is at least one mile away. In order to determine the position of laid egg masses, *M. annulifera* females, which had been fed on rabbit blood, were kept in a cage with *Salvinia*, and laid their egg masses four days later (Fig. 43). They were laid at the edge of both the upper and lower surfaces, just below the water line, as has been found in *Pistia*. Due to the vertical position of the leaf, the mosquito has to hold its body either vertically just above the place where the egg mass is being laid, or perhaps it may stand on the opposite side of the leaf and bend its abdomen around the edge of the leaf at an angle. For some reason,

possibly because of the spongy nature of the *Salvinia* leaf, the attached egg mass is not stuck as firmly to the leaf as on smoother leaves such as those of *Pistia*, *Eichhornia*, *Lemna*, and others. Slight handling of the *Salvinia* leaves may jar the egg masses loose from the cement by which they are attached to the leaf.

SUMMARY.

Continuing the study of attachments of *Mansonia uniformis* aquatic stages to host plants occurring in *Pistia* tanks in Kerala State, South India, it was found that the larvae attached readily to the roots and rootlets of three grasses, *Leersia hexandra*, *Pseudechinolaena polystachya*, and *Brachiaria eruciformis*, as well as to the roots of the leafy, aquatic herb *Colocasia antiquorum*. First-instar larvae, being very small, were seen to attach themselves to fine roots of *Lemna polyrrhiza*, and to fronds of the minute flowering plant *Wolffia arrhiza*. Older larvae attached to the nodes, and branches especially, of the stonewort *Chara wallichii* and the hornwort *Ceratophyllum demersum*. It was established, after months of observation that, attachment to leaves is a *normal*, and not an unusual form of attachment, as demonstrated in *Pistia stratiotes* and *Salvinia auriculata*, as an alternative to the root type of attachment for obtaining oxygen and giving up carbon dioxide. Forty-three photographs by the author illustrate in detail the nature of the plants and the attachments described.

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Indian Journal of Malariology, **14**, 2, *June 1960*.

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

IV. OBSERVATIONS ON LARVIVOROUS ACTIVITIES OF VARIOUS FISHES IN FILARIAL AREAS OF KERALA STATE, SOUTH INDIA.

BY

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[March 3, 1960].

WHILE conducting surveys for *Mansonioides* larvae and pupae in tanks or ponds containing water lettuce (*Pistia stratiotes*) and water hyacinth (*Eichhornia speciosa*) in Kerala State, South India, numerous fishes were seen swimming in the tanks, even in those which had considerable breeding of *Mansonia annulifera* and *M. uniformis*. The question then arose as to the feeding habits of the various fishes, not only on free-swimming larvae and pupae, but also on those which were attached and apparently motionless.

Fishes were seen in practically every tank from which *Mansonioides* was obtained. Since they tend to react to motion, it stood to reason that they would attack free-swimming larvae, which are worm-like in general shape. Questioning of householders revealed that there were numerous local names for the various fishes in the tanks, indicating that quite a few species were involved. Since a major part of the life of larvae and pupae of *Mansonioides* consists in being attached to roots or leaves for the purpose of effecting gaseous exchange within the air chambers therein contained, the determination of those fishes which would regularly attack such attached forms was deemed to be of primary importance because of their vulnerability. Also, since *Culex fatigans* is the primary vector of filariasis in India, it was considered useful to determine those fishes which regularly and readily ate this species also. One would think that any fish which would eat *Mansonioides* larvae and pupae while swimming, would also eat *Culex fatigans* larvae and pupae, unless the species had an objectionable taste.

This study covers observations on 14 species of fishes taken from tanks and ditches, plus *Gambusia affinis* which had been brought from a stocked tank at the Malaria Institute of India, Delhi. The 14 species included : *Macropodus cupanus, Ophicephalus gachua, Ophicephalus striatus, Tilapia mossambica, Puntius vittatus, Amblypharyngodon melettinus, Aplocheilus lineatus, Mystus tengara, Anabas testudineus, Etroplus maculatus, Etroplus suratensis, Chanda (Ambassis) dayi, Glossobius giuris*, and *Megalops cyprinoides*. Identifications were made by consulting Hora and

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

Mukerji (1953), Axelrod and Schultz (1955), and Day (1958), and where identifications were not certain the specimens were sent to the Central Inland Fisheries Research Station, Barrackpore near Calcutta, for accurate determinations of species.

The word "larvivorous' is usually used in connection with the eating of larvae, but in this study it was also considered useful to determine whether the fishes involved would also eat pupae, especially in view of the chitinized, hard exoskeleton of the latter as compared to the soft bodies of the larvae. In *Mansonioides* the pupae were practically always attached, except for the brief interval just prior to the emergence of the adult, whereas in *C. fatigans* the pupae were usually quite active and offered motile targets to the fishes.

One of the accepted means of biological control of mosquito larvae, particularly those of anophelines which rest primarily on the surface, is by stocking the pond, well, ditch, or other water area with the top-feeding minnow *Gambusia affinis*, or other known larvivorous fish. The usefulness of this method is generally confined to artificial or ornamental pools, or in wells under certain circumstances.

Since *Gambusia affinis* was available in Delhi, it was decided to compare its larvivorous habits with those of fishes which occur naturally in the tanks and ditches in Kerala, particularly in the area around Cochin and twenty-five miles south to Shertallay and Alleppey. When it was discovered that various stagnant ditches in Mattanchery and Fort Cochin had fishes in them, their feeding habits on *Culex fatigans* were taken into account. A number of the fishes occurring in the odoriferous, stagnant ditches also occurred in the relatively clean water of tanks or ponds, showing that an adaptation had occurred to the various water conditions. The observations recorded here were made over a period of four months, from October, 1959 through February, 1960. Due to difference in strains of the fishes, and to local breeding conditions, it is entirely possible that the larvivorous habits of the various fishes considered herein may be different in other countries or in other parts of the same country. For instance the Indian top-feeding minnow, *Aplocheilus lineatus*, has been recorded as being of importance against mosquito larvae in the past [Hora and Mukerji (1953), p. 40], but in the present study this fish was one of the least important, eating only those larvae which were in its feeding line of direction, and never deviating from this habit, whether in the tanks, or in the laboratory. Some younger specimens, however, appeared to be more active, especially, where vegetation was sparse in the tank.

Due to the fact that a *Pistia*- or *Eichhornia*-covered tank does not ordinarily permit one to observe the exact details of larvivorous habits of fishes, particularly when the specimens are attached to the roots beneath the leaves, many of the observations were made in large glass containers in which the fish had been kept for some time to acclimate them to the container, or in large galvanized buckets, and in the field where possible. The fishes were photographed in a rectangular, clean glass jar, using an Edixa (German) 35 mm. camera having a Takumar (Japanese) f. 2·2, 55 mm. lens mounted on extension rings. Most of the pictures were taken at a speed of 1/500 second in sunlight. These photographs support the statements made

PLATE I.

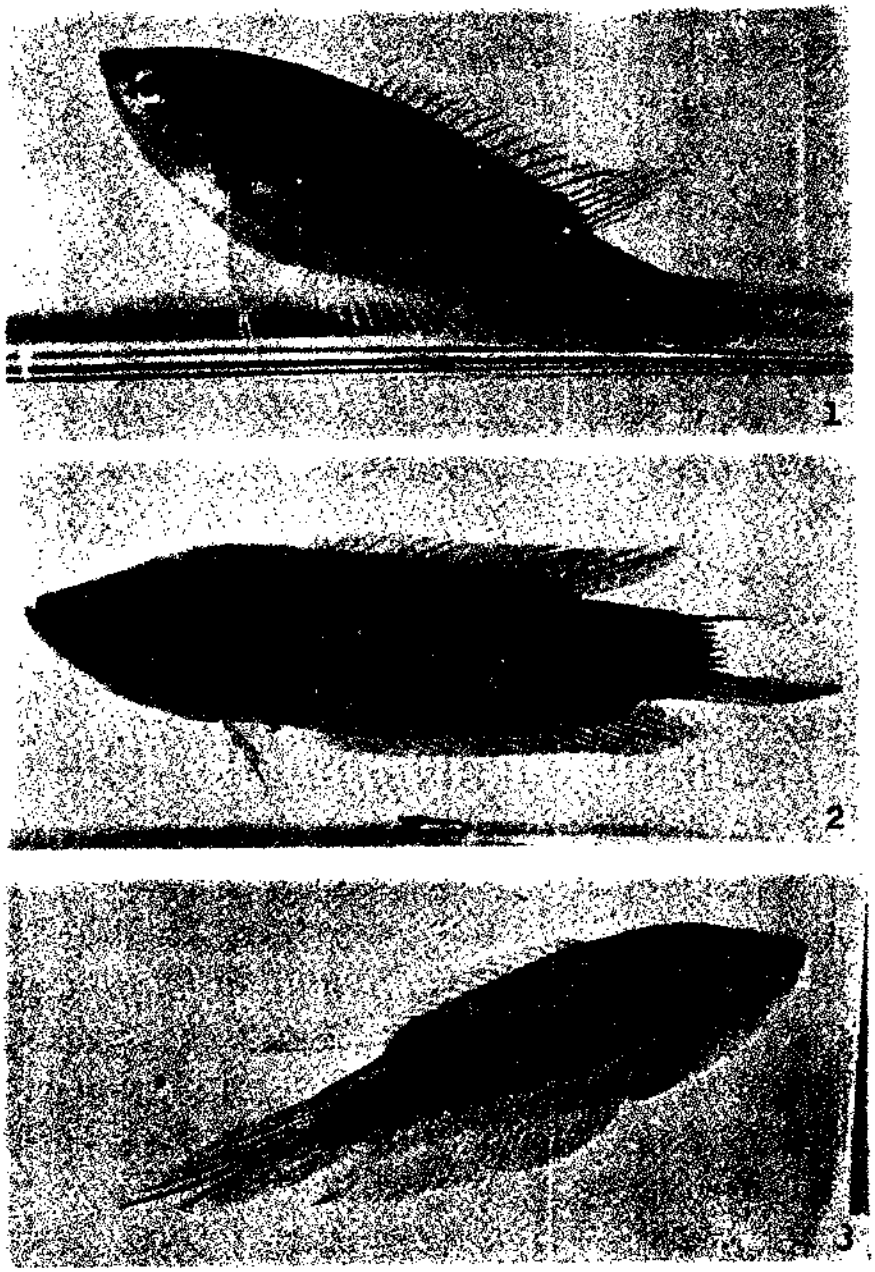


FIG. 1. Light-coloured form of the spike-tailed paradise fish, *Macropodus cupanus*. (×4)

FIG. 2. *Macropodus cupanus*, black form. (×4)

FIG. 3. *Macropodus cupanus*, variety with two long parallel, black horizontal bars. (×4)

PLATE 9

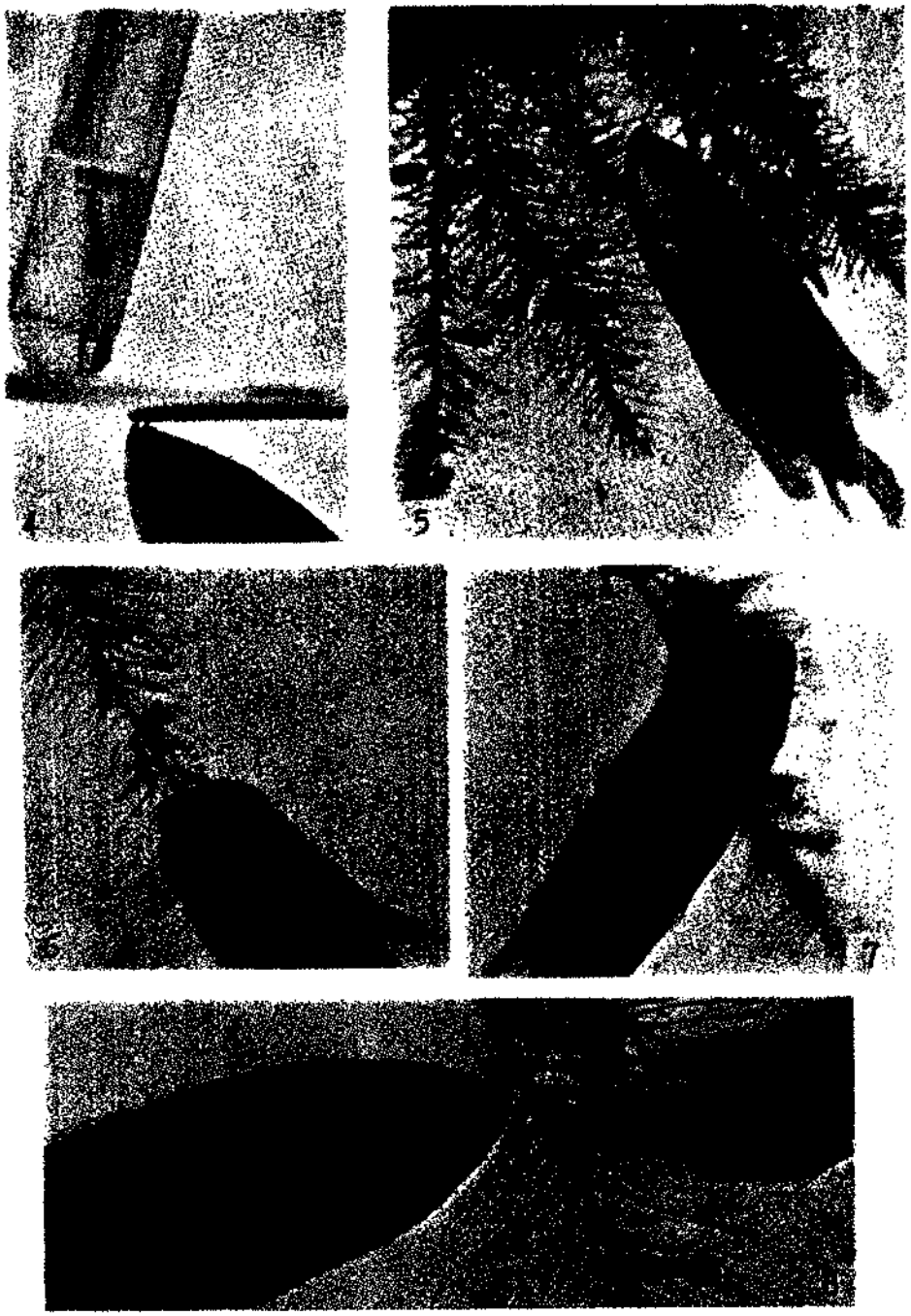


FIG. 4. *Macropodus cupanus* awaiting release of *M. uniformis* larva from pipette.
FIG. 5. *Macropodus cupanus* eyeing attached *M. uniformis* larva.
FIG. 6, 7 and 8. *Macropodus cupanus* eating *M. uniformis* larvae which are attached to main roots.

concerning larvivorous habits of the fishes described, especially the eating of the attached larvae and pupae. Every fish listed has been photographed so as to show its chief characteristics, for comparison with the descriptions furnished.

Since the reactions of the fishes tended to be constant at each feeding, the observations recorded here are valid enough to give an indication as to the relative larvivorous usefulness of the different fishes. A sequel to this study might involve a detailed study of the stomach contents of the fish in order to gauge the relative percentages of the different types of food ingested.

Each fish studied has been described below separately, after first giving its classification based primarily on Axelrod and Schultz (1955).

MACROPODUS CUPANUS (CUVIER AND VALENCIENNES), SPIKE-TAILED PARADISE FISH (FAMILY ANABANTIDAE).

This fish was found to be the most larvivorous one tested. Because of its variable colouration it was thought at first that several different species were involved. It appears variously as yellowish-brown, dark-brown, greenish-brown, and even black, with a single more or less definite longitudinal line laterally, or no line at all, or else two very dark or black lines. The outstanding colour manifestation is seen in the bright red pelvic fins, but sometimes the colour is pink. The tail is drawn out to a fine point, hence the name "spike-tailed." The dorsal and anal fins, often aquamarine in colour, are very long, generally more than half the length of the body excluding the tail. It grows to three inches. The male is more colourful than the female and has a longer and more pointed dorsal fin.

The Malayalam names for the variously coloured forms found along the Malabar coast are: *karup* when the fish is very light in colour (Fig. 1 and 17), *karinganah* when the colour is very dark (Fig. 2), and *karinganachi* when the variety is one with bright red, colourful fins and two very strong, black, lateral lines (Fig. 3). The eyes usually are red peripherally, with a tinge of fluorescence behind. There is a large black spot at the base of the tail. During the breeding season the dorsal and anal fins are sprinkled with tiny red dots. The body may be smoky, with a coppery and somewhat bluish reflection.

This fish was found in very large numbers in *Pistia* tanks and in stagnant drains, therefore it can live both in clean and dirty water. It is very hardy, some specimens still being alive in the laboratory after four months in large glass jars containing green algae. Many of the specimens caught had the tails partially or totally chewed away (Fig. 2), and in several cases the entire posterior part of the body was missing (Fig. 17) but the fishes still swam about and fed on larvae.

The readiness with which *Macropodus cupanus* eats attached *Mansonioides* larvae and pupae is shown in Fig. 5, 6, 7, 8, and 30. Just as soon as every swimming larva has been eaten, the fish swims around for a while, looks at the attached, motionless larvae and pupae, then bores in rapidly, picking them off one by one. Some of these fish were recorded to eat over 50 such attached forms at one feeding,

including the one with the posterior part of the body gone, the latter also eating over 75 free-swimming larvae at one feeding. It is interesting to note that these fishes can react to a conditioned reflex. After it realizes that the food is dropped from a pipette held just over the surface of the water, the fish waits patiently just below the pipette for its next meal (Fig. 4), swallowing it swiftly in one gulp.

Since *Macropodus* attacks and eats both free-swimming and attached *Mansonioides* larvae and pupae, and those of *Culex fatigans* just as quickly (Fig. 29), it should be seriously considered for breeding and stocking of tanks and ditches, especially in view of its ability to survive in dirty, odoriferous water. It has not been seen to eat any fishes which are also larvivorous, apparently just chewing at each other's tails.

OPHICEPHALUS GACHUA HAMILTON, YOUNG, A MURREL (FAMILY CHANNIDAE : SERPENT-HEADED FISHES).

The second best larvivorous fish was found to be the murrel, *Ophicephalus gachua*, which possesses suprabranchial cavities or accessory respiratory devices for aerial respiration. In this fish the walls of the chamber above the gills are rich in capillaries, and consequently gaseous exchange can occur directly when the fish is out of the water. It can travel for some distance on land, aided chiefly by the pectoral and caudal fins. If the aquarium jar or tank is not covered, this fish jumps out easily and later on may be found under some article of furniture, or in a corridor, or even attempting to scale a wall. Its Malayalam name is *vattan*. It grows to a length of 13 inches.

The resemblance of the head of *Ophicephalus* to that of a snake can be seen in Fig. 9 and 14. The fish is generally dark-brown in colour. The dorsal and anal fins are very long and are margined with orange. When on land the pectoral fins are squeezed together so as to become linear or leg-like, thus aiding in terrestrial locomotion, advancing in short hops or jerks with the aid of the tail. In the posterior part of the dorsal fin in young specimens there is a black spot or ocellus within an orange circle. The tail is rounded and has an orange margin. A pair of short, fleshy barbels protrude above the mouth. The dorsal and anal fins have no spines, and the spineless pelvic fins are six-rayed.

The rapidity with which this fish attacks *Mansonioides* larvae and pupae, especially those that are attached, is shown in Fig. 13 and 14. Like *Macropodus cupanus* it first eats swimming larvae in one gulp ; then, seeing the attached forms it swiftly attacks and swallows them one by one, and can deplete a *Pistia* or *Eichhornia* plant of larvae or pupae within a half-hour to an hour. As with *Macropodus*, one gets the impression that the fish *sees*, rather than smells the attached forms.

The chief disadvantage of the murrel is that it eats many other fish which themselves may be larvivorous. Its habit of leaving the water whenever it wishes in order to travel on land to some other water area, is also a disadvantage, for one could not then depend on this fish to rid a particular infested place of mosquito

PLATE III.

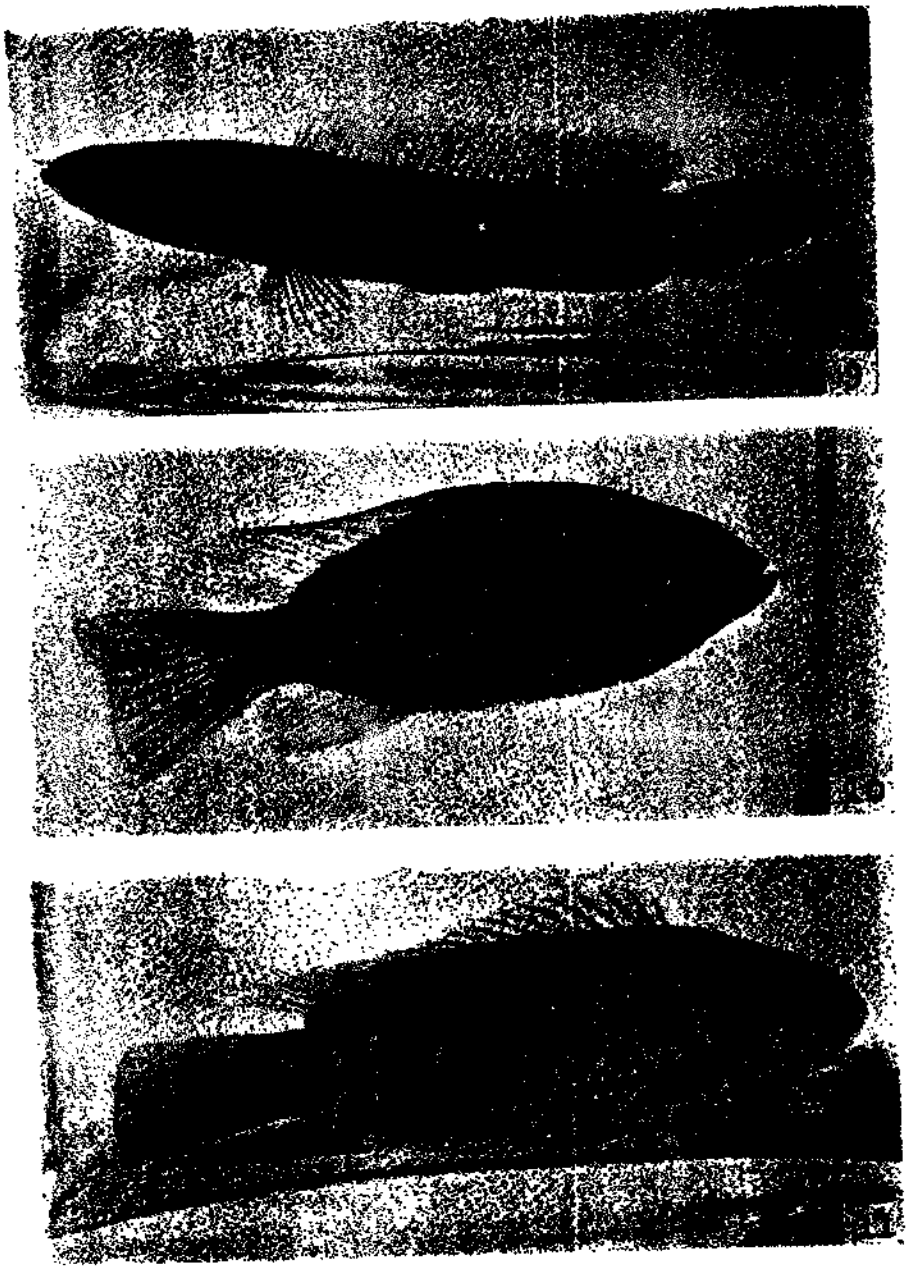
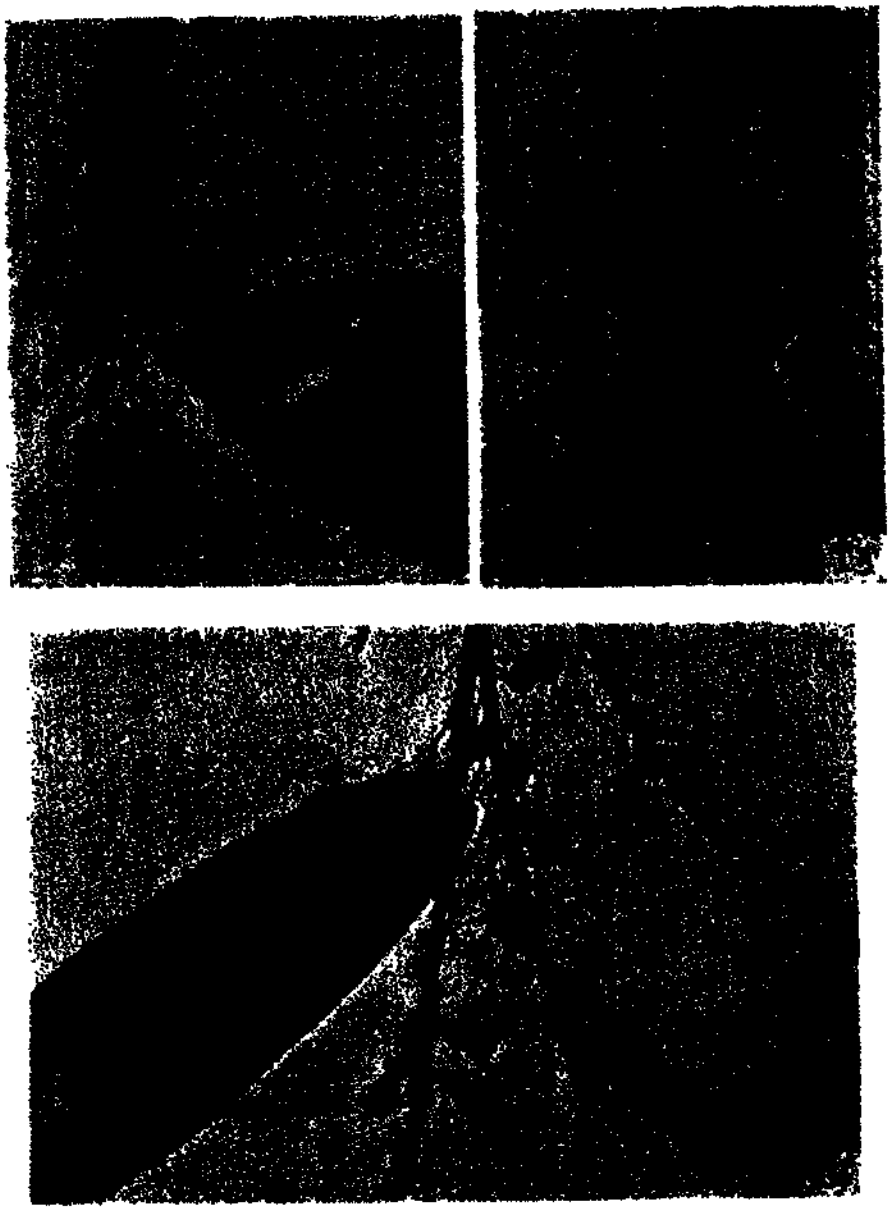


FIG. 9. The marrel, *Ophiocephalus gachua*. (Original size.)
FIG. 10. The Mozambique mouthbreeder, *Tilapia mossambica*. (Original size.)
FIG. 11. The walking or climbing fish, *Anabas testudineus*. (Original size.)

PLATE IV.



[illegible] *Aplocheilus lineatus* about to eat attached *M. uniformis* larva.

[illegible] *Ophiocephalus striatus* about to eat attached *M. uniformis* larva.

larvae indefinitely. It can live in stagnant, odoriferous water quite easily, and attacks *Culex fatigans* larvae and pupae with the same readiness that it attacks *Mansonia* aquatic stages.

OPHICEPHALUS STRIATUS BLOCH, A MURREL (FAMILY CHANNIDAE : SERPENT-HEADED FISHES).

This fish, similar to but longer than the preceding one, is also found in tanks and drains in Kerala, and its feeding and living habits are similar to those of *Ophicephalus gachua*. It likewise can live out of water for some time, and is also predaceous on other larvivorous fish. Its Malayalam name is pronounced *verarl* or *verahrl*. The dark-grey or blackish colour of this fish is extended ventrally into stripes which are easily visible against the white abdomen (Fig. 42). The posterior end of the dorsal fin has some spots and bars, and as in the preceding species the young may have an ocellus or eye spot at the end of the dorsal fin. Considerably larger than *O. gachua*, this species may grow to three feet or longer, presenting a formidable appearance. It is also found in swamps and paddy fields.

TILAPIA MOSSAMBICA (PETERS), MOZAMBIQUE MOUTHBREEDER (FAMILY CICHLIDAE).

Known in Kerala as *tilopia* and *filopia*, this fish was introduced into India from East Atrica, and can be found in many tanks. It grows to a length of 6 inches. Its body appears pale in sunlight, but looks dark-speckled and even striped indoors or in the shade, due to the indistinct black lateral bar (Fig. 10). The outer edge of the operculum has a black spot, above which is a coppery spot. The upper lip is fringed with a blue fluorescence. The eye has a thin gold ring and a gold spot above. The specimens caught in Cochin and Shertallay did not have the wide orange band on the tail which is characteristic of the male ordinarily.

This fish is called a mouthbreeder because at breeding time the male and female lock jaws on each other's lips, this being a means of selecting a mate. After some days the male fertilizes the eggs which the female has dropped, and picks them up in his mouth for incubation until they hatch. The lips of this fish are very thick, and the mouth opens and closes incessantly.

Tilapia attacks swimming and attached *Mansonia* or *C. fatigans* larvae and pupae readily when hungry (Fig. 12, 22 and 26), and does not feed on any other fish. It can be considered as a useful larvivorous fish, but was not as predaceous as the preceeding three species.

GAMBUSIA AFFINIS AFFINIS (BAIRD AND GIRARD), SPOTTED GAMBUSIA, TOP-MINNOW, MOSQUITO FISH (FAMILY POECILIIDAE : LIVE-BEARING TOOTHED CARPS).

This fish has for many years been considered to be an outstanding larvivorous fish, particularly against anopheline larvae. The specimens studied here were not found in Kerala, but were brought from a stocked tank at the Malaria Institute in Delhi to compare with naturally-occurring fish. Although many specimens were

studied under different conditions, even when starving this fish was not as larvivorous against *Mansonioides* or *Culex fatigans* as any of the preceding fish. It was not seen to eat any attached forms or any pupae, and as far as surface-resting or free-swimming larvae were concerned *Gambusia* was not found to be a ready feeder. It was found over and over again that the larger *Gambusia* fed less readily than the medium-sized or smaller ones, and that when many larvae were introduced into jars or buckets containing starving *Gambusia*, only a few out of each dozen fish would eat the larvae readily. Also, this fish did not especially remain at the surface, even when kept in a well ; it spent most of its time below the surface.

Gambusia grows to a length of 2½ inches. The dorsal and caudal fins have fine, black dots, and the rays are forked (Fig. 18). The mouth has a pouty appearance, with a suggestion of a snout. The body is brownish-green, the belly pale yellowish-white, the operculum has a prominent green spot, and the ventral part of the head and the scales surrounding the eye exhibit some fluorescene.

While photographing *Gambusia* in a jar out in the sun, all of them began to die as soon as the temperature of the water reached 41 degrees Centrigrade. This did not occur with any other fish under the same conditions, despite the heated water. Perhaps the fact that the *Gambusia* tested is not native to the hot climate of Kerala, has something to do with this inability to stand heated water; although in Delhi, where this fish is stocked, the temperatures during May and June far exceed those of Kerala during the year.

PUNTIUS VITTATUS (DAY), A BARB (FAMILY CYPRINIDAE).

This fish, called *kaipoo* or *kaipu* in the Malayalam language, grows to two inches. It is a highly nervous, darting, active fish which is in motion most of the time. The body has a silvery to pale golden sheen, the upper third being brownish-green. There is a large black spot at the base of the tail (Fig. 19). The dorsal fin has an orange forward edge and a black stripe which tends to be somewhat V-shaped. There are some tiny black dots on the sides. The tail is deeply forked.

These small fishes were seen to feed on swimming *Mansonioides* and *C. fatigans* larvae which happened to be nearby, but they did not actively chase the larvae from a distance. Fig. 31 shows this fish getting ready to feed on some *C. fatigans* larvae, but not very aggressively. It was not seen to ingest any pupae. Because of its own size it preferred larvae up to the third-instar in size. It was also found in dirty, stagnant drains in Mattanchery, indicating that it can live under various conditions of pollution.

AMBYLPHARYNGODON MELETTINUS GUNTHER (FAMILY CYPRINIDAE).

The water lettuce tanks in Palluruthy in the Cochin area are rather heavily stocked with the young of this fish, ranging in size from ½ to ¾ inch. Its Malayalam name is *nandan podi*. The body is greenish above and greenish bluish-silvery below, and is somewhat transparent, showing some of the internal organs

PLATE V.

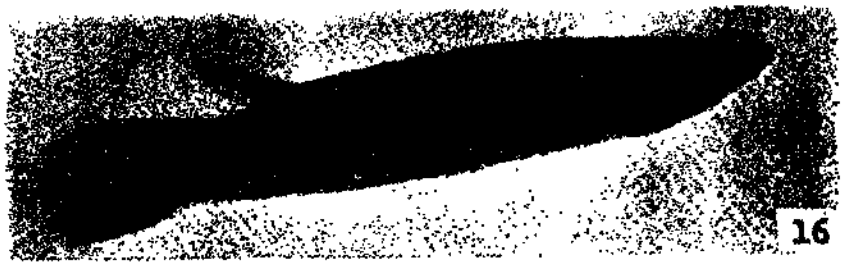


Fig. 15. *Ophiocephalus gachua* eating *M. malinus* pupae which are attached high up on [illegible]
Fig. 16. Male of the Indian top-minnow, *Aplocheilus lineatus* or striped panchax. [illegible]

PLATE VI.

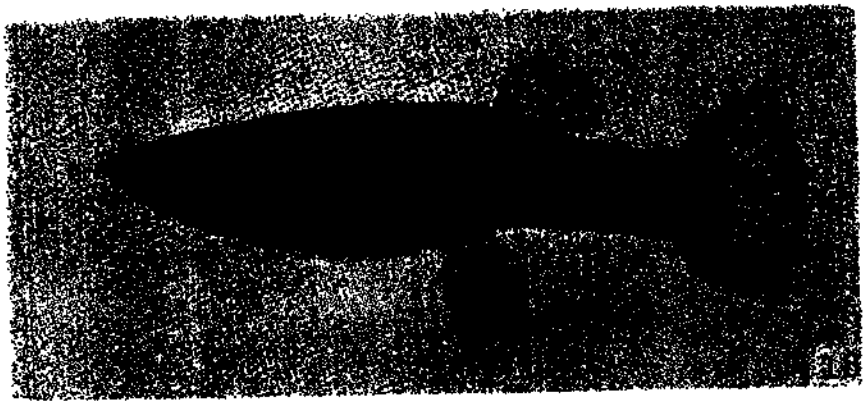


FIG. 17. *Macrognathus aculeatus* with tail eaten away by its own larva. (×4)

FIG. 18. The top-minnow, *Gambusia affinis affinis* (female). (×4)

FIG. 19. The barb, *Puntius vittatus*. (×7)

readily. The abdomen is rounded, the scales are small, the lateral line is incomplete, and there is no upper lip. A bright greenish-yellow band separates the dorsal and ventral colorations. The tail is deeply emarginate or forked.

These small fish attempted to attack third-and fourth-instar *Mansonioides* and *Culex fatigans* larvae very aggressively, but could not swallow them because of the small mouth ; however, first- and second-instar larvae are readily chased after and rapidly ingested. It is probable that this small fish, because of its huge numbers in the tanks, consumes millions of first- and second-instar larvae. As might be expected, it is, however, easily and readily eaten by various other fish, particularly by the murrels *Ophicephalus gachua* and *O. striatus*.

APLOCHEILUS LINEATUS (CUVIER AND VALENCIENNES), INDIAN TOP-MINNOW, STRIPED PANCHAX (FAMILY CYPRINODONTIDAE ; TOP-MINNOWS).

Known in Kerala as *poonjan*, this fish is easily identified by its habit of remaining almost constantly at the surface with the body at an angle and the head quite prominent, and by the very obvious silvery-white occipital spot on top of the flattened head. It grows to four inches in length. The whitish spot is sometimes rubbed off in older specimens. The female is usually darker than the male, with 8 to 10 vertical bars (Fig. 23), whereas the male has 5 to 6 bars (Fig. 16). The dorsal and anal fins are more pointed in the male. The female has a dark spot at the base of the dorsal and anal fins ; the latter, and the tail, are bordered with yellow and red. The body is generally greenish with a purplish-blue fluorescence. There is a golden green spot in the centre of each scale and on the eye. The young are brilliantly coloured ; the body is golden with a lateral reddish fine line, and there is a yellow tail with bright reddish-orange markings.

Although *Aplocheilus lineatus* is a top-feeding minnow, this term was found to be somewhat misleading as regards feeding on mosquito larvae. Hundreds of these specimens were observed in the tanks and in buckets in the laboratory, and only on a few occasions did they eat mosquito larvae, and only when the larvae happened to be directly in the direction of feeding (Fig. 27). In not a single case did this fish chase any larva. Apparently it is content merely to ingest whatever is in its line of feeding, whether it be plankton, algae, or anything else. It is realized that this fish has been reported in the past as being larvivorous, but as far as *Mansonioides* and *Culex fatigans* larvae are concerned, based on present observations, this fish can only be considered as unimportant.

MYSTUS TENGARA (HAMILTON) YOUNG, A CATFISH (FAMILY MYSTIDAE).

This catfish was found, along with many other species of fish, in a stagnant drain in the Chullikel area, Ward Number 26, in Mattanchery (Fig. 33). It is called *koori* in Malayalam. It grows to eight inches. The body is silvery gray, darker dorsally. The high dorsal fin has an anterior spine, and the tail is deeply

forked. There are four pairs of barbels around the mouth : short nasals, very long maxillaries, and the inner and outer mandibulars. This fish tended to lie quietly on the bottom, the mouth opening and closing rapidly, but occasionally it went to the surface. It drew in, by its mouth movements, *Culex fatigans* larvae which happened to swim in front of it, but it did not pursue any larvae or show any interest in them when far away (Fig. 34).

ANABAS TESTUDINEUS BLOCH, WALKING FISH, CLIMBING FISH (FAMILY ANABANTIDAE).

This fish was present in large numbers in water hyacinth and water lettuce tanks in Ernakulam, particularly in the former. Its local name is *karup*, a term applied to several other fish. Like *Ophicephalus* it possesses accessory respiratory devices for direct ærial respiration in the form of a suprabranchial cavity containing a special labyrinthform organ. The latter consists of thin, bony, concentric plates covered with a very vascular mucous membrane for direct absorption of atmospheric oxygen. *Anabas* can live out of the water and travel on land for long periods, having well-developed aerial vision, and can even climb trees. It grows to a length of ten inches.

When young the colour is light-brown, silvery below, with large coarse scales, and 6 to 8 blackish, vertical stripes (Fig. 11). The eyes are orange-ringed and there is a faint reddish colouration on top of the head. The operculum bears a black spot posteriorly. The tail end is rather vertical. The dorsal fins of the male are more pointed than in the female.

This fish rests on the bottom with its mouth closed most of the time. It was observed to ingest only a few swimming *Mansonioides* and *C. fatigans* larvae which swam in front of it. Like *Ophicephalus*, its habits of being predaceous on other fish and leaving its habitat at will makes its usefulness as a larvivorous fish very limited on these factors alone. Its walking movement on land is very jerky, supporting itself with its paired fins while the tail is swirled laterally. It is often eaten by the local people.

ETROPLUS MACULATUS (BLOCH), ORANGE CHROMIDE (FAMILY CICHLIDAE).

This fish appears in Kerala under several colourations : a darker, more striped form known in Malayalam as *karimeen*, and a lighter and more dotted form called *pallathy* or *pullattay*. The younger stages, $\frac{3}{4}$ to $\frac{7}{8}$ inch long, were found in the wide, stagnant ditch in Mattanchery pictured in Fig. 33. The adults were found in the brackish backwaters in Palluruthy. The young are greenish-yellow and resemble the adults in their markings, but the prominent rows of bright orange dots are not apparent. Evidently it is difficult to separate the sexes due to similarities in markings, but the male is said to have a redder eye and more intense colouration.

The general body colouration of the adults is yellowish with a green back. The more striped form (Fig. 39) has three clear blackish stripes transversely,

PLATE VII

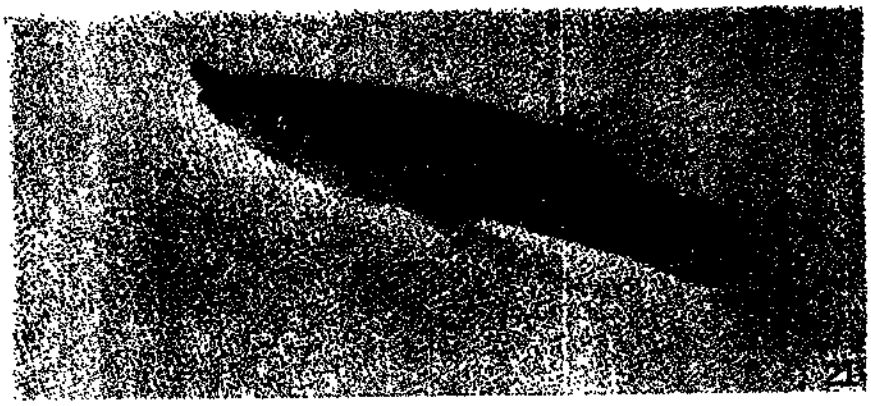
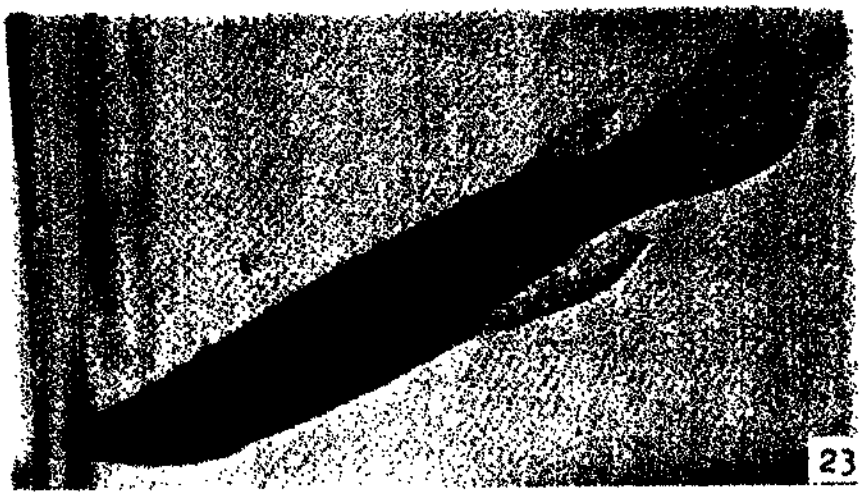


FIG. 20 and 21. *Gambusia affinis* eating a larva of *M. uniformis*.
FIG. 22. *Tilapia mossambica* about to eat *M. uniformis* larvae.

PLATE VIII.



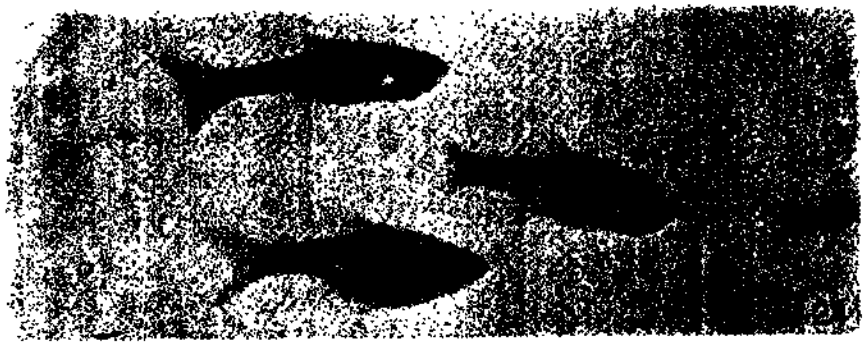


Fig. 23. Female of Indian Sapo [illegible] *Ophiocephalus striatus*. (×4·5)
Fig. 24. Young of *Amblypharyngodon melettinus*. (×4)
Fig. 25. Young of *Chanda* (*Ambassis*) *dayi*. (×4)
Fig. 26. *Tilapia mossambica* eating attached *M. uniformis* larva.

which are enlarged into dots along the lateral line, the middle dot being the most prominent. There are weaker stripes anteriorly and posteriorly. Apparently food and light conditions produce the variations in background body colour and striping effects as seen in the Cochin area. The tail is black at the base, yellow posteriorly, and is slightly forked. The dorsal fin is long, extending from the operculum opening to the base of the tail. The ventral or anal fin is $\frac{2}{3}$ to $\frac{3}{4}$ the length of the dorsal. The pelvic fins are black, the dorsal fin is smoky, and the anal fin is black and yellow. There is a blue fluorescence around the eye and a bluish horizontal stripe in the middle of the operculum. This fish is called the orange chromide because of the rows of bright golden-orange rather evenly spaced dots, each in the centre of a scale, which cover the body lengthwise from the tail to the pectoral fins, there being approximately 17 such rows extending from the dorsal fin to the abdomen.

The lighter form (Fig. 40) has the same rows of bright orange dots. There are three strong blackish-blue dots with weaker ones anteriorly and posteriorly. As in the more striped form, the central dot is the largest. This tail is edged with orange posteriorly, the dorsal fin has dark dots, and the anal fin is edged with yellow. There is a faint blue fluoresence below the eye. The operculum has two faint pale blue fluorescent horizontal stripes.

This fish grows to about 3 inches long and $1\frac{3}{8}$ inches wide. The female is slightly smaller than the male.

E. maculatus was observed to eat *Culex fatigans* larvae readily, chasing them until they were caught, particularly the younger fish (Fig. 28). It survives transportation very badly, often dying within one hour after being collected in the adult stage ; however, any of the younger forms which live after the first day of captivity generally live for some days.

ETROPLUS SURATENSIS BLOCH, GREEN CHROMIDE (FAMILY CICHLIDAE).

This fish has the same shape as *E. maculatus*. The body is smoky in colour, with a dark greenish tinge. There are eight vertical bars extending from the posterior end to just over the eyes. Also very apparent are the pale bluish, pale greenish, or pearly scattered tiny dots, each in the centre of a scale. Many black dots are lined up on the abdomen. The base of the tail is smoky green. There is a large black or purplish-blue spot at the base of the mustard-coloured pectoral fins. The pelvic fins are black, the dorsal fin is smoky with white spots, and the anal fin is of a smoky, dark purplish hue. This fish is called *karimeen* in Malayalam (apparently striped fish are called by this name), and it grows to at least 12 inches (Fig. 35).

E. suratensis was caught in the brackish backwaters of Palluruthy and is said to also inhabit *Pistia* tanks there, close to the backwaters. Its larvivorous habits are the same as those of *E. maculatus*. It is eaten in Kerala. As with *E. maculatus*, it cannot stand transportation and dies within a few hours after being caught, even when kept under favourable conditions.

CHANDA (AMBASSIS) DAYI BLEEKER, GLASSFISH, (FAMILY AMBASSIDAE OR CENTROPOMIDAE).

This fish was found in the young stages in the above-described fresh-water ditch in Mattanchery (Fig. 33). It is transparent, hence its name of "glassfish". There are two dorsal fins, the first being spiny and the second soft-rayed, the two being connected at the base. The largest specimen taken was 1¼ inches long, but it grows to 7 inches in length. Its Malayalam name is *kypoo*, which is also applied to the barbs. The anterior edge of the first dorsal fin (between the second and third spines) is black, and so are the dorsal and ventral edges of the body posteriorly, giving a partial outline to the body (Fig. 25). The edges of the second dorsal fin, the anal fin, and the tail are somewhat dark. The tail is deeply forked. The body is silvery with a broad, lateral brownish band. It is very active, with nervous, rapid movements typical of the barb, *Puntius vittatus*, but it remains at the surface for long periods of time, and feeds readily on small *C. fatigans* larvae which it encounters.

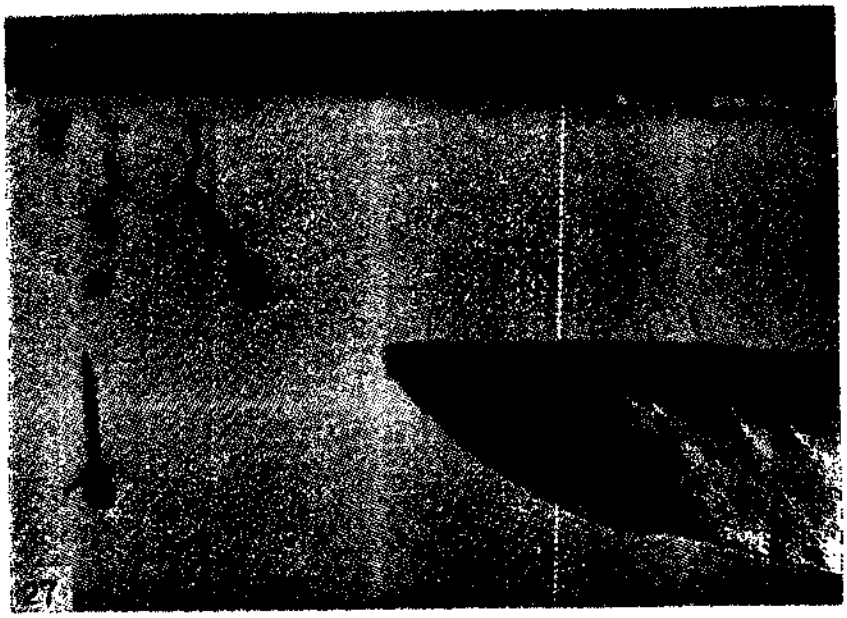
GLOSSOBIUS GIURIS (HAM. BUCH), SLEEPER OR GOBY (FAMILY GOBIIDAE).

This fish, called *poolan* in Malayalam, is very quiescent, remaining in the same place for many hours, sucking in only what happens to be in front of it, ingesting *C. fatigans* larvae this way. It grows to 6 or 7 inches in length, and is said to eat other fish. The body is dark-brown, with a mottled appearance due to many random placed dots and short stripes. There are two dorsal fins with several rows of dots. The first dorsal spine has a prominent black spot. The longer lower jaw gives this fish a pugnaceous appearance. The tail is rounded, with 4 more or less prominent dark bands. The eyes bulge from the head. The pectoral fins are long, and the pelvic fins are fused, cup like, and scallop-shaped, with 2 prominent black dots at the base. The anal fin is without markings. Like any other fish which is very quiescent and remains at the bottom most of the time, this fish is relatively unimportant as a larvivorous fish.

MEGALOPS CYPRINOIDES BROUSSONET, A HERRING (FAMILY CLUPEIDAE).

This silvery, shining herring, is very common in fresh water tanks in Fort Cochin, the specimen pictured here having been 7 inches long and was taken from a tank 6 feet in diameter. The tail is deeply forked. The body is dark bluish-green dorsally, and the tail, posterior operculum, and anterior edge of the dorsal fin are also darker in colour. The scales are large, and the scale margins, sides of the head, and eyes are silver. The anal fin is emarginate, the upper fork extending to near the base of the tail. This fish is called *kanni* or *kunnay* in Malayalam. Like any other large fish, it feeds on smaller, swimming organisms, including mosquito larvae, but is not particularly selective as regards mosquito larvae.

PLATE IX.



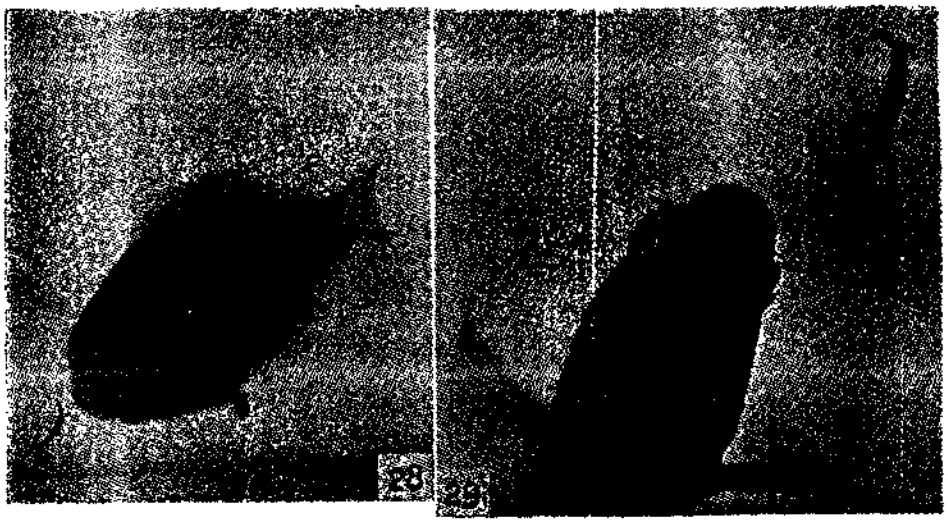


FIG. 27. Indian top-minnow, *Aplocheilus lineatus*, getting ready to ingest *C. fatigans* larva which lies in its direction of swimming.

FIG. 28. Young of *Etroplus maculatus* about to ingest *C. fatigans* larva.

FIG. 29. *Macropodus cupanus* about to ingest *C. fatigans* larva.

PLATE X.

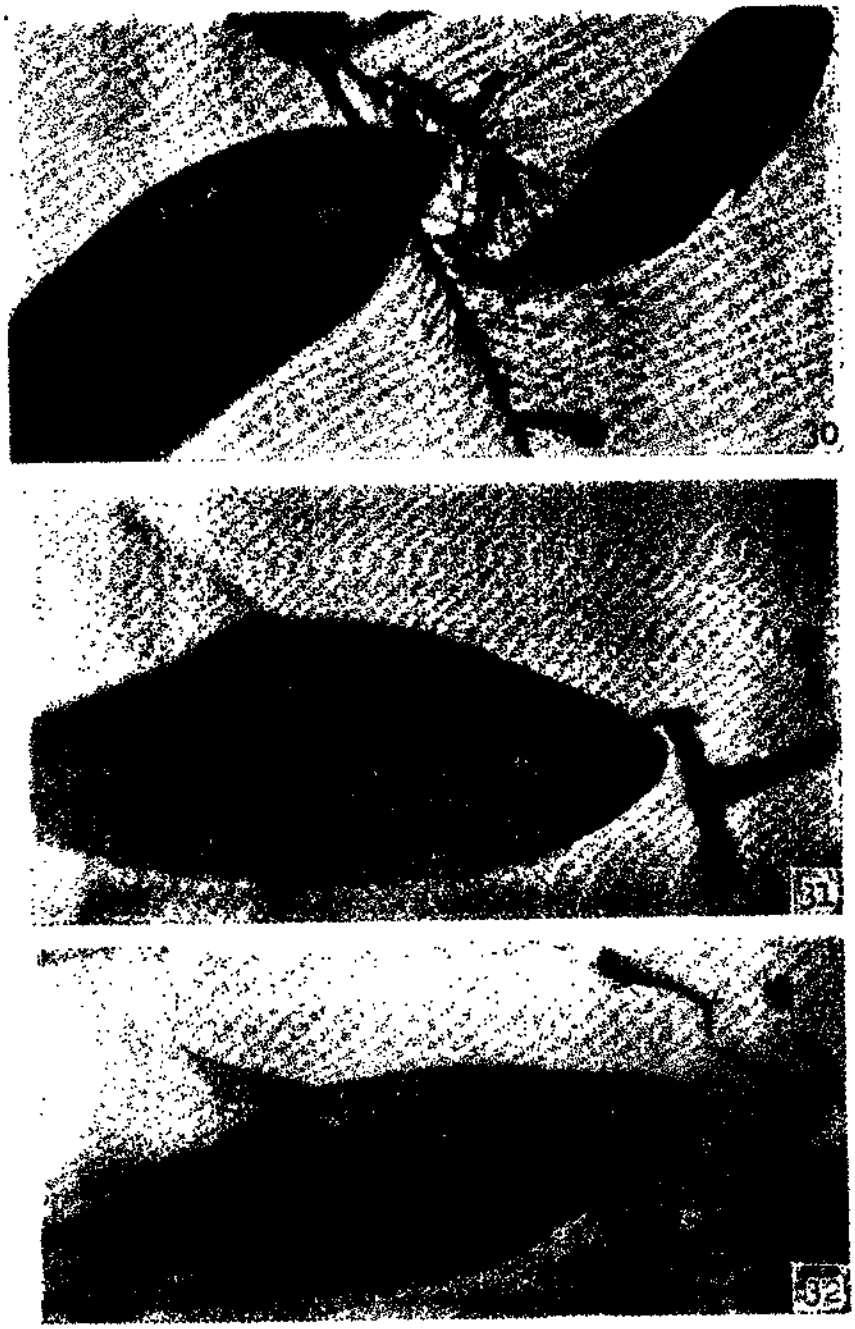


FIG. 30. *Macropodus cupanus* attacking attached *M. annulifera* larva.
FIG. 31. The barb. *Puntius vittatus* about to attack *C. fatigans* larva.
FIG. 32. *Amblypharyngodon melettinus about to eat C. fatigans* larva.

PLATE XI.

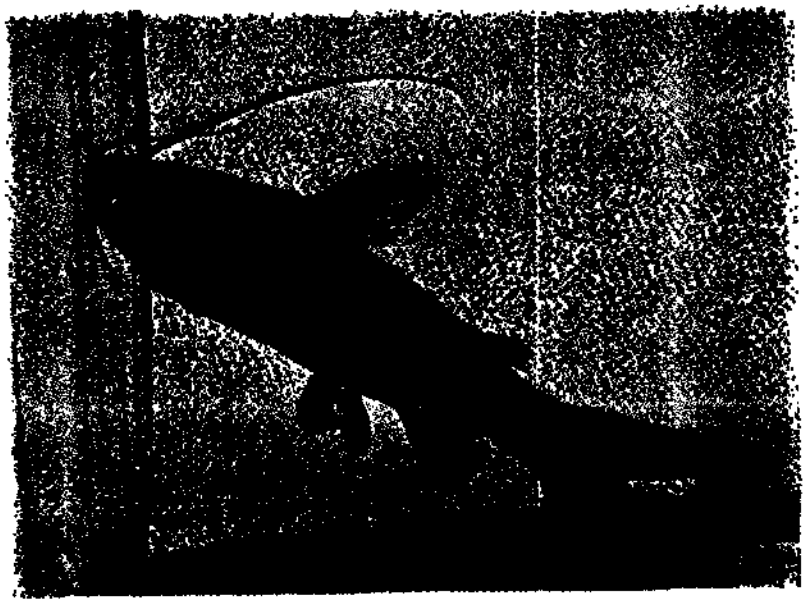


Fig. 33. Stagnant fresh water ditch in the Chuttikad area, Ward Number 26 of Mattancherry, Cochin, in Kerala, which contained many species of fish, and was free of *C. fasciatus* breeding.

Fig. 34. The catfish, *Mystus tengara*. (Original size).

PLATE XII.

Fig. 36. The green chromide, *Etroplus suratensis*. (Original size).

Fig. 36. The sleeper-goby, *Glossobius giuris*. (Original size).

Fig. 37. Darker, striped, young form of the orange chromide, *Etroplus maculatus*. (×1·5)

Fig. 38. Lighter, dotted, young form of the orange chromide, *Etroplus maculatus*. (×1·5)

PLATE XIII

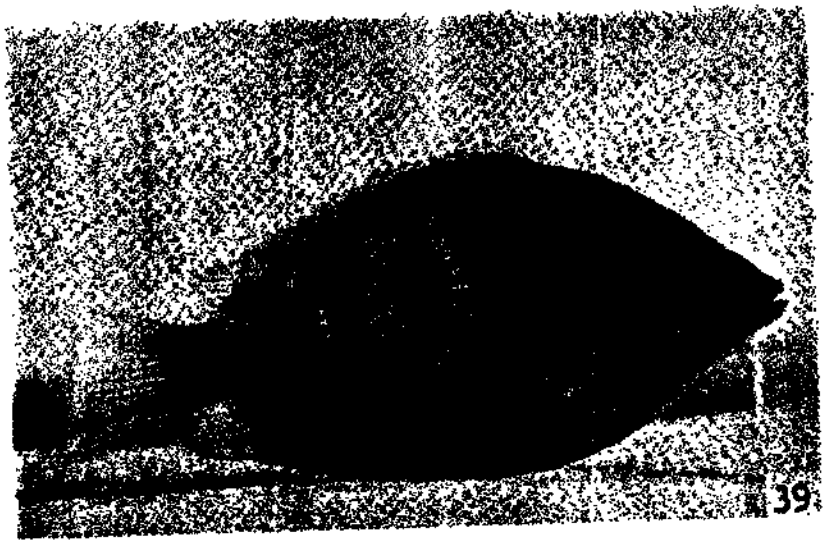


Fig. 38. Darker, more striped adult of the orange chromide, *Etroplus maculatus* (×1·3).
Fig. 39. Lighter, more dotted-appearing adult of the orange chromide, *Etroplus maculatus* (×1·3).

PLATE XIV.

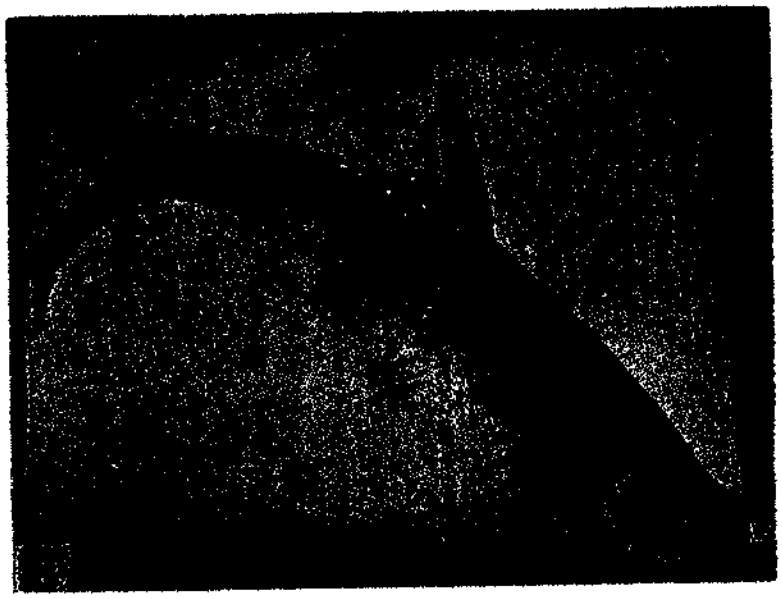


Fig. 41 A herring, *Megalops cyprinoides*. (×0·7)
Fig. 42 Shevaurel, *Ophiocephalus striatus*. (×0·8)

SUMMARY.

Observations were made on the larvivorous habits of fourteen species of fish collected from tanks or ponds containing water lettuce (*Pistia stratiotes*) and water hyacinth (*Eichhornia speciosa*), and from fresh water, stagnant ditches, along the Malabar Coast from Cochin to Shertallay, South India. The three most larvivorous fish which readily ate attached and unattached larvae and pupae of *Mansonia uniformis* and *Mansonia annulifera*, and larvae and pupae of *Culex fatigans* were, in order of importance : *Macropodus cupanus* (spike-tailed paradise fish), *Ophicephalus gachua* (murrel or serpent-headed fish), and *Tilapia mossambica* (Mozambique mouthbreeder). Another murrel, *Ophicephalus striatus*, had habits similar to those of *O. gachua*.

The younger stages of *Etroplus maculatus* (orange chromide), *Etroplus suratensis* (green chromide), and *Amblypharyngodon melettinus*, as well as *Puntius vittatus* (barb), were all good larvivorous fishes, chasing after and eating the mosquito larvae quite readily.

The top-minnow, *Gambusia affinis affinis*, which had been brought from the Malaria Institute of India, Delhi, was found by comparison to be only moderately effective against culicine larvae, and did not attack any attached forms of *Mansonioides*. The Indian top-minnow or striped panchax, *Aplocheilus lineatus*, was found to be a general plankton feeder and of little importance. Another top-feeder, the glassfish *Chanda* (*Ambassis*) *dayi* fed on *C. fatigans* larvae to a better degree.

The fishes which remained quiescent at the bottom, ingesting only larvae which swam in front of them, were *Mystus tengara* (catfish), *Glossobius giuris* (sleeper or goby), and *Anabas testudineus* (walking or climbing fish). These are of little importance as larvivorous fishes. *Megalops cyprinoides* (a herring), collected from tanks in Fort Cochin, was a general feeder, ingesting mosquito larvae only incidentally.

In Mattanchery and Fort Cochin, some odoriferous, stagnant, scum-covered ditches contained large numbers of the following fish, and appeared to be quite free of mosquito larvae, particularly of *C. fatigans* : *Macropodus cupanus*, *Ophicephalus striatus*, *Glossobius giuris*, *Chanda dayi*, *Mystus tengara*, *Etroplus maculatus*, *Tilapia mossambica*. and *Puntius vittatus*.

If a program of biological control were to be undertaken in Kerala against the larvae and pupae of *Mansonioides* and *Culex fatigans*, the best all round larvivorous fish for stocking ponds and ditches is *Macropodus cupanus*, and the second best is *Tilapia mossambica*. The first one is a native fish, but the second has been introduced from East Africa. Both feed readily on attached and unattached larvae and pupae of *Mansonioides* and on *C. fatigans* larvae and pupae. They can also withstand living in dirty, stagnant water such as in drains. Although it is in the same category, the murrel *Ophicephalus gachua* is not as desirable because it eats smaller, larvivorous fishes, and, because it has an accessory respiratory device for direct aerial respiration, it can live out of the water and travel on land for a while ;

however, it can live in extremely foul water. Fourteen plates and 42 photographs by the author illustrate the fishes studied and their larvivorous activities.

ACKNOWLEDGEMENT.

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OBSERVATIONS ON MASS THERAPY WITH DIETHYLCARBAMAZINE AT BARABANKI, UTTAR PRADESH, UNDER THE NATIONAL FILARIA CONTROL PROGRAMME.

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[November 24, 1959.]

INTRODUCTION.

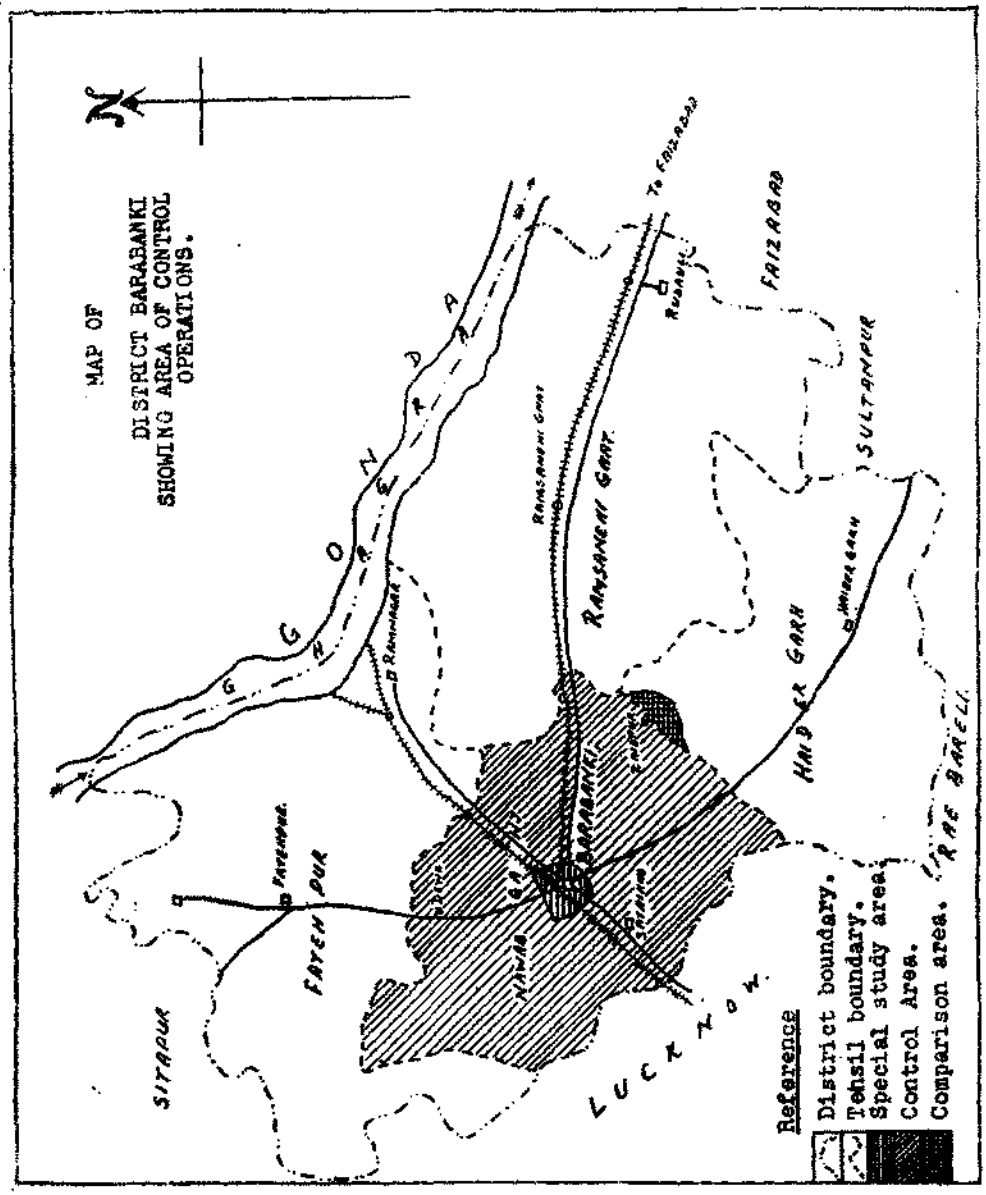
CONTROL of filariasis under the National Filaria Control Programme (Raghvan, 1955) was started at Barabanki and its neighbourhood in June, 1957. The present paper deals with results of mass therapy with diethylcarbamazine carried out in the control area from June, 1958 to the middle of December, 1958. The results described are necessarily interim as follow-up observations are to continue for a number of years before the value of the control is fully assessed.

AREA, CLIMATE AND PEOPLE.

The N. F. C. P. unit covers an area of about 358 square miles with a population of 3,07,543. Its headquarters, the town of Barabanki, is 17 miles north-east of Lucknow City. The urban component of the area consists of 4 small towns with a population of 37,936 while the rural areas are formed by 360 villages with a population of 2,69, 607 (Map 1). The people are mostly agrarian in occupation. Some are engaged in small scale cottage industries.

The climatic conditions of this area are favourable for filarial transmission throughout the year. The average annual rainfall is about 44 inches (111·76 cm.). The temperature ranges between 38°F. (3·3°C.) and 112°F. (44·4°C.) and the relative humidity averages about 60 per cent.

MAP 1.


MAP OF
DISTRICT BARABANKI
SHOWING AREA OF CONTROL
OPERATIONS.
N
G O N D A
SITAPUR
FATEH PUR
LUCKNOW
RAE BARELI
HARDOI
SULTANPUR
FAIZABAD
RAMSANEHI GHAT
BARABANKI
To FAIZABAD
Reference
District boundary.
Tehsil boundary.
Special study area.
Control Area.
Comparison area.

METHODS AND MATERIAL.

Before the mass therapy was started, a night blood survey was conducted by house-to-house visit in about 15 per cent of persons in an area with a population of 30,087 persons, hereafter called the Special Study Area. All age-groups, both sexes, people belonging to different economic, social and religious groups were included in the sample. A total of 4,957 blood slides, with approximately 20 c. mm. of blood in each, were collected between 8 p. m. and midnight from the special study area.

Similarly, 5,547 blood smears were collected from a comparable area (Population 31,256) outside the zone of control operations, hereafter designated as the Comparison Area.

Prior to the launching of mass therapy, the population of the entire area under control was enumerated in detail by the enumerators. Diethylcarbamazine tablets of 100 mg. each were administered by trained persons by door-to-door visit in the following daily doses :

Age-Group.	*Number of Tablets.*
2 – 6 years	Half (50 mg.)
7 – 12 years	1 (100 mg.)
13 – 18 years	1½ (150 mg.)
Above 18 years	2 (200 mg.)

The drug was given in a single daily dose and attempts were made to give the drug to all the persons for 5 consecutive days. The drug was never administered to persons on empty stomach. It was withheld from children below 2 years of age, pregnant women, mental delinquents and acute and chronically ill persons. Advance intimation was given to each group of persons who were to receive mass therapy and they were warned that during the course of therapy, some might develop mild reactions like nausea, vomiting, diarrhoea, headache and body pains. Auxiliary help of local doctors, sanitary personnel, health visitors and village midwives was available in some measure. Publicity and propaganda for the success of the therapy were carried out by loudspeakers, projection of cinema slides, film on filariasis, distribution of pamphlets and by organising group meetings in towns and villages.

Closely following the mass therapy, the parasitological indices were determined in the Special Study Area again during the period July, 1958 to May, 1959. Night blood examination was carried out in respect of as many as possible of those who were examined before starting mass therapy. Similar parasitological indices were determined in the comparison area.

RESULTS.

The results of survey in the Special Study Area (before mass therapy) and in the Comparison Area showed an infection rate of 10·0 per cent in the former,

and 10·6 per cent in the latter area. The average infestation per 20 c. mm. of blood was 14·3 in the Special Study Area and 12·1 in the Comparison Area. The youngest age at which infection was noted, was a two years old male child, in both the areas. The disease manifestation was predominantly hydrocele in the males with some lymphoedema of extremities in both the areas. The infection prevalent was *W. bancrofti* only, transmitted by *C. fatigans*. The infection rates in *C. fatigans*, determined by dissection of 6,879 and 4,068 *C. fatigans* mosquitoes in the Special Study Area and the Comparison Area, were found to be 1·5 and 0·8 per cent respectively. It would thus be noted that filariasis was moderately endemic in the Special Survey and the Comparison areas.

It will be seen from Table I that only 2,48,940 persons, out of 3,07,543 persons in the area, took the therapy for periods varying from 1 to 5 days. It should, however, be stressed that 1,74,791 persons or 59 per cent of the eligible population, received the full course of therapy, i.e., for 5 consecutive days. About 84·5 per cent of the eligible population took the drug for varying number of days.

Reactions such as fever, headache, body pain, nausea, vomiting, diarrhoea and constipation were noted amongst nearly 11 per cent of the population under therapy. A few cases of urticaria and pruritus were also noted. A very small number of persons receiving the drug developed excessive salivation and sweating, occular irritation and nodular swellings on body surface. In the majority of the persons with reactions, they were of a mild type and subsided with symptomatic treatment or by themselves within a week. A small number of cases had severe reactions, e.g., hyper-pyrexia (105°F.), vomiting, diarrhoea and severe type of urticaria. Such reactions were got over by anti-allergic and other symptomatic treatment. A fairly good number of people taking diethylcarbamazine had precipitation of various filarial manifestations which were probably latent before, e.g., lymphangitis, epididimo-orchitis, hydrocele and swelling of the limbs. Almost all such manifestations disappeared in about a fortnight to a month by treatment with antibiotics and sulpha drugs.

The percentage incidence of the various reactions, following administration of diethylcarbamazine, are given in Table II. It will be observed that the highest percentage of such reactions come under fever and precipitation of local filarial manifestations.

Results of blood examination of the persons examined both during the initial and the follow-up surveys after mass therapy, irrespective of whether they took the drug or not in the Special Study Area, and the results of blood examination of the persons similarly followed up in the Comparison Area, are given in Table III.

It would be observed from Table III that in the Special Study Area the infection rate was reduced from 10·0 per cent to 4·59 per cent, while reduction of the average infestation was from 14·3 to 6·27 per c. mm. of blood, i.e., a reduction of about 69 per cent. In the Comparison Area where no drug was administered to the population, these indices remained almost the same as before.

TABLE I.

Number of persons in each age-group who took the drug for varying number of days.

Total population in the area.	Number of persons exempted from taking the drug.	PERSONS AVAILABLE FOR MASS THERAPY.		Per cent persons who took drug.	PERCENTAGE THAT TOOK DRUG FOR					Per cent persons who refused therapy.	Per cent persons who discontinued therapy.
		Age-group (Years).	Number		5 days	4 days	3 days	2 days	1 day		
307,543	13,209	2– 6	26,934	85·1	67·4	12·3	8·3	6·3	5·6	14·9	27·7
		6–12	40,531	87·4	67·6	12·5	7·8	6·6	5·4	12·6	28·3
		13–18	34,359	83·6	68·1	12·2	8·0	6·5	5·2	16·4	26·7
		above 18	192,510	84·1	71·5	10·7	7·1	6·1	4·6	15·9	23·9
307,543	13,209		294,334	248,940	70·2	11·2	7·4	6·2	4·9	45,394	74,149

TABLE II.

Percentage of various types of reactions developed during mass therapy with diethylcarbamazine.

Reaction.	Fever.	Headache.	Body-pain.	Nausea and vomiting.	Diarrhoea and constipation.	Precipitation of filarial disease manifestation.
Per cent	27·4	19·4	17·7	8·8	3·5	23·0

TABLE III.

Results of post-mass-therapy survey.

Descriptions.	Special Study Area.	Comparison Area.
Number of persons examined.	4,008	4,544
Infection rate, per cent.	4·59	8·59
Average infestation/20 c.mm.	6·27	12·22

DISCUSSION.

Practical experience in the conduct of mass therapy amongst a fairly large population, free from any form of regimentation, has shown that in a filarious area many people initially voluntarily cooperate towards the success of such operation. However, a part of the population were either exempted (4 per cent) or refused to take the drug (14 per cent), thus constituting a sizeable reservoir of infection not affected by the mass therapy. Amongst the persons on therapy, 29·7 per cent did not complete the five-day schedule. These constitute the obvious limitations of mass chemotherapy.

Further, mass therapy with diethylcarbamazine in a *bancroftian* area is associated with reactions which, however, are manageable under field condition. These undesirable reactions tend to discourage the acceptibility of full course of therapy by the entire community, thus leaving an additional reservoir of untreated infection which reflects on control measures.

However, the reduction in the number of infected persons in the locality, associated with a lowering of the average infestation compared to the pre-therapy conditions, is likely to lesson the quantum of transmission. To what extent a single round of mass therapy, with vector control, would control the incidence of infection and disease, can only be assessed after a number of years of observations. These points are under active observation.

In the Comparison Area, both infection rate and average infestation remained almost the same during both the surveys.

SUMMARY AND CONCLUSIONS.

1. Practical experience on mass therapy with diethylcarbamazine in a large population is described and the immediate follow-up results are recorded.

2. (a) The findings observed go to show that mass therapy with diethylcarbamazine to an endemic filarial community, has obvious limitations. In addition to administrative handicaps, technically there are some other handicaps such as, some people are deliberately omitted, while others refuse on various grounds, yet others do not have a full course of therapy due to initial reactions. These limitations result in a sizeable residual reservoir of infection.

(*b*) A lowered transmission rate is to be expected even with a single round of mass therapy, due to lowering of infection rate in the community, i.e., number of positive persons and the greater reduction of average infestation per positive person.

3. The extent to which such lowering of the transmission with a single course of mass therapy, as carried out presently, and recurrent vector control measures ; may result in, the control of filariasis is under study.

ACKNOWLEDGEMENT.

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

XXVII. DURATION OF PATENT PRIMARY PARASITAEMIA NECESSARY FOR THE DEVELOPMENT OF MEASURABLE ACQUIRED IMMUNITY, IF ANY, IN THE ALBINO MICE†.

BY

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[December 3, 1959].

GALLIARD and Lapierre (1954) have demonstrated that serum of infected mice, para-amino-benzoic acid, vitamin-B complex (B_1, B_2, nicotinic acid, calcium pento-thenate), protein extract, had no effect on the immunity status of mice. Fabiani and Orfila (1954) have not been able to determine the existence of any protective immunity in seven mice in which the infection had been present for two weeks. Fabiani (1954) reported the absence of immunity in mice. He considered the possibility of its appearance under the influence of milk diet or sulphonamide therapy. The author (Satya Prakash, 1957) demonstrated a prolongation of the period of patent primary parasitaemia in mice fed on a diet with a high proportion of casein.

Repeated doses of nivaquine, during primary parasitaemia, resulted in a latent infection and the animal was able to control a parasitic relapse for a brief period without further treatment (Galliard and Lapierre, 1954 *loc. cit.*). The present study was undertaken to determine if, in mice, any measurable anti-parasitic immunity occurs at all and if so the minimum period of patent primary parasitaemia necessary for the same.

METHODS AND MATERIALS.

The methods employed in these experiments were the same as in the similar previous studies on rats (Satya Prakash, 1959). In the present series, albino mice were used instead of the albino-rats. In order to ensure that the animal was definitely cured of infection, each animal was administered 1·2 mg. sulphadiazine per 20 gm. body-weight once a day intraperitoneally for three consecutive days. Each animal was given at least 15 days' rest to eliminate the drug before being subjected to any further experimental procedure.

*The strain of *P. berghei*, used in the experiment, is being maintained by blood passage in albino rats at the Malaria Institute of India, Delhi, since 1952. It 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 Panjab University.

Sergent (1954) pointed out the need for a total infectivity test before declaring an animal completely cured. The technique recommended by him consists of inoculating the total mass of blood and homogenised internal organs of the animal under test into susceptible animals. As such, the same animal cannot be used to determine the immunity status attained by it. To overcome this, a group of seven albino mice were inoculated every time; one of them was kept as control, three were subjected to total infectivity test to demonstrate complete cure, and the other three to demonstrate the presence or absence of immunity to a challenge homologous infection.

The albino mice, used in the experiments, were adults from the colony* at the Institute. Forty-nine mice were used in these investigations. For the total infectivity tests, inoculations were made into domestic rats (*R. Rattus.*). The susceptibility of domestic rats has been established by Vincke and Lips (1948) and Ramaskrishnan and Satya Prakash (1950) At least six animals were utilized for sub-inoculation from each albino mouse. Blood smears from inoculated domestic rats were examined every day for at least ten days. Approximately 100 specimens of domestic rats were used.

RESULTS.

Table I shows the course of primary infection in the experimental animals, and the chemotherapy. Out of the 49 mice used, 21 died before the final stages of the experiment. Twenty-eight animals were treated at intervals from 3 to 19 days of primary parasitaemia. Twelve of these were subjected to total infectivity tests. The schedule of treatment adopted resulted in a radical cure of infection irrespective of the day of commencement of treatment, as all the twelve cured animals, submitted to the total infectivity test, were found to be radically cured (Table II).

Fourteen of the sixteen cured animals, when given a challenge inoculation, became positive. The course of daily parasitaemia, after challenge inoculations, varied in each group of animals as shown in Table III. It was observed that all the animals, after challenge infection in each group, showed decidedly a lower daily parasitaemia than the untreated controls (Table IV).

Untreated *P. berghei* infections in mice were invariably fatal with usually high parasitaemia of over 2,000 parasites per 10,000 erythrocytes before death (Table IV).

*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.
			100 parts.

TABLE I.

Protocols of albino mice, cured from 3rd to 19th day of parasitaemia, showing the results of challenge infection and total subinoculation.

Number of animals used.	Sex:		Day of patent parasitaemia on which treatment commenced.	Date of inoculation.	Date of commencement of treatment.	Number of animals used:		Result, number of animals positive or negative:		Remarks.
	Male	Female				For challenge inoculation.	For sub-inoculation.	For challenge inoculation.	For sub-inoculation.	
7	..	7	3	Apr. 2, 1956	Apr. 5, 1956	3	3	3/0	0/3	One control died on 13th day of patency.
7	3	4	5	Apr. 26, 1956	May 1, 1956	3	3	3/0	0/3	One control died on 20th day of patency.
7	7	..	9	May 6, 1956	May 15, 1956	3	3	2/1	0/3	One control died after 26 days' patency.
7	3	4	13	May 22, 1956	June 4, 1956	2	1	2/0	0/1	Four animals died before completing the experiment.
7	7	..	14	June 14, 1956	June 28, 1956	3	1	3/0	0/1	Three animals died before completing the experiment.
7	7	..	19	July 5, 1956	July 24, 1956	2	1	1/1	0/1	Four animals died before completing the experiment.
7	7	..	20	*	*	*	*	*	*	
Total: 49	34	15				16	12	14/2	0/12	

*All died before completion of cure or rest period, i.e., before the experimental procedures could be started.

TABLE II.

Protocols of albino mice given challenge re-infection or submitted to total infectivity test after radical cure of primary parasitaemia on successive days after the primary inoculation.

Number of albino mice 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.	
7	3	16	3/0	0/3	One died
7	5	18	3/0	0/3	One died
7	9	19	2/1	0/3	One died
7	13	18	2/0	0/1	Four died
7	14	18	3/0	0/1	Three died
7	19	18	1/1	0/1	Four died
Total : 42			14/2	0/12	

It was noticed that only the mice, cured on the third day of primary patent parasitaemia, succumbed to challenge re-infection. But even in these, the average daily parasitaemia was never more than 668. Moreover, it showed a downward trend on the tenth day of parasitaemia followed by a rise again to 533 parasites per 10,000 red blood cells before death (Table III).

In animals cured on the thirteenth and fourteenth day of primary parasitaemia, the maximum period of patent parasitaemia, after the challenge infection, was fifteen days. Mice cured on the nineteenth day of primary parasitaemia, when given the challenge infection, showed parasitaemia for six days and a peak of 120 parasites per 10,000 erythrocytes only.

Most of the mice did not succumb to the challenge homologous infection, except for the group cured on the third day of patent parasitaemia. In the experience of the author since 1951 this is of unknown occurrence in untreated *P. berghei* infections in thousands of albino mice.

The average prepatent period, following the primary inoculation in normal animals (Table V), was less than twenty-four hours. It was similar in the case of challenge re-infection in three animals cured on the third day of patent parasitaemia, indicating that any anti-parasitic immunity, gained in three days of parasitaemia, was negligible. The average prepatent period was three days in mice cured on the fifth, ninth and fourteenth days. An exception was found in animals cured on the thirteenth day of primary infection in which the prepatent period, after re-inoculation, was one day. The reasons for this are not known. In mice cured on the nineteenth day, however, the average prepatent period, after the challenge inoculation, was seven days (Table V).

TABLE V.

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

Number of animals.	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 primary inoculation.	Average prepatent period in days after challenge inoculation.	Remarks.
7	3	16	0	0	
7	5	18	0	3	
7	9	19	0	3	
7	13	18	0	1	
7	14	18	0	3	
7	19	18	0	7	
Total : 42					

Animals which survived the challenge infection were cured again and given rest. They were given a second challenge homologous inoculation. It was observed that animals of the thirteenth day group, when given a second challenge dose, showed no parasitaemia for fifteen days' observation (Table VI). They were subsequently sacrificed. Those of the fourteenth-day group showed parasitaemia for five days after a pre-patent period of 10 days. Parasitaemia, however, was only 124 parasites per 10,000 RBC's. (Table VII).

TABLE VI.

Average prepatent period after primary, challenge and re-challenge inoculation in albino mice radically cured after primary challenge and re-challenge parasitaemia in each group.

Number of animals used.	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 primary inoculation.	Average prepatent period in days after challenge inoculation.	Interval in days between completion of cure after challenge infection and rechallenge inoculation.	Average prepatent period in days after rechallenge inoculation.
7	3	16	0	0	..	..
7	5	18	0	3	..	..
7	9	19	0	3	..	..
7	13	18	0	1	16	15 days parasitaemia nil.
7	14	18	0	3	16	10
7	19	18	0	7	..	..
Total : 42						

DISCUSSION.

A limitation of the experiments has been that in albino mice it was not possible to determine immunity status beyond 19 days of patent parasitaemia, as within that period all the mice died (Table I). The groups of mice, cured after 13th and the 14th days of primary parasitaemia, showed some anti-parasitic immunity conferred on them during this period (Table VII).

From the observations it appeared that some degree of antiparasitic immunity is acquired by infected mice if adequately treated to prevent death. The animals treated on the third day of primary parasitaemia showed the least amount of such immunity; the prepatent period of the challenge infection was nil, and all the animals died on the sixteenth day of parasitaemia after re-inoculation. In other groups, the prepatent period varied from one to ten days. The average daily and peak parasitaemias were low, and most of the animals did not succumb to the challenge infection. It appeared that at least some anti-parasitic immunity was present to enable the animals to overcome the challenge infection and save them from death. As already stated such survival is not known in albino mice with untreated *P. berghei* infection (Table IV).

It was further observed that animals infected twice did show an increased immunity. The prepatent period was very much prolonged in mice in which the primary parasitaemia was allowed to run its course for 13 to 14 days. This was further supported by as short a patent period as 5 days after second challenge inoculation which resulted in prepatent period of 10 days in the group of mice cured on the fourteenth day of primary parasitaemia. In the group that had 13 days of primary parasitaemia, the second challenge inoculation showed no parasites at all during fifteen days of observations (Table VII).

SUMMARY.

Experiments were conducted to determine the period of primary patent parasitaemia (*P. berghei*) in albino mice essential for producing immunity, if any.

It was found that some degree of immunity was developed in albino mice after 3 days of patent parasitaemia and was correspondingly increased up to 19th day of patent parasitaemia. But it was never as complete as seen in albino rats after eleven days or more of patent parasitaemia.

Second challenge homologous infection, increased the quantum of immunity.

It was impossible to determine the immunity status developed after more than nineteen days of initial patent parasitaemia, as all animals had died by that time.

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RECORD OF MORPHOLOGICAL VARIATIONS IN *A. ANNULARIS* VAN DER WULP NEAR DHANBAD.

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THERE are several records of different species of anophelines showing morphological variations or abnormalities from the type form, in the egg, larval and adult stages. In adults the abnormalities generally relate to the ornamentation of the maxillary palpi, the proboscis, the wings and the tarsi of the hind legs. The present paper puts on record morphological variations observed in the ornamentation of the palpi, hind tarsi and wings in ten specimens of *A. annularis* (adult females) collected from five villages in the Dhanbad area during the period November 27 to December 16, 1959.

Routine adult mosquito collections are being carried out from fixed catching stations in sprayed villages in the Jharia Coalfield area. The collections are brought to the laboratory at Jagjiwan Nagar, Dhanbad, the same day for identification and record. During the period mentioned above, 41 specimens of *A. annularis* were collected from the catching stations out of which ten specimens showed the morphological variations indicated in Table I and in Plate I, Figs. 1-3.

Among the morphological variations mentioned above, the dark scales on the proximal end of third hind tarsal segment in two specimens and on the 5th vein of the right of one specimen are cases of winter melanic variation as mentioned by Christophers (1933), Puri (1938), Bhatia and Kalra (1957) and Bhatnagar *et al.* (1958). The presence of pale scales on the palpi in *A. annularis*, as observed in the specimens mentioned here, has not been recorded previously.

During the period of observation (November 27 to December 16, 1959) when these ten specimens with the morphological abnormalities were recorded, thirty-one specimens of typical form of *A. annularis* were also collected from these villages. However, the normal and abnormal forms were not recorded on the same day.

Attempts are being made to follow up the development of these abnormal forms and find out if any morphological variations occur in the larval stages as well.

TABLE I.

Morphological variations in A. annularis in Jharia Coalfield Area, Dhanbad.

Serial number.	Date of collection.	Village.	Number of specimens	Nature of variations from the type form.	
1	Nov. 27, 1959	Mathkuria	1	Palpi : (Fig 1, *a*)	Rows of white scales on all the dark bands.
2	Dec. 3, 1959	Saraidhala	1	Palpi (Fig. 1, *b*)	2 to 3 rows of white scales on all the dark bands.
3	Dec. 4, 1959	Dhokra	4	Palpi (Fig 1, *c-f*)	1 specimen (Fig. 1 *c*) with more white scales in regular pattern on the dark bands of the left palp whereas few scales on the right palp. The scattered scales on the sub-apical dark bands on right palp and those on the proximal dark band of left giving speckled appearance.
					1 specimen (Fig. 1, *d*) with two rows of white scales in the middle dark band.
					1 specimen (Fig. 1, *e*) with one row of white scales in the sub-apical and middle dark band.
					1 specimen (Fig 1, *f*) with 1 to 2 rows of white scales on the sub-apical and middle dark band and a few scales on the proximal dark band of right palp. And with a few scattered white scales on the sub-apical dark band of left palp.
4	Dec. 10, 1959	Chiragora	1	Palpi (Fig. II, *a*)	Rows of white scales present on the three dark bands.
				Tarsi (Fig. II, *b*)	Only two hind tarsal segments completely white.
		Mathkuria	1	Palpi (Fig. II, *c*)	Row of white scales on the pre-apical and middle dark bands.
				Tarsi (Fig. II, *b*)	Only two hind tarsal segments completely white.
				Wing	The right wing with dense dark scales. The 5th vein and its lower branch without any pale interruption.
5	Dec. 16, 1959	Nuthundi	2	Palpi (Fig. III, *a,b*)	White scales present in a row on the middle dark band and a few white scales present on one of the pre-apical dark bands.

PLATE I.

Variations in the palpi and hind tarsi of A. annularis *Van Der Waip.*

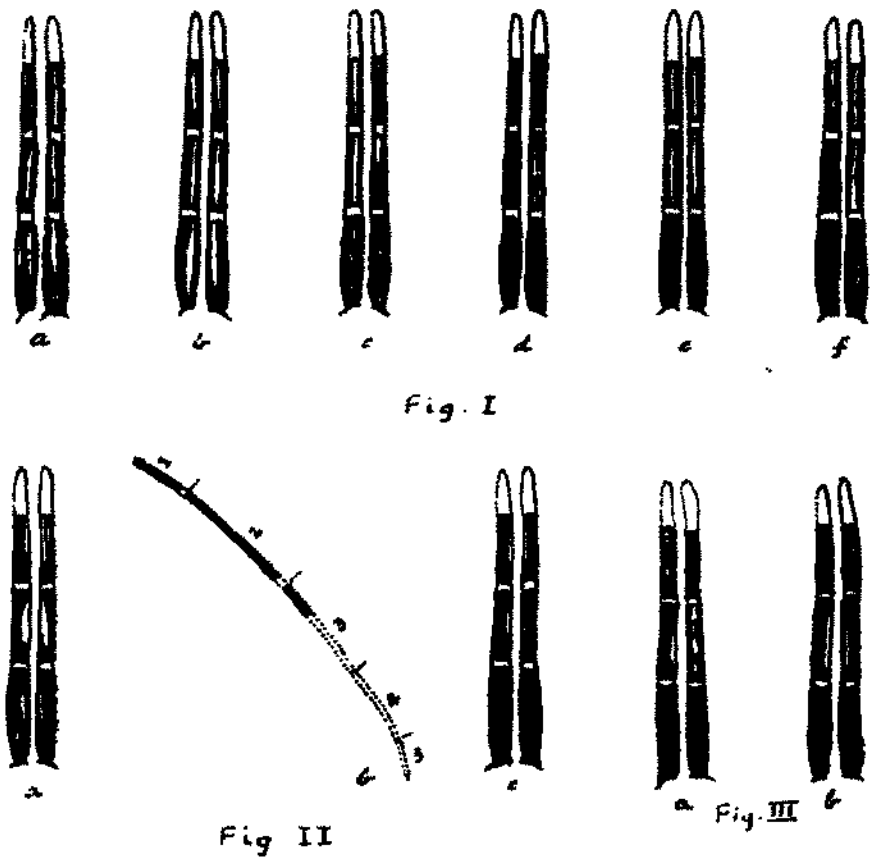

FIG. I : (*a*) Palpi of specimens from Mathkuria village.
(*b*) Palpi of specimens from Saraidhala village.
(*c*, *d*, *e*, and *f*) Palpi of specimens from Dhokra village.

FIG. II : (*a*) Palpi of specimens from Chiragora village.
(*b*) Hind tarsi of the same specimens from the two villages.
(*c*) Palpi of specimens from Mathkuria village.

FIG. III : (*a*, *b*) Palpi of specimens from Nuthundi village.

SUMMARY.

Ten specimens of *A. annularis*, collected from five different villages near Dhanbad and showing certain morphological variations from the type form, are described.

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A NOTE ON DDT-RESISTANT *CULEX FATIGANS* WITH CROSS-RESISTANCE TO GAMMA BHC AND DIELDRIN*.

BY

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[November 5, 1959.]

INTRODUCTION.

THE selection of a DDT-resistant colony of *C. fatigans*† for studies on the various aspects of resistance has been briefly described before (Mohan, 1955 and Dikshit *et al.*, 1956). Subsequent selection of the strain resulted in a very high degree of resistance in the mosquitoes. Studies were carried out with this strain to assess the degree of resistance to DDT and cross-resistance to other insecticides both with mosquito larvae and adults. The results obtained are presented in this publication.

SUSCEPTIBILITY OF ADULT MOSQUITOES TO INSECTICIDES.

(i) *Results obtained with the Busvine-Nash method (as adopted by the Expert Committee on Malaria of the World Health Organization. Technical Report Series No. 80, 1954).*—Tests were performed on mosquitoes of the 65th and 66th generations. The insects were fed on a fowl at night and tested the next morning. At the highest concentration of DDT (4 per cent technical grade), with the maximum allowable exposure period of 48 hours, the mortality did not exceed 5 per cent in the case of the resistant strain. The median lethal concentration (MLC) of DDT for the normal strain was 0·724 × 24 (per cent insecticide concentration × hours of exposure period).

(ii) *Results obtained with topical application of insecticide.*—Tests were carried out on gravid females of 78th generation by means of 'Agla' micro-syringe needle, using benzene solution of p-p' DDT. The percentage mortality was nil in all the three lots of resistant *C. fatigans* at dosage levels of 16, 32 and 64 micrograms of the insecticide per mosquito. The highest dosage of 96 micrograms of DDT contained in 0·6 microlitre of 16 per cent solution per insect, caused only 26 per cent mortality. With 32 per cent strength of the solution, topical applications could not be made satisfactorily because of the freezing of the solution inside the needle of the micro-syringe. In contrast, normal *C. fatigans* showed 100 per cent mortality when treated in the same manner with 0·6 micrograms of p-p' DDT per mosquito.

*The investigations were financed by the Indian Council of Medical Research.

†The strain of *C. fatigans* originated from Kallar, Eastern Nilgiris, South India, in 1951, prior to the institution of DDT-spraying programme in that area.

It may also be stated that applications with mineral oil solution of DDT (Busvine, 1951 : 1957) were not made because the maximum volume of the solvent (kerosene or white oil) that could be safely tolerated by *C. fatigans*, was found to be less than 0·2 microlitre, the minimum measurable quantity by 'Agla' syringe. Other apparatus for delivering smaller quantities was not available.

(*iii*) *Studies on cross-resistance to gamma BHC and dieldrin.*—The DDT-resistant strain of *C. fatigans* showed clear-cut resistance to gamma BHC and dieldrin, without ever having been exposed to these insecticides, as shown by the median lethal concentration (MLC) values given in Table I.

TABLE I.

Details regarding the susceptibility of DDT-resistant C. fatigans *females (77th generation) to gamma BHC and dieldrin*.*

Insecticide.	Median lethal concentration (per cent) :		Remarks.
	DDT-resistant strain.	Normal strain.	
Gamma BHC (lindane)	0·45×1†	0·054×1†	Period of exposure in hours†.
Dieldrin (technical grade)	1·99×18†	0·63 ×2†	

* Total number of mosquitoes exposed to each concentration varied from about 150 to 200.

† Continued selection of the strain with DDT resulted in increased cross-resistance to gamma BHC and dieldrin (Ramakrishnan and Mohan, 1959 ; Mohan, 1960). In generations 82 and 83, the DDT-resistant strain was about 24 times more resistant to gamma BHC than the normal strain (Ramakrishnan and Mohan, 1959).

SUSCEPTIBILITY OF MOSQUITO LARVAE TO DDT§.

Test suspensions in parts per million (p.p.m.) were obtained by adding 1 c.c. of p-p' DDT solution in acetone of a given strength to 200 c c. of tap water in glass beakers.

The percentage mortalities obtained after 24 hours exposure in 4th-instar larvae of the normal strain, together with the median lethal concentration in p.p.m., are shown in Table II.

TABLE II.

Details regarding the susceptibility of normal C. fatigans *larvae to DDT‡.*

p-p'DDT concentration in p.p.m.	0·125	0·0625	0·03125	0·015625	LC 50 in p.p.m.
Per cent mortality	96	63	19	2	0·056

‡ Total number of mosquito larvae exposed to each concentration was about 200.

To obtain a critical range or mortality in resistant larvae, higher concentrations had to be used. But such concentrations were defective because of the precipitation§§ of DDT. It was obvious that without proper dispersion of the

§Resistance was found to extend to the larvae also, although selection was done only with the adults by exposing them to DDT deposits in each successive generation.

§§Attempts made to remedy this defect by decreasing the strength and correspondingly increasing the volume of the DDT solution were unsuccessful.

insecticide, results would be grossly exaggerated. Thus, mortality in resistant larvae of 66th generation did not exceed 59 per cent at 100 p.p.m. in which plenty of DDT crystals had precipitated and were thickly sticking to the glass rod used for stirring the water.

Studies to determine the degree of cross-resistance to gamma BHC and dieldrin, in the case of larvae of DDT-resistant strain, were not undertaken.

STUDIES ON THE MAINTENANCE OF THE RESISTANT STRAIN IN CONTINUOUS CONTACT WITH DDT DEPOSITS.

Due to the very high level of DDT-resistance in the strain, it was possible to maintain the strain in continuous contact with DDT deposits by stocking the adults in a colony cage (2 feet × 2 feet × 2 feet) coated with the insecticide at the rate of 200 mg. per square foot on all sides except the front (upper half glass) and the bottom. Experiments were first tried with one-day old males and females and then with adults emerging from pupae (73rd generation) placed inside the treated colony cage.

Except for the earlier and higher mortality, particularly among the males, the activities of the mosquitoes inside the treated colony cage appeared to be similar to and synchronised with those of the 'comparison' group of the same generation kept under identical conditions in the untreated colony cage.

The surviving mosquitoes seemed to exercise no discrimination in selecting the resting sites inside the treated colony cage. They swarmed, mated, imbibed blood feed and developed ova. Of the mosquitoes that hatched out inside the treated colony cage from 1,000 pupae, 38 females became gravid. Of these, 8 laid viable egg-rafts on the third night after imbibing blood meal when the experiment was terminated.

DISCUSSION.

C. fatigans is stated to have low susceptibility to insecticides, and resistant strains of its population get selected quickly after exposure to DDT and dieldrin or gamma BHC. It is also well known now that, as far as the chlorinated hydrocarbon insecticides are concerned, insecticide resistance in mosquitoes can be of two categories, viz., resistant to DDT and its analogues but susceptible to gamma BHC and dieldrin, and resistant to gamma BHC and dieldrin but susceptible to DDT and its analogues. In the present studies, the resistant strain of *C. fatigans* selected with DDT alone showed high degree of cross-resistance to gamma BHC and dieldrin, without ever having been exposed to any of these insecticides. No instance of a similar nature in the case of laboratory strains of mosquitoes was traceable in the available literature. Meltzer (1958), however, noticed high resistance in houseflies, to gamma BHC and dieldrin in the strain selected with DDT. Under field conditions, similar findings have been recorded in some species of anopheline mosquitoes. In Java, dieldrin resistance was reported in *A. vagus*, *A. barbirostris* and *A. subpictus malayansis* from a village sprayed only with

DDT (Chow, 1958). Similarly, dieldrin-resistance in *A. splendidus* was also reported from an area of Ranchi District of Bihar where only DDT was used (Patil and Ramalingam as quoted by Pal, 1958).

It has been stated that selection of resistant strains by exposure of adults or larvae alone does not confer the same degree of insecticide tolerance on one stage as it does on the other (Sharma, 1958). For example, the degree of resistance was found to be not of the same magnitude in adults and larvae of *A. subpictus* (Delhi) and *A. stephensi* (Madras) where insecticide selection was exerted on adults in the former and larvae in the latter. DDT-resistant *A. subpictus* adults showed a greater degree of tolerance to DDT as compared to larvae, whereas *A. stephensi* larvae were more tolerant to the insecticide than adults (Sharma and Krishnamurthy, 1957*a*, 1957*b*, and Mammen, 1958 as quoted by Sharma, 1958 *loc. cit.*). In the present studies on the DDT-resistant strain of *C. fatigans*, the degree of resistance of both larvae and adults to the insecticide, compared to the normal strain, could not be determined precisely due to limitations of the test methods employed, but larvae of *C. fatigans* did show a very high tolerance to DDT though selection of the strain was made by exposing only the adults to the insecticide. The different findings observed in *C. fatigans* and the two species of Anopheles may be due to various factors such as generic characters, different environmental conditions existing in the field and laboratory and the like. Further investigations are indicated to elucidate the point.

SUMMARY.

Results of susceptibility to DDT, gamma BHC and dieldrin, in a resistant strain of *C. fatigans* selected with DDT by exposing the adult mosquitoes to insecticide, have been presented.

Adult mosquitoes of the DDT-resistant strain of *C. fatigans* showed resistance to DDT as well as to gamma BHC and dieldrin.

The selection of *C. fatigans* adults by DDT also conferred resistance in the larval stage.

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FURTHER OBSERVATIONS ON SPOROGONY CYCLE OF *PLASMODIUM RELICTUM* IN A DDT-RESISTANT STRAIN OF *CULEX FATIGANS**.

BY

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[November 25, 1959.]

In a previous publication, the author (Mohan, 1955) had shown that the susceptibility of a DDT-resistant strain of *C. fatigans* to *P. relictum* infection was the same as of normal *C. fatigans*. The sporogony cycle of the parasite in the resistant mosquitoes remained unaltered even when they were exposed to DDT during the extrinsic incubation period. Subsequently, the degree of resistance† to DDT of the mosquitoes of the colony increased to such a limit that they could be maintained in continuous contact with the insecticide (Mohan, 1960). The sporogony cycle of *P. relictum* in these highly resistant mosquitoes was investigated. The results are herein reported.

COMPARATIVE INFECTIONS IN NORMAL AND RESISTANT STRAINS OF *C. FATIGANS* WITH *P. RELICTUM*.

C. fatigans of the normal and resistant strains were fed simultaneously on the same infected sparrows. Females, which had fed to repletion, were selected and kept in separate cages under identical conditions for the determination of sporozoite infection in the salivary glands. The results of dissections are contained in Table I.

In brief, 651 mosquitoes, 372 of the normal and 279 of the resistant strain, were given comparable blood feeds in five different lots. The results of dissections furnished no evidence of any increase or decrease in the vectorial capacity of resistant *C. fatigans*. In all the series, mosquitoes of both the strains were about equally infected, in three instances to the extent of 100 per cent.

EFFECT OF EXPOSURE TO DDT ON SPOROGONY IN RESISTANT *C. FATIGANS*.

In order to study the effect of DDT exposure on sporogony, if any, mosquitoes were kept in continuous contact with DDT during the entire extrinsic

*The investigations were financed by the Indian Council of Medical Research.

†MLC value of the resistant strain could not be determined due to the limitations of the test methods used. By the Busvine and Nash method (World Health Organization, Technical Report Series No. 80, 1954) at 4 per cent concentration of DDT with 48 hours' exposure the mortality in the resistant strain did not exceed 5 per cent, whereas the MLC of the normal strain was found to be 0·724 per cent with 24 hours' exposure.

incubation period. Feeding was carried out in exposure chamber (6 inches×6 inches×6 inches) lined on the inside, including movable lid, with butter paper. The entire exposed surface of the paper was treated uniformly with acetone solution of DDT to give a deposit of 200 mg. of the insecticide per square foot. The gametocyte-carrying sparrow, confined in a small wire-cage, was placed inside the exposure chamber. Mosquitoes were then introduced into it through the central aperture of the lid and left undisturbed for 12 hours.

TABLE I.

Comparative infections in normal and resistant C. fatigans *with* P. relictum.

Lot number.	Normal *C. fatigans*:			Resistant *C. fatigans*:		
	Number dissected.	Sporozoite positive.		Number dissected.	Sporozoite positive.	
		Number.	Percentage.		Number.	Percentage.
I	60	53	88	9	7	*
II	131	34	26	93	21	23
III	65	65	100	50	50	100
IV	63	62	98	55	55	100
V	53	53	100	72	72	100
Total	372	..	..	279	..	..

*The sparrow died when only a small proportion of the mosquitoes had ingested blood meal; percentage not given because of the wide disparity in the numbers of the normal and resistant mosquitoes dissected.

Note.—(i) Resistant *C. fatigans* used in lots I to III were of 78th generation and in lots IV and V of 79th generation.

(ii) Besides 'unexposed' mosquitoes of the normal and resistant groups shown against lots IV and V, there were also two other groups, one normal and one resistant, each of which was separately given the opportunity to imbibe infective blood meal during exposure to 200 mg. of DDT per square foot in exposure chamber with the same sparrow enclosed. Since these groups fall under different series of experiments, the results are included in Table IV.

Mosquitoes thus enclosed along with infected sparrow in exposure chamber for 12 hours, could imbibe infective blood meal at any time. At the end of this prolonged exposure, fully engorged females were transferred into a colony cage (2 feet×2 feet×2 feet) coated with 25 mg. of DDT per square foot on all sides except the front and bottom, wherein they were fed on glucose solution until dissections. The results are recorded in Table II.

In Lot I sporozoite infection was 75 per cent, while in Lot II it was 89 per cent. The development of sporozoites took about 8 days in both the comparison and DDT-exposed groups under the prevailing laboratory conditions. The sporozoites appeared normal in fresh and stained preparations. The results supplement and confirm the previous findings (Mohan, 1955).

It seems reasonable to conclude that DDT itself had no effect on the sporogony cycle of *P. relictum* in resistant *C. fatigans* even though the mosquitoes

remained in continuous contact with the insecticide throughout the extrinsic incubation period.

TABLE II.

Development of sporogony in resistant C. fatigans *in continuous contact with DDT deposits from before imbibing infective blood meal to the formation of sporozoites in the salivary glands or longer.*

Lot number.	RESISTANT *C. fatigans*: With continuous exposure to DDT. Infective blood meal imbibed during 12-hour exposure period while enclosed in exposure chamber treated with 200 mg. of DDT per sq. ft. and thereafter transferred into a colony cage coated with 25 mg. of DDT per sq. ft. until dissections.			RESISTANT *C. fatigans*: Without exposure to DDT. Comparison group.		
	Number dissected.	Sporozoite positive.		Number dissected.	Sporozoite positive.	
		Number.	Percentage.		Number.	Percentage.
I	12	9	75	..	..	..
II	27	24	89	18	12	67

Note :— (i) Resistant *C. fatigans* used in Lot I were of 71st generation, and in Lot II of 74th generation.

(ii) Lot II also included another group of resistant *C. fatigans* exposed to DDT deposits after imbibing infective blood feed. Since this group falls under different series of experiments, the results are included in Table III.

EFFECT OF EXPOSURE TO DDT ON SPOROZOITE INFECTION RATE IN RESISTANT *C. FATIGANS* AFTER IMBIBING INFECTIVE BLOOD MEAL.

Mosquitoes which had fed to repletion were partly kept for comparison (unexposed group) and partly exposed to 200 mg. of DDT per square foot. The exposure period was 24 hours in Lot I and 48 hours in Lot II. Results of dissections are shown in Table III.

Sporozoite infections in lots I and II were 67 and 53 per cent respectively for the comparison group and 70 and 50 per cent respectively for the groups exposed to DDT deposits. The findings indicated that once the infective blood meal was ingested, subsequent exposure to DDT brought about no alteration in the infection rate (Mohan, 1955).

EFFECT OF EXPOSURE TO DDT ON SPOROZOITE INFECTION RATE IN RESISTANT *C. FATIGANS* IMBIBING INFECTIVE BLOOD MEAL DURING 12-HOUR CONTACT PERIOD.

Different groups of mosquitoes in each lot were fed separately on the same infected sparrow, confined in a small wire-net cage. The untreated (comparison)

TABLE III.

Effect of exposure to DDT on sporozoite infection rate in resistant C. fatigans *after imbibing infective blood meal.*

Lot number.	RESISTANT *C. fatigans*:					
	Exposed to 200 mg. of DDT per sq. ft. for 24 or 48 hours after imbibing infective blood meal.			Without exposure to DDT. (Comparison group).		
	Number dissected.	Sporozoite positive.		Number dissected.	Sporozoite positive.	
		Number.	Percentage.		Number.	Percentage.
I	13	9	70	18	12	67
II	38	19	50	17	9	53

Note :—Resistant *C. fatigans* in both the lots were of 74th generation.

groups were fed in their respective bobbinet cages. Next, the treated groups, first the normal and then the resistant, were given the opportunity to get engorged during exposure to 200 mg. of DDT per square foot in exposure chamber with the same sparrow enclosed. The period of exposure for normal mosquitoes was one hour in Lot III and 45 minutes in Lot IV. Resistant mosquitoes were exposed for 12 hours. While there was little or no mortality among resistant *C. fatigans*, all the normal mosquitoes were knocked down with the exception of 2 specimens which had become partially engorged during 45 minutes exposure. The results of dissections are set out in Table IV.

It may be seen from Table IV that the sporozoite rates in the comparison groups (no exposure to DDT) of both the normal and resistant strains were 99·1 per cent and 91·5 per cent respectively. Similarly the sporozoite rates of 100 per cent (only two mosquitoes survived the extrinsic incubation period and they were found positive for sporozoite on dissection) and 91 per cent were observed in the normal and resistant strains respectively when they were exposed to DDT. These differences in the infection rates in mosquitoes of two strains and groups are not statistically significant. Therefore, from these results it may be concluded that (i) the two strains did not differ in respect of their vectorial capacity and that (ii) exposure to DDT did not have any effect on the development of malaria parasite in the surviving mosquitoes.

DISCUSSION.

A question of fundamental importance posed by the findings reported in this paper is whether the gene(s) responsible for physiological resistance in mosquitoes is the same or different from that for the susceptibility to infection with malaria parasites. Before attempting an answer to this question, it is desirable to review briefly the concepts of resistance and host-parasite relationship in vector species.

TABLE IV.

Effect of exposure to DDT (200 mg. per sq. ft.) on sporozoite infection rate in resistant C. fatigans imbibing infective blood meal during 12 hour contact period in treated exposure chamber with infected sparrow enclosed.

Lot number.	Resistant *C. fatigans*: Exposed to 200 mg. of DDT per sq. ft. Infective blood meal imbibed during 12-hour contact period in exposure chamber with sparrow enclosed.			Resistant *C. fatigans*: Without exposure to DDT. (Comparison group).			Normal *C. fatigans*: Exposed to 200 mg. of DDT per sq. ft. Infective blood meal imbibed during 45 minutes contact period in exposure chamber with infected sparrow enclosed.			Normal *C. fatigans*: Without exposure to DDT. (Comparison group).		
	Number dissected.	Sporozoite positive. Number.	Sporozoite positive. Percentage.	Number dissected.	Sporozoite positive. Number.	Sporozoite positive. Percentage.	Number dissected.	Sporozoite positive. Number.	Sporozoite positive. Percentage.	Number dissected.	Sporozoite positive. Number.	Sporozoite positive. Percentage.
I	27	24	80	18	12	67	..	..	..	..	..	..
II	24	20	83	20	12	60	..	..	..	..	..	..
III	15	15	100	55	55	100	All knocked down, none fed.			63	62	98
IV	12	12	100	72	72	100	2	2	..	53	53	100
Total	78	71	91·0	165	151	91·5	2	2	100	116	115	99·1

Note :—(i) Resistant *C. fatigans* used in lots I and II were of 74th generation and in lots III and IV of 79th generation.

(ii) In Lot III all the normal mosquitoes were knocked down at the end of one hour exposure to 200 mg. of DDT per square foot while enclosed with sparrow in exposure chamber. In contrast, none of the resistant *C. fatigans* was knocked down, although 79 per cent of the specimens did not feed on sparrow during 12-hour exposure.

(iii) In Lot IV, three specimens of the normal strain were found to have taken infective blood meal partially during 45-minutes exposure.

Brown (1958) has reviewed the articles of several workers and has shown that resistance is a pre-adaptive phenomenon. Insecticide pressure kills the susceptible insects in a heterogeneous population, while the individuals which are naturally most resistant are able to survive and breed, and in the course of time become dominant.

Huff (1927 : 1930) has studied the question of susceptibility to bird malaria in *C. fatigans* and has demonstrated the existence of a natural factor which determines susceptibility or non-susceptibility of the individuals within the same species. By selection of the susceptible and non-susceptible individuals, the same author (Huff, 1929 : 1931) has further shown that susceptibility and non-susceptibility are hereditary attributes, behaving as a dominant Mendelian character.

C. fatigans, used in the present investigation, was selected for resistance to DDT and not for susceptibility or insusceptibility to infection with *P. relictum* in local sparrows. If the factors making for resistance were the same as those which determine malarial susceptibility or non-susceptibility, the resistant strain of *C. fatigans* should have also automatically become either highly susceptible or non-susceptible and, therefore, one should have expected to find either extremely high or extremely low infection rate, particularly in the lots in which less highly resistant individuals, if any, were weeded out as a result of the prolonged contact with DDT deposits. But this was obviously not the case. On the other hand, both the normal and resistant *C. fatigans* were about equally infected, whether the grade of infection was low, medium or high. It, therefore, seems reasonable to infer that the *genetic basis* of resistance is different from the genetic basis of mosquito susceptibility or non-susceptibility to infection with malaria parasites.

SUMMARY AND CONCLUSIONS.

1. Normal and highly resistant *C. fatigans* were about equally infected with sporozoites of *P. relictum*, in three lots to as high a rate as 100 per cent.

2. Sporogony cycle was completed in a normal manner in resistant *C. fatigans* kept in continuous contact with DDT deposits throughout the extrinsic incubation period.

3. Resistant *C. fatigans* exposed to DDT deposits for 24 or 48 hours, after ingesting infective blood meal, showed practically the same sporozoite infection rate as that found in the untreated (comparison) groups of the same strain.

4. There was no alternation in the vectorial capacity of resistant *C. fatigans* for *P. relictum* and DDT itself had no effect on the malaria parasites.

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IN MEMORIAM.

DR. D.K. VISWANATHAN

(1901-1960).

DR. DHARMAVADANI KRISHNIER VISWANATHAN, Senior Regional Malaria Adviser to the Regional Office for South-East Asia, World Health Organization, died at Madras on March 27, 1960, at the age of 59.

Dr. D.K. Viswanathan was born on March 1, 1901, in Tirunelveli (Madras State). He got his medical degree at the Medical College in Madras in 1923, and five years later, his Diploma in Public Health at the same Institution. In 1937, he obtained the degree of Master of Public Health from the Johns Hopkins University.

For a few years, he was the State Malaria Officer of Madras State. He then went to Assam as the Research Officer of the Assam Medical Research Association. During the Second World War, in 1943 he was commissioned as a Major in the Indian Army Medical Corps and acted as the first Deputy Assistant Director of Malariology in the Indian Army in India. He was specially released within a few months of service to take up the appointment of Assistant Director of Public Health with the Government of Bombay State. He held this post from 1943 until 1952 when he became the Director of Public Health Service of the same State. In 1956, he joined the World Health Organization as Senior Regional Malaria Adviser to the Regional Office for South-East Asia.

Dr. Viswanathan was a Member of the Committees of the Indian Council of Medical Research, the Council of Scientific & Industrial Research, the W.H.O. Expert Advisory Panel on Malaria and a Fellow of the Indian Academy of Sciences.

The death of Dr. Viswanathan came as a rude shock to all his friends in India and abroad. The Public Health field in general, and the Malariology world in particular, has lost a great savant in Dr. Viswanathan. May his soul rest in peace !

—EDITOR.

DR. D. K. VISWANATHAN.

THE LATE DR. D. K. VISWANATHAN

GLIMPSES OF THE MAN.

DR. D.K. VISWANATHAN was known to many in the field of malariology, all over the world. The most formal contact with him, was formal only for a matter of minutes and led straightaway to deep friendship. Immediate antagonism came to be replaced later by admiration. He was irresistible. An aggressive public health worker, in thought, word and deed, he found in malariology of the last 3 decades a *milieu* most suited to his aspirations, vision, and to expend his inexhaustible energies. Where malaria control—and eradication later—was concerned, he was all combined in one, the Chief of Staff, the Commander-in-Chief and the front line soldier with bayonet drawn all the time. Yet, there have been occasions when he expressed disappointment at his recognition only as a malariologist despite his no less profound contributions in fields of public health other than malariology.

An outstanding feature of the late Dr. D.K. Viswanathan, the man, was his quickness of thought and action. He was quick to see situations, perhaps anticipate them, and equally quick in exploiting them to fit in with his scheme of things. In 1944, Mahatma Gandhi was stricken with malaria in Poona. Dr. D.K. Viswanathan, the then malariologist of Bombay State, collected mosquitoes in the Aga Khan Palace where Gandhi was staying and found one with oöcysts. He carried the dissected specimen and the microscope to the Mahatma to explain the source of the infection in him. Dr. Viswanathan quotes the Mahatma in his book, as having expressed his doubts of the mosquito having given him the infection or he himself having infected it? This introduction of practical malariology was perhaps responsible later for Gandhi conveying his blessings telegraphically to the inauguration of the then largest malaria control programme in the rural areas of Bombay State with DDT. The telegram established the requisite public relations as nothing else could have done.

Dr. Viswanathan combined in himself the qualities of a planner, an executive and a research worker. As he himself has recorded, his research was purposeful. The problems that arose in the field, formed the basis for his research which he successfully prosecuted and advanced the frontiers of knowledge.

Uniquely, he was an extremely good speaker as well as a writer. There, perhaps, has not been a single conference out of the innumerable ones he participated in, where he did not leave a forceful impression of what he had to say, not to mention of a positive contribution to the subject of discussion. To many in this country who have attended the malaria meetings of the Indian Council of Medical Research, year after year, Dr. Viswanathan's contributions were always stimulating. The void left by him will be keenly felt during these meetings hereafter. His speeches on such occasions combined the depth of knowledge, the fervour of a savant, the lucidity of exposition of a top criminal lawyer and the language of an accomplished orator. His repartee was quick. He was once asked by an inmate of Gandhi's ashram whether spraying of the walls of the houses with DDT to kill mosquitoes was not "Himsa" (cruelty) and, therefore, wrong. His

spontaneous reply was that a man was not a murderer if a thief got electrocuted while crossing the electric burglar-trap with which he had surrounded his house.

His writings in the field of malariology have been prolific and constitute milestones in the control of malaria for the future historian. His writings, however, have not been confined only to this speciality. He even wrote a short story in his mother tongue (Tamil) years ago which won a prize in a competition. A result of years of study of Hindu philosophy, was the book written by him in English which has merited the attention of other thinkers in that field. One has heard of authors like Edgar Wallace dictating entire books for publication. Dr. D.K. Viswanathan rarely wrote and most of his published papers and books were the result of dictation. Deservedly, he used to pride himself that rarely had he to revise a sentence or two of the typed draft.

For a practical man and executive of a high calibre and a scientist, Dr. Viswanathan was intensely religious. His religion had no orthodoxy or form. Yet, he had not eschewed altogether the formal practices of his religion. His was not a tolerance of other religions, but an absence of prejudice against them. His religion had a touch of mysticism in it.

It was not possible for Dr. Viswanathan to relax for long. He had no vacations except for a month in Europe not long ago. Even then, between times spent at the W.H.O. library, he was organising the Divine Life Society in Geneva and spread the message of his Guru, the Swami Shivananda. Perhaps a contradiction was that he enjoyed mystery fiction and detective stories. This was indeed a consolation to lesser people like me. Reading of detective fiction is generally associated with ordinary folks.

Like many well known scientists, Dr. Viswanathan was fond of music. Characteristically his enjoyment of music was serious and energised and not relaxed. On occasions, when the music was exquisite, he would close his eyes and listen to it, as it were, with his whole body.

The late Dr. Viswanathan was a sincere friend of a large number of people at home and abroad. He was generous to the extreme and has helped many unstintedly in various ways. Having received his guidance many times, once I asked him how I could ever repay his help which on many occasions had made heavy inroads into his busy and valuable time. His reply was characteristic. The only way I could repay the debt was to help others similarly when the occasion arose. It has been an advice, never to be forgotten.

He was a proud father. Deeply affectionate to all members of his family and relations, he had praise for every one of them. His was a four generation family—his father, his children and grand children. His energetic but tender ministrations, a few months before his demise, to his ailing infant grandson afflicted with encephalitis, achieved almost a miracle. At that time Dr. Viswanathan himself was ill and was receiving whole blood transfusions to combat his extreme anæmia. The grandson, named after the grandfather, made a total recovery.

The late Dr. Viswanathan was affectionately referred to as D.K. He had a child-like simplicity and enjoyed openly the implicit as well as explicit compliments and tributes paid to him by anyone. His frankness was disarming. He would heatedly argue a technical point and at times his criticism of the opposition was scathing, but never involved personalities. He was open to convincement. The public health component in him was severely against any large-scale use of antimalarials by the public health service in malaria control programmes. The prejudice lingered even when the concept of eradication came. But soon he was convinced and swung over to the other extreme and became a staunch exponent of the use of antimalarials as part of the eradication programme.

It was a treat to see him at work. Whether as a national or later an international public health expert, he had no occasion to refer to any notes even for the minutest details of any country's programme. His memory was phenomenal. Where others required a slide-rule or a calculating machine, he was ready with complicated mental calculations in a matter of seconds.

It has been said that modern malariology is not confined to localities, areas or even one country as in the past. The malariologist today has to have a global concept, understanding of the epidemiology, logistics of operations and assessment from a global stand point of view. The late D.K. was truly a global malariologist. His faith in eradication was profound. Yet this did not overwhelm his approach to the subject of malariology.

His thoroughness of approach to a problem on hand makes it difficult to classify him. In the early days his competency as a general physician was great. He has himself often recounted the tale as to how in a remote village he had to face a medical emergency. The child whom he was called to see was blue with diphtheria. He performed a tracheotomy with a kitchen knife on the patient lying on the floor. Till the motives of his action, resulting in a dramatic recovery of the child, were clear to the parents and the community, his own life was in danger ! Who can tell whether D.K. would not have become a great legendary general practitioner. As he often quoted the first principle of public health, "The greatest good to the largest number of people with available resources", his contributions were larger in the field of preventive medicine than what they could have been in medical practice.

Dr. D.K. Viswanathan departed from this world in March this year. In his own words, in a letter written shortly before death, he was "ready to meet his Maker". His memory will live long after his contemporaries are no more, and his work has a permanent place in the history of public health.

The global malaria eradication programme has lost a great leader. India has lost one of its greatest public health exponents. This Journal will no more see his brilliant and frequent contributions.

S.P. RAMAKRISHNAN

Delhi,
June 30, 1960.

Indian Journal of Malariology, **14**, 3, September 1960.

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

V. FURTHER STUDIES ON ATTACHMENT OF *MANSONIA ANNULIFERA* LARVAE TO HOST PLANTS OCCURRING IN TANKS CONTAINING *PISTIA STRATIOTES* IN KERALA STATE, SOUTH INDIA.

BY

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[*Entomologist (Filariasis), U.S. Technical Cooperation Mission to India.*]

[April 19, 1960.]

In the first study in this series (Burton, 1959), attachments of *Mansonioides* larvae were noted on roots, leaves, or other plant parts of the following eleven plants: *Pistia stratiotes, Eichhornia speciosa, Bacopa monnieri, Commelina salicifolia, Trapa bispinosa, Marsilea quadrifoliata, Limnophila gratioloides, Azolla pinnata, Lemna minor, Nelumbo nucifera,* and *Nymphaea* species. In a subsequent study (Burton, 1960), *Mansonia uniformis* larvae were seen to attach to roots or other plant parts of nine additional plants: *Brachiaria eruciformis, Leersia hexandra, Pseudechinolaena polystachya, Chara wallichii, Colocasia antiquorum, Wolffia arrhiza Lemna polyrrhiza, Ceratophyllum demersum,* and *Salvinia auriculata.* Although most attachments took place on roots, it was established by the second study that attachment to leaves lying in or on the water was a normal, rather than an unusual occurrence, as an alternative means of effecting gaseous exchange.

Continuing the observations on attachment of *Mansonioides* larvae to host plants occurring in *Pistia stratiotes* tanks in Kerala, South India, it was found in the present study that *M. annulifera* larvae attached to roots, leaves, or stems of the following fourteen plants: *Isachne dispar, Ilysanthes rotundifolia, Cyperus haspan, Cyperus compressus, Hydrilla verticillata, Elodea* (*Anacharis*) *canadensis, Monochoria vaginalis, Limnophila racemosa, Limnophila roxburghii, Ceratopteris thalictroides, Asteracantha longifolia, Limnanthemum cristatum, Alternanthera triandra,* and *Nitella furcata.*

As in the previous studies, photographs were taken on Kodak Panatomic X black-and-white film with an Edixa (German) 35 mm. camera having a Takumar (Japanese) f. 2·2, 55 mm. lens, plus extension rings for close-up shots. Enlargements, through the microscope, were made by mounting the reflex camera body on an adapter which was then fitted over the top of the body tube of the microscope, and the lenses of the microscope then replaced the camera lens.

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

Cross sections of roots or stems have been included as before, to show where the air chambers lie within the tissues, thus accounting for any particular behaviour on the part of the larvae during attempts to attach. The plants are presented below in the order in which they were studied.

1. *ISACHNE DISPAR* TRIN., A GRASS (FAMILY GRAMINEAE).

This plant is very common in *Pistia* tanks in Kerala, particularly around the edges, in the water and on moist banks. The stems are about 1/25 to 1/16 inch in diameter. The leaves are solitary, sessile, alternate, lanceolate, up to two inches long, smooth, 3/8 inch wide, with fine parallel longitudinal veins. The panicle is about two inches high, its branches extending at right angles up to 7/8 inch in length. The spikelets are short-pedicelled, 1 mm. wide by 1½ mm. long. The roots are white and ½ to 1 mm. in diameter.

A cross section of a root shows fifteen main chambers containing air, and a number of secondary chambers (Fig. 2). *M. annulifera* larvae attached in very large numbers to roots, stems, and leaves, burying their serrated plates on the siphons deeply into the tissues (Fig. 1 and 3).

The leaf attachments of *M. annulifera* larvae to this plant are extraordinary and unusual. A cross section of a leaf shows the complete absence of air chambers, there being the usual motor cells, sclerenchyma, chlorophyllous layers, and vascular bundles ; yet leaves which fell beneath the surface of the water, had dozens of attached larvae which remained in position effecting gaseous exchange for hours and even several days in some instances. Attachments were made both into upper and lower surfaces (Fig. 4 and 5). An *Isachne dispar* leaf consists of a midrib and 28 other longitudinal ribs (on each side of the medial rib), every fourth rib being stronger by having more xylem cells. Due to the absence of air chambers, it is assumed that the supply of oxygen within the chlorophyllous tissue is so great in this plant that the larvae sense this and therefore attach in large numbers to these leaves.

2. *LYSANTHES ROTUNDIFOLIA* BENTH., FALSE PIMPERNEL (FAMILY SCROPHULARIACEAE).

This annual, aquatic plant was found floating erect or recumbent in some *Pistia* tanks in Palluruthy, Kerala (Fig. 6). The squarish stems are 6 to 10 inches high, with opposite, sessile, broadly ovate, entire or toothed, 3-5 nerved leaves. The internodes are 1 to 1¾ inches apart except at the apex. The branching is axillary. The white flowers are short-pedicelled and axillary, the corolla being ¼ inch in length.

The roots are fine and hairlike. Attachment of *M. annulifera* larvae took place in large numbers at the origins of the main roots (Fig. 7) as well as along the roots, and also to the leaves in large numbers (Fig. 8). A cross section of a smaller main root (Fig. 9) shows the air chambers enclosed within a substantial cortex, thus enabling the terminal hooks of the siphon to maintain their grasp in the tissues.

PLATE 1.

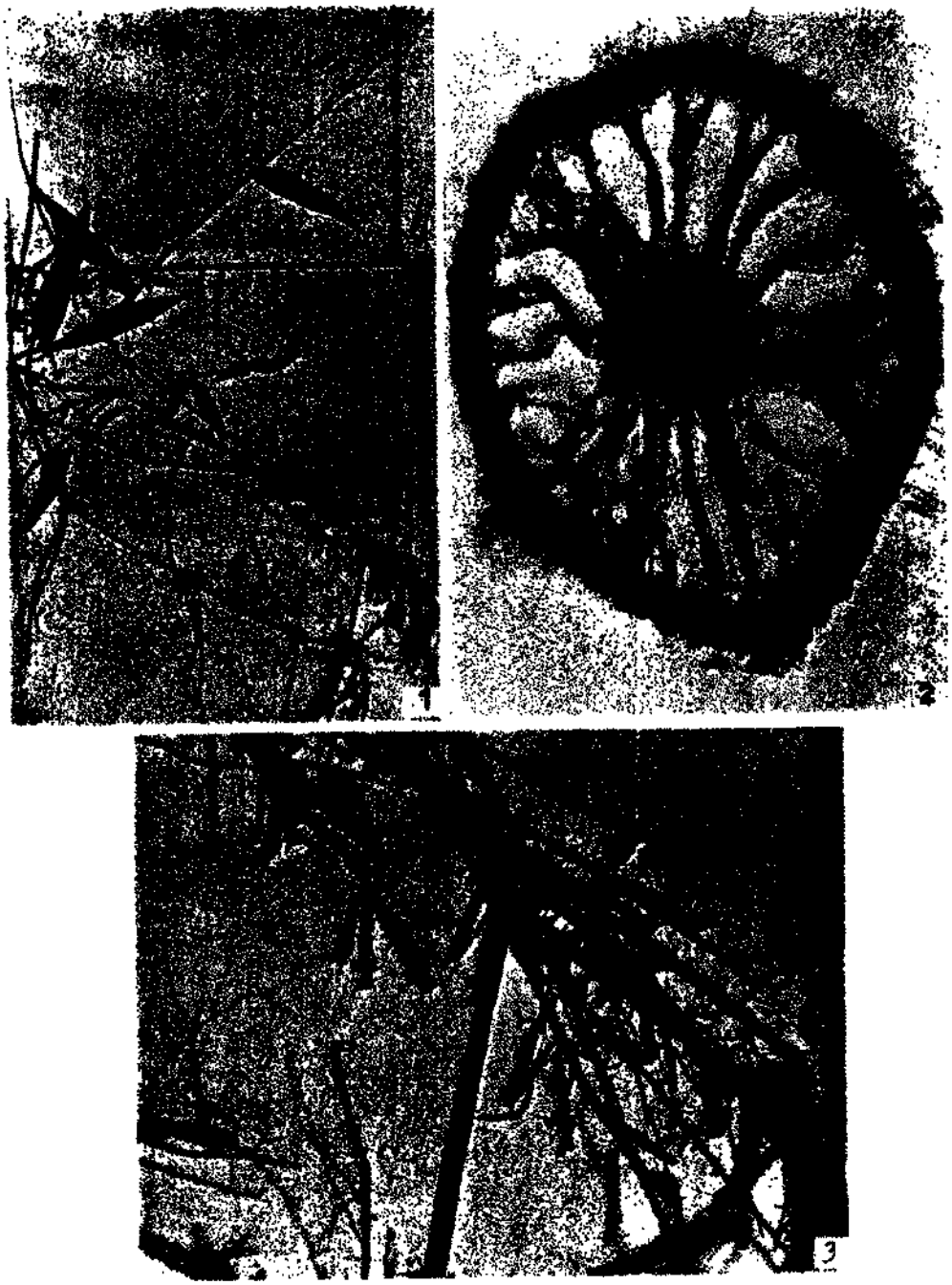
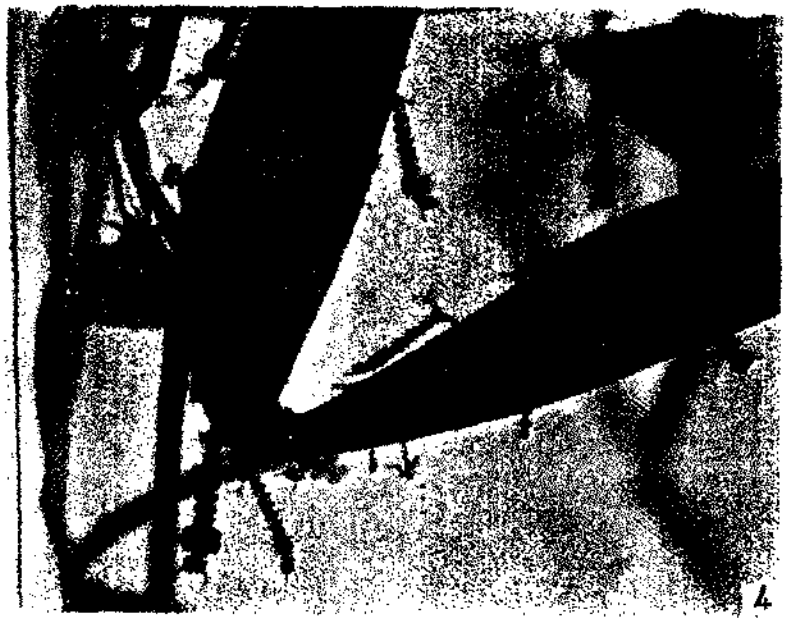


FIG. 1. *Isoetes dispar* with attached *Mansonia annulifera* larvae.
FIG. 2. Cross section of root of *I. dispar* showing air chambers.
FIG. 3. *M. annulifera* larvae attached to main roots of *I. dispar*.

PLATE II.



FIGS. 4 and 5. *M. annulifera* larvae attached to leaves of *Eichhornia diversifolia*. Note attachment on both surfaces of blades.

PLATE III.

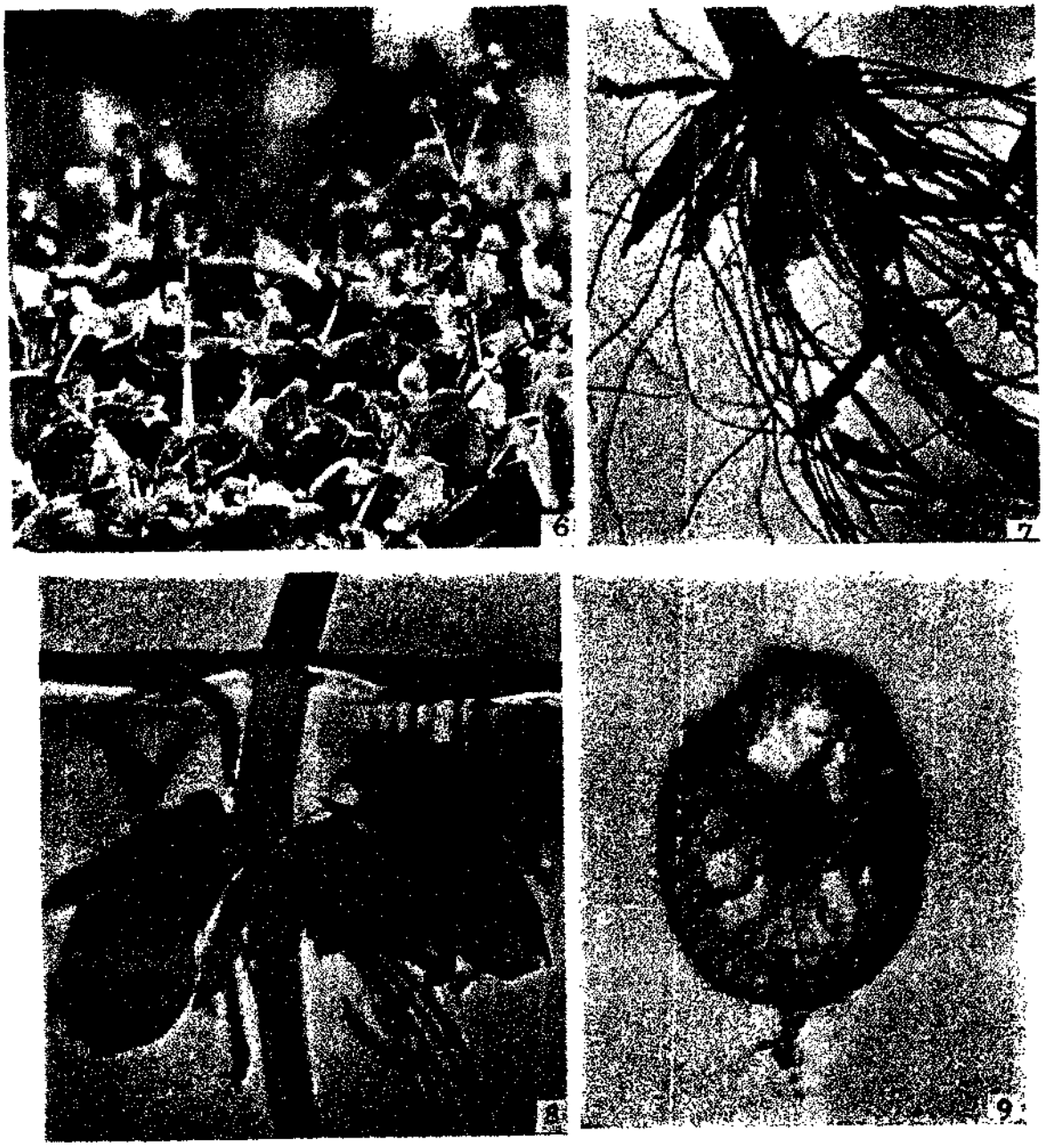
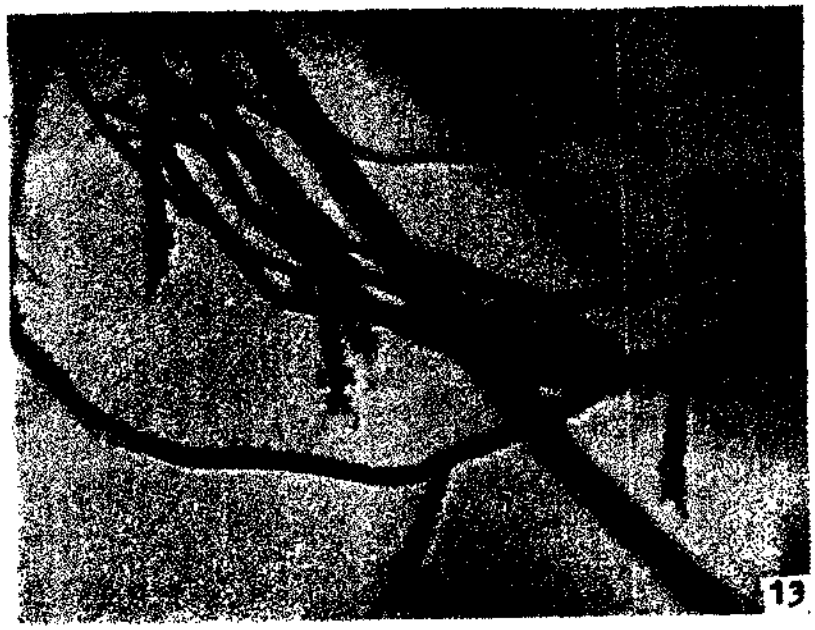


FIG. 6. *Hydrocharis rotundifolia* plants, erect and floating among *Pistia stratiotes* plants in a tank.
FIG. 7. *M. annulifera* larvae attached at origins of roots of *L. rotundifolia*.
FIG. 8. *M. annulifera* larvae attached to leaves of *L. rotundifolia*.
FIG. 9. Cross section of root of *L. rotundifolia* showing air chambers.

PLATE IV.

FIG. 10. *Cymbalus haspan* with attached *M. annulifera* larvae.
" 11. Cross section of *C. haspan* root showing few large air chambers.
" 12. *M. annulifera* larvae attached to main roots of *C. haspan*.

PLATE V.



FIGS. 13 and 14. Close-ups showing *M. annulifera* larvae attached to stem roots of *Cynanchum laeviga*

PLATE VI.

FIG. 15. *Cyperus compressus* with *M. annulifera* larvae attached to main roots.
FIG. 16. Close-up of *M. annulifera* larvae attached to main roots of *C. compressus*.

3. *CYPERUS HASPAN* LINN., A SEDGE (FAMILY CYPERACEAE).

This tufted, perennial herb may be 4 to 18 inches high, and may or may not have an underground rhizome. The stem is 3/16 inch wide, and the roots are 1 mm. in diameter with fine lateral rootlets. The leaves are few and linear, often reduced to membranous sheaths. The inflorescence at the top of the stem is a diffusely branched compound, spreading umbel, there being about 11 filiform rays, one short leaf and one long leaf (Fig. 10). The spikelets are narrowly linear. The fruit is small, round to ovoid, and pale brown. This plant is called *chali thuga* in the Telegu language.

Mansonia annulifera larvae attached in very large numbers to roots of this plant (Fig. 10 and 12), remaining so attached for days. A cross section of a root shows a peripheral tissue ring composed of the epidermis and a narrow cortex, with a dense core of tissue internally, the air chambers being few in number and very large (Fig. 11). Penetration into an air chamber in this root is therefore relatively simple (Fig. 13 and 14).

4. *CYPERUS COMPRESSUS* LINN., A SEDGE (FAMILY CYPERACEAE).

This sedge resembles that of the preceding species, but the umbel is somewhat different, there being three long leaves, some shorter leaves, and the spikelets are grouped as shown in Fig. 15. This plant was 7 inches high, the stem being somewhat triangular. The roots are numerous, ⅜ inches long, ½ mm. in diameter, and are reddish and white in colour, with many fine rootlets. Attachment of *M. annulifera* larvae took place readily, but many of them preferred to attach near the origins of the roots (Fig. 15 and 16). In cross section, the root was found to be identical with that of *C. haspan*.

5. *HYDRILLA VERTICILLATA* CASP. (FAMILY HYDROCHARITACEAE).

This branched, annual, aquatic herb is usually submerged, forming wide mats or masses. The leaves are in whorls of 4—8 at each node, about ⅛ to 1 inch in length, but averaging ⅛ inch, being minutely-toothed and linear-oblong. The internodes quite short. The lower leaves are opposite and longest. The flowers are ⅛ to ¼ inch long, sessile, and axillary. The long-stalked fruit may be smooth or covered with minute spinules. The leaves are usually green, but older specimens may present a brownish appearance. Rooting frequently takes place at the node.

M. annulifera larvae attached in large numbers to the stem, mostly at the nodes, but also into the internodes (Fig. 17 and 18), remaining in place for many hours. A cross section of the stem shows about a dozen air chambers with sufficient supporting tissue for embedding of the siphon tip (Fig. 19).

6. *ELODEA* (*ANACHARIS*) *CANADENSIS* MICHX., WATER-WEED, (FAMILY HYDROCHARITACEAE).

This perennial, slender, submerged plant may be confused with *Hydrilla*, but its leaves are longer than those of the preceding species. The lower leaves are

opposite and the upper are whorled, both kinds being sessile and single-nerved. The leaves are linear to oblong, ¾ inch long, or longer, and are minutely serrulate. The flowers are sessile and solitary. Long, silky-looking roots come off at the nodes, being about ½ mm. wide by one inch long. The fruit is carried on an elongate stalk due to prolongation of the calyx tube.

As in *Hydrilla*, attachment of *M. annulifera* larvae occurred mostly at the nodes, but some larvae also attached to the leaves (Fig. 20). A cross section of the stem (Fig. 21) and of a fine root (Fig. 22), showed the same kind of air chambers as are present in *Hydrilla*.

7. *MONOCHORIA VAGINALIS* PRESL. (FAMILY PONTEDERIACEAE).

This plant belongs to the same family as *Eichhornia speciosa*, the water hyacinth, one of the main differentiating characters being the absence of inflated petioles in *Monochoria*. The latter flowers during the monsoon season, being common in tanks, ricefields, and marshes. It is a perennial herb with a creeping, suberect rhizome. The leaves are somewhat heartshaped, about 2 inches wide and 3 inches long, on 6—12 inch petioles, the veins parallel and converging at the tip (Fig. 23). The blue, red-spotted, ½ inch wide flowers are short-pedicelled and arranged in racemes arising from the leaf sheath ; the petiole bearing the inflorescence is enlarged in the middle. The ellipsoid fruit is less than ½ inch in length. This plant is known as *kakapola* in Malayalam, *neelolpalam* in Sanskrit, and *neer thamarai* in Tamil.

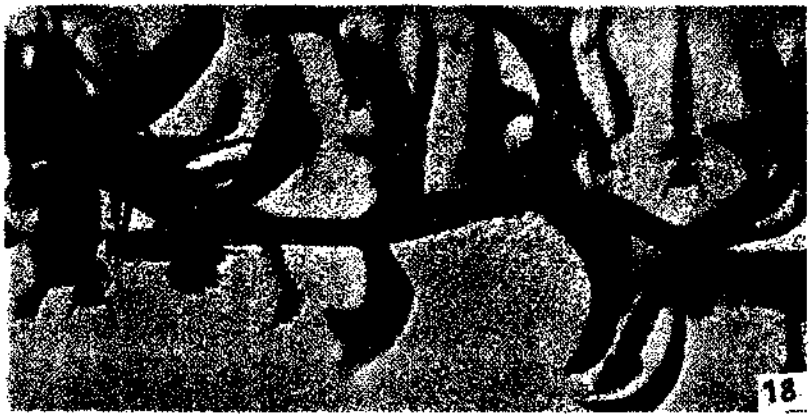
The coarse, white roots are 1/16 to 3/32 inch in diameter, with silky, hairlike, lateral roots. *M. annulifera* larvae attached readily to the main roots in varying positions (Fig. 25 and 26), probably correlated with the very soft, yielding root tissues as seen in the cross section in Fig. 24. The strong anchorage of the hooks in roots of other plants is prevented in this species by the loose, soft tissues, making it necessary for the larva to twist itself in order to gain a favourable position. As is then to be expected, the hooks and serrated portion of the siphon are embedded rather deeply into the root.

8. *LIMNOPHILA RACEMOSA* BENTH. (FAMILY SCROPHULARIACEAE).

This plant is often quite abundant in *Pistia* tanks (Fig. 27), the submerged, finely divided leaves filling the entire tank. It is readily discernible by the characteristic green cylinder-shaped or ball-like masses of capilliform, multifid, needle-like leaves which are completely submerged (Fig. 29). The stem continues aerially from the apex of an ovoid or cylindrical mass to form an erect shoot having sessile, somewhat ovate, serrulate leaves. The bluish or purplish flowers are in terminal, erect recemes.

Monsonia annulifera first-instar larvae attached in large numbers to the submerged, needle-like leaves, penetrating readily with their cylindrical siphon tips (Fig. 33), and remaining attached for many hours. Many larvae also attached to

PLATE VII.



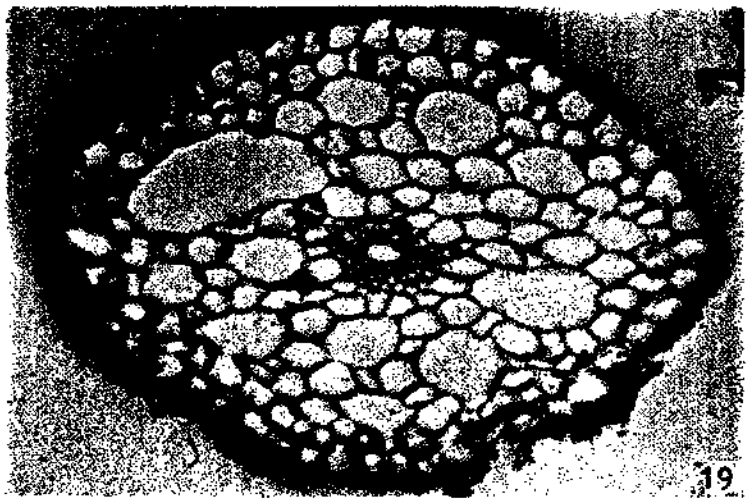


FIG. 17 and 18. M. *annulifera* larvae attached to stem and at nodes of *Hydrilla verticillata*.
FIG. 19. Cross section of stem of *H. verticillata* showing nature of air chambers.

PLATE VIII.

FIG. 20. *M. annulifera* larvae attached to leaves and at nodes of *Elodea canadensis.*

FIG. 21. Cross section of a fine root of *Elodea canadensis* showing similarity to section of stem of *Hydrilla verticillata.*

FIG. 22. Cross section of stem of *Elodea canadensis.*

PLATE IX.

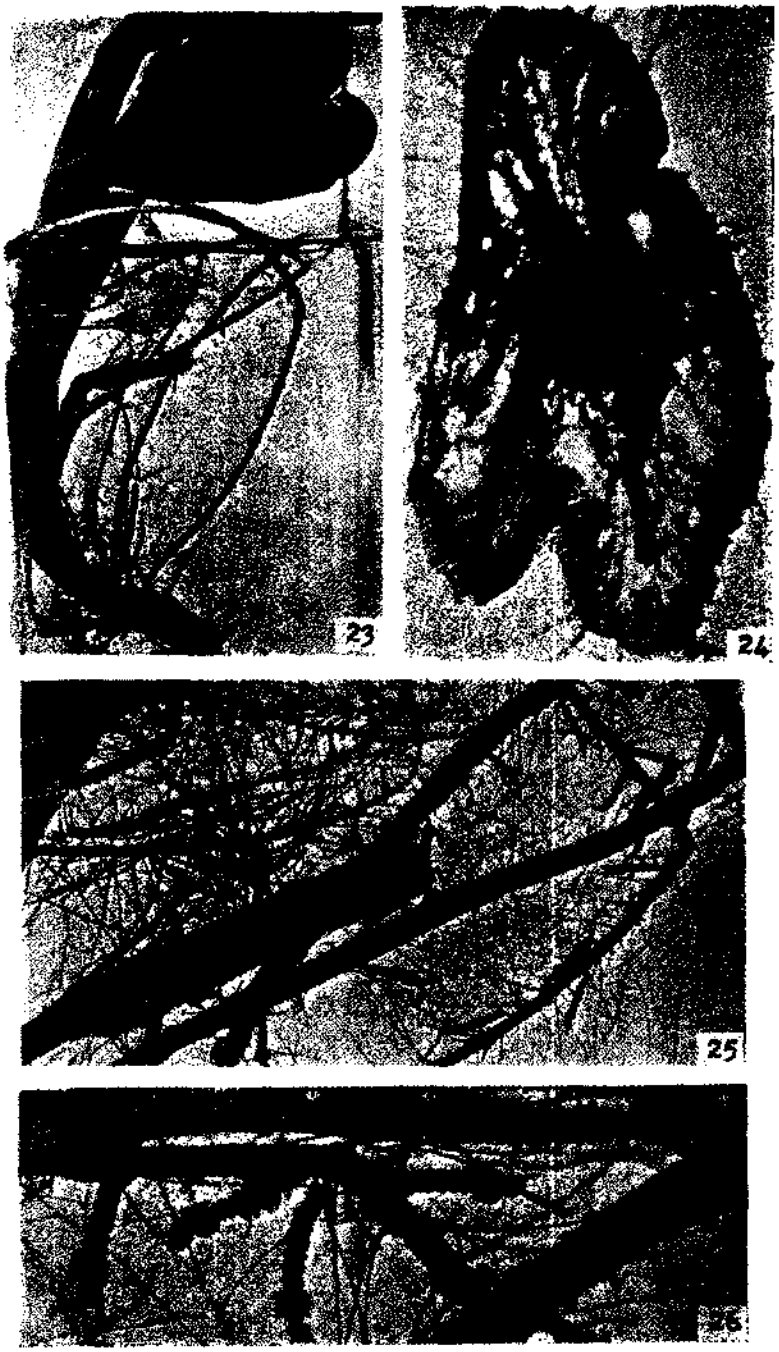


FIG. 23. *Monochoria vaginalis* with *M. annulifera* larvae attached to main roots.

FIG. 24. Cross section of a main root of *M. vaginalis* showing air chambers and soft tissues.

FIG. 25 and 26. Close-ups showing *M. annulifera* in attachment positions on main roots of *M. vaginalis*.

PLATE X.

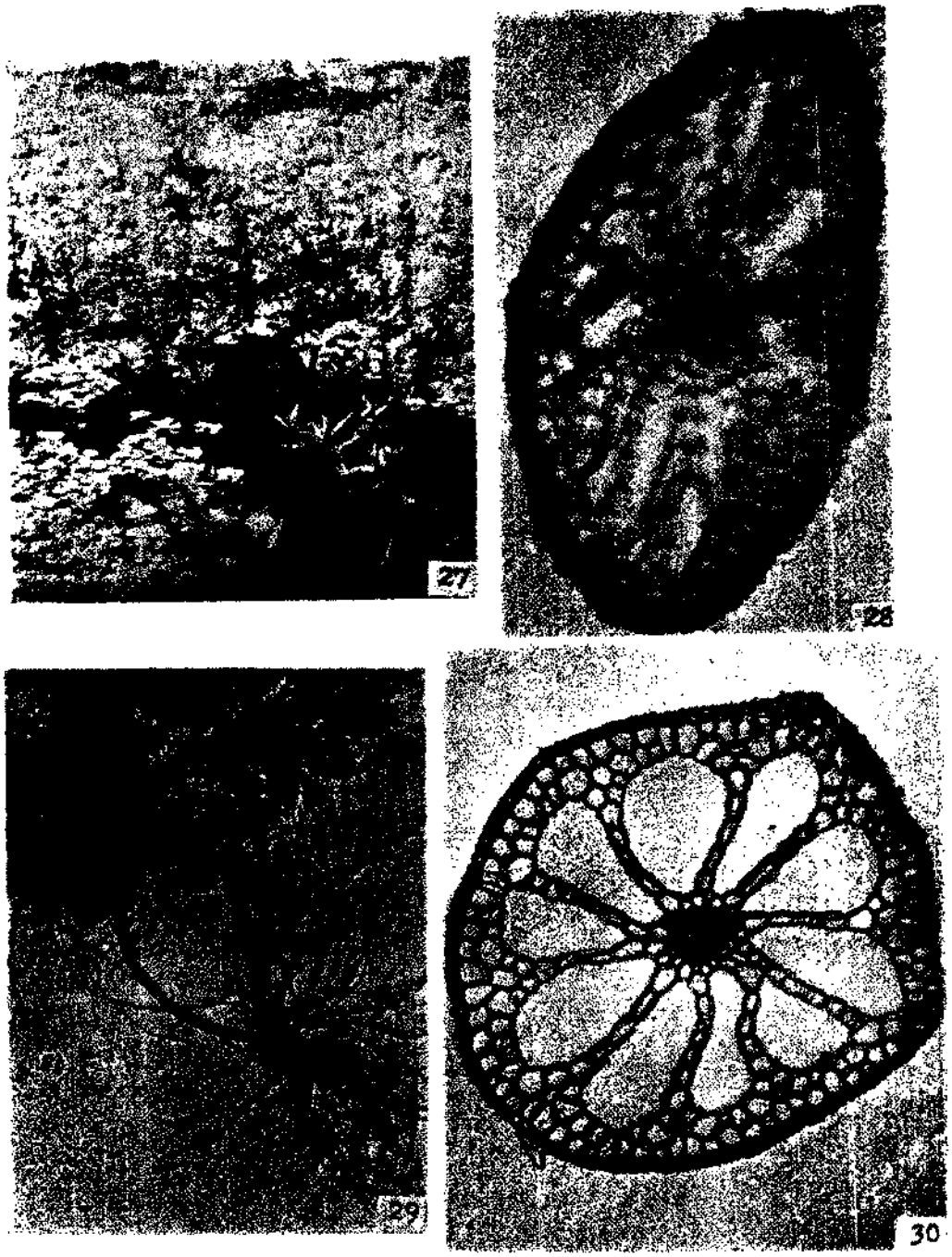


FIG. 27. *Lemnophila racemosa* erect and submerged portions in *Pistia stratiotes* tank.

FIG. 28. Cross section of root of *L. racemosa* showing air chambers.

FIG. 29. Roots and multipinnatified submerged leaves of *L. racemosa* with attached *M. annulifera* larvae.

FIG. 30. Cross section of submerged stem of *L. racemosa* showing clear-cut air chambers.

the submerged stem (Fig. 31) and to the roots (Fig. 32). A cross section of a stem (Fig. 30) shows 10 clear-cut, large air chambers within a soft cortex, and a cross section of a root (Fig. 28) shows a similar type of structure, but with narrower, more numerous air chambers.

9. *LIMNOPHILA ROXBURGHII* G. DON. (FAMILY SCROPHULARIACEAE).

This species differs from the preceding one in that there are no submerged whorls of pinnatified or multifid leaves. It was found in large numbers associated with *Pistia stratiotes* in Alleppey, Kerala (Fig. 34). There are many stout stems with opposite, short-petioled, elliptic or ovate, serrate or crenulate leaves which are punctate beneath and have prominent veins. The violet, sessile or short-petioled flowers are grouped in terminal, leafy spikes.

The white roots are 1/16 to 3/32 inch in diameter, growing to four inches in length, with many fine lateral roots. *M. annulifera* larvae attached themselves to the main roots, especially where they originate from the stem (Fig. 35, 36, and 37), as in *Cyperus compressus* and *Ilysanthes rotundifolia*. A cross section of a main root (Fig. 38) shows the air chambers and adjoining tissue to be identical with those of *L. racemosa*.

10. *CERATOPTERIS THALICTROIDES* (L.) BROGN., A WATER FERN (FAMILY POLYPODIACEAE).

This annual or perennial plant is a rather succulent, aquatic fern with two types of fronds—one linear and reproductive by means of sori, and the other broad-lobed. The fertile fronds are pinnately 2—3 branched, with the terminal segments forked and somewhat pod-like. The forked fronds have a resemblance to the branching of stag horns. In a *Pistia* tank some of the plants may be directly in the water and others may be at the edge (Fig. 39). The stem tends to be 4-sided or quadrate. The leaf stalk or stipes is inflated, being filled with large air spaces which keep the plant afloat. The long, green stems meet at the base, and the finely feathered, long roots have almost invisible lateral rootlets.

Being a water fern, this plant has characteristic sori on two or three veins running longitudinally along the fertile pinnae The sporangia are sessile and subglobose. This plant is called *manganari* in Malayalam and *kuttra* in Hindi.

M. annulifera larvae were seen to attach instantly and tenaciously to the fine main roots and to the leaves (Fig. 41). Young *C. thalictroides* plants float at the surface like those of *Pistia* (Fig. 42), with roots dangling downwards and resembling rootlike structures of *Salvinia auriculata*. The larvae attach instantly to the main roots of these young plants in large numbers (Fig. 44 and 45). A cross section of a main root (Fig. 43) shows a soft epidermis and cortex enclosing at least a dozen large air chambers, thus accounting for the ease of attachment. At least half of the serrated plate of the siphon is buried in the cortex of the root during attachment.

11. *ASTERACANTHA LONGIFOLIA* NEES (FAMILY ACANTHACEAE).

This is a stout, erect, hairy herb (Fig. 46), 1–5 feet high, whose hairy stems have sharp spines or thorns ½ to 1½ inches long, at the nodes. The lanceolate, sessile, opposite leaves, which can grow to 7 inches in length, have wavy margins, taper at the base, and are covered with tiny, white hairs. They are pseudo-whorled in sixes. The 1¼ inch long flowers, which are in sessile, axillary whorls, are blue, bluish-purple, rose or white in colour. The tubular corolla has a bi-lobed upper lip and a tri-lobed, orange-streaked lower lip. The ½ inch long fruit is a linear-oblong capsule. This plant is called *vayalchulli* or *voyal-chullai* in Malayalam ; *kailaya, gokshura, talmakhana,* or *kantakalia* in Hindi ; *nirmuli* in Tamil ; and *kokilakshi* or *neeru-gobbi* in Telugu.

The white roots are 1/20 to 3/16 inch in diameter, and the longer roots have lateral branches without small rootlets as such (Fig. 46). The plant is loosely rooted in the tank, with the upper, white roots floating or hanging down.

M. annulifera larvae attached readily to the large and small roots (Fig. 47 and 49), and also to the underwater leaves and stem (Fig. 50), remaining so attached for very long periods of time. A cross section of a large root, as shown in Fig. 48 shows at least 40 air chambers of varying sizes, with a relatively loosely packed cortex. The serrated portion of the siphon is deeply buried within the cortex, and the larvae hang at various angles. When attached to leaves, the larvae tend to hang dorsal side up (Fig. 50) rather than upside down.

12. *LIMNANTHEMUM CRISTATUM* GRISEB., FLOATING HEART (FAMILY GENTIANACEAE).

This is a perennial water plant rooted in the mud with round, heart-shaped entire or sinuate leaves, 3—4 inches in diameter (Fig. 51). These leaves float at the surface at the ends of petiole-like branches. The delicate, white flowers are small, on long, clustered peduncles about an inch below the blade, where the stem also produces roots (Fig. 54). There are 5—6 entire petals having a longitudinal fold down the middle. The capsule is 1/5 inch in diameter, and is subglobose. The plant is called *anthara thamara* in Telugu.

Larvae of *M. annulifera* attached readily to the large green pedicels and roots near the nodes (Fig. 54). It is interesting to note that many of these larvae pupated (Fig. 52) while so attached, the adults emerging later on. A cross section of a main root (Fig. 53) shows a radiating pattern of parenchymatous cells, the cortical ones having numerous chloroplasts, but there are no air chambers as such. Apparently there is an abundance of oxygen within this tissue, the loosely packed cells permitting gaseous exchange to take place, as evidenced by pupation and subsequent emergence of the adult.

13. *ALTERNANTHERA TRIANDRA* LAMK. (FAMILY AMARANTHACEAE).

This is a much branched, prostrate or semi-erect herb having lanceolate or somewhat ovate entire, opposite leaves up to 2½ inches in length. The stems are

PLATE XI.

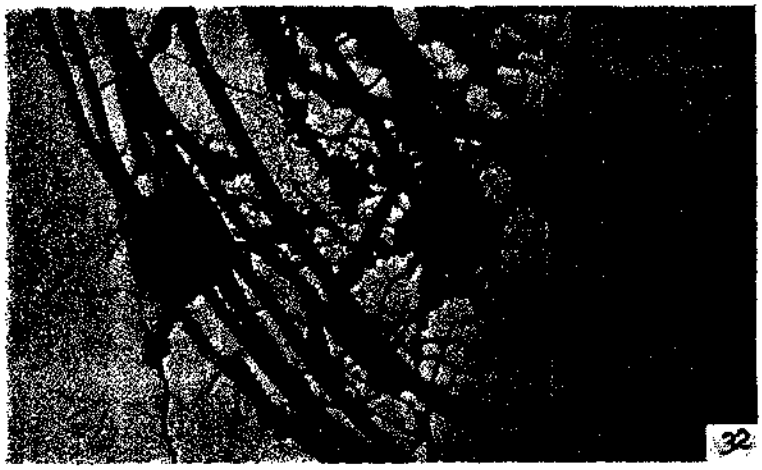


FIG. 31. *M. annulifera* larvae attached to roots and submerged stem of *Limnophila racemosa*.
FIG. 32. *M. annulifera* larvae attached to main roots of *L. racemosa*.
FIG. 33. First-instar larvae of *M. annulifera* attached to fine, filiform, submerged leaves of *L. racemosa*.

PLATE XII.

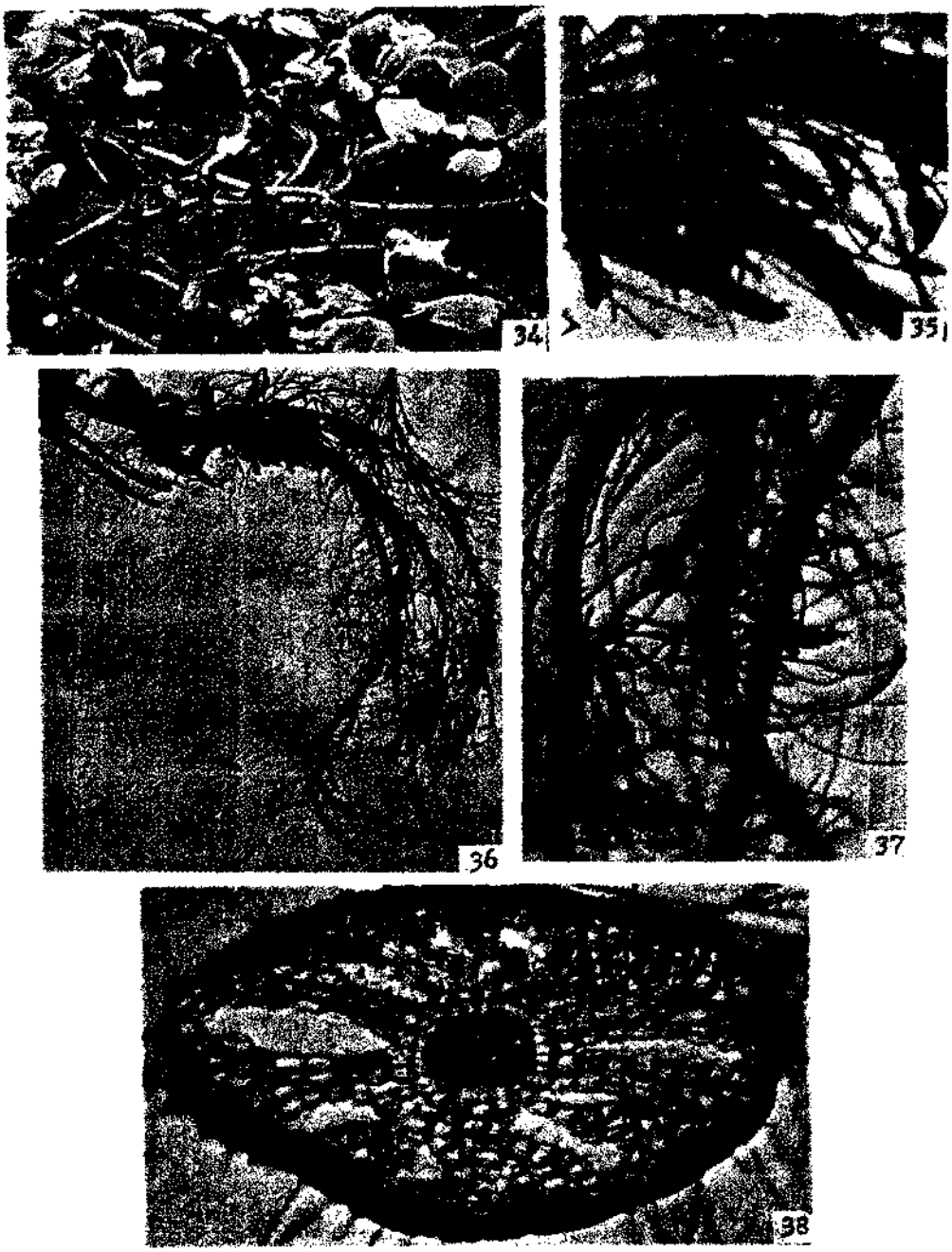


FIG. 34. *Lemnophila roxburghii* submerged stems in *Pistia stratiotes* tank.

FIGS. 35, 36 and 37. *M. annulifera* larvae attached all along main roots of *L. roxburghii*.

FIG. 38. Cross section of main root of *L. roxburghii*. Note similarity to root structure of *L. racemosa*.

PLATE XIII.

FIG. 39. Erect plants of *Ceratopteris thalictroides* in *Pistia* tank.

FIG. 40. Single unit of *C. thalictroides* showing *M. annulifera* larvae attached to main roots and to some leaves.

FIG. 41. Close-up of *M. annulifera* first-instar larva attached to pinnatified leaf of *C. thalictroides*.

PLATE XIV.

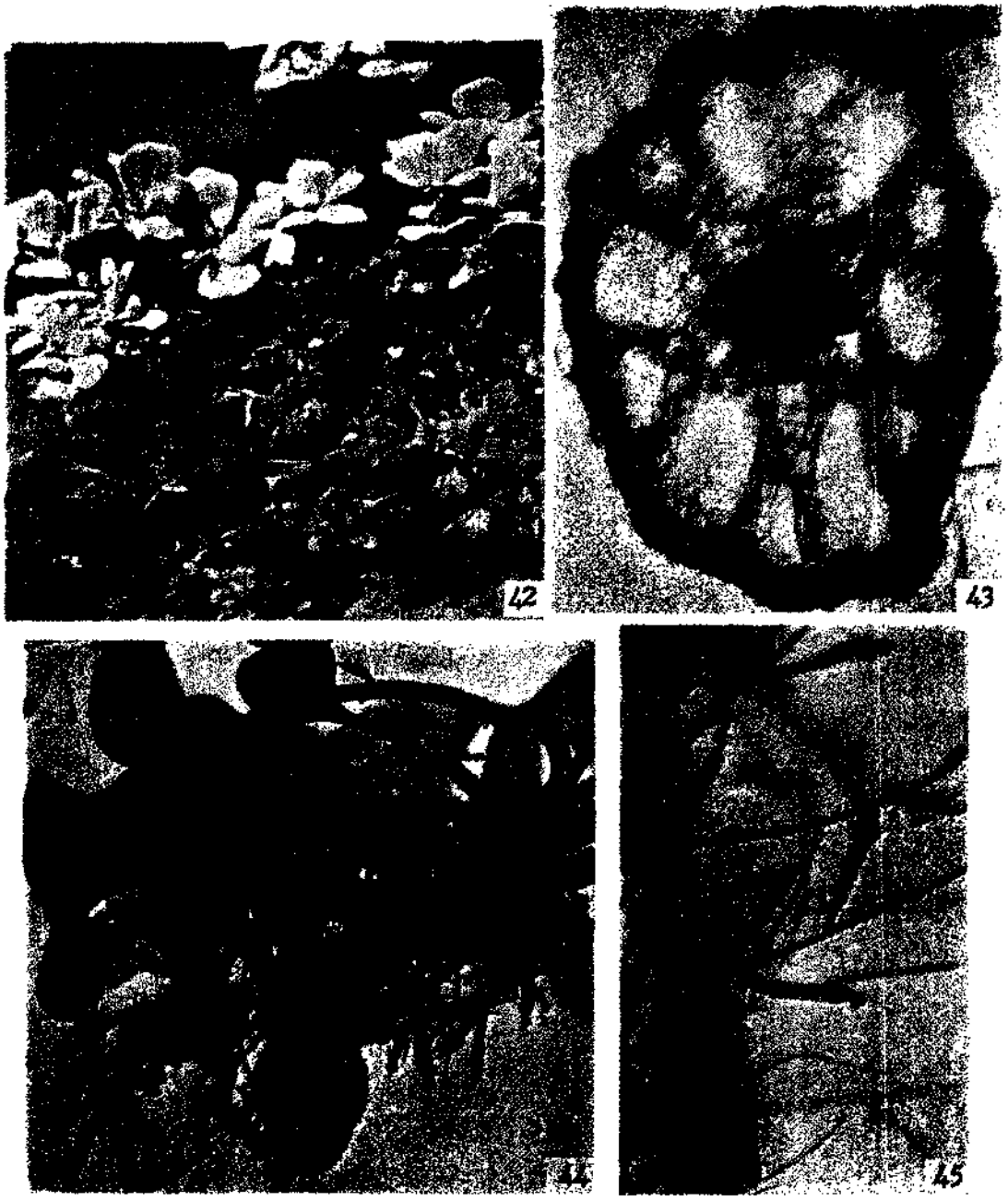


FIG. 42. Young plants of *Ceratopteris thalictroides* floating in *Pistia* tank.

FIG. 43. Cross section of main root of *C. thalictroides* showing large air chambers.

FIG. 44. Young, floating *C. thalictroides* plant showing many *M. annulifera* larvae attached to leaves and main roots.

FIG. 45. Close-up of *M. annulifera* larvae attached to main roots of *C. thalictroides*.

PLATE XV.

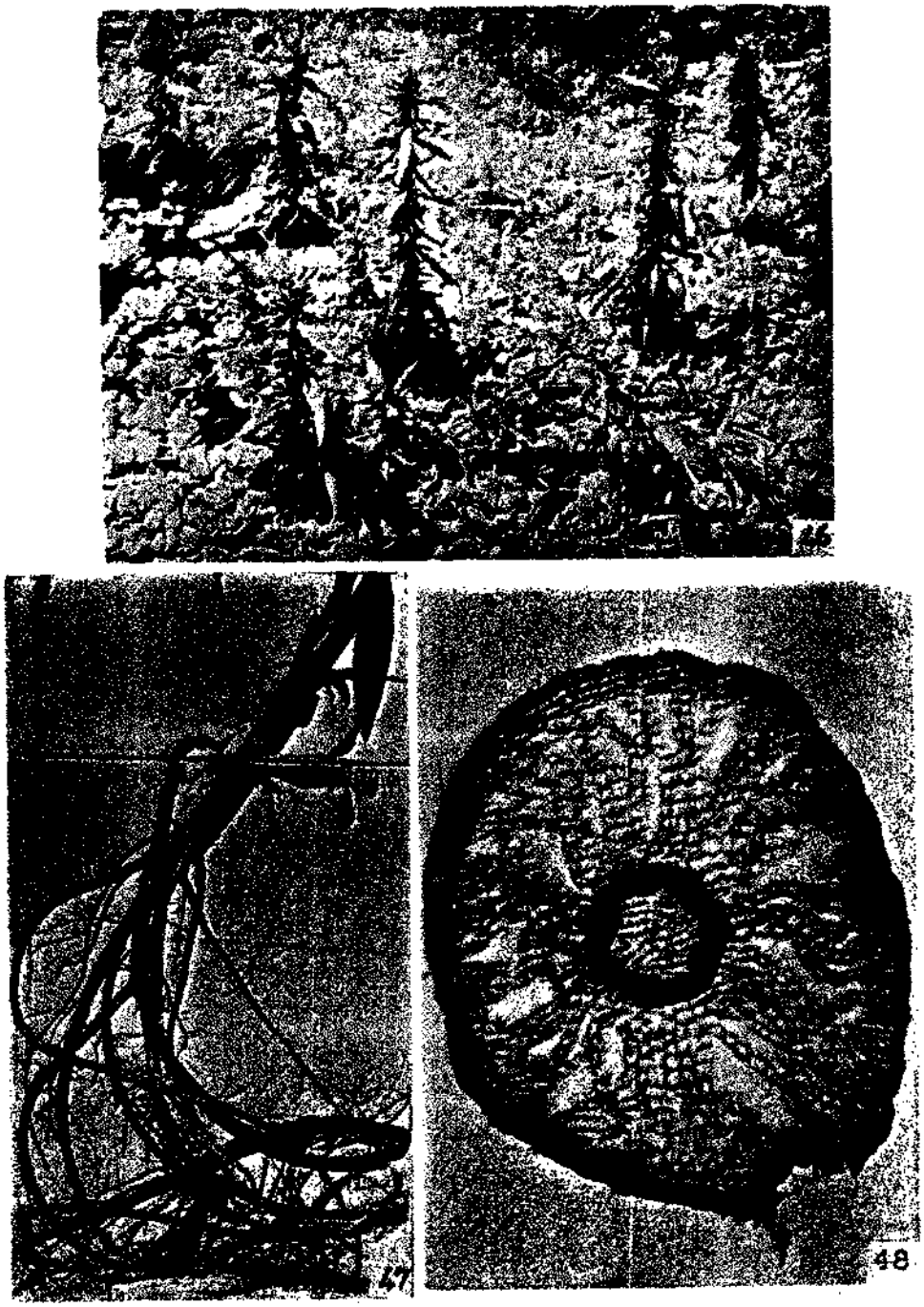


FIG. 46. Erect *Asteracantha longifolia* plants in *Pistia stratiotes* tank.
FIG. 47. *A. longifolia* roots with attached *M. annulifera* larvae.
FIG. 48. Cross section of large main root of *A. longifolia* showing many air chambers.

PLATE XVI.

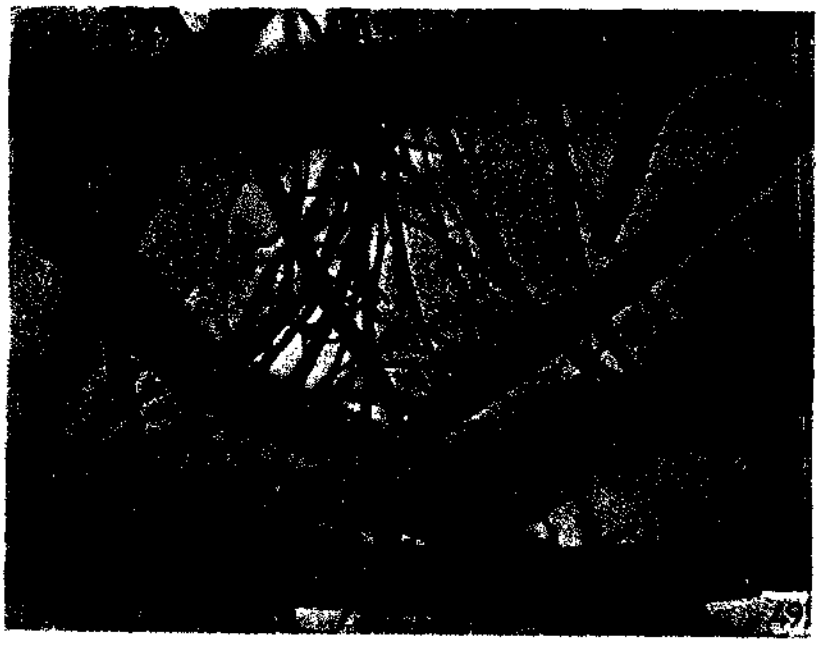


FIG. 49. Close-up of *M. annulifera* larvae attached to main roots of *Asteracantha longifolia*.
FIG. 50. *M. annulifera* larvae attached to stem and leaves of *A. longifolia*.

PLATE XVII.

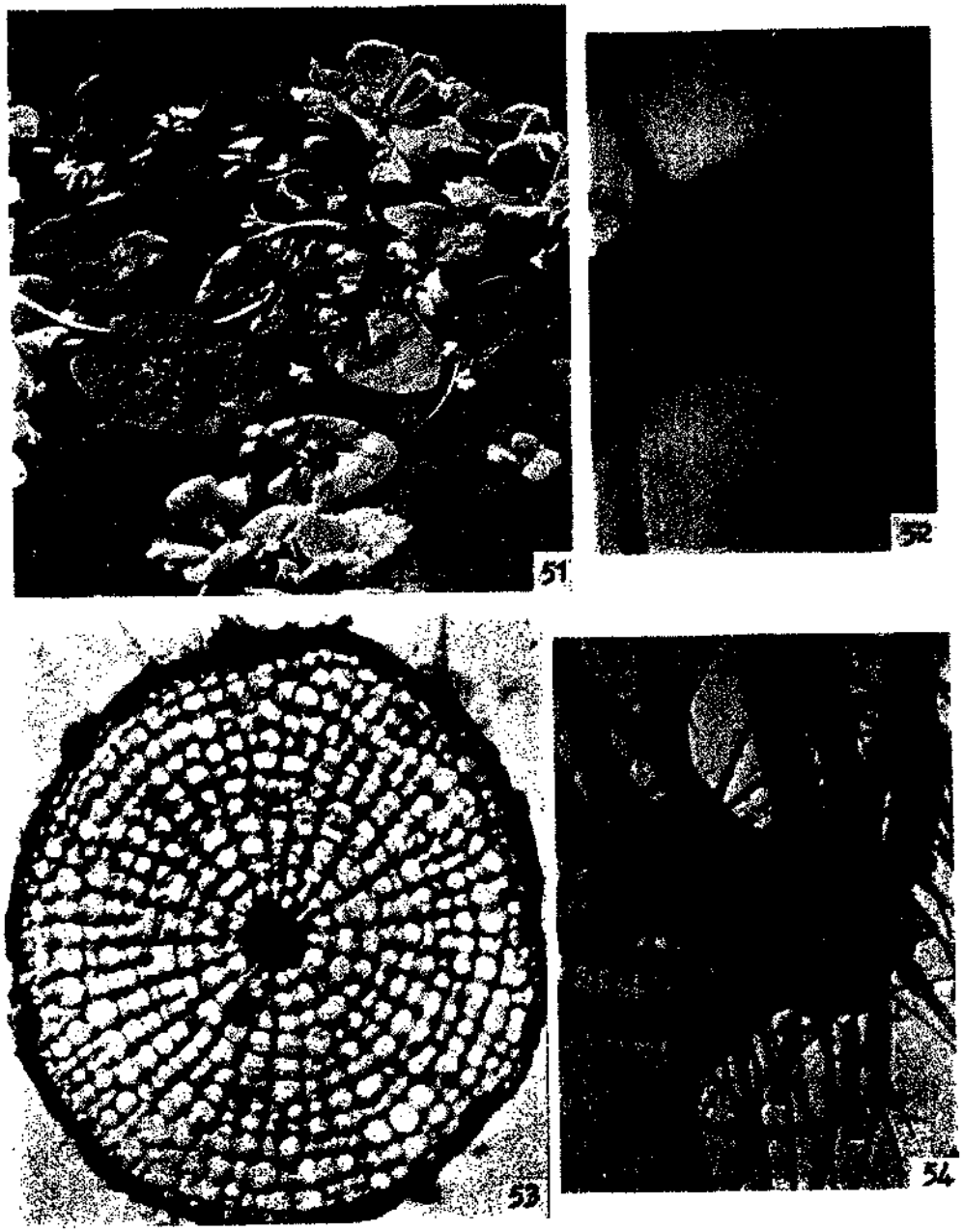
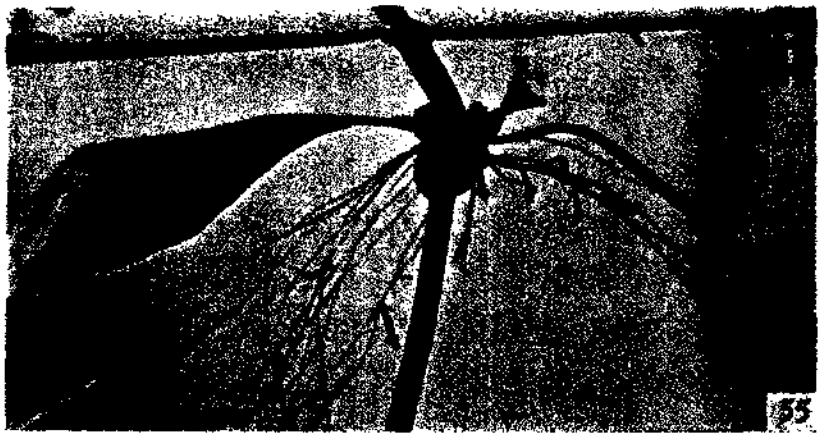
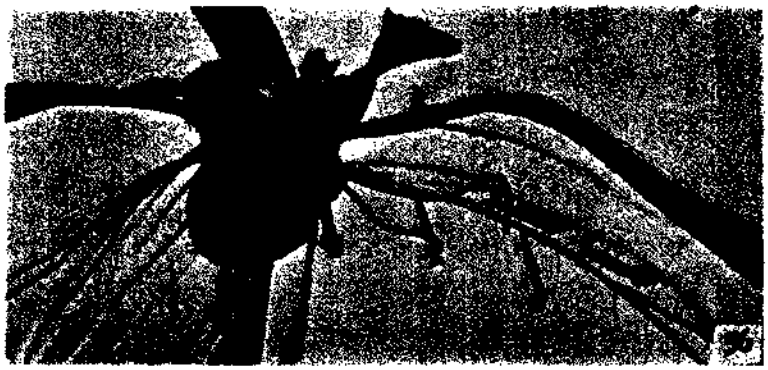
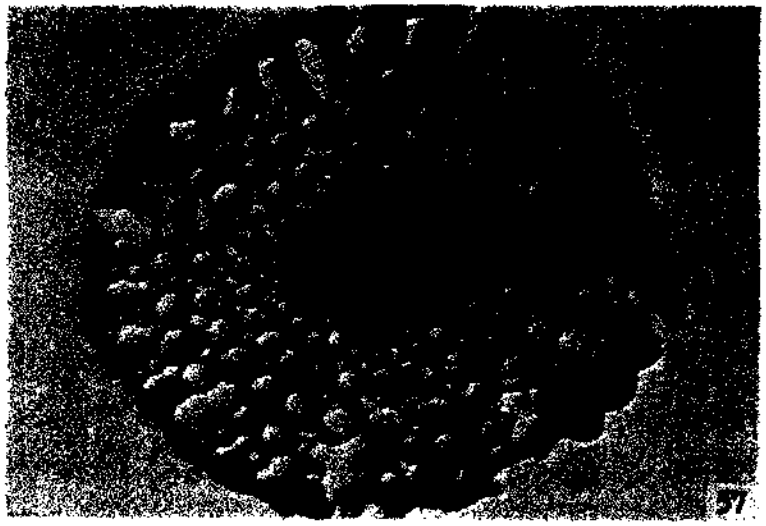


FIG. 51. *Limnanthemum cristatum* plants associated with *Pistia stratiotes* in a tank.
FIG. 52. Pupa of *M. annulifera* attached to main root of *L. cristatum*.
FIG. 53. Cross section of *L. cristatum* main root.
FIG. 54. Close-up of *M. annulifera* larvae attached to main roots of *L. cristatum*.

PLATE XVIII.







FIGS. 55 and 56. M. annulifera larvae attached to main roots of *Alternanthera triandra.*
FIG. 57. Cross section of main root of *Alternanthera triandra* showing peripheral air chambers.

PLATE XIX.

FIG. 58. Nature of plant body of *Nitella furcata*.
FIG. 59. Close-up showing two *M. annulifera* larvae attached to stem of *Nitella furcata*.
FIG. 60. Characteristic branchlets and dactyles of *N. furcata*.
FIG. 61. Close-up of stem of *N. furcata* showing alignment of chloroplasts.

quadrate, 1/8 to 3/16 inch wide. From the nodes white roots originate, 1 mm. in diameter and 3—4 inches long, with fine, lateral rootlets. The internodes are about 4 inches long. The flowers occur in dense, axillary spikes. The plant is called *ponnanganni keerai* in Tamil, *ponnanganni koora* in Telugu, and *kozhuppa* in Malayalam.

M. annulifera larvae attached readily to the main roots and in some cases to the leaves (Fig. 55 and 56). A cross section of a main root (Fig. 57) showed the small air chambers to be peripherally arranged in the cortical region of the root.

14. *NITELLA FURCATA* (ROXB.) AGARDH, A STONEWORT (FAMILY CHAROPHYCEAE).

The stem of this filamentous plant is 588—672 microns thick, the internodes being 1½ times as long as the branchlets which are 5—6 whorled and 3—4 times furcate (Fig. 58). The dactyles are two-celled, the proximal one slightly tapering and the distal cell conical and measuring about 70 × 42 microns (Fig. 60). A closeup of the stem shows the chloroplasts peripherally packed in rough alignments (Fig. 61). Within the central vacuole the cell sap is in constant motion, moving down one side and up the other, into the branches. No air chambers are present in this plant ; nevertheless, as in the case of *Limnanthemum cristatum* and in the previously considered stonewort *Chara wallichii*, the *M. annulifera* larvae attached themselves to the chloroplastid layer where oxygen is in abundance, remaining so attached for many hours (Fig. 59).

SUMMARY.

The attachment behaviour of *Mansonia annulifera* (Theobald) larvae to an additional fourteen species of plants occurring in *Pistia stratiotes* tanks or ponds in Kerala State, South India, was studied. The larvae were seen to attach to roots, stems, and leaves of *Limnophila racemosa* and *Asteracantha longifolia* ; to roots and leaves of *Isachne dispar*, *Ilysanthes rotundifolia*, *Ceratopteris thalictroides*, and *Alternanthera triandra* ; to roots and stems of *Limnanthemum cristatum* ; to leaves and stems of *Elodea* (*Anacharis*) *canadensis* ; to stems alone of *Hydrilla verticillata* and *Nitella furcata* ; and to roots alone of *Cyperus haspan*, *Cyperus compressus*, *Monochoria vaginalis* and *Limnophila roxburghii*. The greatest amount of leaf attachment took place in *Isachne dispar*, gaseous exchange being possible in the absence of air chambers because of the loosely-packed tissue containing oxygen. The attachment to leaves in 7 of the 14 species studied once more substantiates the author's contentions that leaf attachments represent normal and not unusual behaviour, as an alternative method of effecting gaseous exchange. Nineteen plates containing 61 photographs by the author illustrate the plants studied and their attachment behaviour.

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STUDIES ON THE EFFECT OF HETRAZAN* ON *CONISPICULUM GUINDIENSIS.*

BY

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[January 14, 1960.]

THE discovery of the lizard filarial worm, *Conispiculum guindiensis* (Pandit *et al.*, 1929*a*), was expected to open a new era in the chemotherapeutic studies on filariasis in India as it made available a locally procurable and convenient experimental animals with a natural infection. The vector, course of infection and life cycle of this parasite being comparable to those of human filariasis (Pandit *et al.*, 1929*b*; Menon *et al.*, 1944) raised hopes regarding the suitability of this infection for routine screening of filaricides (Hawking, 1946). Following this, Bhattacharya and Ajoy Gupta (1947) studied the effect of Neostibosan (an antimonial compound) against *C. guindiensis* and reported favourable results comparable to those of Culbertson and Rose (1944) on *L. carinii*.

Hetrazan* which has been found to be an efficient microfilaricide and to a degree active against adult worms also of *L. carinii*, *D. immitis* and *Wuchererian* infections (Jaswant Singh and Raghavan, 1957), was tested for its efficacy against *C. guindiensis* and the results are presented.

MATERIAL AND METHODS.

Wild-caught naturally infected garden lizards (*Calotes versicolor*), used in these studies, were obtained from Madras†. Microfilarial counts of infected lizards were determined before drug administration, and on alternative days thereafter. During the early trials, a measured quantity of blood (3 c.mm.) was drawn from the cut end of the tail and the microfilariae counted from the smear made from that amount of blood after staining with JSB. I. (Jaswant Singh and Bhattacharji, 1944). In the later trials, however, the infected blood was drawn into a W.B.C. pipette with sterile 2 per cent citrate saline as a diluting fluid, and the microfilariae counted directly with a haemocytometer.

* Hetrazan is the trade name of 1-diethyl carbamyl piperazine dihydrogen citrate, of Messrs Lederle Laboratories Ltd., New York.

† Obtained through the courtesy of the Director, King Institute of Preventive Medicine, Guindy, Madras.

The drug was administered by means of a fine rubber tube passed through the gullet of the reptile to a distance of about 2 inches below the throat to ensure that the drug went into the stomach. The dosage regimes tried varied from 5 mg. to 50 mg. per kilogram weight of the animal per day for 7 days. The Hetrazan tablet was suspended in water and dilution so adjusted that the quantity to be given to each animal was about 1 c.c. which was easy to administer and retained by the animal.

The animals were inspected daily in the morning and evening. When an animal was found to be moribund or dead, it was opened up by a ventral incision and a blood smear from the heart was taken. Adult worms were also taken out and examined. The surviving animals were sacrificed on the tenth day of treatment and subjected to similar post-mortem examination. The results are given in Table I.

TABLE I.

Microfilarial counts in lizards receiving hetrazan.

Dose of Hetrazan mg./kg.	Microfilarial count per c.m.m. prior to drugging.	MICROFILARIAL COUNTS ON DAYS FOLLOWING COMMENCEMENT OF THERAPY.				
		2	4	6	8	10
5	140	172D	..	..	..	..
10	91	150	86	35	180	200S
20	35	210	195	170S	..	..
40	248	312	680	860	D	..
50	380	590	600	720D	..	..

D=died S=sacrificed.

A marked, progressive and persistent fall alone was taken to indicate definite microfilaricidal action, as fluctuations in the microfilarial counts generally occur even in untreated animals.

Live adult worms are usually pearly white in colour and generally free from any fibrinous adhesions. Following treatment with Neostam, Bhatacharya and Ajoy Gupta (1947 *loc. cit.*) found inactive yellowish worms with fibrinous adhesions in the abdomen of the lizards. A similar change was looked for during the current trials to assess the action of the drug on the adult worms.

RESULTS.

The blood examinations, following therapy, showed normal active microfilariae. No changes were noticed either in the morphology or staining characters. The microfilarial density in the peripheral blood (Table I) showed that the drug did not have any microfilaricidal action.

The adult worms obtained from the treated animals were normal in appearance and activity. The embryos, issuing from the adult female worms examined, were noted to be viable and uncoiled themselves into active microfilariae.

From these findings, it appeared that the Hetrazan had no effect either on the adult worms or on the microfilariae of *C. guindiensis*.

DISCUSSION AND CONCLUSIONS.

Differences in the action of Hetrazan on the different species of filarial worms, and their different stages of development have been noted by earlier workers. Hawking (1950) considered that the microfilaricidal effect of Hetrazan was due to its opsonising effect and rendering the microfilariae easily susceptible to the phagocytes. The studies reported here showed that *C. guindiensis* was not susceptible to the action of Hetrazan during any of the stages of its develoment in the vertebrate host.

The drug is believed to affect the genital anlage in the adult worms, resulting in sterility. The observations reported on *C. guindiensis*, however, showed that the drug obviously did not have any such action on this parasite, as neither the circulating microfilariae, nor the adults, or the embryos inside the latter, were in any way affected by the heavy doses of Hetrazan administered. Doses, much smaller than those tried here, have been found to produce dramatic reduction in the numbers of microfilariae of *W. bancrofti*.

C. guindiensis, however, responded to organic antimonials like Neostam (Bhattacharya and Ajoy Gupta, 1947) which acts mainly on the somatic tissue of the worms.

The diverse response of the parasite to the different filaricides makes it difficult at present to assess the value of this reptilian filarial infection in chemotherapeutic 'screening' of such drugs. Further trials with various other known filaricides, and studies on the mode of action and excretion of these drugs, will have to be carried out before judging the utility of this infection in chemotherapeutic studies.

SUMMARY.

Studies on the effect of Hetrazan on *Conispiculum guindiensis* are reported.

Doses, as large as 3 to 10 times that recommended for human infection, produced no effect on either the circulating microfilariae or the adult worms or the embryos in the latter.

The infection would appear to have restricted use in the routine screening of filaricides.

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Indian Journal of Malariology, **14**, *3, September 1960.*

FILARIASIS IN KERALA STATE.

VI. FILARIA SURVEY OF FORT COCHIN MUNICIPALITY.

BY

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[February 12, 1958.]

COCHIN in Kerala State, consists of two adjacent municipal towns, i.e., Mattancheri and Fort Cochin (Map 1). Prior to re-organisation of States in October, 1956, the former belonged to the erstwhile State of Travancore-Cochin and the latter to Madras Province. A filariasis survey of Fort Cochin Municipality was conducted during August-September, 1957 and the findings are reported in this paper.

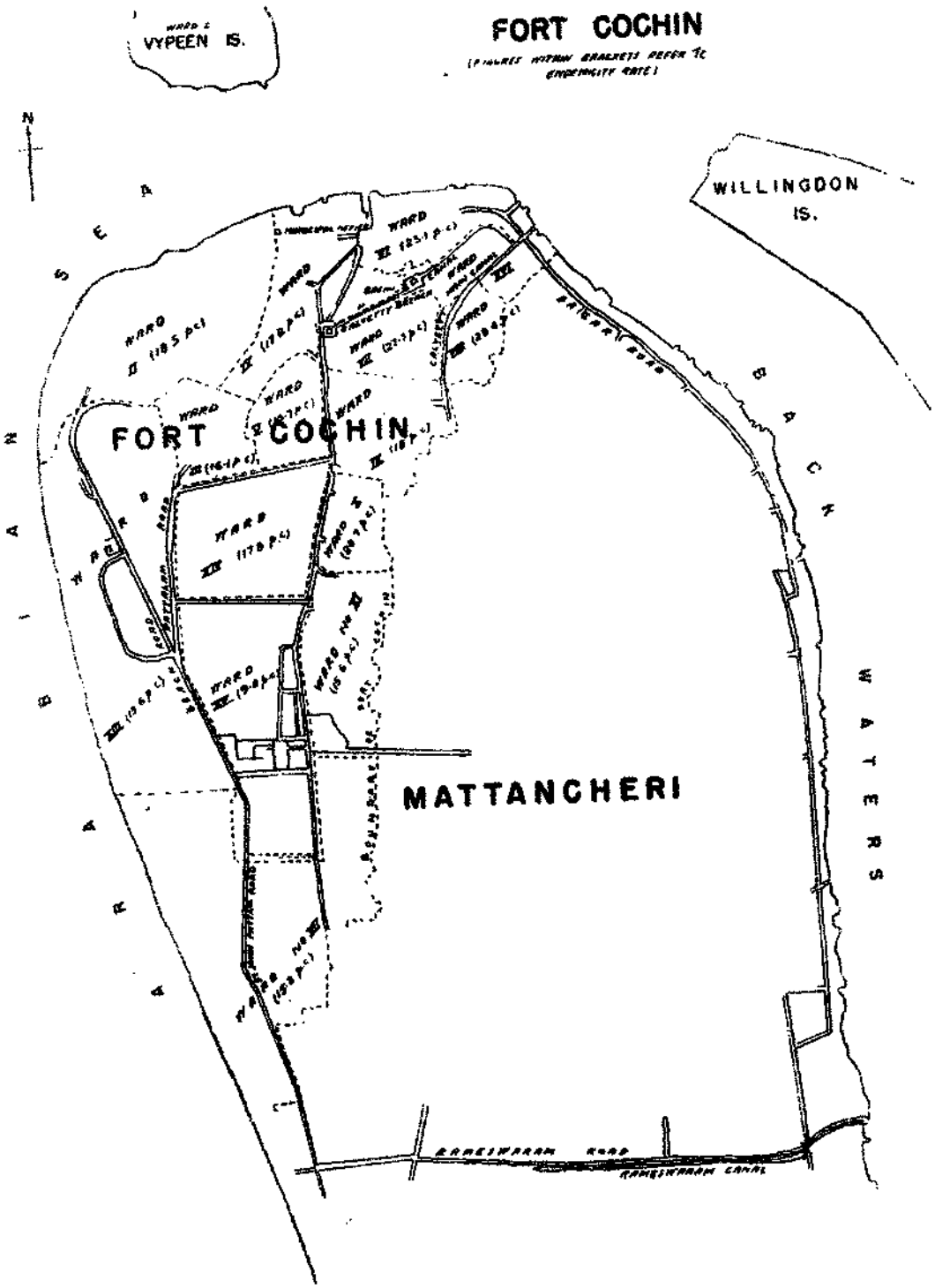
Fort Cochin Municipality has an area of about one square mile, and is a town of great commercial and strategic importance. The municipality is divided into fifteen wards for administrative purposes. Ward I, the Vypeen Island, is separated from the mainland by about 500 yards by the channel connecting the sea with the Cochin harbour. The municipality has a population of 29,856 (1951 census) composed of Christians, Hindus and Muslims. The general literacy rate of the population is high, but the economic level low.

Most of the houses are brick-built or made of coconut leaves. There is neither proper drainage system in the municipal area, nor a satisfactory method for disposal of sewage. Most of the drains are kutcha, but at places are lined by stone or laterite blocks. There are two main canals connected with the backwaters (Basin and Calvettiy canals), both subject to tidal effects. The soil is sandy and the subsoil water level very high. The town has a protected water supply from the Alwaye River, but the supply is very meagre, and is supplemented by wells and ponds.

The average annual rainfall in Cochin is 133·22 inches. Both the monsoons contribute to this precipitation. The annual mean temperature ranges between 64°F. and 93°F. The relative humidity remains high almost throughout the year.

The Municipality has been carrying out antilarval operations on a limited scale since 1942. The operations consist of oiling and small scale use of D.D.T., but the municipality does not have either adequate staff or material.

MAP 1


VYPEEN IS.
FORT COCHIN
WILLINGDON IS.
N
ARABIAN SEA
BACK WATERS
FORT COCHIN
MATTANCHERI
RAMESWARAM ROAD
RAMESWARAM CANAL

FILARIASIS IN FORT COCHIN.

From the description of Clarke (1709) of 'Cochin legs', it would appear that the disease has been endemic here at least for over two centuries. Cruickshank and Wright (1914) surveyed Fort Cochin area, then known as British Cochin. They recorded a microfilaria rate of 20·9 and elephantiasis rate of 12·9 per cent. The exact localities surveyed by them, however, are not known and thus a comparison of their data with those of the present survey is not possible.

The Regional Malariologist, Coimbatore (Madras State), surveyed Fort Cochin in 1949 covering 3 per cent of the population, and recorded a microfilaria rate of 16·5, disease rate of 2·5 and endemicity rate of 19·0 per cent. (Table I). Karani *et al.* (1956) surveyed only one ward of the municipality and recorded a microfilaria rate of 11·1 per cent. Jaswant Singh *et al.* (1956) conducted a filaria survey of the adjacent areas of Mattancherry Town and recorded microfilaria, disease and endemicity rate of 9·2, 14·4 and 21·7 per cent, respectively.

TABLE I.

Results of previous filaria surveys of Fort Cochin.

Year.	Microfilaria rate.	Elephantiasis rate.	Endemicity rate.	Reference.
1914	20·9	12·9	..	Cruickshank and Wright (1914).
1949	16·5	2·5	19·0	Regional Malariologist Coimbatore (1949).
1955-56	11·1	Not recorded.	..	Karani *et al.* (1956). (Vypeen island)

METHODS AND MATERIAL.

Representative areas of all the wards in the municipality, except the first, were surveyed during the present survey.

The filaria surveys were conducted between 8·30 p.m. and 12·30 a.m. by house-to-house visits in the areas selected at random. As far as possible, all the members of the family, including infants, were examined in each of the houses visited. Approximately 20 c.mm. of blood, was collected from each person and made into thick smears on clean glass slides. On the following day, the air dried smears, after dehaemoglobinisation, were stained with JSB. I. (Jaswant Singh and Bhattacharji, 1944) for five minutes. The slides were then examined for microfilariae, and in positive smears the species was identified and the number of microfilariae counted. At the time of night survey, the disease manifestations in the persons examined were also noted. In working out the disease rate, only persons with chyluria, hydrocele and chronic elephantoid swelling of the extremeties, scrotum and breast, were taken into consideration.

Entomological survey was conducted between 7 a.m. and 9 a.m. All female mosquitoes were dissected and examined for filarial infection. In positive specimens, the stage and number of developmental forms as also the site of their location were recorded.

FINDINGS RECORDED DURING THE SURVEY.

Microfilaria rate.—A total of 8,581 persons (4,087 males and 4,494 females), representing 28·7 per cent of the total population, was surveyed. 969 (11·3 per cent) were positive for microfilariae. Of these, 931 (or 96·1 per cent) were *Mf. bancrofti* and 33 (or 3·4 per cent) were *W. malayi* infections. The remaining 5 (or 0·5 per cent) had both the infections. The average infestation was 20·2 per positive smear of approximately 20 c.mm. of blood.

The microfilaria, disease and endemicity rates and the average infestation for the different wards surveyed, are set out in Table II and for the different age-groups in Graph I.

TABLE II.

Endemicity of filariasis in different Wards in Fort Cochin.

Ward number.	Number examined.	Number with microfilariae.	Infection rate. (per cent)	Average infestation.	Number with disease.	Disease rate. (per cent)	Number with both disease and infection.	Endemicity rate. (per cent)
II	275	34	12·4	32·5	19	6·9	2	18·5
III	553	66	11·9	19·2	24	4·3	1	16·1
IV	507	47	9·3	9·7	40	7·9	..	17·2
V*	910	60	6·6	11·7	37	4·1	..	10·7
VI*	529	77	14·6	13·0	48	9·1	3	23·1
VII*	708	124	17·5	16·3	73	10·3	1	27·7
VIII	232	38	16·4	19·0	29	12·5	1	28·9
IX	473	49	10·4	22·3	36	7·6	..	18·0
X*	774	120	15·5	42·0	42	5·4	2	20·7
XI	717	85	11·9	18·4	28	3·9	1	15·6
XII	448	33	7·4	11·2	35	7·8	..	15·2
XIII*	741	73	9·9	12·3	28	3·8	..	13·6
XIV.	947	117	12·4	24·6	52	5·5	..	17·8
XV*	767	46	6·0	9·2	29	3·8	..	9·8
Total	8,581	969	11·3	20·2	520	6·1	11	17·2

*Only *Mf. bancrofti* prevalent.

The infection rates in various age-groups for both sexes are shown in Table III. The lowest infection rate of 3·2 per cent was recorded in the age-group 0-5, which gradually increased to 13·3 per cent in the age-group 21-30 and remained almost static in the higher age-groups.

The disease rate for both sexes was 6·1 per cent. Table III sets out the number of persons with disease manifestations in both sexes in different age-groups. Disease manifestation was lowest (0·4 per cent) in the 0-5 years age-group and gradually increased with advancing age and reached the maximum of 15·2 per cent in the age-group over 50 years.

An analysis of the cases of filarial disease recorded, is given in Table IV.

It would be seen that swelling of the limbs constituted 56·5 per cent., while genito-urinary lesions (especially hydrocele) constituted nearly 12 per cent of the disease manifestations.

GRAPH 1.

Incidence of filaria disease, infection, average infestation among both sexes, Fort Cochin.

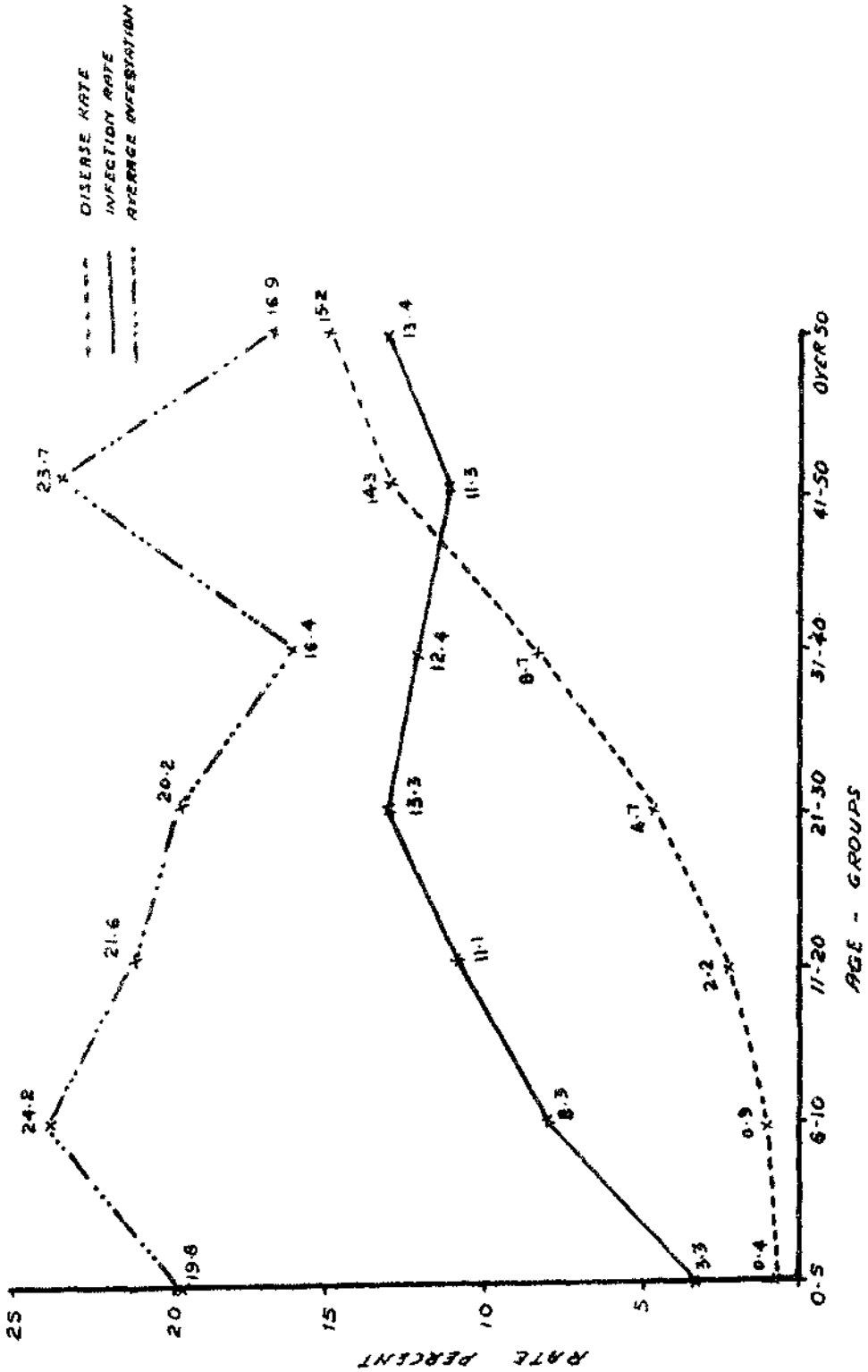


TABLE III.

Incidence of filarial infection and of filarial disease in the different age-groups—Fort Cochin.

Age-group (years).	Number examined.	Number with infection.	Infection rate per cent.	Number with disease.	Disease rate, per cent.	Number with infection or with disease, or both.
0—5	499	16	3·2	2	0·4	18
6—10	931	77	8·3	8	0·9	85
11—20	2,122	237	11·1	47	2·2	283
21—30	1,925	256	13·3	92	4·7	347
31—40	1,410	175	12·4	122	8·7	293
41—50	905	102	11·3	129	14·3	229
Above 50	789	106	13·4	120	15·2	223
Total	8,581	969	11·3	520	6·1	1,478

TABLE IV.

Classification of filarial disease manifestations recorded in Fort Cochin.

	Elephantiasis of leg, hand or both.	Elephantiasis of scrotum, hydrocele or chyluria.	Elephantiasis of breast.	Lymphangitis.
Number of cases	453	98	1	249
Percentage incidence	56·5	12·2	0·1	31·1

An analysis of the persons, with and without disease, examined during the survey is given below :—

Group.	*Number examined.*	*Number with Mf.*	*Mf. rate per cent.*
With filarial disease	520	11	2·1
Without filarial disease	8061	958	11·9

A majority of the microfilaria positive persons were symptomless people. The occurrence of microfilaremia in persons with established filarial disease was comparatively low. This is in conformity with the findings reported by Iyengar (1938 *loc. cit.*) and Jaswant Singh *et al.* (1956 *loc. cit.*), and brings out the importance of dealing with the symptomless microfilaria carriers who are mainly concerned in the maintenance of transmission of the disease in an endemic community.

The earliest age of appearance of filarial infection and of filarial disease.

The youngest age at which *W. bancrofti* occurred was in a boy as well as in a girl, both aged about 3 years, with microfilarial counts of 102 and 91, respectively, in 20 c.mm. blood samples.

A boy aged five was the youngest to show swelling of the left leg. He also gave a history of frequent attacks of filarial fever.

Microfilarial infestation at different ages.—Microfilarial counts in the 969 positive smears in Fort Cochin have been classified (Table V) to bring out the

variations in the average infestation in the different age-groups. The average infestation of 19·8 per 20 c.mm. in the youngest age-group (0-5 years) rose to 24·2 per 20 c.mm. in the next age-group of 6-10 years. In the higher ages, the count was fluctuating below this level. Almost all the persons examined are the permanent residents of the locality. There appears to be no correlation between the microfilarial count and the duration of residence in the area.

TABLE V.

Average infestation among the different age-groups of both sexes.

Age-group (years)	Number examined.	Number positive.	Average infestation.
0–1	10	..	..
2–5	489	16	19·8
6–10	931	77	24·2
11–20	2,122	287	21·6
21–30	1,925	256	20·2
31–40	1,410	175	16·4
41–50	905	102	23·7
Above 50	789	106	16·9
Total	8,581	969	20·2

Entomological surveys.—In all, 548 mosquitoes were collected out of which *C. fatigans* constituted 443 (81·8 per cent). The per man-hour density for this species was 60. The other mosquitoes present in the area were :—

C.gelidus
C.sitiens
C.vishnui
C.whitmorei
M.annulifera
A.barbirostris
A.subpictus
A.vagus.

All these species were dissected but only *C.fatigans* showed developing stages of filarial infection. Out of 348 female *C. fatigans* dissected, 48 (13·7 per cent) showed different stages of filarial infection and 12 (3·4 per cent) showed infective larvae. One to six infective larvae were found among the latter and the average was 2·75 per infective mosquito. Only one *Mansonioides* mosquito was dissected and was negative.

Breeding places of *C.fatigans* were mostly domestic cesspits, cemented drains, ponds, air-raid-precaution tanks dug during the last war, roadside drains and ditches.

Pistia stratiotes was noticed in some of the ponds in Wards III, IX, XI, XII, XIV and XV. Lemna and water hyacinth were present in some of the larger water collections in these wards. Search, made for breeding of *Mansonioides* in some of the ponds, did not reveal the aquatic stages of these mosquitoes.

DISCUSSION AND CONCLUSIONS.

The present survey reveals that filariasis continues to be endemic in Fort Cochin area, though in comparison with the observations recorded earlier [Cruickshank and Wright, (1914 *loc. cit.*) and Report of the Regional malariologist, Madras (1949)] this appears to be on the decline. While the overall microfilaria rate shows a fall. the relative incidence of *W. bancrofti* infection has recorded an increase. The Regional Malariologist observed that *W. bancrofti* formed about 67 per cent of the microfilaria positive cases, the remaining being *W.malayi* ; while during the present survey, the incidence of *W.bancrofti* was recorded to be 96·1 per cent. Cruickshank and Wright (1914 *loc. cit.*) "met with only one variety of microfilaria, namely, *bancrofti*" during their survey ; but *W. malayi* was described only in 1927 by Brug (Brug, 1927) and the first record of mf. *W. malayi* in India was that of Korke (1929). It is likely that the possible presence of any *W. malayi* infection was missed during the survey of Cruickshank and Wright (1914). This increase of *W. bancrofti* is probably due to the gradual urbanisation that has taken place in the last decade and also to some degree due to reduction in the extent of breeding grounds for *Mansonioides* species as many of the tanks, which were formerly having *Pistia* in them, have now been filled up.

It is noted that the incidence of filariasis has continued to remain high during the last over 40 years. The changes in the topographical and filariogenic conditions, consequent on the urbanisation of Fort Cochin Municipality, appear to have resulted in a reduction of the *W. malayi* problem, only to enhance the transmission of *W. bancrofti* infection. The control measures, that have been in force, have not been able to touch the fringe of the problem as is evidenced by the high density of *C. fatigans*, 60 per man-hour, recorded during the survey.

SUMMARY.

A filariasis survey of Fort Cochin (Kerala State) was carried out during August-September, 1957.

8,581 persons were examined for infection and disease, covering 28·7 per cent of the population. The infection, disease and endemicity rates were 11·3, 6·1 and 17·2 per cent, respectively.

96·1 per cent of the microfilaria carriers harboured *W. bancrofti*. 3·4 per cent had *W.malayi* infections ; 0·5 per cent had mixed infections.

The youngest age at which infection and disease were noted was three years and five years, respectively.

Of 520 persons with filarial disease, 2·1 per cent had microfilaria in their blood ; while out of 8,061 apparently healthy persons, 958 (or 11·9 per cent) were positive for microfilariae.

C. fatigans was the vector of *W. bancrofti* with an infection rate of 13·7 per cent and infectivity rate of 3·4 per cent.

ACKNOWLEDGMENT.

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FILARIASIS IN CENTRALLY ADMINISTERED AREAS.

Part I.

FILARIA SURVEY OF PONDICHERRY SETTLEMENT.

BY

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(*Malaria Institute of India.*)

[December 24, 1959.]

AFTER about 280 years of French occupation, the State of Pondicherry was transferred to India on November 1, 1955. Since this *de facto* transfer, the territory is being administered by a Chief Commissioner appointed by the Government of India.

The incidence of filariasis in Pondicherry Settlement was investigated in the month of March, 1957 and the findings of the survey are given in this paper.

PHYSIOGRAPHIC CONDITIONS.

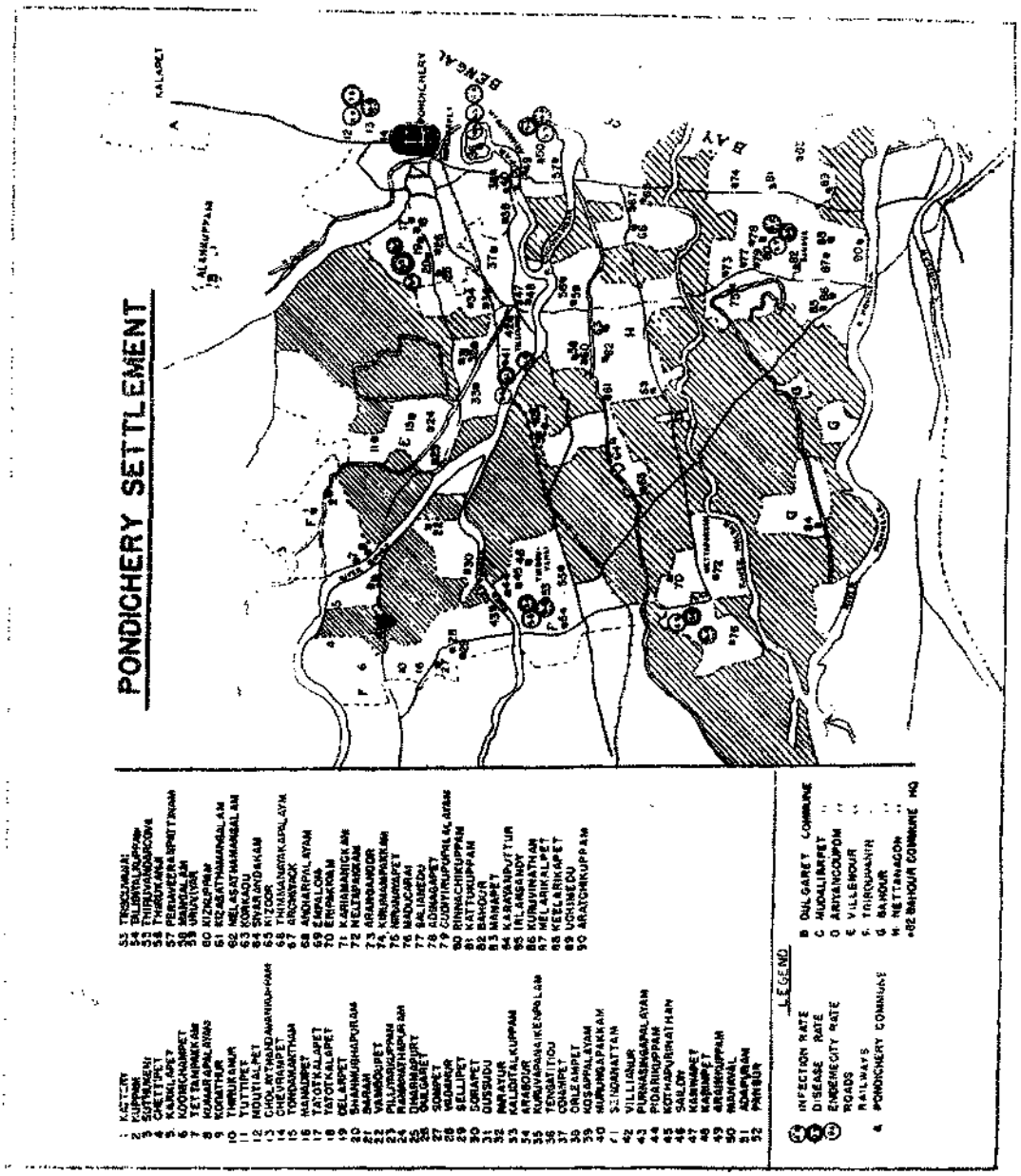
This State consists of four Settlements, viz., Pondicherry, Karaikkal, Yanom and Mahi. Of these, Pondicherry Settlement is the biggest with an area of 115 square miles and population of about 2,32,772 (1948 census). It lies on the east coast of India, about 89 miles south of Madras City. This Settlement (Map 1) is intercepted by a number of enclaves belonging to Madras Territory. The only major town in this Settlement is Pondicherry (Map 2) with a population of about 60,000 (1948 census). Excluding Pondicherry Town, the adjoining territory is divided into eight municipal communes covering 97 groups of villages. Towns falling within these communes are Ariancoupom, Bahour, Villenour and Mudaliarpet with a population of about 10,642, 5,500, 5,500 and 5,768 respectively. Two main rivers, Gingee and Pambayar, traverse through these Settlements and drain into the Bay of Bengal.

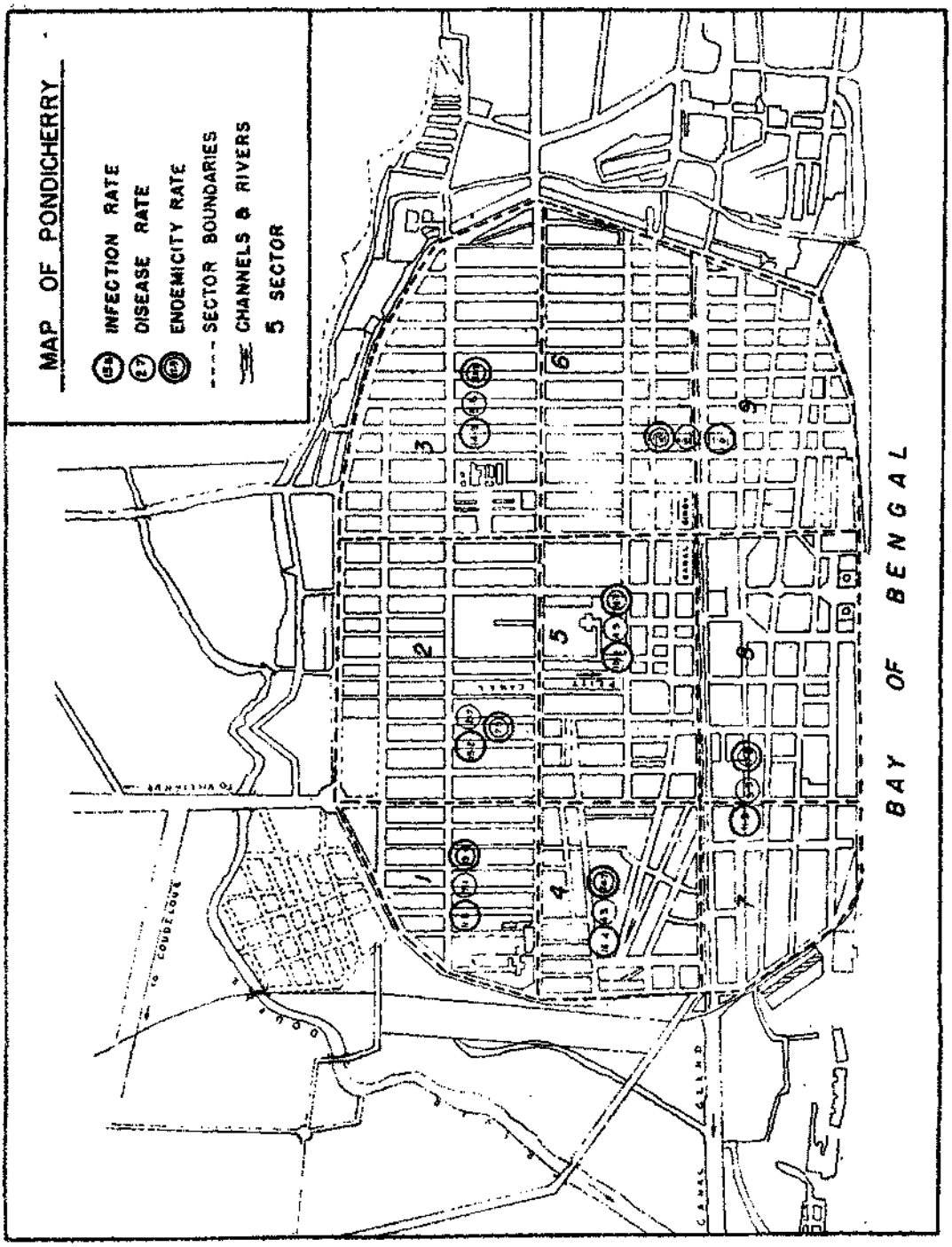
Pondicherry has a port with a road-stead, which is the best on the entire coast. The Settlement has a number of roads linking all important villages of Pondicherry Settlement. Excepting the main road in Pondicherry area, the rest of the roads are kutcha. There is a railway line running from Villupuram, a junction on the Southern Railway, connecting some villages in the Settlement on the way.

SOCIO-ECONOMIC ASPECTS.

The houses in the town, excepting in the slum areas, are of brick masonry. In the villages, the houses are mostly built with mud walls and thatched with coconut leaves.

Map I.


PONDICHERY SETTLEMENT
KALAPET
ALIANKUPPAM
PONDICHERY
BENGAL
BAY
1 KATERY
2 KUPPAM
3 SOTTANKENI
4 CHETTIPET
5 KANNILAPET
6 KOOREHAMPET
7 TETTAMMAKAM
8 KUMARAPALAYAM
9 KORATTUR
10 THIRUKANUR
11 TUTTIPET
12 MOULAR THANDAVANKUPPAM
13 CHEVRAPPET
14 TONDAMARTHAN
15 HANADPET
17 TAYOTKALAPET
18 TAYOTKALAPET
19 DELARPET
20 SHANMUGHAPURAM
21 BARAM
22 VANDOUPET
23 PILLAIYARKUPPAM
24 PILLAIYARKUPPAM
25 DALGARET
26 SOMPET
27 VIDANUR
28 SELLIPET
29 SORAPET
30 DUSSUDU
31 MATATUR
32 KALDITALKUPPAM
33 ARABOUR
34 KURUVANAIKENPALAM
35 TENGAITTIDU
36 CHAMPET
37 ORLAMPET
38 NOSAPPALAYAM
39 MURUNGAPAKKAM
40 SENGARATTAN
41 VILLIANUR
42 PUNNAIMADAPALAYAM
43 PORANGANDAM
44 KOTHANDARAMATHAN
45 SALON
46 KARUMPET
47 KARUMPET
48 ARAIMUPPAM
49 MANAVAL
50 ADAPURAM
51 PAKUR
52 PAKUR
53 TIRUKANUR
54 TALAIYALAPPAM
55 THIRUVANDARCOVE
56 THIRUKANUR
57 PERUMEERAMPETTINAM
58 MANGALAM
59 URUVIYAR
60 KIZALPIRAM
61 KOMARATHAMANGALAM
62 MELASATHAMANGALAM
63 SORKADU
64 SOMAPALAYAM
65 KITTOOR
66 THIRUMANGALAPALAYM
67 ARCHAVACK
68 ANDHARPALAYAM
69 EMPALOOR
70 ERIPAKKAM
71 KARIAMANICKAM
72 MELTAPAKAM
73 ARANGANDOR
74 KIRUMAMPAKKAM
75 NIRAMAPET
76 MADUCARAI
77 BALIMEDU
78 ADIMAGAPET
79 CUDDITIRUPPALAYAM
80 RIMMACHIKUPPAM
81 KATTUKUPPAM
82 BAHOUR
83 MANAPET
84 KARAVANPUTTUR
85 INLANGANDY
86 KURUVINATHAM
87 MELARIKALPET
88 KEELARIKALPET
89 UCHIMEDU
90 ARATCHIKUPPAM
LEGEND
INFECTION RATE
DISEASE RATE
ENDEMICITY RATE
ROADS
RAILWAYS
PONDICHERY COMMUNE
B DULGARET COMMUNE
C MUDALIARPET
D ARIYANCOUPOM
E VILLENOUR
F. THIROUVANDIR
G BAHOUR
H NETTIANGON
82 BAHOUR COMMUNE HQ


MAP OF PONDICHERRY
INFECTION RATE
DISEASE RATE
ENDEMICITY RATE
SECTOR BOUNDARIES
CHANNELS & RIVERS
5 SECTOR
BAY OF BENGAL
TO COUDELOUR
CANAL
1
2
3
4
5
6
7
8
9

MAP 2.

Education is free up to the secondary stage in the urban areas and only up to the elementary stage for the rural population. Language spoken is mostly Tamil, but majority of the people know French also.

OCCUPATION.

The population consists of agriculturists (about 1,71,000) and non-agriculturists (about 52,000). About 7,545 persons are employed in the textile mills and some persons are engaged in other minor industries, such as coir or soap making.

WATER SUPPLY.

There is a protected water supply in Pondicherry Town. The source of water is from lakes in the neighbouring communes. Piped water supply is being attempted in some places, especially Bahour, Madukarai and Thirubouvanin. In rural areas, ordinary wells and tube-wells are the only source of water supply.

DRAINAGE.

There are two open cemented drains, running within the town, and a small stretch of sub-soil drain just at the periphery of the western limits of the town to collect the waste water including sewage of the town. These drains discharge into either a wide open cemented drain on the northern part or a slow moving more or less choked kucha drain in the southern part of the town, just outside the municipal limits. Before the sullage and sewage finally drain into the Bay of Bengal through these drains, it forms vast sheets of stagnating water, especially on the southern side. Apart from these drains described above, there is no drainage of any description in the rest of the town areas. The drainage thus provided is inadequate and in many parts of the town, the sullage water from houses flows along the sides of the streets, some of which are stone-paved.

METEOROLOGICAL CONDITIONS.

The monthly mean meteorological data for the period 1945 to 1954 are depicted in Graph 1. The total rainfall is about 38 inches and the precipitation is mostly during the months of August to December. Being very close to the sea coast, the relative humidity is high throughout the year. Wind direction is mostly eastern or south-eastern in the months of September to May and western or south-western in June, July and August.

PREVIOUS RECORDS OF FILARIA INCIDENCE IN THE SETTLEMENT.

There is no record of any previous filaria survey carried out in the Settlement.

Record of cases treated for filaria in the Pondicherry Hospital during the period 1946 to 1955, is shown in Table I.

GRAPH 1.

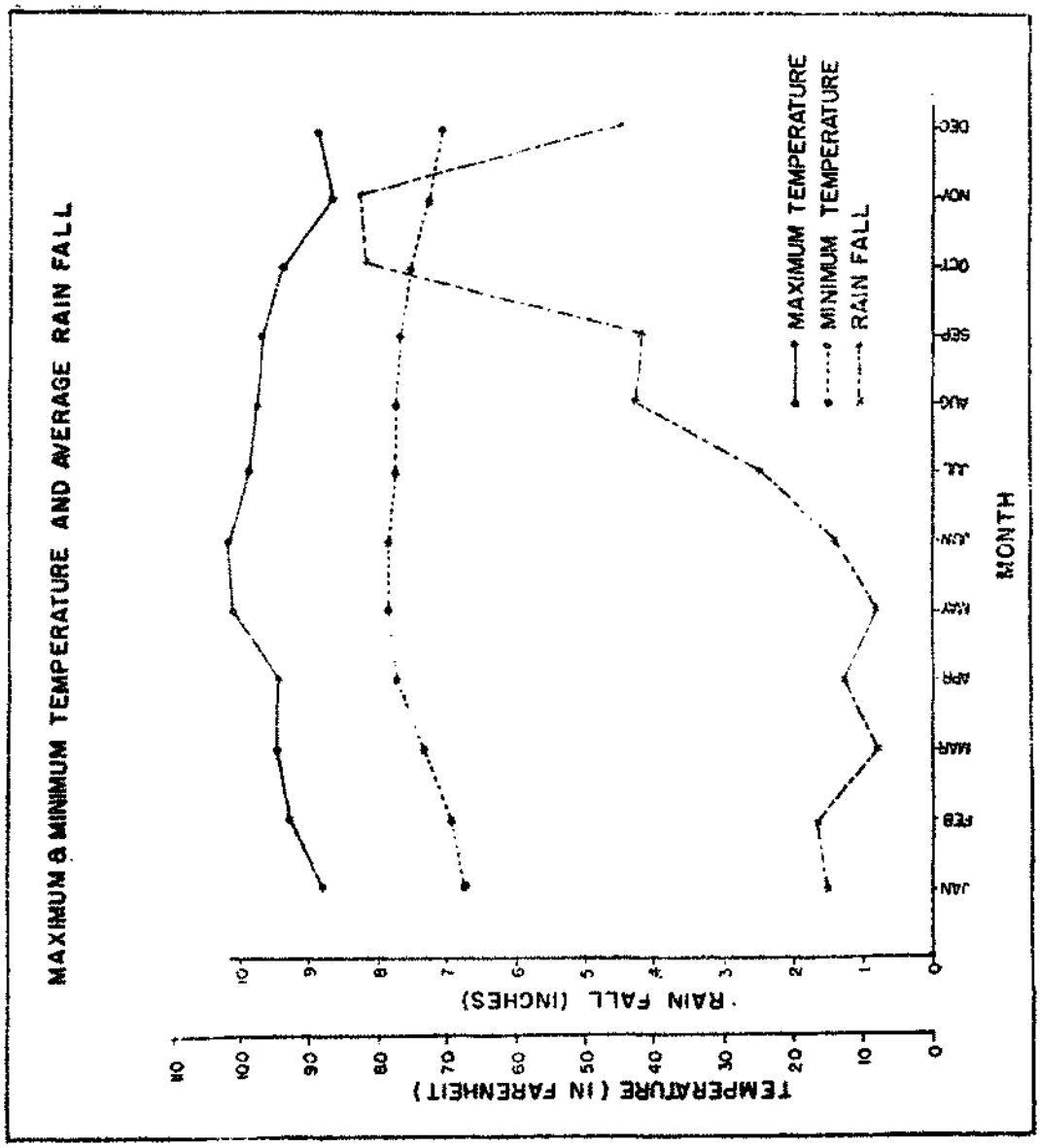

MAXIMUM & MINIMUM TEMPERATURE AND AVERAGE RAIN FALL
MAXIMUM TEMPERATURE
MINIMUM TEMPERATURE
RAIN FALL
MONTH
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
RAIN FALL (INCHES)
TEMPERATURE (IN FARENHEIT)

TABLE I.

Number of filaria cases treated in the Pondicherry Hospital.

Year.	Total cases treated for filaria.		Chyluria.	Hydrocele.	Elephantiasis :	
	Out-patient.	In-patient.			Genital.	Limbs.
1946	232	31	..	29	8	..
1947	258	34	..	63	9	..
1948	123	19	1	64	13	..
1949	43	23	..	23	17	1
1950	139	54	..	83	7	..
1951	164	34	..	120	20	..
1952	285	66	..	56	9	..
1953	NA	NA	NA	NA	NA	NA
1954	NA	NA	NA	NA	NA	NA
1955	337	22	3	135	1	14

NA=not available.

PRESENT SURVEY.

(*a*) *Night blood.*—Surveys were conducted in different sectors of the town and the communes. Firstly, certain parts of the towns and some villages in the communes were selected at random, and representative samples of the population in these town areas and in the villages were covered, by making house-to-house visits between 8 p.m. and 1 a.m. and examining all the inmates. Approximately, 20 c.mm. of blood was collected from a finger prick of each person examined, and made into thick smears. As far as possible, these smears were stained the next day with JSB stain and examined for microfilaria, and details, such as the species and their numbers, were recorded in positive smears. Enquiries were made to record the incidence of various manifestations of filarial disease, especially genital lesions and elephantoid swelling of the limbs.

(*b*) *Entomological.*—Adult mosquitoes were collected both during morning and night time from houses and cattle-sheds from different parts of the town as well as the communes. After identification, these were dissected for detecting the presence of any of the stages of the filarial infection. Search was made in as many water collections as possible to trace the breeding of the vector species.

(*c*) *Survey of domestic animals.*—Blood smears were collected during day time from some of the common domestic animals in the areas to note whether any of them was positive for filarial infection.

RESULTS OF THE SURVEY.

(A) HUMAN SURVEY.

Details in regard to the population of Pondicherry Town and of the different communes and percentage population surveyed in these places, are furnished in Table II. On the whole, 8·21 per cent of the total population in the Settlement was surveyed.

TABLE II.

Percentage population of the town and different communes surveyed.

Serial number.	Area (Town or Commune.)	Number of sectors/villages surveyed.	Total population.	Population surveyed.	Percentage surveyed.
1	Pondicherry* ...	9	59,835	7,184	12·0
2	Pondicherry† ...	7	10,200	1,081	10·6
3	Oulgaret† ...	13	35,311	2,210	6·3
4	Mudaliarpet† ...	8	22,396	1,058	4·7
5	Ariancoupom† ...	7	18,793	825	4·4
6	Villenour† ...	15	27,991	1,773	6·3
7	Tribouvanin† (Manadipet)	16	23,232	2,376	10·2
8	Bahour† ...	8	20,591	1,253	6·1
9	Nettapacom† ...	8	14,423	1,364	9·5
..	Total ...	91	2,32,772	19,124	8·21

*Town †Commune

The parasitological and clinical aspects of the disease, as gathered during the survey, are given below :—

(a) *Parasitological findings.*—The number of persons found positive for microfilariae, the estimated infection rate and average infestation in the different sectors of the town and in the communes, are given in Table III.

TABLE III.

Infection and average infestation in pondicherry settlement.

Serial number.	Places surveyed.	Total number examined.	Number with microfilaria.	Infection rate. (per cent)	Average infestation.* (number)	Average density per individual in the community surveyed. (number)
1	Pondicherry Sector I	693	79	11·4	24·8	2·8
2	Pondicherry Sector II	1,007	107	10·6	12·1	1·3
3	Pondicherry Sector III	1,337	209	15·6	22·9	0·5
4	Pondicherry Sector IV	981	94	9·6	16·7	1·6
5	Pondicherry Sector V	1,123	79	7·0	14·2	0·99
6	Pondicherry Sector VI	956	62	6·5	16·6	1·1
7	Pondicherry Sector VII and VIII	1,087	112	10·3	25·5	2·6
8	Pondicherry Commune	1,081	84	7·8	18·2	1·4
9	Oulgaret Commune	2,210	72	3·3	14·8	0·5
10	Mudaliarpet Commune	1,058	31	2·9	15·0	0·4
11	Ariyancoupom Commune	825	87	10·5	23·6	2·5
12	Villenour Commune	1,773	80	4·5	17·6	0·8
13	Tribouvanin Commune	2,376	56	2·4	14·6	0·3
14	Bahour Commune	1,253	32	2·6	12·0	0·3
15	Nettapacom Commune	1,364	22	1·6	8·2	0·1
Total for Pondicherry Town		7,184	742	10·3	18·9	1·9
Total for Pondicherry Communes		11,940	464	3·9	15·5	0·6
Total for Pondicherry Settlement		19,124	1,206	6·3	17·1	1·1

*Density per positive (per 20 cmm.)

While in the communes, the average infection rate was 3·9 per cent., in the Pondicherry Town the rate was 10·3 per cent. On the average, the infection in the whole Settlement worked out to 6·3 per cent. No commune was free from this infection. The average infestation of microfilariae per 20 c.mm. was 17·1. The species of filarial infection in all cases was *W. bancrofti*.

The microfilaria rate and average infestation observed among males and females in Pondicherry Town and in the different communes, are recorded in Table IV.

TABLE IV.

Microfilaria rate and average infestation among males and females in Pondicherry Town and different communes.

Place.	MALES:				FEMALES:			
	Number examined.	Number with microfilaria.	Microfilaria rate (per cent).	Average infestation (Number)	Number examined.	Number with microfilaria.	Microfilaria rate (per cent).	Average infestation (Number)
Pondicherry town	3,780	429	11·3	20·1	3,404	313	9·2	19·2
Pondicherry commune	600	54	9·0	18·4	481	30	6·2	17·9
Oulgaret*	1,153	39	3·4	18·4	1,057	33	3·1	10·3
Mudaliarpet*	571	47	8·2	31·2	487	40	8·2	14·6
Villenour*	1,170	61	5·2	28·2	603	19	3·2	16·2
Ariancoupom*	471	18	3·8	14·8	354	13	3·7	15·2
Tribouvanin*	1,444	34	2·4	12·8	932	22	2·4	18·0
Bahour*	656	19	2·9	13·6	597	13	2·2	9·7
Nettapacom*	830	16	1·9	10·0	534	6	1·1	5·3
Total for communes	6,895	288	4·2	18·4	5,045	176	3·5	13·4
Total for communes and Pondicherry Town.	10,675	717	6·7	18·6	8,449	489	5·9	14·0

*Communes.

The microfilaria rate was found to run almost parallel among males and females in all the places. Slight variation was, however, noted in the average infestation, being 14·0 in females and 18·6 in males. The incidence of the microfilaria carriers and the average infestation in the different age-groups of the two sexes, separately and combined, are analysed and shown in Table V and VI respectively.

Fifteen infants were examined and all of them were negative for microfilariae. In all other age-groups, there was microfilaria infection. A progressive rise in microfilaria rate was noticeable up to the age of 20, after which it remained almost static with minor fluctuations. This phenomenon was noticeable in both sexes. A similar finding was also noted with regard to the average infestation ; a progressive

rise up to the age-group 11-20 and maintaining a level thereafter slightly less than this in the other ascending age-groups without any appreciable variation on the whole. In the age-group 11-20, the infection rate was 7·8 to 7·9 and the same was applicable to both sexes. The average infestation for this particular age-group was recorded as 25·7, 24·7 and 25·2 for males, females and both sexes respectively. In general, the range in the microfilaria count in different individuals varied between 1 and 283. The youngest age at which infection was detected was 2 years.

TABLE V.

Microfilaria rate and average infestation in different age-groups of the two sexes separately.

Age-group (years).	MALES:				FEMALES:			
	Number examined.	Number with micro-filaria	Micro-filaria rate (per cent).	Average infestation (Number).	Number examined.	Number with micro-filaria.	Micro-filaria rate (per cent).	Average infestation (Number).
0—1	9	..	..	..	6	..	..	..
2—5	595	8	1·3	5·0	495	7	1·4	15·6
6—10	1,067	42	3·9	15·8	845	42	4·9	14·2
11—20	2,179	171	7·8	25·7	1,399	126	7·9	24·7
21—30	2,395	181	7·6	18·6	2,203	135	6·1	12·2
31—40	1,730	110	6·4	20·6	1,435	75	5·2	15·9
41—50	1,302	100	7·8	16·3	951	49	5·2	16·6
Above 50	1,398	105	7·5	16·2	915	55	6·0	16·4
Total	10,675	717	6·7	16·9	8,449	489	5·8	16·7

TABLE VI.

Microfilaria rate and average infestation in different age-groups of both sexes combined.

Age-group.	Number examined.	Number with microfilaria.	Microfilaria rate. (per cent)	Average infestation (Number).
0—1	15	..	..	..
2—5	1,090	15	1·4	10·3
6—10	1,912	84	4·4	15·0
11—20	3,778	297	7·9	25·2
21—30	4,598	316	6·9	15·9
31—40	3,165	185	5·8	18 2
41—50	2,253	149	6·6	16·4
Above 50	2,313	160	6·9	16·3

(*b*) *Clinical findings.*—The disease manifestations, including and excluding filarial fever in the town and the different communes, recorded during the survey, are shown in Table VII. These data are further analysed and the incidence of these manifestations among the two sexes in different sectors of Pondicherry Town is tabulated in Table VIII.

Highest disease rates of 7·8 per cent including filarial fever, and 5·6 excluding it, were noted in both sexes in the Pondicherry Commune adjacent to the Pondicherry Town. The next worst affected place was in Sector I of the town itself, with 7·1 per cent including filarial fever, and 5·6 excluding filarial fever,

TABLE VII.

Incidence of filarial disease in the two sexes in Pondicherry Town and different communes.

Area.	MALES:					FEMALES:					BOTH SEXES				
	Number examined.	Number with disease including filarial fever.		Number with disease excluding filarial fever.		Number examined.	Number with disease including filarial fever.		Number with disease excluding filarial fever.		Number examined.	Number with disease including filarial fever.		Number with disease excluding filarial fever.	
		Number.	Per cent.	Number.	Per cent.		Number.	Per cent.	Number.	Per cent.		Number.	Per cent.	Number.	Per cent.
Pondicherry Town	3,780	172	4·6	139	3·7	3,404	168	4·9	132	3·9	7,184	340	4·7	304	4·2
Pondicherry Commune	600	61	10·2	50	8·3	481	24	5·0	11	2·3	1,081	85	7·8	61	5·6
Oulgaret Commune	1,153	78	6·8	66	5·7	1,057	40	3·8	29	2·7	2,210	118	5·3	95	4·3
Mudaliarpet Commune	571	51	8·9	41	7·2	487	18	3·7	8	1·6	1,058	69	6·5	49	4·6
Ariyancoupom Commune	471	22	4·7	13	2·8	354	20	5·6	15	4·2	825	42	5·1	28	3·4
Villenour Commune	1,170	57	4·9	52	4·4	603	15	2·5	6	0·99	1,773	72	4·1	58	3·3
Tribouvanin Commune	1,444	56	4·9	49	3·4	932	16	1·7	7	0·75	2,376	72	3·0	56	2·4
Bahour Commune	656	34	5·2	31	4·7	597	10	1·7	8	1·3	1,253	44	3·5	39	3·1
Nettapacom Commune	830	40	4·8	37	4·5	534	9	1·7	6	1·1	1,364	49	3·6	43	3·2
Total	10,675	571	5·3	511	4·8	8,449	320	3·8	222	2·6	19,124	891	4·7	733	3·8

TABLE VIII.

Incidence of filarial disease manifestations in the different sectors of Pondicherry Town.

Area.	Males:					Females:					Both sexes:				
	Number examined.	Number with disease including filarial fever.		Number with disease excluding filarial fever.		Number examined.	Number with disease including filarial fever.		Number with disease excluding filarial fever.		Number examined.	Number with disease including filarial fever.		Number with disease excluding filarial fever.	
		Number.	Per cent.	Number.	Per cent.		Number.	Per cent.	Number.	Per cent.		Number.	Per cent.	Number.	Per cent.
Sector I	367	25	6·8	22	5·9	326	24	7·4	17	5·2	693	49	7·1	39	5·6
Sector II	526	15	2·9	14	2·7	481	12	2·5	8	1·7	1,007	27	2·7	22	2·2
Sector III	690	39	5·7	31	4·5	647	39	6·0	34	5·3	1,337	78	5·8	65	4·9
Sector IV	486	20	4·1	15	3·1	495	22	4·4	19	3·8	981	42	4·3	34	3·5
Sector V	570	20	3·5	16	2·8	553	30	5·4	24	4·3	1,123	50	4·5	40	3·6
Sector VI and IX	527	17	3·2	14	2·7	429	12	2·8	12	2·8	956	29	3·0	26	2·7
Sector VII and VIII	614	36	5·9	27	4·4	473	29	6·1	18	2·8	1,087	65	5·9	45	4·1
Total	3,780	172	4·6	139	3·7	3,404	168	4·9	132	3·9	7,184	340	4·7	304	4·2

TABLE

Incidence of filarial disease manifestations

Age-group (Years)	Pondicherry Town:			Pondicherry Commune:			Oulgaret*:		
	Number examined.	Disease rate.		Number examined.	Disease rate.		Number examined.	Disease rate.	
		Including filarial fever.	Excluding filarial fever.		Including filarial fever.	Excluding filarial fever.		Including filarial fever.	Excluding filarial fever.
0 — 5	328	1·8	0·3	78	2·6	1·3	142	4·2	0·7
6 — 10	687	2·0	1·0	97	2·1	2·1	280	1·4	0·7
11 — 20	1,505	1·8	0·7	184	8·7	5·9	537	3·2	2·2
21 — 30	1,817	4·3	3·5	252	7·1	5·2	468	5·6	4·5
31 — 40	1,162	5·6	4·4	188	12·2	9·0	373	7·8	6·2
41 — 50	813	6·1	5·0	130	6·2	4·6	201	6·9	6·9
Above 50	872	11·5	10·4	152	10·5	7·2	209	10·5	10·5

TABLE IX (*Concld.*)

Age-group (Years)	Tribouvani*:			Bahour*:			Nettpacom*:		
	Number examined.	Disease rate.		Number examined.	Disease rate.		Number examined.	Disease rate.	
		Including filarial fever.	Excluding filarial fever.		Including filarial fever.	Excluding filarial fever.		Including filarial fever.	Excluding filarial fever.
0 — 5	184	..	..	68	..	..	90	..	..
6 — 10	191	0·5	0·5	177	1·7	0·6	147	..	..
11 — 20	410	1·2	0·7	212	0·5	0·5	253	2·7	2·4
21 — 30	590	4·7	3·4	304	4·3	3·6	295	4·4	3·7
31 — 40	390	3·6	2·6	199	3·2	2·7	248	4·0	3·6
41 — 50	350	3·2	2·3	130	6·9	6·9	163	3·0	3·0
Above 50	261	6·5	5·4	163	7·9	6·7	168	7·7	7·7

*= Commune

IX.

in the different age-groups.

MUDALIARPET* :			ARIANCOUPOM* :			VILLENOUR* :		
Number examined.	Disease rate.		Number examined.	Disease rate.		Number examined.	Disease rate.	
	Including filarial fever.	Excluding filarial fever.		Including filarial fever.	Excluding filarial fever.		Including filarial fever.	Excluding filarial fever.
49	2·0	2·0	75	..	..	91	..	..
103	7·8	4·9	92	..	..	138	1·4	0·7
227	4·8	3·1	141	6·4	4·3	309	1·6	0·9
261	6·5	4·6	199	4·5	3·0	412	4·8	3·9
147	9·5	7·5	122	11·5	8·2	336	4·5	3·6
135	7·4	5·2	83	6·0	3·6	248	7·2	6·0
136	8·8	6·6	113	4·6	2·7	239	5·0	4·6

TOTAL :		
Number examined.	Disease rate.	
	Including filarial fever.	Excluding filarial fever.
1,105	1·4	0·4
1,912	1·8	1·0
3,778	2·6	1·7
4,598	4·7	3·8
3,165	6·0	4·6
2,253	5·8	4·8
2,313	8·9	7·9

(Contd.)

The average incidence of disease manifestations for the whole Settlement was 4·7 per cent including filarial fever, and 3·8 per cent excluding it. In the town and in the different communes combined, the incidence among the females was 3·8 per cent if filarial fever was also included, and 2·6 per cent if filarial fever was not included, while among the males the corresponding figures were 5·3 and 4·8 per cent respectively. Among males, the incidence was slightly higher than among the females.

The incidence of filarial disease manifestations among the different age-groups is shown in Table IX for the whole Settlement and the different communes separately. The general trend of increase in the filarial disease manifestations with advancing age was very evident. The lowest age at which disease manifestation was observed was in a child aged 4 years.

The incidence of various disease manifestations in the different age-groups among the two sexes is shown in Table X. 2·73 per cent of the males surveyed, had hydrocele. The occurrence of hydrocele steadily increased up to the age-group 21-30, after which it remained constant and maintained almost the same level. With regard to the swelling of the limbs, the incidence was directly proportionate to the ascending order in the age-group and the same was true in the two sexes. Further,

TABLE X.

Incidence of various disease manifestations in the different age-groups.

MALES.

Age-groups.	Number Examined.	Lower limbs.	Upper limbs.	Both limbs.	Filaria fever.	Hydrocele.	Genital.	Chyluria.	Swelling limb as well as genital.
		Rate per cent.	Rate per cent.	Rate per cent.	Rate per cent.	Rate per cent.	Rate per cent.	Rate per cent.	Rate per cent.
0—5	604	0·2	..	..	1·0	0·3	..	..	..
6—10	1,067	0·4	..	..	0·8	0·8	..	0·1	..
11—20	2,179	0·5	..	..	1·0	1·9	..	0·04	0·1
21—30	2,395	1·2	0·04	0·04	0·9	3·5	0·04	0·2	0·3
31—40	1,730	0·7	0·1	0·2	1·3	3·7	..	0·1	0·1
41—50	1,302	1·8	..	..	1·0	3·8	..	0·2	0·2
51 UP	1,398	3·8	0·1	0·1	0·9	3·8	..	..	0·9
Total	10,675	1·3	0·04	0·05	0·9	2·73	0·01	0·1	0·3

FEMALES.

0—5	501	0·2	..	..	0·9	..	..	..	..
6—10	845	0·4	..	0·1	0·6	..	0·1	0·1	..
11—20	1,599	0·7	0·2	0·1	0·7	..	0·2	0·1	..
21—30	2,203	1·5	0·1	0·04	1·1	..	0·13	0·5	0·1
31—40	1,435	2·8	0·4	0·3	1·5	..	0·3	0·6	..
41—50	951	2·6	0·4	..	0·9	..	..	..	..
51 UP	915	5·6	0·3	0·1	1·3	..	0·2	0·9	..
Total	8,449	1·9	0·2	0·09	1·04	..	0·2	0·3	0·01

TABLE XI.

Incidence of different disease manifestation among the persons with clinical history and the extent of concomitant filarial disease and infection among them.

Disease manifestations.	Incidence among persons with filarial disease :				Cases showing microfilaria :					
	Males. (571)		Females. (320)		Males.		Females.		Both sexes.	
	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	Number.	Percentage.
Lower limb	133	23·1	163	50·9	4	3·0	8	4·9	12	4·1
Upper limb	4	0·7	18	5·7	1	25·0	1	5·6	2	9·1
Both limbs	5	0·9	8	2·5	..	..	..	..	..	..
Filarial fever	103	17·9	88	28·0	7	6·8	..	..	7	3·7
Hydrocele	293	50·8	..	..	18	6·1	..	..	18	6·1
Genital	1	0·2	14	4·5	..	..	..	..	..	..
Chyluria	11	1·9	22	7·0	2	18·2	2	9·1	4	12·1
Swelling limb as well as genital	27	4·7	1	0·3	..	..	..	..	..	..

with regard to this clinical manifestation, i.e., swelling of the limbs, no apparent difference in the rate was observed among the two sexes. Actually the slight increase in the overall endemicity rate observed in males, as compared to females (*vide* Table XIII), appears to be due to the fact that hydrocele manifestation is peculiar only to males.

Incidence of the different types of manifestations in the two sexes and also the percentage of such people with disease who showed blood infection as well, are indicated in Table XI. Among males, 50·8 per cent of the disease manifestations were cases of hydrocele. Next to that was swelling of lower limbs which accounted for 23·1 per cent and filarial fever 17·9 per cent. 4·7 per cent of the males with disease had both swelling of limbs as well as genital manifestations. In females, 50·9 per cent of the cases were due to the swelling of lower limb, 28 per cent due to filarial fever and 7 per cent due to chyluria (as per statement on interrogation). Out of 891 cases with various disease manifestations (males and females combined), 4·82 per cent had the concomitant microfilaraemia as well.

(*c*) *Endemicity.*—In Table XII, the endemicity rate for filaria is given for the town as well as the different communes separately. Likewise, the rates for the different age-groups are tabulated in Table XIII. The highest endemicity rate recorded was in Sector III of Pondicherry Town (Table XII). Next to that, in the order of incidence, came Sectors I, VII and VIII in the town, Pondicherry Commune, Ariayamcoupom Commune, etc. With regard to the rates in relation to the different age-groups, there was a progressive rise with age just like the disease rate and the same was found true in both the sexes.

TABLE XII.

Endemicity of filariasis in the different sectors of Pondicherry Town and in different communes of Pondicherry Settlement.

Area surveyed.		Number examined.	Endemicity rate.
Pondicherry Sector I	...	693	18·3
Pondicherry Sector II	...	1,007	13·1
Pondicherry Sector III	...	1,337	20·8
Pondicherry Sector IV	...	981	13·6
Pondicherry Sector V	...	1,123	11·4
Pondicherry Sector VI and IX	...	956	9·3
Pondicherry Sector VII and VIII	...	1,087	15·9
Pondicherry Commune	...	1,081	15·2
Oulgaret Commune	...	2,210	8·4
Mudaliarpet Commune	...	1,058	8·9
Ariayamcoupom Commune	...	825	14·4
Villenour Commune	...	1,773	8·4
Tribouvanin Commune	...	2,376	5·3
Bahour Commune	...	1,253	5·9
Nettapacom Commune	...	1,364	5·1
Total for Pondicherry Town	...	7,184	14·8
Total for Pondicherry Communes	...	11,940	8·3
Total for Pondicherry Settlement	...	19,124	10·7

TABLE XIII.

Filarial endemicity in the different age-groups.

Age-group. (Years)	Endemicity rate:					
	Males.		Females.		Both sexes.	
	Number examined.	Endemicity rate.	Number examined.	Endemicity rate.	Number examined.	Endemicity rate.
Infants.	0	..	6	..	15	..
1—5	595	2·8	495	2·6	1,090	2·8
6—10	1,067	6·0	845	6·2	1,912	6·2
11—20	2,179	10·6	1,599	9·6	3,778	10·5
21—30	2,395	13·4	2,203	9·4	4,598	11·6
31—40	1,730	12·0	1,435	11·0	3,165	11·8
41—50	1,302	14·4	951	8·0	2,253	12·4
Above 50	1,398	16·7	915	13·3	1,413	25·6
Total	10,675	11·8	8,449	9·4	19,124	10·7

(B) ENTOMOLOGICAL SURVEY.

The following species of mosquitoes were collected during the survey of the Settlement :—

1. *C. fatigans.* 2. *C. vishnui.* 3. *C. gelidus.* 4. *C. sitiens.* 5. *C. bitaeniorhynchus.* 6. *Armigeres obturbans.* 7. *Aedes aegypti.* 8. *M. uniformis.* 9. *A. subpictus.* 10. *A. vagus.* 11. *A. hyrcanus.* 12. *A. culicifacies.* 13. *A. tessellatus.* 14. *A. aconitus.*

The man-hour density of mosquitoes in the town and the communes, the number of mosquitoes dissected, the mosquito infection rate, average number of developmental forms per mosquito, etc., are shown in Table XIV. Infection was found only in *C. fatigans* and the same is taken as the vector of this disease in the area. The man-hour density of *C. fatigans* for the whole Settlement was about 16·2. Working out separately for the town and communes, the same was found to be 18 and 15 respectively. The infection rate in this species of mosquito varied from 2·9 to 33·3, but the average was 8·96 per cent. This includes forms found with or without sheath in the stomach of the mosquito. If such forms are excluded altogether for the calculation of infection rate in the mosquitoes, the average works out only to 7·3 per cent.

The breeding places of *C. fatigans* were traced in the town in street drains (practically with no free flow of water), improperly constructed septic tanks, sullage water channels, swampy areas in the immediate vicinity of the town filled with refuse water of the town, etc. In the villages, the household waste water, collected in small soakage pits invariably in the backyard of the individual houses, formed the chief source of breeding of the vector species. In some of the communes very close to Pondicherry Town, *Pistia stratiotes* plants were found in some of the water collections, especially in those subjected to tidal effects, but limited search made during the survey did not show the breeding of *Mansoniodes* species.

TABLE XIV.

Mosquito density and dissection records.

Particulars.		Pondicherry.*	Pondicherry.†	Oulgaret.†	Ariyancoupom.†	Mudaliarpet.†	Villenour.†	Tribouvani.†	Bahour.†	Nettapacom.†	All areas.
Man-hour density species mosquito.	All mosquitoes.	28	28	51	46	47	120	48	17	49	48
	C. fatigans.	18	2	5	5	12	15	34	5	21	16·2
C. fatigans	Number dissected.	184	59	19	8	30	34	72	3	26	435
Number showing infection in	Abdomen only.	2	1	..	1	..	..	1	1	1	7
	Thorax only.	9	10	..	1	1	..	4	..	4	29
	Head and proboscis only.	1	..	..	..	1	1	..	..	..	3
Infection rate for all stages (per cent).		6·9	18·6	..	25·0	6·6	2·9	6·9	33·3	19·2	8·96
Infection rate for infective larvae (per cent).		2·2	..	..	..	3·3	2·9	..	..	..	1·4
Other culicines	Number dissected.	56	6	179	57	72	107	42	3	8	530
	Number with infection.	..	..	..	..	..	..	..	..	..	..
Anophelines	Number dissected.	93	18	92	50	19	184	135	13	43	647
	Number with infection.	..	..	..	..	..	..	..	..	..	..

* = Town † = Commune.

(B) ANIMAL SURVEY.

Blood smears of 158 animals, as per details given below, were examined. Out of these, only one bull was found to be harbouring microfilaria.

TABLE XV.

Type of animal.	Number surveyed.	Number positive for microfilaria.	Infection rate.
Bull ...	45	1	2·2
Cow ...	51	..	..
Goat ...	15	..	..
Dog ...	14	..	..
Fowl ...	8	..	..
Heijuj Cal ...	21	..	..
Pigeon ...	3	..	..
Emu ...	1	..	..

CONCLUSIONS.

The whole of Pondicherry Settlement is endemic for *W. bancrofti* filariasis. Incidence of the disease is comparatively higher in Pondicherry Town and in the neighbouring communes, such as Pondicherry and Ariyamcoupom, as compared with the rest of the places in the Settlement.

Only 15 infants could be examined during the survey, and none of them showed microfilaria in the night blood. In all the other age-groups, blood infection as well as disease was evident. The incidence of infection rose up gradually with the ascending age up to 20 years, and thereafter remained static with minor fluctuations. The infection rate and average infestation ran almost parallel in all the age-groups of the two sexes.

The incidence of filarial disease showed an ascending order with advance in age. The above findings are similar to those observed by Jaswant Singh *et al.* (1956), Krishnaswami (1955) and Nair *et al.* (1960).

SUMMARY.

The incidence of filariasis in Pondicherry Settlement, having an urban population of about 60,000 and rural 1,50,000 (1948 census), was investigated in March 1957. 8·21 per cent of the total population was surveyed. An overall infection rate of 6·3 per cent, disease rate (excluding filarial fever) of 3·8 per cent and endemicity rate of 9·9 per cent were recorded during the survey. In Pondicherry Town, the incidence was higher and the indices worked out to 10·3, 4·2 and 14·1 per cent for infection, disease and endemicity respectively. The vector of filariasis was *C. fatigans*. The mosquito infection rate was 8·96 per cent.

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STUDY OF AN OUTBREAK OF EPIDEMIC MALARIA IN ARASIKERE TALUK, HASSAN DISTRICT, MYSORE STATE.

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INTRODUCTION.

THE earliest available record of the existence of malaria in Arasikere Taluk of Hassan District, Mysore State, dates back to 1929, when Sweet (1929) reported that the spleen rate in the taluk ranged from 13·3 to 40.3 per cent and considered that the taluk was a low endemic area with a few definite foci. However, as no major epidemic of malaria had been reported in the area till 1954, no antimalarial measures had been undertaken in the taluk.

But in 1954, the conditions were peculiarly favourable for the occurrence of a large outbreak of malaria. The majority of the children below twelve years of age had been little, if at all, exposed to malaria and the level of immunity against malaria was thus at a very low point. A series of years of deficient rainfall had preceded the year of the epidemic, whilst the unusually heavy rainfall in 1953 was responsible for the filling up of all tanks in the taluk. From January, 1954, there was a steady increase in malaria cases attending the dispensaries but the matter was only reported during the end of May, 1954, when the epidemic had already attained its peak. Nevertheless, during the period June 2 to 23, 1954, intensive studies were conducted in the area before undertaking control measures with D.D.T. spraying. This paper records the results of certain observations conducted during the epidemic and post-epidemic periods.

DESCRIPTION OF THE AREA.

Arasikere is the biggest taluk in Hassan District, Mysore State, and is about 2,645 feet above the mean sea level. In its northern half is located the Malekal Tirupathi Range of hillocks. The taluk slopes gently towards the north-west,

*Delay in publication of this paper is due to change of press with effect from March, 1959. Consequently, manuscripts of some papers, including this one, had to be withdrawn from the previous press.—*Editor*

which forms part of the basin of the River Vedavati. There are a few seasonal streams surrounding the hillocks and a number of valleys along the course of which are situated 34 major tanks and 142 minor tanks (Map I). The whole taluk is a semi-dry tract of light-red ferruginous soil.

The taluk has an area of 475 square miles and a population of 1,48,745, distributed in 323 main villages and 133 hamlets. Most of the houses are mud-walled structures with thatched roofs and poor ventilation. There are two towns—Arasikere and Banavar. The former is the bigger of the two, with a population of 15,860 and has a major Municipality. It is also the taluk headquarters. The five *hoblies** of the taluk are Arasikere, Banavar, Gandasi, Javagal and Jayachamarajapura.

In the taluk, the area under cultivation is 1,32,872 acres of which only 2,025 acres are under wet cultivation. The major crops are millets and coconuts. Rainfall is scanty, the annual average rainfall being about 18·0 inches, September and October usually receiving the maximum precipitation. But in 1953 there was abnormal rainfall, amounts varying from 26·0 to 33·0 inches being recorded in different parts of the taluk.

PRE-EPIDEMIC CONDITIONS.

No observations were made during the pre-epidemic period as the abnormal increase in the number of outpatients for malaria, attending the five dispensaries in the taluk, was reported only during the middle of May, 1954. Rainfall data for the period January, 1951 to May, 1954, are shown in Table I. As mentioned earlier, the year 1953 was marked by abnormally heavy rainfall, the maximum precipitation being in September and October. Records of maximum and minimum temperatures and relative humidity for the taluk are not available but the figures recorded at the observatory at Hassan, 24 miles away to the south-west, show that during November, 1953 to March, 1954 the relative humidity at 08·30 hours was above 70·0 per cent.

THE EPIDEMIC PERIOD.

(*a*) *Morbidity.*—Dispensary statistics for the four hospitals at Arasikere, Banavar, Javagal and Jayachamarajapura for the period 1949 to 1953 are shown in Table II. The number of patients treated weekly during 1953-1954 for all diseases, and for malaria, at the five hospitals at Arasikere, Banavar, Gandasi, Javagal and Jayachamarajapura, are represented in Table III and Graph I. No epidemics had been reported during the years 1949, 1950, 1951 and 1952. It will be seen that the percentage of malaria cases varied from 27·8 to 52·3 and during 1953 it was actually at the lowest level in Banavar and Jayachamarajapura. Weekly figures indicate that the increase in malaria cases starts from November and reaches its peak in March and April. After a slight drop in May, it again rises in June and then gradually declines till November when again there is a steady increase.

Hobli means a revenue circle.

MAP 1.

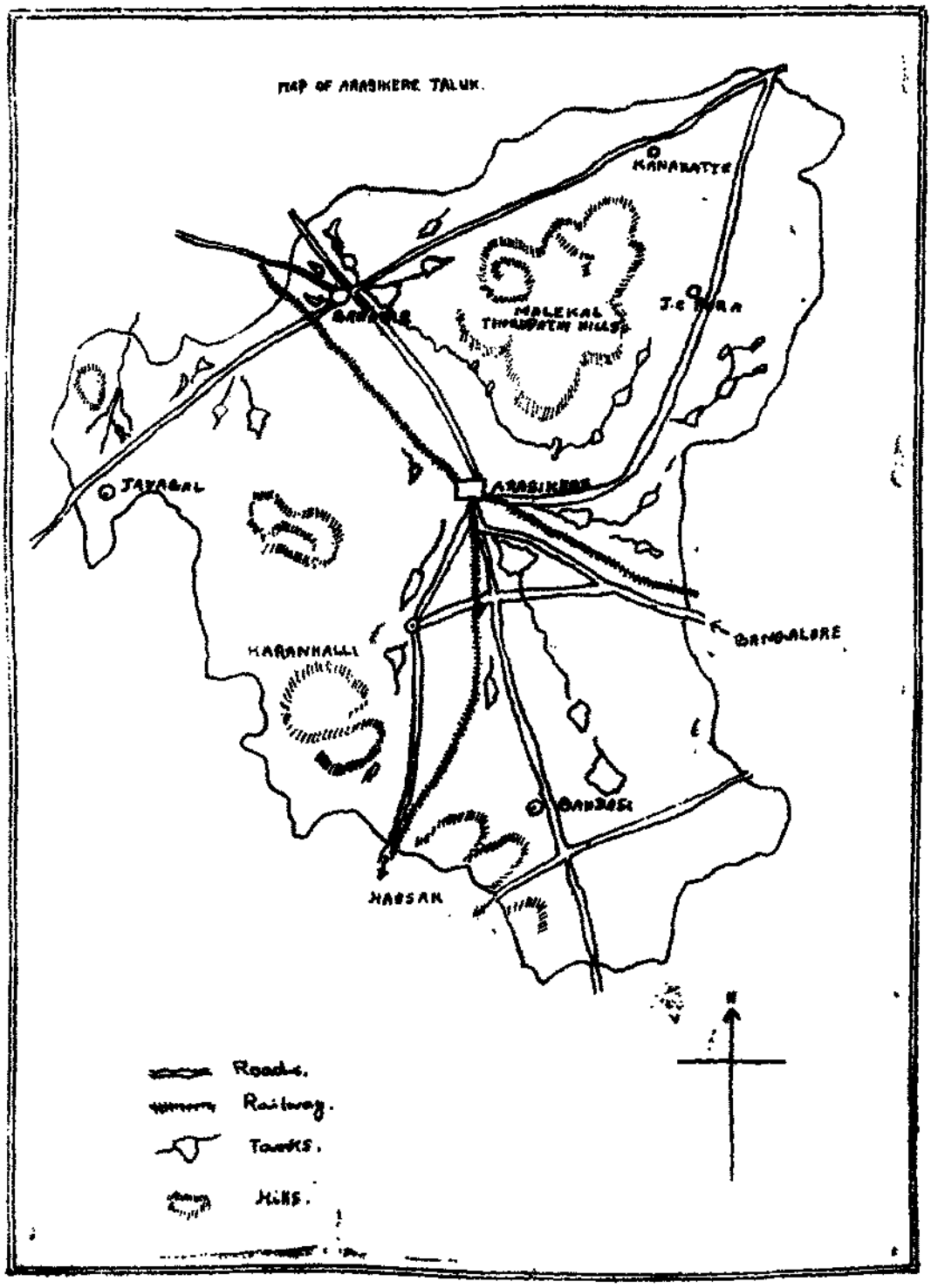
MAP OF ARASIKERE TALUK.
KANABATTE
MALEKAL THIMMATHI HILLS
JAVAGAL
ARASIKERE
BANGALORE
KARANHALLI
HASSAN
Roads.
Railway.
Towns.
Hills.

TABLE I.

Rainfall data for Arasikere Taluk, Hassan District Mysore State, January, 1951 to May, 1954.

Year and month.	RAINFALL IN INCHES :			
	Arasikere.	Banavar.	Gandasi.	Kanakatte.
1951 January	0·00	0·00	Data not available.	0·00
February	0·00	0·00		0·00
March	0·00	0·30		0·30
April	0·60	1·33		2·12
May	4·43	3·29		3·60
June	1·40	1·80		0·00
July	1·70	2·34		1·56
August	0·45	0·20		0·23
September	10·50	6·55		7·25
October	2·77	3·10		1·75
November	0·50	0·00		0·00
December	0·00	0·00		0·00
1951 Total	22·35	18·91		16·81
1952 January	0·00	0·00	0·00	0·00
February	0·19	0·00	0·00	0·27
March	0·00	0·00	0·00	0·00
April	1·17	1·00	0·00	0·84
May	2·75	1·90	0·00	1·20
June	0·65	0·00	0·00	0·45
July	1·58	1·05	0·65	2·16
August	2·49	1·11	2·30	0·98
September	1·71	1·50	1·13	3·57
October	10·61	0·63	7·80	2·88
November	0·00	0·00	0·00	0·00
December	3·25	0·00	2·83	1·92
1952 Total	24·40	7·19	20·71	14·27
1953 January	0·00	0·00	0·00	0·00
February	0·00	0·00	0·00	0·00
March	0·00	0·10	0·00	0·00
April	2·32	3·35	2·40	2·40
May	0·24	0·00	0·00	0·00
June	3·39	0·89	1·23	3·00
July	4·68	8·06	3·11	7·62
August	1·60	3·52	0·58	1·12
September	4·44	2·72	4·10	3·05
October	16·02	12·13	14·80	15·20
November	0·00	0·00	0·00	0·00
December	0·00	0·00	0·00	0·00
1953 Total	32·69	30·77	26·22	32·39
1954 January	0·12	0·00	0·50	0·00
February	0·00	0·00	0·00	0·00
March	0·00	0·15	0·00	0·00
April	2·20	1·88	0·75	0·63
May	5·92	7·70	5·23	2·98

TABLE II.

Dispensary Statistics—Arasikere Taluk, Hassan District, Mysore State
Years 1949 to 1953.

Year.	ARASIKERE			BANAVAR			JAVAGAL			JAYACHAMARAJAPURA		
	Total cases treated.	Total malaria cases treated.	Percentage of malaria cases to total.	Total cases treated.	Total malaria cases treated.	Percentage of malaria cases to total.	Total cases treated.	Total malaria cases treated.	Percentage of malaria cases to total.	Total cases treated.	Total malaria cases treated.	Percentage of malaria cases to total.
1949	30,449	9,832	32·2	29,295	8,068	27·8	9,271	2,821	30·4	12,835	4,389	44·2
1950	31,900	8,927	28·0	22,298	4,629	20·8	9,067	536	5·9	12,800	3,876	30·3
1951	38,018	11,689	30·8	19,275	3,333	17·2	7,947	746	9·4	13,628	7,134	52·3
1952	38,960	9,896	25·5	13,848	2,510	14·8	8,570	800	9·3	11,962	5,025	42·0
1953	51,949	15,558	30·0	24,495	2,400	9·8	10,473	1,789	16·1	10,570	2,516	24·0

TABLE III.

Number of patients treated weekly at the five dispensaries in Arasikere Taluk, Hassan District, Mysore State, during the years 1953 and 1954.

Week ending.	ARASIKERE			BANAVAR			GANDASI			JAVAGAL			JAYACHAMARAJAPURA		
	Total cases treated.	Total malaria cases treated.	Percentage of malaria cases to total.	Total cases treated.	Total malaria cases treated.	Percentage of malaria cases to total.	Total cases treated.	Total malaria cases treated.	Percentage of malaria cases to total.	Total cases treated.	Total malaria cases treated.	Percentage of malaria cases to total.	Total cases treated.	Total malaria cases treated.	Percentage of malaria cases to total.
1953 January															
3	164	60	36·6	109	12	11·0	36	2	5·5	400	16	4·0	96	20	20·8
10	379	158	41·7	248	29	11·7	54	5	9·2	380	19	5·0	204	49	24·0
17	368	117	31·7	164	15	9·1	95	12	12·6	413	31	7·5	229	39	17·0
24	394	120	30·5	105	13	12·4	114	7	6·1	584	34	5·9	212	53	25·0
31	312	116	37·2	97	9	9·3	24	2	8·3	786	39	4·9	212	41	19·3
February															
7	330	108	32·7	105	8	7·6	20	1	5·0	161	3	1·8	166	32	19·3
14	403	128	31·7	100	14	14·0	15	3	20·0	401	12	2·9	131	29	22·1
21	462	145	31·4	105	11	10·5	42	8	19·0	609	14	2·3	190	33	17·4
28	445	170	38·2	116	11	9·5	18	3	16·7	791	17	2·1	162	25	15·4
March															
7	569	156	27·4	107	14	13·1	44	4	9·1	193	4	2·1	169	24	14·2
14	621	213	34·3	160	33	20·6	38	10	26·3	336	9	2·6	209	30	14·4
21	564	163	28·9	219	21	9·6	75	8	10·7	490	12	2·5	182	25	13·5
28	730	245	33·5	213	23	10·8	22	4	16·2	652	21	3·2	169	34	20·1
April															
4	755	287	38·0	229	19	8·3	84	10	11·9	132	3	2·3	187	32	11·7
11	742	289	38·9	240	19	7·9	68	18	26·5	261	7	2·7	230	37	16·1
18	825	327	39·6	179	21	11·7	95	13	13·7	400	10	2·5	203	36	17·7
25	948	389	41·0	222	17	7·6	119	19	15·9	559	23	4·1	207	43	20·8
May															
2	951	364	38·2	259	23	8·8	140	14	10·0	38	2	5·3	173	44	25·4
9	1,019	426	41·8	289	37	12·9	184	24	13·0	201	21	10·4	223	56	25·1
16	1,037	388	37·4	320	50	15·6	109	16	14·8	406	43	10·6	210	62	29·5
23	967	400	41·4	211	30	14·2	53	18	33·9	629	70	11·1	192	53	27·6
30	964	356	36·9	268	37	13·8	51	18	35·3	792	93	11·8	174	58	33·3

1953 June															
6	935	379	36·9	243	34	13·9	108	12	11·1	117	18	15·4	189	54	28·6
13	1,124	317	28·2	275	32	11·6	126	15	11·9	383	46	12·0	190	49	25·8
20	908	290	31·9	240	46	19·2	114	24	21·1	603	79	13·1	148	64	43·2
27	907	280	30·8	256	38	14·8	102	18	17·6	760	88	11·6	141	63	44·6
July															
4	889	250	28·1	208	30	14·4	59	5	8·5	87	14	16·1	160	53	33·1
11	795	261	32·8	207	33	15·9	109	10	9·2	196	25	13·3	172	42	24·4
18	957	282	29·4	275	37	13·4	85	8	9·4	341	43	12·6	186	38	20·4
25	883	313	35·8	288	41	14·3	112	19	16·9	562	61	10·9	200	35	17·5
August															
1	912	319	34·9	216	31	14·2	54	8	14·8	422	30	7·2	124	32	25·8
8	929	374	40·2	272	27	9·9	69	9	13·0	167	13	7·8	133	30	22·5
15	724	239	33·0	280	36	12·8	74	14	18·9	322	25	7·8	134	33	24·6
22	966	314	32·5	292	34	11·6	66	4	6·1	516	49	9·5	158	39	24·7
29	1,042	385	36·9	387	50	12·9	50	3	6·0	743	84	11·3	172	36	20·9
September															
5	1,270	364	28·4	393	40	10·2	98	8	8·2	136	18	13·2	175	32	18·3
12	1,192	310	26·0	337	35	10·4	113	12	10·6	308	43	13·9	198	37	18·7
19	1,457	451	30·9	372	38	10·2	124	22	17·7	546	79	14·5	223	41	18·4
26	1,338	379	28·3	446	49	10·9	128	12	9·4	786	134	17·0	223	38	17·0
October															
3	1,525	450	29·5	439	55	12·5	88	6	6·8	107	24	22·4	228	48	21·1
10	1,436	374	26·0	373	75	20·1	104	14	13·5	337	74	21·9	308	55	17·8
17	1,214	333	27·4	394	79	20·1	144	22	15·3	601	140	23·3	252	48	19·1
24	1,302	316	24·3	350	82	23·4	84	10	13·3	829	218	26·3	285	54	18·9
31	1,476	317	21·1	316	83	26·3	33	3	9·1	1,148	334	28·9	247	46	18·6
November															
7	1,487	405	27·2	508	130	25·6	101	14	13·8	273	116	42·5	257	54	21·0
14	1,506	452	30·0	456	110	24·1	109	16	14·7	553	239	43·2	220	46	20·9
21	1,431	415	29·0	504	113	22·4	98	12	12·2	802	341	42·5	274	47	17·1
28	1,124	342	30·4	324	100	30·9	116	23	19·8	1,029	422	41·0	267	52	19·5
December															
5	1,316	368	27·9	415	117	28·2	50	14	28·0	164	56	34·1	265	103	38·8
12	1,101	322	29·2	363	120	33·1	88	16	18·2	408	81	19·8	240	98	40·8
19	1,147	331	28·9	371	90	24·2	134	29	21·6	603	143	23·7	273	123	45·1
26	1,087	322	29·6	292	80	27·4	83	16	19·3	792	270	34·1	260	100	38·5
1954 January															
2	1,223	252	20·6	269	82	30·5	46	14	30·4	49	9	18·4	197	63	31·9
9	995	332	33·4	251	50	19·9	98	25	25·5	210	34	16·2	189	55	29·1
16	940	307	32·6	243	50	20·5	89	20	22·5	351	60	17·1	202	68	33·6
23	946	328	34·6	230	46	20·0	44	15	34·1	516	91	17·6	158	48	30·4
30	1,017	405	39·8	243	33	13·6	37	10	27·0	654	116	17·7	160	56	35·0

TABLE III (*Contd.*)

Week ending	Arasikere :			Banavar :			Gandasi :			Javagal :			Jayachamarajapura :		
	Total cases treated.	Total malaria cases treated.	Percentage of malaria cases to total.	Total cases treated.	Total malaria cases treated.	Percentage of malaria cases to total	Total cases treated.	Total malaria cases treated.	Percentage of malaria cases to total	Total cases treated.	Total malaria cases treated.	Percentage of malaria cases to total.	Total cases treated.	Total malaria cases treated.	Percentage of malaria cases to total.
1954 February															
6	1,053	320	30·4	221	40	18·1	74	28	37·8	93	10	10·8	132	40	30·3
13	1,027	318	30·9	263	56	21·3	85	32	37·7	234	25	10·7	167	48	28·7
20	931	227	29·8	272	36	13·2	94	40	42·5	250	43	12·3	159	43	27·0
27	1,050	299	38·8	277	41	14·8	67	12	17·9	519	59	11·4	174	50	28·7
March															
6	1,085	272	25·1	267	63	23·6	85	40	47·1	173	25	14·4	219	65	29·6
13	991	397	40·1	268	89	33·2	104	51	49·0	384	71	18·5	238	104	43·7
20	1,258	397	31·6	296	126	42·5	94	46	48·9	622	128	20·6	233	112	48·1
27	1,302	384	21·8	374	141	37·7	98	34	34·7	873	220	25·2	321	173	53·9
April															
3	1,301	397	30·5	379	197	51·9	280	145	51·8	113	52	46·0	294	169	57·5
10	1,248	369	29·6	370	196	52·9	149	109	73·2	391	160	40·9	346	215	62·1
17	1,264	396	31·3	467	182	38·9	198	121	61·1	648	271	41·8	338	215	63·6
24	1,403	426	30·3	538	191	35·5	119	69	57·9	892	357	40·0	362	246	67·9
May															
1	1,537	456	29·6	541	163	30·1	209	132	63·1	35	9	25·7	513	346	67·2
8	1,330	406	30·5	506	109	21·5	164	119	72·6	239	99	41·5	298	264	88·6
15	1,299	386	29·7	515	131	25·4	174	114	65·5	460	174	37·8	402	288	71·6
22	1,333	365	27·4	375	141	37·6	198	113	57·1	637	226	35·5	377	267	70·8
29	1,418	462	32·6	314	126	40·1	127	88	69·3	770	262	34·0	387	240	62·0
June															
5	1,133	380	33·5	279	124	44·4	94	27	28·7	114	18	15·8	338	201	59·4
12	1,042	401	38·5	240	92	38·3	134	35	26·1	257	52	20·2	308	156	50·6
19	1,173	424	36·1	253	84	33·2	148	44	29·7	430	90	20·9	290	148	51·0
26	1,243	403	32·4	384	98	25·5	165	56	33·9	564	123	21·8	313	156	50·0
July															
3	1,161	386	33·2	495	66	13·3	106	24	22·6	103	24	23·3	339	143	42·2
10	972	309	31·8	467	64	13·7	98	22	22·5	357	100	28·0	233	104	44·6
17	981	288	29·4	418	62	14·8	112	28	25·0	574	129	22·5	231	69	29·5
24	1,073	261	24·3	410	58	14·1	88	14	15·9	805	164	20·4	167	58	34·7
31	1,027	201	19·6	323	44	13·6	102	12	11·7	1,097	201	18·3	159	40	25·1

1954 August															
7	1,031	199	19·3	282	28	9·9	101	22	21·7	305	34	11·1	151	64	42·4
14	867	158	18·2	366	41	11·2	94	14	14·9	547	69	12·6	189	44	23·3
21	1,065	185	17·4	366	33	9·0	85	10	11·8	813	103	12·7	184	32	17·4
28	1,088	186	17·0	337	27	8·0	108	16	14·8	1,116	152	13·6	149	21	14·1
September															
4	916	130	14·2	286	24	8·4	96	14	14·6	89	17	19·1	168	32	19·0
11	1,068	164	15·4	321	28	8·7	84	12	14·3	306	44	14·4	171	26	15·2
18	1,234	202	16·4	290	25	8·6	109	18	16·5	584	70	11·9	199	29	14·6
25	1,140	200	17·8	360	28	7·7	91	16	17·6	868	95	10·9	213	52	24·4
October															
2	1,109	199	17·9	326	25	7·7	105	12	11·4	191	9	8·9	126	43	29·4
9	986	140	14·2	320	25	7·8	95	9	9·5	354	49	13·6	190	38	20·0
16	10,90	201	18·4	283	26	9·2	84	17	20·2	622	75	12·1	174	38	21·8
23	883	144	16·3	271	22	8·1	76	6	7·9	824	98	11·9	161	27	16·8
30	1,062	169	15·9	340	28	8·2	54	10	18·5	1,001	116	11·5	186	46	24·7
November															
6	1,458	203	13·9	363	25	6·9	104	18	17·3	201	29	14·4	177	47	26·5
13	1,208	234	19·4	408	33	8·1	92	16	17·4	505	68	13·5	193	38	19·7
20	1,083	185	17·1	374	28	7·5	108	20	18·5	776	90	11·6	244	46	18·8
27	1,064	172	16·1	354	45	12·9	114	26	22·8	962	101	10·5	243	36	14·8
December															
4	1,099	185	16·8	388	46	11·6	98	28	28·6	147	19	12·9	213	50	23·5
11	1,096	198	18·1	342	51	14·9	108	24	22·2	383	31	8·1	257	43	13·1
18	986	184	18·7	293	28	9·5	112	26	23·2	642	60	9·4	199	33	16·6
25	990	166	16·8	235	20	8·5	81	16	19·7	889	82	9·1	191	29	15·2
1955 January															
1	985	142	14·4	248	25	10·1	102	22	21·5	22	6	27·3	163	35	21·4

During 1953, from the first week of November there was a steady increase in the number of malaria cases, the peak attendance reaching in April and May, 1954 with two drops in the last weeks of February and March. Banavar, Gandasi and Jayachamarajapura appeared to be the worst affected areas with 53·0, 73·0 and 89·0 per cent malaria morbidity respectively at the peak periods.

(*b*) *Mortality.*—A peculiar feature of this outbreak of malaria was the absence of any obvious increase in mortality, the highest epidemic figure (Christophers, 1911) recorded being only 2·1, though during the epidemic itself there was a slight increase in the number of still-births.

(*c*) *Entomological data.*—During the actual epidemic period itself no surveys were carried out. But surveys carried out between June 2 and 23, 1954 are dealt under "Post Epidemic Period", as by that time post-epidemic conditions had begun.

(*d*) *Spleen and parasite surveys.*—Spleen and parasite surveys were conducted in all the five hoblies of the taluk simultaneously by five different teams. 195 villages were visited and 9,836 children between 2 and 12 years of age were examined for splenomegaly. Of these, 5,831 showed enlarged spleens, the spleen rate and average enlarged spleen for the whole taluk being 59·3 per cent and 2·3 respectively. Details of spleen survey are set out in Table IV.

TABLE IV.

Childhood spleen rates in Arasikere Taluk, Hassan District, Mysore State, during June, 1954.

Name of hobli.	Number examined.	Number positive.	Enlarged spleens:					Spleen rate.	Average enlarged spleen.
			1	2	3	4	5		
Arasikere	1,436	893	258	349	224	53	9	62·1	2·1
Banavar	935	546	157	255	108	26	0	58·4	2·0
Gandasi	1,159	527	234	206	75	12	0	45·4	1·7
Javagal	3,146	2,025	844	785	296	78	22	64·4	1·8
Jayachamarajapura	3,160	1,840	320	1,003	400	102	15	58·2	2·2
Total	9,836	5,831	1,813	2,598	1,103	271	46	59·3	2·3

Besides the childhood spleen rate, in 31 villages out of the 195 villages visited adult spleen rates were also obtained to find out whether malaria had been recently initiated or the area was really hyperendemic. Out of 661 adults examined, 349 had enlarged spleens, the spleen rate and average enlarged spleen being 52·8 and 2·2 per cent respectively, thus indicating epidemic conditions.

1,155 blood smears were collected from all age-groups from the 195 villages visited. More smears were not taken as this number was considered adequate to give an idea of the parasite rate and species prevalence. 766 smears were positive for malaria parasites, giving a parasite rate of 66·3 per cent. The species prevalence was *P. falciparum* 41·7 per cent, *P. vivax* 38·3 per cent, *P. malariae* 5·6 per cent

and mixed infection 14·4 per cent. Out of a total of 766 smears with parasites, 321 or 41·8 per cent of the slides examined showed gametocytes. Parasites were encountered in all age-groups, the maximum incidence being in the 6-20 years group. Details of blood examinations are shown in Table V to VIII.

TABLE V.

Parasite incidence by age-group.

Age-group (years)	Examined	Positive	Per cent positive
0	5	2	40·0
1	12	8	66·7
2	52	41	78·8
3	79	63	79·7
4	82	60	73·2
5	77	60	77·9
6—10	466	303	65·0
11—20	220	146	66·4
21—50	132	67	50·8
Over 50	8	3	37·5
Not known	22	13	59·1
Total	1,155	766	66·3

TABLE VI.

Gametocyte incidence by age-groups.

Age-group (years)	Examined	Positive	Number with gametocytes	Gametocyte rate
0—5	307	243	108	46·1
6—20	686	449	194	43·2
Over 20	149	70	17	24·2
Not known	22	13	2	15·4
Total	1,155	766	321	41·8

TABLE VII.

Correlation of age and incidence of different species of parasites.

Age group (years)	Total examined	Total positive.	*P. falciparum*		*P. malariae*		*P. vivax*		MIXED INFECTION	
			Number positive.	Per cent positive.	Number positive.	Per cent positive.	Number positive.	Per cent positive.	Number positive.	Per cent positive.
0— 1	17	10	2	20·0	0	0·0	6	60·0	2	20·0
2— 5	290	224	64	28·5	9	4·0	111	49·5	40	17·8
6—10	466	303	142	47·1	16	15·2	100	33·0	45	14·8
11—20	220	146	78	53·4	9	6·2	41	28·1	18	12·3
Above 20	140	70	31	44·3	4	5·7	31	44·3	4	5·7
Not known	22	13	3	23·0	5	38·4	4	3·8	1	7·0
Total	1,155	766	320	41·7	43	5·6	293	38·3	110	14·3

TABLE VIII.

Age and incidence of single and mixed infections.

Age group. (years)	*P. falciparum.*	*P. malariae.*	*P. vivax.*	*P. falciparum*+ *P. malariae.*	*P. falciparum*+ *P. malariae*+ *P. vivax.*	*P. malariae* +*P. vivax.*	*P. falciparum*+ *P. vivax.*
0—1	2	0	6	1	0	1	0
2—5	64	9	111	5	1	8	26
6—10	142	16	100	8	2	9	26
11—20	78	9	41	2	1	5	10
Over 20	31	4	31	0	0	2	2
Not known	3	5	4	0	0	0	1
Total	320	43	293	16	4	25	65

Paludrine and Resochin tablets were distributed to the suffering population during the surveys in all the 456 villages of the taluk by house-to-house visits, as an immediate measure of relief, pending the arrival of the spraying squads with insecticides.

POST EPIDEMIC PERIOD.

Entomological data.—Entomological surveys were carried out, both during day and night, between June 2 and 23, 1954. In the course of this survey in 31 villages of the five hoblies of the taluk, 6,876 anopheline mosquitoes were collected in 174·7 man-hours, yielding a gross per man-hour figure of 39·3. Out of the fourteen anopheline species recorded, 2,739 (39·8 per cent) were *A. culicifacies.* 202 (2·9 per cent) *A. fluviatilis* and 24 (0·3 per cent) *A. stephensi.* The highest per man-hour figure recorded for *A. culicifacies* was 97·0. Next to *A. culicifacies, A. annularis* was the most abundant species recorded. The anopheline species recorded were *aconitus, annularis, barbirostris, culicifacies, fluviatilis, hyrcanus, jamesi, jeyporiensis, pallidus, splendidus, subpictus, stephensi, vagus* and *varuna.*

By the beginning of May, 1954, all the temporary breeding places had dried up and at the time of the surveys in June, 1954, although all water collections near the villages were searched, only 901 larvae were collected. *A. annularis* was the most abundant species (33·1 per cent) with *A. culicifacies* the next in order of abundance (32·6 per cent). Only solitary specimens of *A. fluviatilis* were met with.

Out of a total of 2,384 mosquitoes dissected, dissections of *culicifacies* occupied the major portion (83·3 per cent of the total dissections which, in turn, constituted 34·6 per cent of all anopelines collected). All *A. fluviatilis* and *A. stephensi* collected were also dissected. Dissections were confined to the salivary glands as the sporozoite rate was considered to be more valuable since it indicated the complete development of the plasmodium in the mosquito. Eight positives for glands, all from *culicifacies,* were recorded, yielding a sporozoite rate of 0·4 per cent. All the positives were from daytime collections in mixed dwellings.

Night time collections were also carried out to procure a large number of blood-fed females for precipitin tests. Out of 86 blood meals tested at the Malaria Institute of India, Delhi, (80 from *culicifacies* and 6 from *fluviatilis*) 30 were positive for human blood (26 from *culicifacies* and 4 from *fluviatilis*). The anthropophilic indices were respectively 32·5 and 66·6 per cent.

Data presented in Table III and Graph I indicate that by the first week of June, 1954, the epidemic was on the decline. Surveys conducted between June 2 and 23, 1954, also indicated that the peak period of the epidemic was over and that post-epidemic conditions had begun. No observations on the natural post-epidemic conditions were possible as further transmission was intercepted by spraying the entire taluk with D.D.T. Side by side with D.D.T.-spraying in the villages, anti-malarial drugs were distributed for a second time, i.e., after two weeks of the first administration. It was gratifying to observe that, during this second visit, the demand for the drugs had considerably fallen and there was a noticeable change for the better in the people.

After spraying, the area was visited every month for a period of one year for collection of infant blood smears and mosquitoes to judge the effectiveness of control measures. From September onwards (three months after spraying and drug distribution) infant blood smears became negative for malaria parasites. Vector species were absent for a period of nearly twelve weeks and their re-appearance after this period was in too low numbers to cause any apprehension. A random spleen survey conducted in November, 1954 in some of the worst affected villages showed that there was a considerable fall in the spleen rate, the figures had come down to 17·0 to 25·0 per cent from 70·0 per cent as is expected during the post-epidemic period particularly after drug administration. From November, 1954 onwards the taluk was included within the ambit of the National Malaria Control Programme.

DISCUSSION.

The five dispensaries in the taluk are under the control of the Medical Department and it is rather unfortunate that the increase in the number of malaria cases noticed from January, 1954 itself was not intimated to the health authorities. Only during May, 1954 when public agitation for immediate relief by D.D.T.-spraying became vehement the matter was reported to the District Health Officer, Hassan, (A.R.S.) who immediately arranged for surveys and distribution of drugs, pending diversion of insecticides for spraying. Consequently, no studies were possible during the pre-epidemic period and the early part of the epidemic period.

The year 1953 was in several respects an abnormal one in some parts of Mysore State. There was excessive rainfall in almost all the plain regions or 'maidan' areas, following successive years of drought leading to famine conditions. As in Kolar District of Mysore State where an epidemic of malaria occurred during March-April, 1954, the conditions in Arasikere Taluk also were very favourable for an outbreak of epidemic malaria. No major epidemic had occurred in the area for a long time so that both adults and children below twelve years of age, had

probably seldom or never been exposed to infection. The unusually heavy rainfall in 1953 and the consequent overflow of all the tanks in the taluk created a state of sustained high atmospheric humidity during November, 1953 to March, 1954. As a result of these conditions; which were very favourable to mosquito life, there was an abnormal increase in the prevalence of anophelines and especially of *A. culicifacies* in the whole taluk.

Judging from dispensary statistics, transmission season during normal years would appear to be from November to March of succeeding year and the whole taluk one of low endemicity for malaria, the percentage of malaria cases treated varying from 10·0 to 30·0 per cent. There are numerous references to occurrence of epidemics in such areas when floods occur due to excessive rainfall. In the present case, although malariometric indices are lacking for the pre-epidemic period and the early part of the epidemic period, the dispensary statistics are, nevertheless, very revealing. There was a steady increase in the number of malaria cases from the first week of March, 1954, the wave of morbidity reaching its maximum by the middle of May and then beginning to decline. This period was also coincident with a high degree of relative humidity.

The high spleen rate and the low average enlarged spleen both in children and adults, indicate epidemic conditions. The first two degrees of enlargement accounted for 75·0 per cent of the spleen positive cases among the children. In the absence of figures for parasite rate during the pre-epidemic period and the first week of the epidemic, one can only assume that gametocyte carriers must have been very few. Covel and Baily (1932) have pointed out that one crescent carrier among 200 in the first week of an epidemic can give rise to 100 crescent carriers during the ninth week. Macdonald and Majid (1931) have also stated that, at the beginning of an epidemic, there is an extreme paucity of gametocyte carriers and a considerable time must elapse before a large population of gametocyte carriers can be built up by a process of geometrical progression. A gametocyte rate of 41·8 per cent was encountered in the present studies during the 14th-16th week of the epidemic. Of this, nearly 45·0 per cent constituted crescent carriers.

The natural post-epidemic conditions could not be studied as further transmission was intercepted by spraying the entire taluk with D.D.T. This was carried out on an emergency basis and eleven teams covered the entire taluk in five days—a remarkable instance of co-operation between the villagers and the revenue officials with the spraying squad. As stated earlier, infant parasite rates became zero three months after spraying and a spleen survey conducted four months after spray and drug distribution indicated that there was a remarkable fall in the spleen rate.

A peculiar feature of this epidemic was the absence of any obvious increase in the mortality rate. This could be probably ascribed to the timely intervention and treatment with antimalarial drugs.

SUMMARY.

Some observations carried out during an epidemic of malaria in Arasikere Taluk, Hassan District, Mysore State, are described. The area was one of low endemicity and consisted of wholly untreated populations. The cause of the epidemic was excessive rainfall following a series of years of drought leading to famine conditions. The epidemic itself followed for the most part the characteristic course described by Christophers (1911) in the case of malaria epidemics in the Punjab. *P. falciparum* was the chief malaria parasite concerned in the epidemic. There was no obvious increase in mortality rate, which is ascribed to timely intervention with drugs and insecticides. Mosquito densities, even at the end of the epidemic, were very high, the maximum prevalence of *A. culicifacies* recorded being 97·0 per man-hour. A sporozoite rate of 0·4 per cent and an anthropophilic index of 32·5 per cent for *A. culicifacies* were recorded. After spraying with D.D.T., infant parasite rates dropped down to zero. The spraying operations and the drug distribution resulted in considerable fall in the spleen rate and mosquito densities.

These observations reveal that occurrence of epidemics in certain areas in years of heavy rainfall is quite common and that public agitation for D.D.T.-spraying should not be construed as a sort of 'fashion'. The importance of epidemic areas in eradication of malaria has been realised in the National Malaria Eradication Programme.

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OBSERVATIONS ON THE TREATMENT OF 678 MALARIA CASES WITH PRIMAQUINE IN AN AREA FREE FROM MALARIA TRANSMISSION IN MYSORE STATE, INDIA.

BY

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[February 1, 1960.]

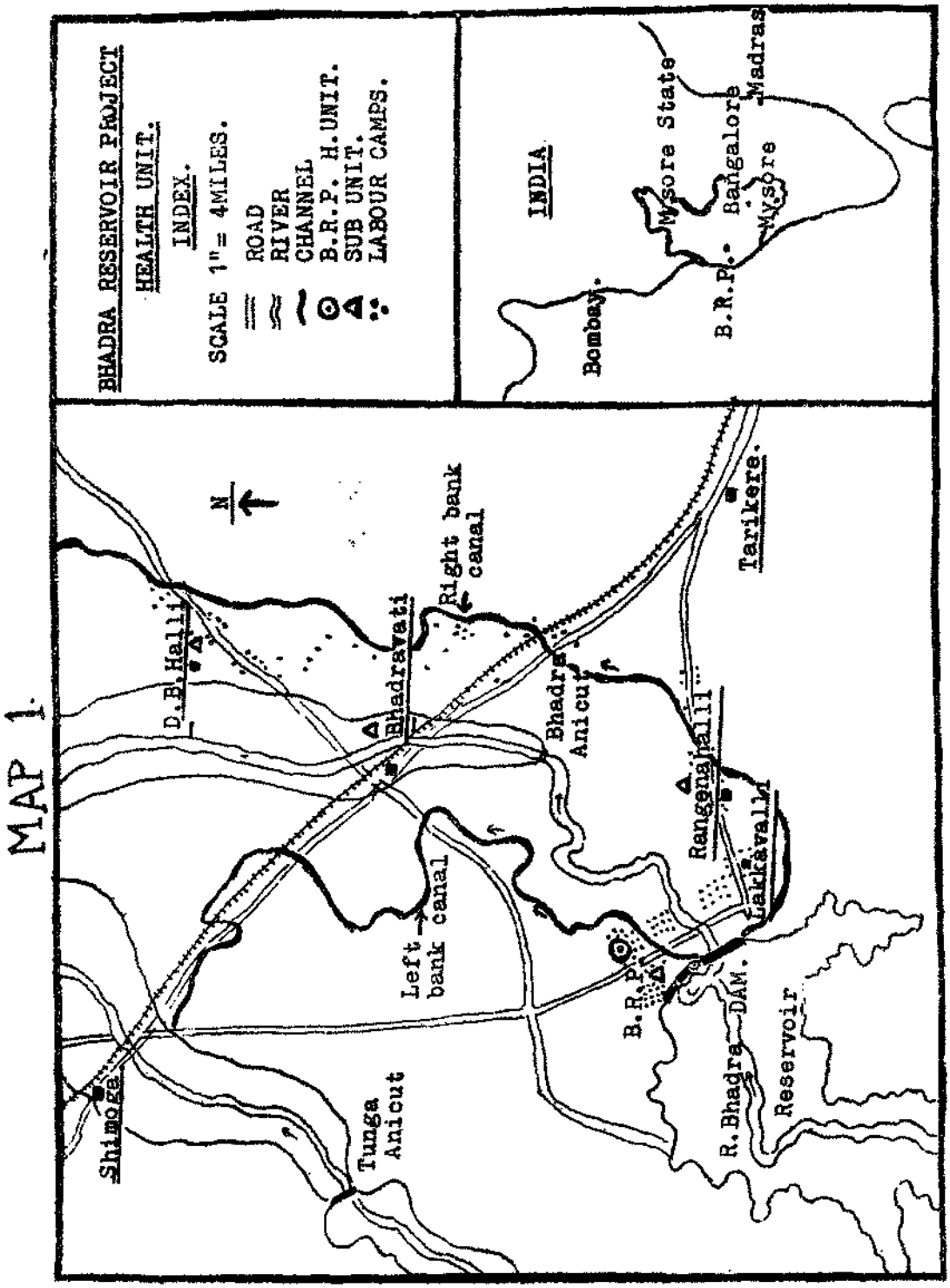
The Bhadra Reservoir Project is a River Valley Project under construction for purposes of irrigation and the generation of electricity. The main dam is in Shimoga District*, Mysore State (Map 1). The Project was commenced in December, 1947. A dam across the River Bhadra, near Lakkavalli, is nearing completion. On either bank of the reservoir, there is an irrigation channel, one 150 miles long on the right bank and the other 50 miles long on the left bank. Both the channels are for irrigation purposes. The main colony of the project is situated in the border of Malanad† area of the State. A special feature of the project, usual in the State, is that the health survey of the area was completed before commencement of its construction. Due to the engineering works involved, a large labour force is employed, a major proportion of it being imported from neighbouring States. A unique feature in the project area for malaria prevention has been the development of a full-fledged Health Unit‡ which was entrusted with, among other functions, house-to-house visits at least once a month to register the details of all the people, follow up and detect malaria cases and treat them for radical cure.

Malaria was hyper-endemic in the area prior to 1949. Residual insecticide spraying of houses in the villages was commenced in 1948 and the malaria incidence was brought down. Spraying of the Project Health Unit houses and labour sheds was commenced in 1954, when large number of labourers came in along with the set up of the Project Health Unit Organisation. The Project Health staff used to spray with 50 per cent water wettable powder, using a dosage of 56 mg. per square foot per round, three rounds in 1954, and one round in 1955. The second round in 1955 was carried out by the National Malaria Control Programme with 75 per cent D.D.T. water wettable powder, using a dosage of 112 mg./sq. ft. per round and was continued with two rounds a year, and since 1958 continued by the National Malaria Eradication Unit (Shama Sastry and Narayana Iyengar, 1959). In addition, antilarval measures have been carried out in the headquarters area of the Project, chiefly to reduce mosquito nuisance. The measures resulted in an abrupt

*Foot-hill

†The Project covers areas in Chikmagalur, Shimoga and Chitradurga Districts.

‡The organisation of the Health Unit and its functions are shown in Appendices I and II, respectively.


MAP 1.
BHADRA RESERVOIR PROJECT
HEALTH UNIT.
INDEX.
SCALE 1" = 4MILES.
ROAD
RIVER
CHANNEL
B.R.P. H.UNIT.
SUB UNIT.
LABOUR CAMPS.
INDIA
Bombay
B.R.P.
Mysore State
Bangalore
Mysore
Madras
N
Shimoga
Tunga Anicut
D.B.Halli
Bhadravati
Left bank canal
Right bank canal
Bhadra Anicut
B.R.P.
R.Bhadra
DAM.
Reservoir
Rangenahalli
Lakkavalli
Tarikere.

reduction of transmission to begin with, and later conceivably a total interruption of the same, as can be seen from Tables I and II.

TABLE I.

Bhadra Reservoir Project Health Unit.

Spleen rate and average enlarged spleen, 1955 to 1959.

		Years:				
		1955	1956	1957	1958	1959
April	Population ...	Spleen survey not carried out.	48,479	30,421	22,127	18,319
	Children examined ...		3,948	3,669	1,227	1,300
	Positives ...		31	18	23	8
	Spleen rate (per cent.)		0·7	0·4	1·8	0·6
	Average enlarged spleen ...		1·4	1·6	1·6	1·6
	Average spleen ...		0·009	0·006	0·02	0·009
October	Population ...	40,490	35,206	20,152	21,348	15·904
	Children examined ...	4,041	3,630	1,210	1,573	1·146
	Positives ...	58	28	44	29	1
	Spleen rate (per cent.)	1·4	0·7	3·6	1·2	0·08
	Average enlarged spleen ...	1·5	1·4	1·5	1·7	1
	Average spleen ...	0·02	0·009	0·05	0·02	0·008

TABLE II.

Percentage of malaria cases treated in the Bhadra Primary Health Unit, catering only villages with indigenous population.

(Situated in the middlle of the Project area and coming under Secondary Health Centre, Shimoga).

	Years:										
	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959
Number of malaria cases treated.	264	383	624	215	71	247	66	93	131	43	5
Total number of cases treated.	5,936	9,219	6,797	9,015	7,612	12,150	11,341	11,335	14,129	9,435	7,695
Percentage of malaria cases.	7·2	5·9	4·6	3·2	1·1	2·0	0·58	0·9	0·92	0·5	0·06

The Bhadra Reservoir Project Health Unit started functioning in the area in 1955. It has been responsible for the maintenance of health and prevention of diseases for all the population of the Project including the itinerant labour population. It is responsible for the detection and spraying of all temporary shelters constructed from time to time by the shifting labour population. All

adequate precautions were taken to detect and spray all structures as soon as they came up anywhere in the area, with least practicable delay. In addition, the Health Inspector in each Sub-Unit has to visit every house/shelter once a month specifically to detect and obtain a blood smear from every fever case, and administer a single dose of 4-aminoquinoline (0·6 gram camaquin per adult). To those of the fever cases, which clinically appeared to be due to malaria, were also administered, along with 4-aminoquinoline drug, a single dose of 8-aminoquinoline (Primaquine diphosphate 15 mg. of the base, which was continued on the second, third, fourth and fifth day (Jaswant Singh *et al.*, 1954). Other fever cases, whose blood smears were found to be positive later, were also treated with the five-day course of primaquine. If the interval for the diagnosis exceeded two months, the person was administered a second dose of 4-aminoquinoline along with primaquine on the first day of treatment. Similar treatment schedule was followed for persons with fever, attending dispensaries and weekly clinics.

The procedure of treating clinically diagnosed cases of malaria with 8-aminoquinoline, before the receipt of results, was adopted in the earlier stages of the programme, due to delays as long as a month in establishing the microscopic diagnosis of the fever cases. Besides, it was observed that the patients' interest in the treatment would be highest during the fever episode than during apyrexial intervals. Later, when rapid diagnostic facilities were established, only the persons whose smears were positive were treated with primaquine. This treatment mostly was carried out personally by the Assistant Medical Officer of Health of the Sub-Unit. The analysis of the persons so treated and the subsequent course of infection in them are presented and discussed later and forms the purpose of the paper.

As referred to earlier, the special problem in the area where autochthonous malaria transmission has been interrupted* and the insecticide spraying is continued, is the malaria that is imported by the migrant labour. Registration of all the people in the area is made along with other relevant information. Every individual, including those that are treated with primaquine, is followed up almost every month and the relevant observations are recorded from August, 1957. This registration, with monthly surveillance entries in the register, helps to follow up the individual as long as he stays in this area. The details of the registration form adopted are shown in Appendix III.

Table III depicts the yearly total population in the area and the number and percentage of malaria cases treated from 1953 to 1959 inclusive.

Table III (A) depicts district-wise origin of labour population, in the Bhadra Reservoir Project Health Unit. Population composition is shown in Tables III (B) and III (C).

*It has been indicated by the author that autochthonous malaria transmission has presumably been interrupted in the area. There is no evidence to prove this in the absence of infant parasite indices which were not determined. Presumably the author has based his statement about the absence of local transmission on the evidence that the malaria cases were restricted to immigrant labour force and were not noted amongst the indigenous population. —EDITOR.

TABLE III.

Bhadra Reservior Project Health Unit.

Malaria Prevalence.

	Years.						
	1953	1954	1955	1956	1957	1958	1959
Population ...	26,320	26,320	51,857	36,282	25,097	21,859	16,178
Total malaria cases treated ...	726	1,277	1,260	2,414	6,009	1,810	400
Malaria prevalance ...	2·7	4·8	2·4	6·6	23·9	8·3	2·4

TABLE III (A).

Bhadra Reservoir Project Health Unit.

Population according to Districtwise origin of labour.

District of origin.		Population (August, 1957).
South Arcot	...	1,268
North Arcot	...	1,963
Coimbatore	...	2,689
Salem	...	993
Cuddappa	...	1,514
Chittore	...	137
Nellore	...	211
Ananthapura	...	214
Madras	...	455
Karnol	...	333
Malabar	...	547
Hyderabad	...	67
Madurai	...	374
Trichi	...	70
Chengalpet	...	20
Mysore State (9 Districts)	...	4,742
Other places	...	371
Unknown	...	856
Total	...	16,824

TABLE III (B).

Age distribution of population in Bhadra Reservoir Project Health Unit, compared with that of India.

	Age-group :				
	0–4 years.	5–14 years.	15–34 years.	35–54 years.	55 and above.
Bhadra Reservoir Project Health Unit. August, 1957	12·8 per cent	22·0 per cent	39·7 per cent	21·9 per cent	3·6 per cent
India : 1951 Census	13·3 per cent	24·2 per cent	34·3 per cent	19·7 per cent	8·5 per cent

(Age composition is more or less the same as that of India as a whole except slightly less in the younger and older group and more in the working adult age-group 15-34 years. But the difference is not much.)

TABLE III (C).

Sex distribution in different age-groups in Bhadra Reservoir Project Health Unit. August, 1957.

	AGE-GROUPS :				
	0–4 years.	5–14 years.	15–34 years.	35–54 years.	55 and above.
Males ...	6·3 per cent	10·9 per cent	20·0 per cent	14·3 per cent	2·0 per cent
Females ...	6·4 per cent	11·1 per cent	19·8 per cent	7·5 per cent	1·5 per cent

(Females are equal to males except in the working adult and the older age-groups.)

The records of malaria cases that had complete course of five-day-treatment has been analysed, from November, 1957 to July, 1959. 2,314 cases have been thus completely treated and observed.

Table IV shows the age and sex distribution of these cases :—

TABLE IV.

Cases treated with primaquine from November, 1957 to July, 1959.

	AGE AND SEX DISTRIBUTION :						
	Age-group in years.						
	0–4	5–14	15–34	35–54	55 and above.	Total.	Grand Total.
Male ...	94	280	480	350	33	1,237	2,314
Female ...	85	226	557	183	26	1,077	

As is seen from Table IV, among these cases, all the age-groups and both the sexes are represented. None of the cases treated with Primaquine showed any toxic symptoms as observed during the treatment and afterwards during the monthly follow-up. Most of the people treated with Primaquine in this area are hard-working labourers with brown to dark complexion and generally with low standards of nutrition.

Out of these 2,314 cases treated with Primaquine, blood smears having been taken before the treatment, 678 cases were found parasite positive. Different species of parasites prevalent, found among the positives, are shown in Table V.

TABLE V.

Species of parasites prevalent among the positives.

NUMBER OF CASES :				
P. vivax.	*P. falciparum.*	*P. malariae.*	Mixed.	Total.
563	110	1	4	678

Age and sex distribution of the positive cases are shown in Table V (*A*).

TABLE V (A).

Age and sex distribution of parasite positive cases found among the cases treated.

Sex.	AGE-GROUPS IN YEARS :						
	0–4.	5–14.	15–34.	35–54.	55 and above	otal.	Grand Total.
Male	22	98	148	89	8	365	678
Female	31	79	141	50	10	313	

Table VI shows the number of parasite positive cases treated with Primaquine and parasite-positive-relapses among them as observed in the monthly follow-up in the area during a period of one year and seven months.

TABLE VI.

Positive cases treated with primaquine and parasite-positive-relapses among them observed after treatment.

Number of positive cases treated with primaquine.	Number of parasite-positive-relapses.
678	39

Different species found in the relapse cases are shown in Table VII.

TABLE VII.

Species distribution of positive cases and percentage relapse among them.

	P. vivax.	*P. falciparum*	*P. malariae*	Mixed.	Total.
Cases treated with primaquine	563	110	1	4	678
Relapse among them	34	5	..	..	39
Per cent relapse	6·03	4·54	..	..	5·75

Note :- Recrudescence in cases of *P. falciparum* has not been differentiated from relapse for the purpose.

Table VIII shows the months in which relapses occurred after the treatment with Primaquine.

TABLE VIII.

Positive relapses found in different months after the treatment with primaquine.

Relapse found.	*P. vivax.*	*P. falciparum.*	Total.
Within 2 months.	7	2	9
In 3rd and 4th months.	10	1	11
In 5th and 6th months.	7	1	8
In 7th and 8th months.	4	..	4
In 9th and 10th months.	1	1	2
In 11th and 12th months.	3	..	3
In 13th and 14th months.	1	..	1
In 15th and 16th months.	1	..	1

Relapse cases are analysed according to age and sex in Table IX.

TABLE IX.

Relapse cases according to age and sex.

Sex	Age-groups in years: 0–4	5–14	15–34	35–54	55 and above.	Total.	Grand Total.
Male	3	9	8	8	..	28	39
Female	..	5	4	1	1	11	

In all, there were 39 cases of relapses out of the 678 positive cases followed up in this area, giving an overall percentage of 5·75. This analysis covers all the age-groups and both the sexes. Majority of the cases followed up were *P. vivax* and next in order were the *P. falciparum* cases. Only one case of *P. malariae* and four of mixed infection were encountered.

Prior to November, 1957, only 4-aminoquinoline was used in this area. Record of follow-up of a few cases was maintained during that period*. The analysis of cases treated only with 4-aminoquinolines between April, 1956 and February, 1957, is discussed below :—

560 clinical malaria cases were treated with only 4-aminoquinoline after taking the blood smear in Rangenahalli Sub-Unit of this Project Health Unit. Out of these, 47 cases were parasite positive. Among these, there were 19 parasite positive relapses in the observation period, giving a relapse rate of 40·4 per cent.

Thus, treatment of parasite-positive malaria cases with 4-aminoquinoline has given a 40 per cent relapse rate; whereas in combination with 8-aminoquinoline, i.e., primaquine diphosphate given for five days, the percentage is only 5·75.

SUMMARY.

Absence of toxicity and feasibility of treatment with Primaquine, with the object of radical cure, has been discussed in this paper. A group of 47 cases treated in the course of a period of 10 months with a single dose of 4-aminoquinoline had a relapse rate of 40 per cent. In another group of 678 parasite-positive cases treated in the course of one year and 7 months with a single dose of 4-aminoquinoline drug, followed by primaquine diphosphate 15 mg. per adult per day for 5 days, the relapse rate was found to be only 5·75 per cent.

*Dr. K.S. Sreenivasa Rao, B.Sc., M.B.B.S., D.P.H., was the Medical Officer of Health of this Health Unit from March, 1956 to April, 1957.

Under his guidance, Dr. K. Rajagopal, Asstt. Medical Officer of Health, and Sri N. Vasudeva Rao, Health Inspector, had maintained the records of follow-up of these cases treated with only 4-aminoquinolines.

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This paper is being published with the permission of the Director of Public Health, Mysore State, to whom the author expresses his thanks.

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APPENDIX I.

ORGANIZATION OF THE BHADRA RESERVOIR PROJECT HEALTH UNIT.

Executive Engineer,
No. 1, B.R.C. Division, Bhadra Reservoir Project.
ADMINISTRATIVE HEAD.

District Health Officer
Chikmagalur,
TECHNICAL HEAD.

MEDICAL OFFICER OF HEALTH,
Bhadra Reservoir Project.

MINISTERIAL AND EXECUTIVE.

1st Division Clerk	... 1
Typist	... 1
2nd Division Clerk	... 1
Peons	... 3 (1 Vacant)
Senior Health Inspector	... 1
Gangmen	... 12
Casual Labourers	... 6

COMBINED HOSPITAL.

Male Section		Female Section	
Asst. Surgeon	... 1	Lady Asst. Surgeon	... 1
Nurses	... 4		
Compounders	... 2		
Ward Attendants	... 2		
Surgery Attendant	... 1		
Midwife	... 1		
Waterman	... 1		
Peon	... 1		
Washerman	... 1		
Cook	... 1		

CRECHES 3

Lady Attendants	6

ISOLATION WARDS.

Sweepers	... 2

SUB UNITS.

(1) Bhadra Reservoir Project.

Asstt. Med. Officer of Health	1
Jr. Health Inspectors	... 2
Midwife	... 1
Lady Attendants	... 2
Peon	... 1
Maistries	... 2
Sweepers	... 30
Scavengers	... 11
Rubbish Cartmen	... 10

(2) Rangenahalli.

Asstt. Med. Officer of Health	1
Jr. Health Inspector	... 1
Peon	... 1
Maistry	... 1
Sweepers	... 5
Scavengers	... 3

(3) Bhadravati.

Asstt. Med. Officer of Health	1
Jr. Health Inspector	... 1
Midwives	... 2
Maistries	... 2
Lady Attendants	... 2
Peon	... 1
Sweep	...15
Scavengers	... 6

(4) D.B. Halli.

Asstt. Med. Officer of Health	1
Jr. Health Inspector	... 1
Midwives	... 2
Lady Attendants	... 2
Peon	... 1

APPENDIX II.

ACTIVITIES OF THE BHADRA RESERVOIR PROJECT HEALTH UNIT.

I. ENVIRONMENTAL SANITATION.

(*a*) Systematic cleaning of the area and disposal of the refusal.

(*b*) Removal of dilapidated structures.

(*c*) Inspection of food establishments.

(*d*) Technical guidance regarding supply of protected water ; chlorination of wells and drinking water sources.

II. CONTROL OF COMMUNICABLE DISEASES.

(*a*) Detection.

(*b*) Isolation and treatment of cases.

(*c*) Vaccinations and inoculations.

(*d*) Other preventive measures.

III. MATERNITY AND CHILD WELFARE.

(*a*) Registration of ante-natals, deliveries and infants, follow-up and domiciliary care by Midwives.

(*b*) Ante-natal and children clinics at the dispensary by the Lady Assistant Surgeon.

(*c*) Domiciliary and dispensary labour services and Maternity Ward.

(*d*) Milk supply to the infants and children.

IV. SCHOOL HEALTH.

Inspection of school childern is carried out by the Assistant Surgeon, during the spleen survey.

V. ANTI-MALARIA WORKS.

(*a*) Anti-larval work in the headquarters sub unit.

(*b*) Adulticidal, D.D.T. spraying work twice a year by the National Malaria Eradication Programme Unit and routine spraying of new and un-sprayed and renovated structures by the Health Unit staff.

(*c*) *Surveillance works* :—Recording individual details of population and detection of cases of malaria and other fever cases and treating with drugs like Resochin, Camoquin or Avlochlor, etc., by paying house-to-house and shed to shed visit and enquiring every individual, at least once a month. Positive cases found by examination of smears and spleen examination are treated with Primaquine diphosphate for five days.

(*d*) *Blood smears collection* :—Blood smears from (1) Infants every month ; (2) Children during spleen survey ; and (3) From all the fever cases.

(2) *Spleen survey* :—It is carried out in April and October each year.

VI. LABORATORY.

A small laboratory for pathological examination and blood smears and urine examination is being maintained.

(*Contd.*)

Appendix II (Concld.)

VII. **CURATIVE MEASURES.**

In the inpatient wards, dispensary and clinics at various places are arranged and ambulance facilities given for patients.

VIII. **CRECHES.**

These are being maintained at the head-work spot for the care of children.

IX. **ISOLATION HOSPITAL.**

It is maintained for infectious diseases.

X. **SURVEYS.**

General and special surveys are conducted for purpose of scientific investigation and for planning the health activities.

XI. **COLLECTION of Vital Statistics.**

XII. **HEALTH EDUCATION** of people is attended to by the staff during the work.

XIII. **TECHNICAL GUIDANCE** is given regarding site selection for the villages and regarding anti-malaria and health points in the planning, and execution of the Project whenever required.

Appendix III.

Headings of the Register containing details of Registration of all the people in the area with monthly follow-up for Surveillance work etc. (Along with the details of sheds).

Name of the Camp..... Sub unit..... Dates of visit.....

Dwelling Number.	Details of DDT spraying.			Serial Number for inmates.	Name of the inmates.	Sex.			Age.		Native place, Taluk and District.	Date of leaving the native place.	Date of arrival at the camp.	Movements in between with places visited and duration of stay.	Previous history of malaria.		January.	February.	and so on.
	Date of spraying.	By whom sprayed.	Details if any.			Male.	Female.	Infant.	Child.	Adult.					Smear number and date of taking the smear.	Result, and treatment if any.			

P.S. In each month of follow-up, the following information should be furnished for each individual.

1. History of malaria or fever in that month.
2. Smear Number and date, clinical malaria (*M*), or pyrexia (*P*), and the treatment given are noted in an abbreviated form, *e.g.*, If with a history of fever and treatment is given, it is denoted as : M. 23/1-6-58. $\frac{\text{R.4/P.10}}{\text{(1-6-58)(1,2,3,4,5 June, 1958).}}$ Alternate days, from 1 week. First attack (or relapse).

   Abbreviations :—M=Malaria, P=Pyrexia, 23=Smear Number and date below. R4 and P.10=Resochin 4 tablets and Primaquine 10 tablets, and date of treatment.
3. When a house visited is found locked, it should be stated "L", meaning the shed was locked at the time of visit.
4. Date of DDT spraying to be noted whenever the house is got sprayed, with the reasons for spraying, *i.e.*, whether structure is new or the walls and the roof are renovated or it was left over in the previous rounds and now sprayed; or refused this time also (*R*). If sprayed, date, by N.M.E.P. or Health Unit staff and reason for spraying *i.e.*, routine spraying due to renovation or being new.
5. If the individual is absent during the visit, mark (*A*) and state that he is "In station". If he has left for some place, "when and to where", is mentioned. If he returns, the date and the places visited by him are noted.
6. If he had fever and not available for treatment or refused treatment it is also noted so as to contact him and to persuade him during the next visit.
7. If there is no history of fever, a dash mark is marked opposite each individual every month.
8. If they leave the place once for all, it is mentioned with a note as to for which place they have left, if the information is available.
9. Any other particulars regarding history of specific disease or A.C. inoculation or vaccinations given, could also be noted in the registers.

   This register, in addition to malaria case finding, helps to record the follow-up of any health activities and observations.

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

XXVIII. THE DURATION OF IMMUNITY DUE TO A SINGLE UNTREATED *P. BERGHEI* INFECTION IN ALBINO RATS†.

BY

SATYA PRAKASH

(Malaria Institute of India, Delhi.)

[April 4, 1960.]

THE normal course of patent primary parasitaemia (Table I) in albino rats lasts for 10 to 20 days (Ramakrishnan and Satya Prakash, 1960). The host either dies due to high parasitaemia or the infection becomes latent in some cases resulting in spontaneous cure later.

It was observed in previous studies by the author that in albino rats no demonstrable immunity was conferred during the first five days of primary patent parasitaemia. When the antigenic stimulus was allowed to operate beyond this period, and the infection was cured after the fifth day of patency, immunity was detectable. It was further observed that it was necessary for the primary parasitaemia to remain patent for a minimum period of 11 days to confer the requisite immunity to totally resist the homologous challenge infection (Satya Prakash, 1960). The object of the present study was to determine the duration of the immunity conferred by an uninterrupted course of a patent primary parasitaemia followed by different periods of latency.

METHODS AND MATERIALS.

The techniques employed in these experiments were the same as followed in the previous studies (Satya Prakash, 1960 *loc. cit.*). For radical cure of *P. berghei* infection, a single dose of sulphadiazine 1·2 mg. per 20 mg. body-weight of animals was found to be sufficient (Ramakrishnan *et al.*, 1951). In order to be certain that sufficient drug was exhibited for definite sterilisation of infection, each animal was administered the above mentioned dose for three consecutive days, i.e., three times the required curative dose. Each animal so treated was given a rest for at least 7 days before being subjected to any further experimental procedures.

Sergent's (1954) technique of total sub-inoculations was employed, in order to demonstrate that the chemotherapeutic measures resulted in complete sterilization of *P. berghei* infection in the host.

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

† This paper forms part of a thesis for the Doctorate degree of the Panjab University.

TABLE I.

Protocols of albino rats showing daily parasitaemia from "O" day of inoculation in normal strain of P. berghei.

Albino rat Number	Daily parasitaemia, Parasites per 10,000 R.B.Cs.																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
R—2133	2	15	135	255	450	710	920	760	650	325	P	4	N	N	N	N	N	N	N	N	N
R—2168	1	8	225	410	490	800	1,040	1,240	1,460	1,080	860	600	450	600	720	240	482	125	P	N	N
R—2200	2	32	300	600	1,120	1,300	1,650	1,860	1,800	2,000	D		..	..	..	..	..	..	..	..	..
R—2210	10	128	350	445	560	880	950	1,070	1,300	1,800	2,000	2,000	2,000	D	..	..	..	..	..	..	..
R—2211	8	98	260	520	540	340	770	890	1,100	1,360	2,000	1,920	2,000	D	..	..	..	..	..	..	..
R—2215	3	8	165	200	240	480	540	810	1,080	350	225	120	15	10	6	5	2	N	N	N	N
R—2216	2	20	290	325	347	370	810	850	960	450	100	65	30	15	10	P	N	N	N	N	N
R—2217	4	12	160	190	235	280	580	920	2,000	890	1,200	712	225	608	1,216	1,600	280	105	N	N	N
R—2225	4	32	100	332	341	350	580	990	1,400	1,000	416	224	32	12	N	N	N	N	N	N	N
R—2228	P	24	260	400	400	790	1,230	997	665	600	500	400	437	200	120	40	22	N	N	N	N
R—2245	P	60	120	460	840	1,020	1,416	1,216	2,400	2,000	1,940	2,000	D	..	..	..	..	..	..	..	..
R—2248	P	75	300	200	302	405	950	1,450	2,000	2,000	1,600	1,680	1,050	685	735	785	225	25	N	N	N
R—2249	20	160	426	300	608	820	1,800	2,000	2,000	2,000	2,740	D	..	..	..	..	..	..	..	..	..
R—2250	12	80	376	450	550	855	1,520	1,800	2,000	2,000	1,950	2,000	2,000	D	..	..	..	..	..	..	..
R—2255	P	12	80	310	750	1,400	890	650	285	332	330	850	1,000	50	N	N	N	N	N	N	N
R—2264	4	48	..	670	720	1,200	1,250	1,800	2,000	2,000	1,650	2,000	D	..	..	..	..	..	..	..	..
R—2267	28	220	350	380	605	620	760	1,800	2,000	2,000	2,000	2,000	..	2,000	2,000	2,000	D	..	..	..	..
R—2272	P	110	370	605	720	980	1,240	1,860	2,000	1,325	1,162	2,000	2,000	2,000	1,310	620	P	N	N	N	N
R—2273	P	135	525	535	430	1,055	1,680	1,480	1,280	260	182	105	20	15	12	10	N	N	N	N	N
R—2286	4	327	650	625	600	1,080	1,510	1,210	800	340	405	470	630	409	270	60	2	1	1	N	N
R—2289	4	227	450	410	370	1,260	1,320	1,600	1,650	1,250	990	730	630	550	420	65	2	N	N	N	N
R—2305	P	22	88	230	320	350	1,240	650	1,320	1,400	2,000	2,000	2,000	2,000	2,000	2,000	D	..	..	..	N
R—2307	3	10	40	70	410	510	610	1,680	1,800	2,000	2,000	2,000	2,000	2,000	2,000	1,180	110	20	P	N	N
R—2308	2	26	110	198	420	960	1,520	1,760	2,000	2,000	2,000	2,000	2,000	2,000	1,840	2,000	720	39	N	N	N
R—2309	2	32	200	267	480	1,040	1,600	1,400	1,850	2,000	2,000	2,000	2,000	2,000	1,400	1,360	200	25	P	N	N

D = Died. N = Negative. P = One parasite in 100 oil immersion fields.

RESULTS.

Group I.

Forty-six albino rats were used in the first group for the observations. Groups of these animals were allowed to experience the latent infection for one, two, three, four, ten, eleven, and fifteen days respectively, before sulphadiazine was administered. Out of the forty-six rats, twenty-three were subjected to total sub-inoculation test and proved to be radically cured. The other twenty-three were given homologous re-infections with negative results. The results of sub-inoculations and super infections are shown in Table II.

Table II.

Protocols of albino rats cured on the first, second, third, fourth, tenth, eleventh and fifteenth days of latency, showing the results of challenge infection and total sub inoculations.

Number of animals used.	Sex:		Day of latency on which treatment was commenced.	Number of animals used:		Result. Number of animals positive or negative:	
	Male	Female		(a) For challenge inoculation	(b) For total sub-inoculation.	(a) Challenge inoculation.	(b) Sub-inoculation total.
10	2	8	1st	5	5	0/5	0/5
10	8	2	2nd	5	5	0/5	0/5
10	9	1	3rd	5	5	0/5	0/5
10	3	7	4th	5	5	0/5	0/5
2	1	1	10th	1	1	0/1	0/1
2	1	1	11th	1	1	0/1	0/1
2	1	1	15th	1	1	0/1	0/1
Total 46	25	21		23	23	0/23	0/23

Group II.

Table III depicts the protocols of the second group which consisted of 51 albino rats. A persistent latent infection was presumed in every case. The infection was uninterfered for periods ranging from 52 to 369 days before curing it radically.

The treatment schedule adopted was successful and never failed to effect a radical cure. Twenty-seven animals were utilized for total sub-inoculation test and all of them proved negative. The remaining twenty-three albino rats were able to totally resist the challenge homologous infection.

Group III.

Four albino rats were allowed to remain latent for 165, 188, 344 and 383 days. They were not tested for the presence of persisting infection. Without any curative treatment, these animals were given a homologous super-infection at the end of these periods (Table IV). None of the animals developed patent parasitaemia for 30 days following the challenge inoculation. This indicated a persistence of immunity and probably a persistence of the injection infection.

TABLE III.

Protocols of albino rats cured after 52 to 369 days of latency, showing the results of homologous challenge infection and total sub-inoculation.

Albino rat Number.	Number of days for which latent.	Cured or not cured.	Number of days' rest after cure.	Result of challenge dose or sub-inoculation.	
				Super positive or/negative.	Total sub-inoculation, positive or negative.
R—1686	368	Cured	7	..	Neg.
R—1699	352	Cured	7	..	Neg.
R—1698	369	Cured	7	Neg.	..
R—2088	176	Cured	7	Neg.	..
R—2089	165	Cured	7	Neg.	..
R—2090	175	Cured	7	Neg.	..
P—2081	179	Cured	7	..	Neg.
R—2082	180	Cured	7	..	Neg.
R—2103	168	Cured	7	..	Neg.
R—2116	141	Cured	7	Neg.	..
R—2125	131	Cured	7	Neg.	..
R—2124	136	Cured	7	Neg.	..
R—2127	130	Cured	7	..	Neg.
R—2128	126	Cured	7	..	Neg.
R—2162	114	Cured	7	..	Neg.
R—2166	112	Cured	7	..	Neg.
R—2216	115	Cured	7	Neg.	..
R—2219	115	Cured	7	Neg.	..
R—2222	115	Cured	7	Neg.	..
R—2215	114	Cured	7	Neg.	..
R—2217	112	Cured	7	Neg	..
R—2221	112	Cured	7	..	Neg.
R—2225	110	Cured	7	..	Neg.
R—2228	110	Cured	7	..	Neg.
R—2239	78	Cured	7	..	Neg.
R—2241	78	Cured	7	..	Neg.
R—2242	78	Cured	7	..	Neg.
R—2273	141	Cured	7	..	Neg.
R—2272	135	Cured	7	Neg.	..
R—2290	136	Cured	7	Neg.	..
R—2256	127	Cured	7	Neg.	..
R—2239	127	Cured	7	Neg.	..
R—2248	160	Cured	7	..	Neg.
R—2255	159	Cured	7	..	Neg.
R—2254	156	Cured	7	..	Neg.
R—2259	54	Cured	7	..	Neg.
R—2261	54	Cured	7	..	Neg.
R—2270	52	Cured	7	Neg.	..
R—2093	275	Cured	←Died after three days' treatment.→		
R—2091	273	Cured	14	..	Neg.
R—2090	271	Cured	14	Neg.	..
R—2100	271	Cured	14	..	Neg.
R—2101	269	Cured	14	Neg.	..
R—2242	161	Cured	14	..	Neg.
R—2303	152	Cured	14	Neg.	..
R—2297	137	Cured	18	Neg.	..
R—2308	166	Cured	18	Neg.	..
R—2292	144	Cured	18	Neg.	..
R—2296	137	Cured	18	..	Neg.
R—2307	166	Cured	18	..	Neg.
R—2294	144	Cured	18	..	Neg.

Neg. = negative.

TABLE IV.

Protocols of uncured albino rats latent from 165 to 383 days, showing results of challenge homologous infection and total sub-inoculation.

Albino rat Numer.	Number of days for which latent.	Cured or not cured.	Result of challenge dose of total sub-inoculation :	
			Super infection, positive or negative.	Total sub-inoculation, positive on negative.
R—1681	383	Not cured	Neg.	..
R—1702	344	Not cured	Neg.	..
R—2051	188	Not cured	Neg.	..
R—2092	165	Not cured	Neg.	..

GROUP IV.

Fourteen albino rats, with latent infections ranging from 93 to 267 days, were divided into three lots as shown in Table V. Lot *A* consisted of five animals with latent infection ranging from 93 to 267 days. These were totally sub-inoculated without any prior curative treatment and proved that even without treatment they had been spontaneously cured. In lot *B*, five uncured albino rats, with latent infection ranging from 106 to 262 days, were given a homologous super-infection. It was observed that none of them showed parasitaemia during thirty days of the follow-up observation. In lot *C*, four albino rats, with latent infection ranging from 100 to 115 days, were cured and given homologous super-infection after fifteen days' rest. No patent parasitaemia was demonstrable in any animal over an observation period of thirty days.

TABLE V.

Protocols of uncured and cured albino rats latent from 93 to 267 days, showing the results of challenge homologous infection and total sub-inoculations.

Albino rat number.	Sex of animal.	Number of days for which latent.	Cured or not cured.	Number of days of rest after cure.	Result of :		Remarks.
					Super infection.	Total sub-inoculation.	
R—2576	M	267	Not cured	..	..	Negative	Lot *A*
R—2933	M	112	Not cured	..	..	Negative	
R—2991	F	97	Not cured	..	..	Negative	
R—3015	F	99	Not cured	..	..	Negative	
R—3036	F	93	Not cured	..	..	Negative	
R—2502	F	262	Not cured	..	Negative	..	
R—2953	F	106	Not cured	..	Negative	..	Lot *B*
R—2996	F	136	Not cured	..	Negative	..	
R—3035	F	133	Not cured	..	Negative	..	
R—3063	M	109	Not cured	..	Negative	..	
R—2942	F	115	Cured	7	Negative	..	Lot *C*
R—2993	F	100	Cured	7	Negative	..	
R—3034	F	109	Cured	7	Negative	..	
R—3053	F	105	Cured	7	Negative	..	

DISCUSSION.

The life span of albino rats is about three years, which may be considered as equal to ninety years of human life (Donaldson, 1924). In regions where there is much humidity with high temperature in summer and extreme cold in winter, few albino rats live more than two years (Farris and Griffith, 1940). Therefore, the observations over periods up to 383 days in albino rats may be considered sufficient for their average life span.

Fifty albino rats with latent infection, the duration of which ranged from 1 to 369 days, twenty-three from 1 to 15 days and twenty-seven from 52 to 369 days, were proved, beyond doubt, to have been sterilised of *P. berghei* parasites after the radical treatment. An equal number, after cure of their latent infection of varying duration, (twenty-three latent from 1 to 15 days, twenty-three from 52 to 369 days and four from 100 to 115 days), totally resisted a challenge homologous super-infection.

The homologous immunity to human plasmodia, as determined by response to super-infection, has been observed to persist as long as a year and may last seven years. This is with reference to *P. vivax* and *P. falciparum* (Boyd and Mathews, 1939), *P. vivax* (Boyd, Stratman-Thomas and Kitchen, 1939) and *P. ovale* (Sinton, Hutton and Shute, 1939). The observations recorded in the current studies indicate that acquired immunity in *P. berghei* in albino rats definitely lasts beyond a period of one year. It was further observed that nine rats, 93 to 383 days after inoculation which did not receive any curative treatment, when given a homologous super-infection, showed no parasitaemia. The anti-parasitic immunity, developed by a single patent primary parasitic episode followed by an undetermined period of latency, lasted over a year.

The immunity was thus patent even after the lapse of over a year (369 days) following the primary episode of patent infection. In these animals, however, latency was allowed to run for periods up to 369 days, before radical cure was effected. While it is known, as already described (Satya Prakash, 1960 *loc. cit.*) that immunity conferred by a minimum period of 11 days of patent parasitaemia was enough to take care of the challenge dose, the duration for which such immunity lasted was not known. In the present series, the immunity built-up has been the result of a complete natural course of primary parasitaemia followed by varying intervals of latency. The latter extended up to the period of 369 days, and the immunity status of albino rats would, therefore, be that resulting from not only the primary episode but the latent infection that followed. It would, however, appear that main bulk of immunity would be the result of the former. It is likely that the sub-patent infection that followed for varying intervals might have reinforced the antigenic stimulus and, therefore, the maintenance or enhancement of the immunity status.

Immunity was demonstrable in the experimental animals following radical cure effected on the 369th day of latency. It has been observed that the occurrence of spontaneous cure in *P. berghei* is not very usual phenomenon;

Ramakrishnan *et al.* (1951) reported that one out of 75 infected animals underwent a spontaneous cure. It has, however, been presumed that parasites continued to remain sub-patent in all the animals used in these studies till they were cleared by sulphadiazine. The persistence of immunity was probably directly due to the continued antigenic stimulus resulting from the latent infection. This is in conformity with the observations of Ramakrishnan *et al.* (1951) who reported that the one rat which had undergone spontaneous cure, responded to the challenge re-infection just as any normal non-immune rat. On the other hand, as seen from Table V, five animals, in whom it was presumed that infection had been latent for periods ranging from 93 to 267 days, proved to have undergone a spontaneous radical cure. The blood and the homogenised viscera of each of the animals, when inoculated into susceptible rats, gave negative results.

A second group consisted of five animals (Table V), animals similar to the above, in which also the infection was originally presumed to have been latent for periods varying from 103 to 262 days. On the basis of the spontaneous cure mentioned above, however, the presumption seemed doubtful. Nevertheless, each of the five animals totally resisted the challenge infection. It seemed, therefore, that the immunity to the challenge infection could have been a residual immunity persisting even after spontaneous cure. This would be an important finding if confirmed by further studies.

Studies on the life of the malaria parasite in the human host, in the absence of re-infection, have assumed great importance in recent years and have given rise to the concept of malaria eradication. *P. falciparum* infection is reported to last usually up to 18 to 24 months, while *P. vivax* lasts up to 3 years. In the case of *P. berghei*, the parasite appears to persist at least over a period of one year—a duration which is very long in comparison with the life span of the host animal. As already pointed out, one year of rat life is equivalent to about 30 years of human life.

If on the other hand, the duration of infection is shorter than presumed as indicated by the five animals (Table V) which had undergone a spontaneous cure, the persisting immunity in five similar animals after a presumed spontaneous cure is of interest. Further studies should be undertaken to determine the duration of immunity after sterilization of the infection.

CONCLUSIONS.

Studies were carried out to determine the duration of the measurable anti-parasitic immunity to homologous super-infection in albino rats as produced by a primary parasitaemia followed by varying periods of presumed latency.

It was observed that the degree of immunity built up by a single episode of primary patent parasitaemia was adequate to totally resist a homologous reinfection up to 369 days of presumed latency.

P. berghei infection appeared to persist in the albino rat at sub-patent levels over a period of about one year at least. In some instances, it appeared that immunity persisted for varying periods after spontaneous cure. This was an important indication well worth for further study.

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OBSERVATIONS ON CERTAIN MORPHOLOGICAL ABNORMALITIES IN TWENTY SPECIES OF INDIAN *ANOPHELES*

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[May 21, 1960.]

In India, the anopheline fauna is represented by forty species. The natural populations of most of these species are known to show variations in their ornamentation from the type form. These variations or abnormalities are usually in the ornamentation of the maxillary palpi, the tarsi of hind legs and the wings and some of these have been recorded in the monumental work on Indian *Anopheles* by Christophers (1933). Recently Ramakrishna (1954) recorded the presence of an additional dark band in the palps of female *A. fluviatilis*; Bhatnagar *et al.* (1958) observed hypomelanic forms of *A. pallidus* and incomplete development of the sixth wing vein in the colony-bred specimens of *A. stephensi stephensi*; and Rajagopal and Chakrabarty (1960) recorded abnormalities in the palpi, tarsi and wing of *A. annularis*.

It has been observed that the variations in ornamentation, sometimes, lead to erroneous identification; therefore, a study of the mosquito collections at the Malaria Institute of India was undertaken to study (*i*) the pattern of abnormalities in the Indian *Anopheles* and (*ii*) the geographic localization of the abnormalities, if any. These observations, it is hoped, will serve as a reference to the workers who may not have recourse to large collections of different species.

Over 31,000 *Anopheles* specimens, comprising all the Indian species, were examined and 154 of these, belonging to the following twenty species, were found to show variations from the type form :

1. *aconitus* (7)*	7. *karwari* (2)
2. *annularis* (34)	8. *maculatus* (10)
3. *culicifacies* (7)	9. *minimus* (7)
4. *fluviatilis* (10)	10. *moghulensis* (1)
5. *hyrcanus* (9)	11. *pallidus* (26)
6. *jamesi* (6)	12. *philippinensis* (4)

*Figures in brackets indicate the number of species found with morphologcial abnormalities.

13. *pulcherrimus* (11)
14. *ramsayi* (1)
15. *splendidus* (7)
16. *subpictus* (7)
17. *theobaldi* (1)
18. *turkhudi* (1)
19. *vagus* (2)
20. *varuna* (1)

The abnormalities observed were in respect of ornamentation on the different parts of the body except in case of one specimen each of *fluviatilis* and *varuna* where one of the maxillary palps was found to be shorter than the other and one specimen of *subpictus* which was entirely pale (hypomelanic form). Many of the abnormalities recorded here are unusual and have not been reported earlier.

The species, along with the nature of abnormalities observed, their dates of collection, and locality are presented in Table I. The illustrated figures in each case include the normal type form and the abnormal form observed in the present study.

TABLE.

Morphological abnormalities in Indian Anopheles *in the collection of Malaria Institute of India.*

Serial number. (1)	Date of collection. (2)	Locality. (3)	Number of specimens. (4)	Nature of variations from the type form. (5)	
		1. *A. aconitus* Donitz, 1902 (Fig. 1).			
1.	March, 1929	Saidpur, Hyderabad.	1	Wing: (Fig. 1, *a,b*)	Both wings without fringe spot at Vein 6, the distal half of Vein 6 without a pale interruption (the latter also reported by Christophers, 1933).
2.	May 3, 1931 March, 1933	Hiriyur, Mysore. Madhubani, Dharbanga.	4 1	Wing:	Both wings without fringe spot at Vein 6.
3.	May 3, 1931	Rampoti, Hyderabad.	1	Palpi: (Fig. 1,*c,d*)	Distal dark band (pre-apical) absent (also Christophers, 1933).
		2. *A. annularis* Van der Wulp, 1884 (Fig 2).			
1.	Feb. 19, 1960	Badshapur, Gurgaon, (Punjab).	1	Wing: (Fig. 2,*a* to *c*)	Left wing with Vein 5 completely pale at the point of bifurcation, right wing similar except for a few dark scales near the base of 5+2.
2.	January, 1928 Dec. 21, 1959 and Jan. 22, 1960 —	Karnal (Punjab). Jharia (Coalfields). U.P. Terrai.	2 2 1	Palpi: (Fig. 2,*d,e*.)	Apical pale area with a patch of dark scales giving an appearance of an incomplete extra dark band.

PLATE I.

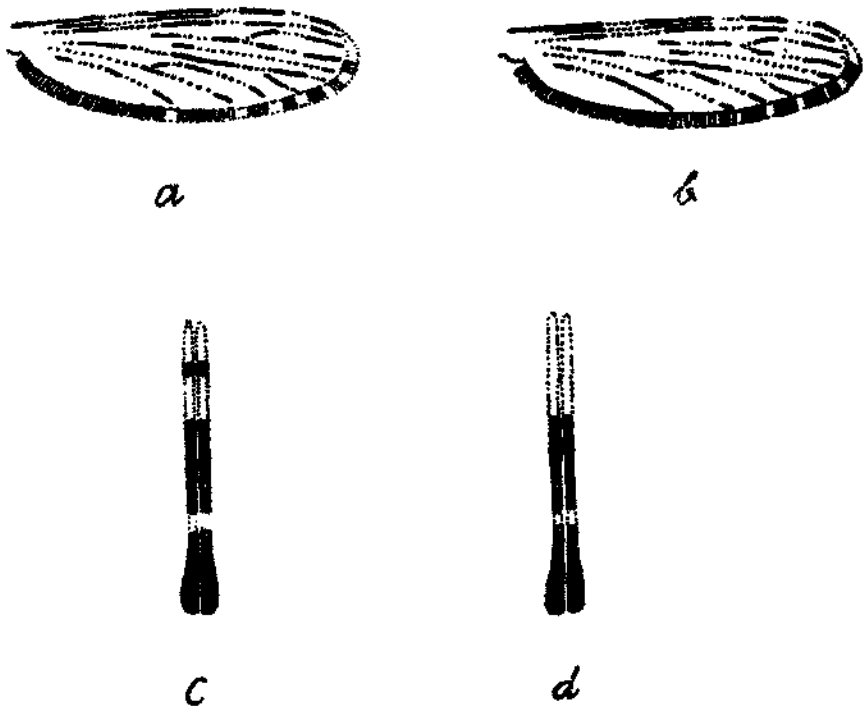

FIG. 1.

FIG. 1. *A. aconitus* (*a*) Normal wing : (*b*) Abnormal wing : (*c*) Normal palpi ; (*d*) Abnormal palpi.

PLATE II.

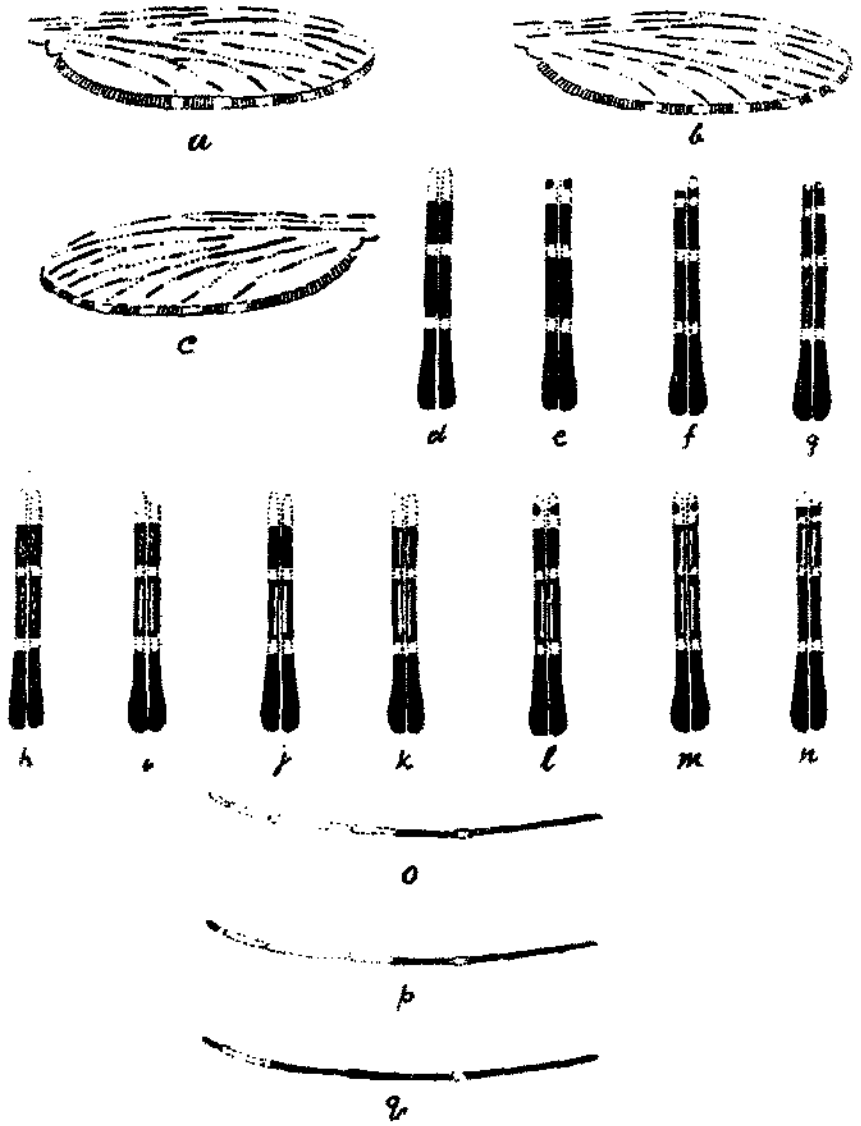

FIG. 2.

FIG. 2. *A. annularis* (a) Normal wing ; (b, c) Abnormal wings ; (d) Normal palpi ; (e—n) Abnormal palpi ; (o) Normal tarsi ; (p, q) Abnormal tarsi.

TABLE (*Contd.*).

Serial number. (1)	Date of collection. (2)	Locality. (3)	Number of specimens. (4)	Nature of variations from the type form. (5)	
3.	March 14, 1927	Karnal.	1	Palpi : (Fig. 2, *f*).	Apical pale area with dark scales forming an extra dark band (also Christophers, 1933).
	January, 1928	Gorakhpur (U.P.)	3		
	January, 1928	Karnal.	5		
	1930	Ambala (Punjab).	1		
	Nov. 26, 1930	Karnal.	1		
	Jan. 21, 1959	Jharia.	1		
4.	Jan. 16, 1959	Moradnagar. (U.P.)	1	Palpi : (Fig. 2, *g*).	Distal 3/4 of the apical pale area covered with dark scales with a few pale scales scattered near the tip. The palpi appear to have three narrow pale bands, three large dark bands, a narrow (distal) dark band and a speckled tip.
5.	Feb. 17, 1959	Jhansi (U.P.)	2	Palpi : (Fig. 2, *h*)	Scattered pale scales arranged in a linear fashion towards the inner margin on the subapical and middle dark bands giving a speckled appearance.
6.	July, 1959	Purnea (Bihar).	1	Palpi : (Fig. 2, *i*)	Scattered linear row of pale scales present on the subapical dark band, and a continuous row of pale scales on the middle dark band, both towards the inner margin.
7.	Feb. 24, 1958	Shimoga (Mysore).	1	Palpi : (Fig. 2, *j*)	A continuous row of pale scales present on the middle dark band towards the inner margin.
	Jan. 22, 1960	Jharia.	2		
	March, 1960	Pilibhit (U.P.)	1		
8.	Oct. 1, 1922	Kashmir.	1	Palpi : (Fig. 2, *k*)	A continuous row of pale scales present on the subapical and middle dark bands.
	Nov. 15, 1957	Arthala (U.P.)	1		
9.	Jan. 22, 1960	Jharia.	2	Palpi : (Fig. 2, *l*)	Apical pale bands with a patch of dark scales towards the outer margin near the centre and the middle dark band with a continuous row of pale scales towards the inner margin.
10.	February, 1959	Ajmer (Rajasthan)	1	Palpi : (Fig. 2, *m*)	As in respect of '9' above but with a row of pale scales both on subapical and middle dark bands.
11.	..	N.W.F.P. (West Pakistan).	1	Palpi : (Fig. 2, *n*)	An extra dark band present on the apical pale area (*see* in respect of '3' above) and the subapical dark band with a continuous row of pale scales towards the inner margin.
12.	December, 1959	Arthala.	1	Tarsi : Fig. 2, *o,p*,)	Apical half of the hind tarsal Segment 5 of both legs dark.

TABLE (*Contd.*).

Serial number. (1)	Date of collection. (2)	Locality. (3)	Number of specimens. (4)	Nature of variations from the type form. (5)	
13.	..	Batala (Punjab)	1	Tarsi : (Fig. 2, *q*)	In both legs, hind tarsal segments 5 and 2 completely dark and Segment 3 dark with a narrow pale area near its joint with 4th.
		3. A. *culicifacies* Giles, 1901 (Fig. 3).			
1.	August, 1921	Belgaum	1	Wing : (Fig. 3, *a,b*)	In both wings, base of the costa without a pale interruption external to humeral cross vein.
	July 30, 1923	Bombay	1		
	..	Andhra Valley	2		
	.	Punjab	2		
	1958	Delhi.	1		
		4. A. *fluviatilis* June, 1902 (Fig. 4).			
1	May 4, 1927	Saharanpur (U.P.)	1	Wing : (Fig. 4, *a,b*)	In both wings a pale interruption present on costa near the base just before the basal dark spot on Vein 1 starts.
	January, 1928	Jeypore Hills	1		
	—	U.P. Terrai	1		
2.	Jan. 12, 1923	Singhbhum	1	Palpi : (Fig. 4, *c,d*)	Sub-apical dark band nearly equal to the apical pale band.
		Silguri.	2		
3.	January, 1913	U.P. Terrai	3	Palpi : (Fig. 4, *e*)	Apical pale band with dark scales forming an extra dark band (also Ramakrishna, 1954)
4.	..	Silguri	1	Palpi : (Fig. 4, *f*)	Two palps unequal, the tip of the left palp reaching about the middle of the sub-apical dark band of the right palp.
		5. A. *hyrcanus* var. *nigerrimus* Giles, 1900 (Fig. 5).			
1.	Nov. 3, 1959	Thana (Bombay)	1	Wing : (Fig. 5, *a,b*)	Both wings without pre-apical and sub-costal pale areas. Costa, sub-costa and Vein 1 evenly dark.
2.	Nov. 3, 1959	Thana (Bombay)	1	Wing :	Right wing normal, left wing, abnormal as in respect of '1' above.
3.	Nov. 3, 1959	Thana (Bombay)	1	Wing :	Left wing normal, right wing abnormal as in respect of '1' above.
4.	Oct. 24, 1957	Nelligere (Mysore)	1	Palpi (Fig. 5, *c,d*)	A continuous row of pale scales present on the proximal dark band towards the inner margin.
	Oct, 15, 1958	Tripura	1		
5.	Oct. 24, 1957	Nelligere	1	Palpi : (Fig. 5, *e*)	A continuous row of pale scales on the proximal dark band as in respect of '4' above and a shorter row of pale scales present towards the base of the next dark band.
6.	Oct. 24, 1957	Nelligere	1	Palpi : (Fig. 5, *f*)	Continuous linear row of pale scales present towards the inner margin on all dark bands except the distal narrow sub-apical band.
	Oct. 15, 1958	Tripura	2		

PLATE III.

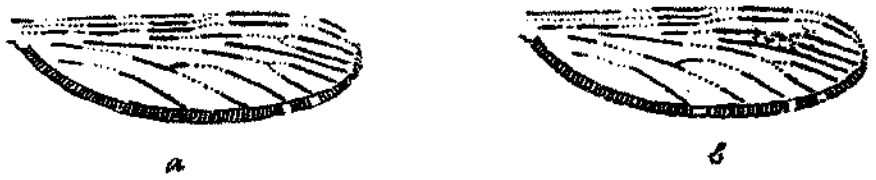

FIG. 3.

FIG. 3. *A. calicifacies* (*a*) Normal wing ; (*b*) Abnormal wing.

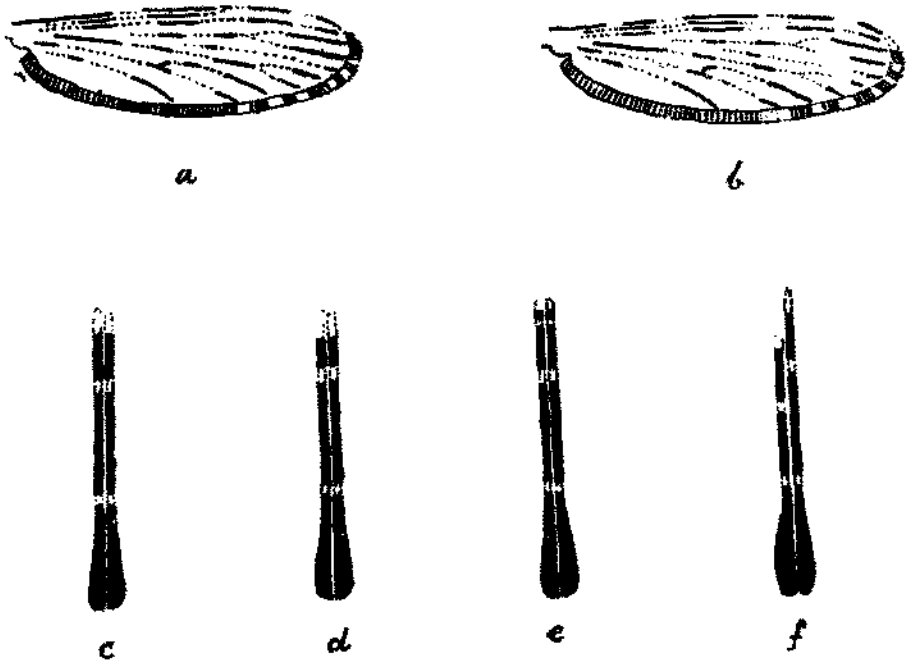

FIG. 4.

FIG. 4. *A. fluviatilis* (*a*) Normal wing ; (*b*) Abnormal wing ;
(*c*) Normal palpi ; (*d—f*) Abnormal palpi.

PLATE IV.

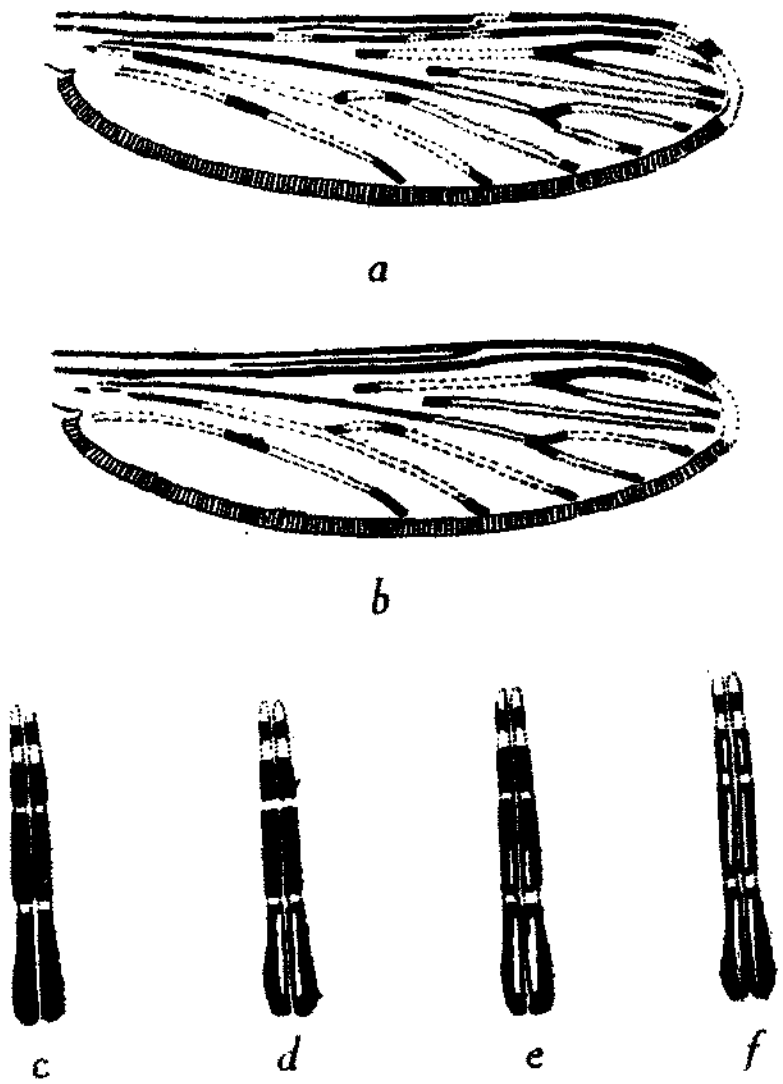

FIG. 5.

FIG. 5. *A. hyrcanus* var *nigerrimus* (*a*) Normal wing ; (*b*) Abnormal wing . (*c*) Normal palpi ; (*d*—*f*) Abnormal palpi.

TABLE (*Contd.*).

Serial number. (1)	Date of collection. (2)	Locality. (3)	Number of specimens. (4)	Nature of variations from the type form. (5)
		6. *A. jamesi* Theobald, 1901 (Fig. 6).		
1.	March, 1930 —	Andhra. Sylhet (East-Pakistan)	1 1	Palpi : (Fig. 6, *a,b*) — Pale apical band with a patch of dark scales towards the left margin in the middle of each palp.
2.	—	Orissa	1	Palpi : (Fig. 6, *c*) — The left palp with an extra dark band in the apical pale area.
3.	— —	Coorg Madras	1 1	Palpi : (Fig. 6, *d*) — Apical pale band with dark scales in the centre forming an extra dark band in both palps.
4.	—	Madras	1	Tarsi : (Fig. 6, *e,f*) — Left hind tarsal segments 2 and 3 completely dark, only 4 and 5 white.
		7. *A. kanwari* James, 1903 (Fig. 7).		
1.	—	Assam	1	Wing : (Fig. 7, *a,b*) — In both wings, basal half of the costa internal to middle dark spot without the usual pale interruptions.
2.	—	Assam	1	Palpi : (Fig. 7, *c,d*) — Palpi without the distal (pre-apical) narrow dark band. The pale apical area nearly 1/3 the length of the palp.
		8. *A. maculatus maculatus* Theobald, 1901 (Fig. 8).		
1.	—	Malaria Institute, Delhi.	1	Wing : (Fig. 8, *a,b*) — Both wings without sub-apical pale spot and sector pale area.
2.	Feb. 12, 1959 Feb. 20, 1959 March 4, 1960	Sikkim Sikkim Simla	1 2 3	Palpi : (Fig. 8, *c,d*) — 2 to 3 patches of white scales on the middle dark band giving it speckled appearance.
3.	March 20, 1960	Sikkim	1	Palpi : (Fig. 8, *e*) — Continuous row of white scales towards the inner margin of the middle dark band. The one on right palp slightly shorter than the one on the left palp.
4.	March 29, 1960	Sikkim	1	Palpi : (Fig. 8, *f*) — Distal dark band (sub apical) absent, the apical pale band nearly 1/3 length of the palp; 2 to 3 patches of white scales present on the middle dark band.
5.	—	Lakhimpur (Assam)	1	Palpi : (Fig. 8, *g*) — Sub-apical dark band absent on left palp.

TABLE (*Contd.*).

Serial number. (1)	Date of collection. (2)	Locality. (3)	Number of specimens. (4)		Nature of variations from the type form. (5)
		9. *A. minimus* Theobald, 1901 (Fig. 9).			
1.	Nov. 10, 1921	Kamrup (Assam)	1	Wing : (Fig. 9, *a,b*)	Both wings without the usual small pale interruption at the inner side of the inner costal spot.
	Nov. 12, 1921	Goalpara (Assam)	2		
	Nov. 13, 1921	Sibsagar (Assam)	1		
	January, 1928	Jeypore (Vizag.)	2		
	October, 1929	Jeypore (Vizag.)	1		
		10. *A. moghulensis* Christophers, 1924 (Fig. 10).			
1.	June, 1921	Kasauli (Simla Hills)	1	Wing : (Fig. 10, *a,b*)	Basal (inner) part of the costa with two pale interruptions on both wings.
		11. *A. pallidus* Theobald, 1901 (Fig. 11).			
1.	Dec. 30, 1922	Singhbum	2	Palpi : (Fig. 11, *a,b*)	Dark scales in the centre of the apical pale area making an extra dark band.
	—	Dharbanga	7		
	—	Purnea	1		
	Feb. 8, 1929	Uttar Pradesh	11		
	August, 1929	Bengal	1		
	Jan. 8, 1959	Burdwan (Bengal)	1		
2.	—	Dharbanga	1	Palpi : (Fig. 11, *c*)	A patch of dark scales in the centre of the apical pale area towards the external margin.
3.	February, 1958	Kanpur (U.P.)	1	Palpi : (Fig. 11, *d*)	An extra dark band in the apical pale area as in respect of '1' above, a row of scattered pale scales towards the inner margin of the two distal broad dark bands.
4.	Jan. 8, 1959	Burdwan	1	Palpi : (Fig. 11, *e*)	A continuous linear row of white scales on the pre-apical and middle dark bands.
		12. *A. philippinensis* Ludlow, 1932 (Fig. 12).			
1.	May, 1927	Cachar (Assam)	1	Palpi ; (Fig. 12, *a,b*)	A patch of dark scales in the centre of the apical pale area towards the external margin.
	—	Purnea (Bihar)	1		
2.	Sep. 5, 1928	Calcutta	1	Palpi :	A patch of dark scales in the centre of the apical pale area of the left palp only.
3.	—	Bengal	1	Palpi : (Fig. 12, *c*)	An extra dark band formed in the centre of the apical pale area.

PLATE V.

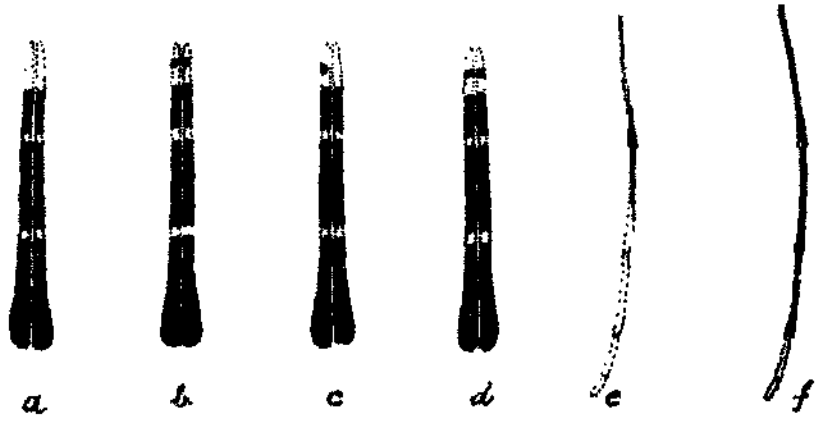

FIG. 6.

FIG. 6. *A. jamesi* (*a*) Normal palpi : (*b*—*d*) Abnormal palpi ;
(*e*) Normal tarsi ; (*f*) Abnormal tarsi.

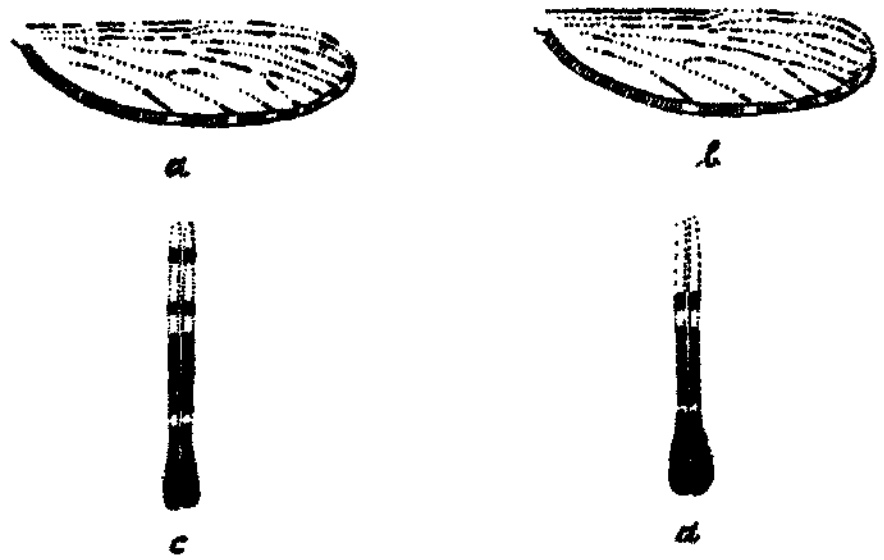

FIG. 7.

FIG. 7. *A. karwari* (*a*) Normal wing ; (*b*) Abnormal wing ;
(*c*) Normal palpi ; (*d*) Abnormal palpi.

PLATE VI.

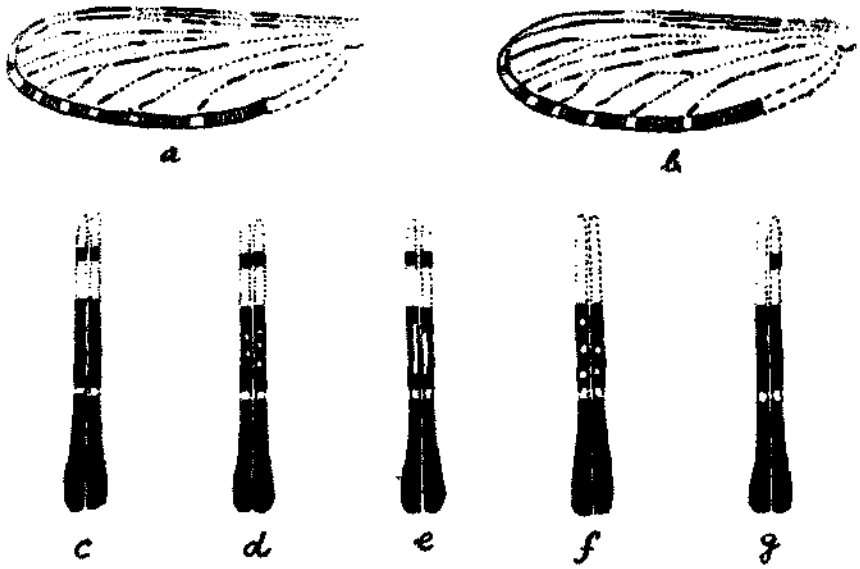

FIG. 8.

FIG. 8. A. *maculatus* (*a*) Normal wing ; (*b*) Abnormal wing
(*c*) Normal palpi ; (*d*–*g*) Abnormal palpi.

FIG. 9.

FIG. 9. A. *minimus* (*a*) Normal wing ; (*b*) Abnormal wing.

PLATE VII.

FIG. 10.

FIG. 10. *A. moghulensis* (*a*) Normal wing ; (*b*) Abnormal wing.

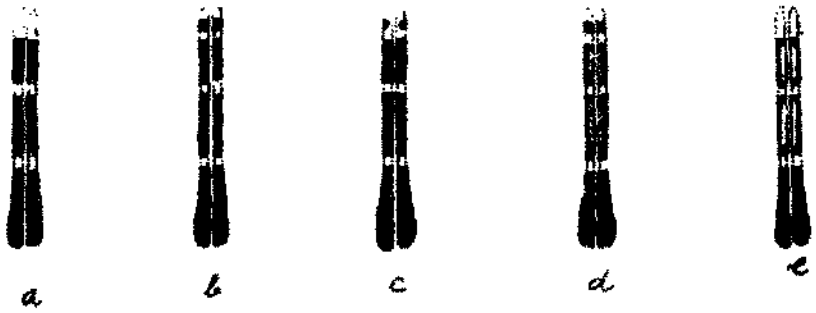

FIG. 11.

FIG. 11. *A. palibdus* (*a*) Normal palpi ; (*b*—*e*) Abnormal palpi.

PLATE VIII.

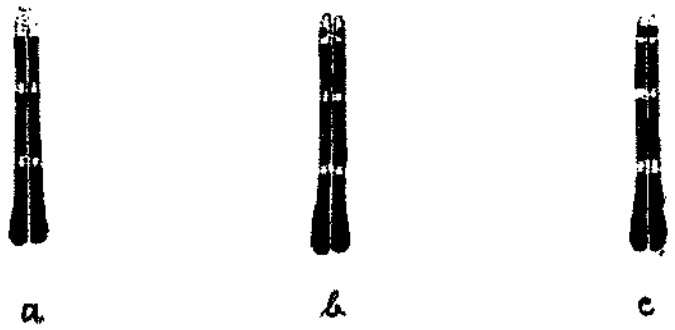

FIG. 12.

FIG. 12. *A. philippinensis* (*a*) Normal palpi ; (*b*, *c*) Abnormal palpi.

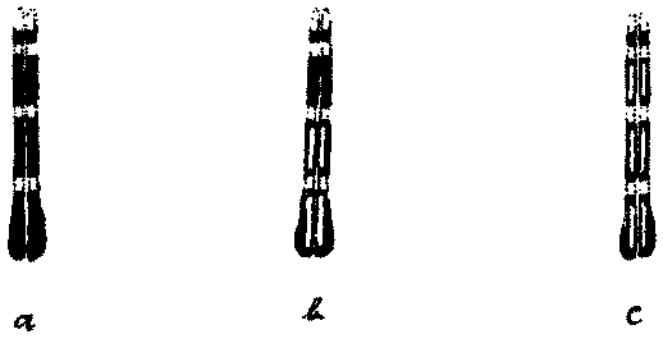

FIG. 13.

FIG. 13. *A. pulcherrimus* (*a*) Normal palpi ; (*b*, *c*) Abnormal palpi.

TABLE (*Contd.*).

Serial number. (1)	Date of collection. (2)	Locality. (3)	Number of specimens. (4)	Nature of variations from the type form. (5)	
		13. *A. pulcherrimus* Theobald, 1902 (Fig. 13).			
1.	March, 1960	Arthala	3	Palpi : (Fig. 13, *a,b*)	A continuous linear row of pale scales on the two proximal broad dark bands towards the inner margin.
2.	March, 1959	Arthala	8	Palpi : (Fig. 13, *c*)	A continuous row of pale scale on the three broad dark bands towards the inner margin; the narrow sub-apical dark band normal.
		14. *A. ramsayi* Covell, 1927.			
1.	October, 1927	Bengal	1	Abdominal scales.	With golden coloured scales on abdominal Segment 8 (characteristic feature of *A. jamesi*).
		15. *A. splendidus* Koidzumi, 1920 (Fig. 14).			
1.	—	Mount Abu (Rajasthan)	1	Palpi : (Fig. 14 *a,b*)	Without sub-apical dark band, resulting in a continuous apical pale band representing the apical and subapical pale areas.
2.	—	Kohat (West-Pakistan)	1	Palpi : (Fig. 14, *c*)	Apical pale area more then 1/3 length of the palp; sub-apical pale band and middle dark band narrow than usual. Proximal and middle dark bands without the usual speckling.
3.	—	Kohat (West Pakistan)	1	Palpi : (Fig. 14, *d*)	The right palp with a broad apical pale area, 3 narrow pale bands, broad proximal dark band and three narrow distal dark bands, the left palp with broad apical pale area, two narrow proximal pale bands of varying size, a broad proximal dark band and two distal dark bands. The left sub-apical pale band picked with a few dark scales in the centre, only proximal dark band with speckling.
4.	—	Kasauli (Simla Hills)	1	Palpi : (Fig. 14, *e*)	A narrow extra dark band just before the tip of the palpi, making the tip appear dark. The narrow apical pale area with a few black scales.
	January, 1960	Jammu	1		
5.	—	Bannu (West-Pakistan)	1	Palpi : (Fig. 14, *f*)	The right palp without the sub-apical dark band, the left palp with a slightly narrow (than usual) sub-apical dark band.
	April, 1921	Madhupur (Punjab).	1		

TABLE (*Contd.*).

Serial number (1)	Date of collection. (2)	Locality. (3)	Number of specimens. (4)		Nature of variations from the type form. (5)
		16. *A. subpictus* Grassi, 1899 (Fig. 15).			
1.	July 16, 1958	Arthala (U.P.)	1	Hypomelanic form :	A uniformly pale yellow specimen lacking the characteristic dark and white areas on wings, palps, and tarsi was caught during the regular indoor catches. The two broad pale bands on the fore tarsi of the right leg could be discerned against the pale yellow background of the leg. Also the dorsum of thorax was covered with hairs. The specimen could be confused with *A. vagus* but the latter species has not so far been recorded in this area (Puri, 1955; Bhatia *et al.*, 1958). Also *A. subpictus* was the predominant species present during the period when this specimen was captured *A. pallidus*, *A. subpictus*, *A. stephensi*, *A. vagus* have been reported by Chistophers (1933) to show this form of extreme hypomelanism. However only description of hypomelanic *A. pallidus* (Bhatnagar *et al.*, 1958) is availabe in literature.
2.	Dec. 31, 1928	Daulatapur, Sind (West-Pakistan)	1	Wing : (Fig. 15, *a,b*)	Costa of both wings without sector pale area.
	March, 1959	Arthala (U.P.)	1		
3.	Dec. 31, 1928	Daulatpur, Sind (West-Pakistan)	1	Wing : (Fig. 15, *b*)	As in respect of '2 above.
				Palpi : (Fig. 15, *c,d*)	Apical pale area about 1/2 length of the sub-apical dark area and with a continuous linear row of dark scales on the left margin, sub-apical pale area represented by a few pale scales only.
4.	Nov. 7, 1958	Hissar (Punjab)	1	Palpi : (Fig. 15 *e*)	A few pale scales present on the sub-apical dark area.
	Jan. 16, 1959	Kanpur (U.P.)	1		
5.	May, 1958	Bellaryard Etawa (U.P.)	2	Palpi : (Fig. 15, *f*)	Dark scales in the centre of apical pale area forming an extra dark band.

PLATE IX.

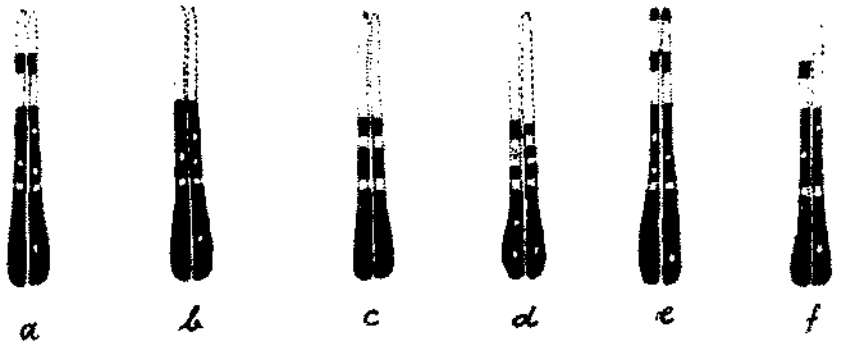

FIG. 14.

FIG. 14. *A. splendidus* (*a*) Normal palpi ; (*b*—*f*) Abnormal palpi.

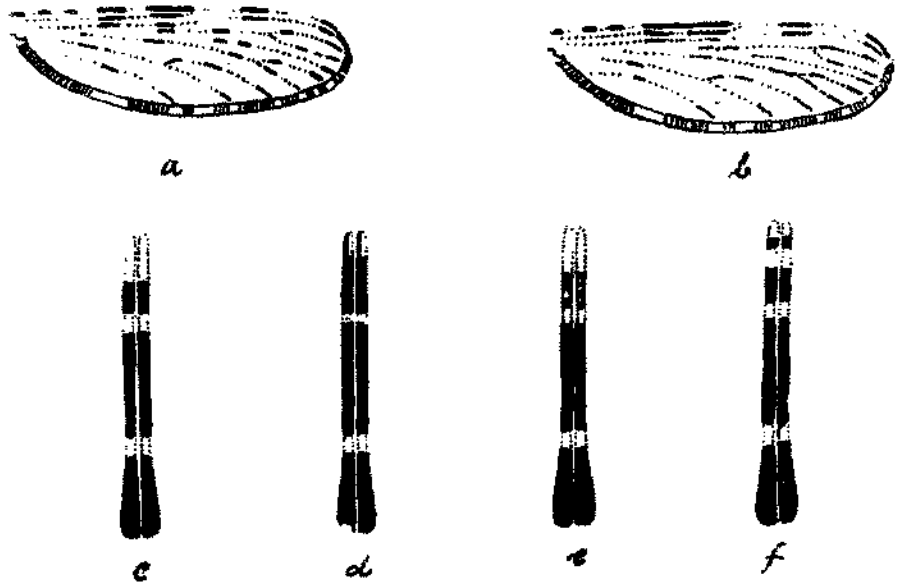

FIG. 15.

FIG. 15. *A. nupicus* (*a*) Normal wing ; (*b*) Abnormal wing ; (*c*) Normal palpi ; (*d*—*f*) Abnormal palpi.

PLATE X.

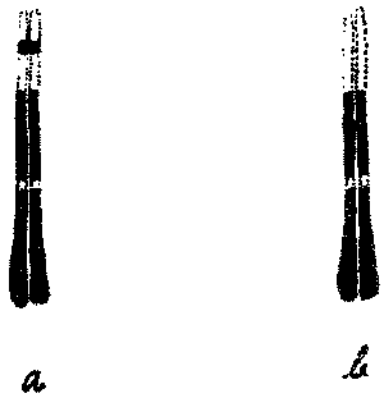

FIG. 16.

FIG. 16. *A. cheobaldi* (*a*) Normal palpi ; (*b*) Abnormal palpi.

FIG. 17.

FIG. 17. *A. turkhudi* (*a*) Normal wing ; (*b*) Abnormal wing.

PLATE XI.

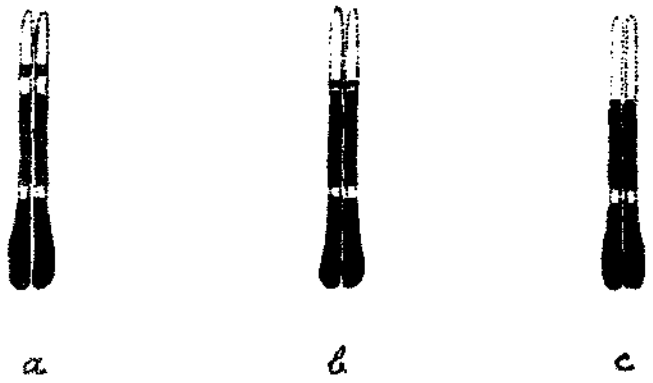

FIG. 18.

FIG. 18. *A. vagus* (*a*) Normal palpi. (*b*, *c*) Abnormal palpi.

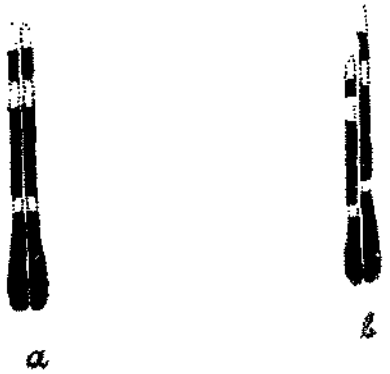

FIG. 19.

FIG. 19. *A. varuna* (*a*) Normal palpi; (*b*) Abnormal palpi.

TABLE (*Concld.*).

Serial number. (1)	Date of collection. (2)	Locality. (3)	Number of specimens. (4)	Nature of variations from the type form. (5)
		17. *A. theobaldi* Giles, 1901. (Fig. 16).		
1.	—	Mount Abu (Rajasthan)	1	Palpi : (Fig. 16, *a,b*) Without the subapical dark band, with only one broad and one narrow pale band.
		18. *A. turkhudi* Liston, 1901 (Fig. 17).		
1.	—	Kasauli, Simla Hills.	1	Wing : (Fig. 17, *a,b*) Left wing without the usual prosector and humeral pale areas on the inner costa, right wing normal.
		19. *A. vagus* Donitz, 1902. (Fig. 18).		
1.	Nov. 7, 1958	Hissar (Punjab)	1	Palpi : (Fig. 18, *a,b*) The subapical dark band narrow and about 1/8 length of the apical pale area, subapical pale band also very narrow.
2.	—	24-Paraganas (West Bengal)	1	Palpi : (Fig. 18, *c*) Without the subapical dark band with only one broad and one narrow pale band.
		20. *A. varuna* Iyengar, 1924 (Fig. 19).		
1.	Nov. 2, 1959	Hyderabad (Andhra)	1	Palpi : (Fig. 19, *a,b*) Palps unequal, left palp nearly 3/4 length of the right palp.

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A NOTE ON A TECHNIQUE FOR QUANTITATIVE ESTIMATION OF SUSCEPTIBILITY OF BED-BUGS TO CONTACT INSECTICIDES.

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INTRODUCTION.

INSECTICIDE susceptibility data in respect of any particular insect, obtained by various workers in different parts of the world, can only be comparable if the same standard technique is used in all the studies. A standard method has not so far been developed for bed-bugs and as such different workers are using different methods. A critical review of the different techniques in vogue (Table I) suggests that none is suitable and satisfactory in all respects for use in the field.

During the period March to April, 1960, insecticide susceptibility tests, using the W.H.O. test kit for body lice, were carried out with bed-bugs in Delhi. The preliminary results obtained are promising. The details of the investigations are reported in this communication so that the technique may also be tried by other workers and, if found suitable, it may be adopted as a standard technique for universal use.

MATERIALS AND METHODS.

For these studies adult bed-bugs (*Cimex hemipterus*) of both sexes were obtained from the colony* maintained at the Malaria Institute of India, Delhi, as well as from the mice colony heavily infested with them†. The latter source could provide a very large number of insects and hence most of the tests were carried out with the material obtained from there. Bed-bugs which had fed on blood were collected and kept in test tubes in batches of 10 to 20. After two hours, damaged specimens, if any, were removed. The healthy insects were exposed to varying concentrations of D.D.T., gamma B.H.C. and pyrethrins.

* The colony was established from a large number of insects collected from Police Lines, Delhi (an unsprayed locality) in the year 1958.

† It is presumed that few bed-bugs accidentally escaped from the insectary and established themselves in the mice colony. In the mice colony room, they had the requisite shelter as well as ready supply of blood feed from the mice. Since these studies were completed, mice colony has been successfully de-bugged.

TABLE I.

Summary of the results obtained with different techniques used for insecticide susceptibility tests of bed-bugs.

Species.	Insecticide and carrier medium.	Substratum.	Mode of application.	Exposure period.	Holding period.	Authority.	Remarks on the performance of the technique.
C. hemipterus and *C. lectularius*	5·0 per cent DDT in Kerosene oil	Glass jars	Dry film	5 minutes	48 hours	Lindquist *et al.* (1946)	Critical range of mortality in insects was not obtained *C. hemipterus* was found more resistant than *C. lectularius*.
C. lectularius	DDT in acetone	Filter paper, wood and glass	Dry film	5 minutes to 24 hours	..	Barnes (1946)	Critical range of mortality in insects was obtained. DDT on filter papers was most toxic, glass and wood came next
C. lectularius	DDT, gamma-BHC and pyrethrins in acetone	Filter papers	Dry film	2 hours-in some cases it varied from 5 minutes to 7 days	..	Busvine and Barnes (1948)	Lc50 values of DDT, gamma-BHC and Pyrethrins were found to be 5 mg., 0·0006 mg. and 0·002 mg. per sq. ft., respectively.
C. hemipterus	DDT in talcum powder.	Filter paper (Filter papers turned over several times on the insecticide powder and then bed-bugs were exposed on them)	Dry film	2-10 minutes	24 hours	Rao and Halgeri (1956)	10 minutes exposure gave critical range of mortality in a strain of insects considered to be susceptible. But when tested after 3 years by increasing the concentration of DDT even up to 100 per cent, critical range of mortality in insects was not obtained. Development of high degree of resistance in bedbugs was stated to be due to DDT spraying in that area.
C. lectularius	DDT, gamma-BHC, dieldrin and diazinon in xylene	Filter paper	Dry film	1 hour	24 hours	Mofidi (1958)	Critical range of mortality in insects was not obtained.

C. lectularius	DDT, chlordane, dieldrin, lindane and malathion in acetone	Filter paper	Dry film	Continuous exposure of insects to insecticidal deposits for varying periods		Burden (1958)	Lc50 values for different insecticides were estimated. Dry film of DDT was found to be more toxic to insects than the oil film.
C. hemipterus	DDT and gamma-BHC in talcum powder	Filter paper	Dry film	10 minutes	24 hours	Menon, Karani and Narasimhan (1958)	Critical range of mortality in insects was observed. Lc50 values for DDT and gamma-BHC were found to be 46·0 per cent and 0·41 per cent respectively. Control mortality rates in insects ranged from 5·0 to 6·7 per cent.
C. lectularius	DDT in odourless distillate and ethylene dichloride	Block of materials commonly used in building construction	Oil film	24 hours	6 days	Barnes (1946)	Critical range of mortality in insects was not obtained after 48 hours of application of insecticides to substratum.
C. lectularius	DDT in oil	Filter paper (WHO)	Oil film	Not stated		Mofidi (1958)	Critical range of mortality in insects was obtained but control mortality rate was high (21 per cent).
C. lectularius and *C. hemipterus*	DDT in oil	Filter papers	Oil film	Continuous exposure of insects to insecticidal deposits for 5 days when mortality in them was recorded.		Busvine (1938)	Critical range of mortality in insects was obtained. Control mortality in healthy young bed-bugs was below 10 per cent.
C. hemipterus	DDT in non-volatile oil	Filter papers (WHO)	Oil film	5 days	2 days	Burden (1958)	Very high mortality in control (47·0 per cent) tests was observed. Oil film was found to be less toxic to insects than dry films.

(Contd.)

TABLE 1 (*Concld.*)

Species.	Insecticide and carrier medium.	Substratum.	Mode of application.	Exposure period.	Holding period.	Authority.	Remarks on the performance of the technique.
Cimex spp.	DDT, gamma-BHC, dieldrin, malathion and diazinon in non-volatile oil.	Filter papers (WHO)	Oil film	1 hour	24 hours	Sen (1959)	Critical range of mortality in insects was not obtained for dieldrin, malathion and diazinon. Only one concentration of gamma-BHC, was tested. 20 and 24 per cent mortality rates in insects were observed with 2 and 4 per cent DDT concentrations, respectively. Control mortality rates in insects not given.
C. lectularius	DDT, gamma-BHC and pyrethrins in refined white oil also dusted in Kaolin	Filter papers (WHO)	Direct spraying or dusting	1 hour	7 days	Busvine (1946)	MLC values for pyrethrins, gamma-BHC were found to be 0·045, 0·051 and 0·56 per cent respectively. Bed-bugs dusted and kept for 7 days also gave encouraging results.
C. lectularius and *C. hemipterus*	DDT, gamma-BHC and pyrethrins in Risella oil	..	Direct spray	..	7 days	Busvine (1958)	MLC values for DDT, gamma-BHC and pyrethrins were found to be 3·9, 0·038 and 0·055 per cent, respectively, for *C. hemipterus* (DDT-resistant strain) and 1·4, 0·1 and 0·04 per cent respectively for *C. lectularius* (susceptible colony strain).
C. lectularius and *C. hemipterus*	DDT and pyrethrins	Bed-bugs fed on rabbits which were fed with DDT and pyrethrins	Stomach poison technique	..	..	Lindquist *et al.* (1944)	Critical range of mortality in insects was not observed. Knock down effect of pyrethrins was found to be faster than DDT.

The insecticidal concentrations used for the present tests were those supplied with the body lice kit. Half gram of a particular concentration of an insecticide was spread uniformly over a piece of cloth (12·5 cm.² supplied in the kit) with the help of a glass slide. Bed-bugs were exposed to the treated cloth pieces for varying time periods and then removed on (only in cases of tests with gamma BHC and pyrethrins) to clean cloth pieces and kept for 24 hours when mortality in them was recorded. During the exposure and holding periods the insects were covered with glass petridishes (3½ inches diameter) to prevent them from escaping. In the case of tests with D.D.T., mortality* counts in insects were made after 24 hours' continuous exposure to the insecticide. For each series of tests, controls were kept by exposing the insects to clean cloth pieces.

The mortality data were analysed by probit analysis method outlined by Finney (1952) and the different parameters were estimated by the maximum likelihood method. Tests for goodness of the *lc-pr* (log concentration probit regression) line and tests of significance were also carried out by following the appropriate biometric techniques.

RESULTS.

The results of experiments on the different aspects of the technique are summarised below under appropriate headings.

(A) LINEARITY OF RESPONSE AND BATCH VARIABILITY.

The results of susceptibility tests against D.D.T., with the bed-bugs collected from the two sources, are depicted in Tables II and III. The probit values of the mortality rates were found to be linearly related with log concentrations (Fig. I, II and III) which suggested that the data was suitable for probit analysis.

The graphically estimated Lc_{50} values of D.D.T., for the bed-bugs, collected from the insectary and mice colony room, were found to be 0·14 per cent × 24 hours† and 0·13 per cent × 24 hours† respectively. Since the Lc_{50} values were almost identical, the data of Tables II and III were combined and statistically analysed.

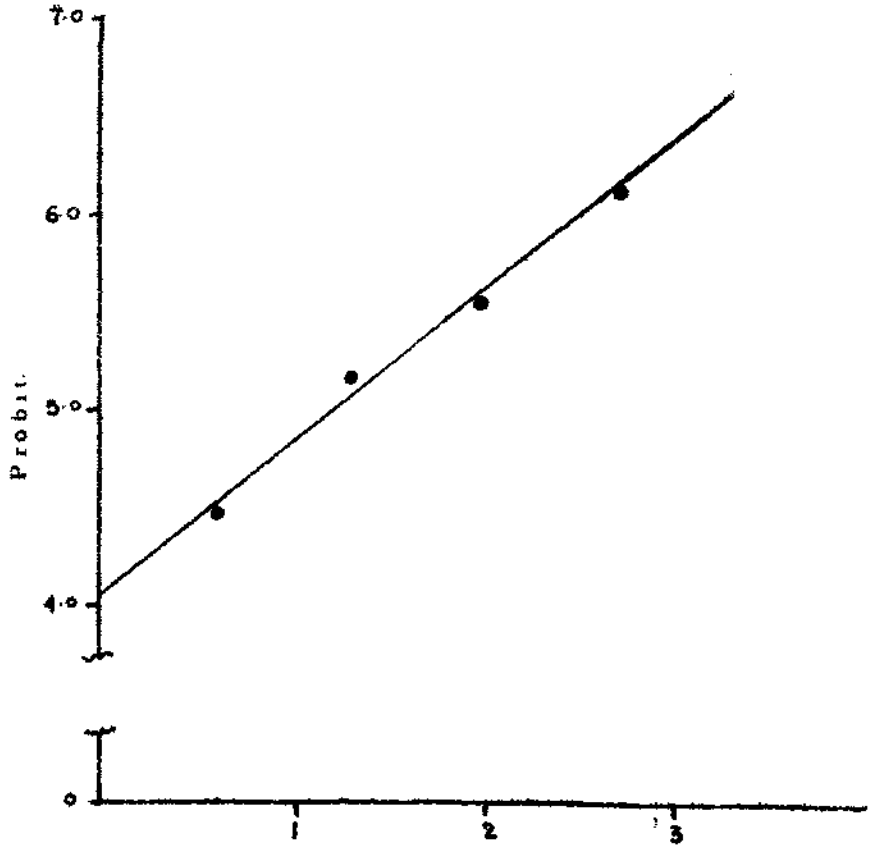
Chi square tests were carried out on the data obtained with the three insecticides. This was to assess the goodness of fit of the *lc-pr* lines and homogenity of response in the bed-bugs of different batches exposed to the same concentration of insecticide (Table IV). The results obtained suggested that the *lc-pr* line was a good fit straight line in all cases and that the differences in mortality rates in different batches of insects, with a particular concentration of insecticide, were not significant. This meant that with the technique employed, consistent results in different replicate tests were obtained.

* Bed-bugs found dead or moribund were counted as dead. Insects found on their backs, though alive yet failing to rectify their position even when stimulated gently with a needle, were considered as moribund.

† Mortality in insects was recorded after 24 hours' exposure of the insects to insecticidal deposits.

GRAPH 1.

Showing the relationship of probit of mortality and log concentrations of DDT (combined data of Tables II and III).



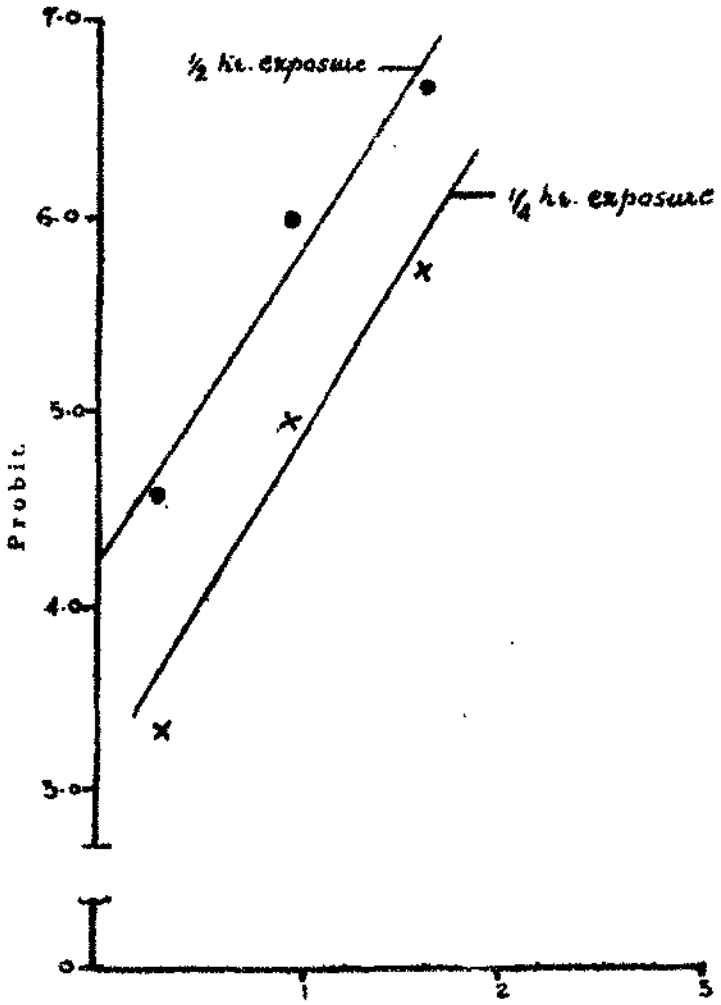
(B) SENSITIVITY OF THE TECHNIQUE.

For a technique to be sensitive enough to measure quantitatively and fairly accurately the insecticide susceptibility of an insect population, the *lc-pr* line obtained with its susceptible strain should be steep. The steeper the line the more sensitive is the test method. To measure the sensitivity of the technique employed in the present studies, regression coefficient values were estimated (Table IV).

The regression coefficient values for D.D.T. and gamma B.H.C. tests were found to be $0{\cdot}8 \pm 0{\cdot}1$ and $1{\cdot}6 \pm 0{\cdot}3$ respectively. Similar values for tests with pyrethrins, with half-an-hour and one hour exposure periods were found to be $3{\cdot}1 \pm 0{\cdot}7$ and $2{\cdot}4 \pm 0{\cdot}4$ respectively. From these data it appeared that the technique was quite sensitive for tests with gamma B.H.C. and pyrethrins though it was

GRAPH 2.

Showing the relationship of probit of mortality and log concentrations of gamma BHC.



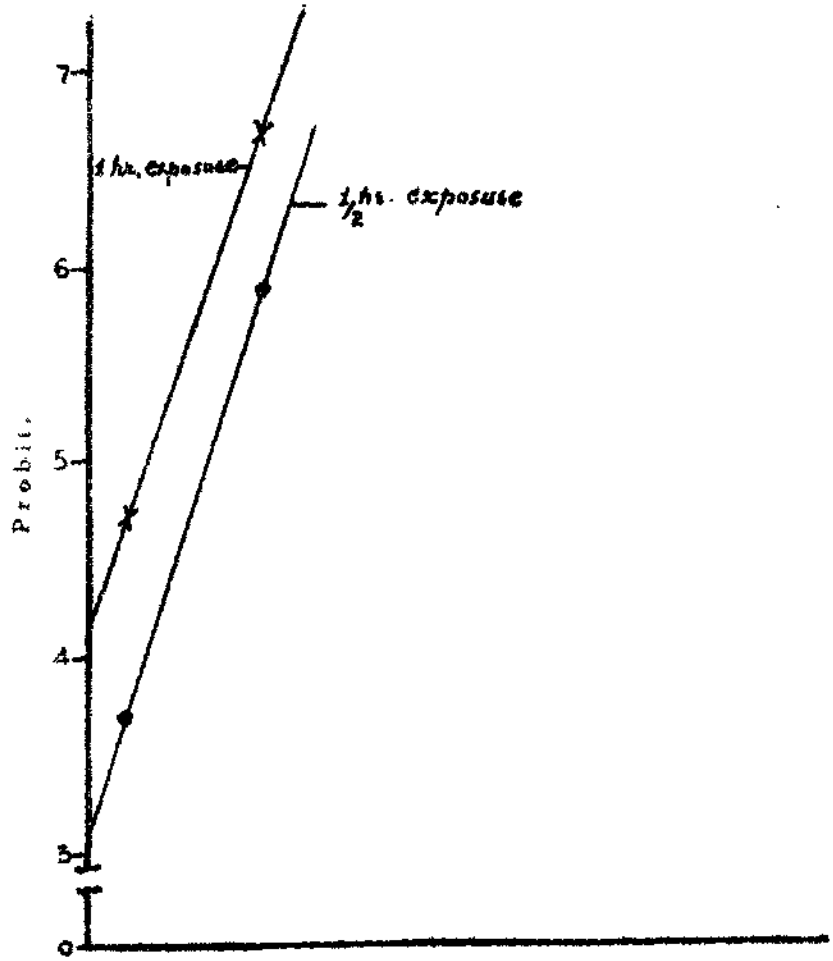
Log 100 concentrations of gamma BHC.

relatively less sensitive* in the case of D.D.T. But even with D.D.T., with the technique critical range of mortality in insects could be obtained with 24 hours' continuous exposure (as against 5 days in the W.H.O. provisional technique) to the insecticide. It could, therefore, be considered sensitive enough to be of practical use in the field.

* The lower value of regression coefficient may also be due to heterogenous (with respect to insecticide susceptibility) composition of the bed-bug population. However, this point needs further investigations.

GRAPH 3.

Showing the relationship of probit of mortality and log concentrations of pyrethrine.



Log 1,000 concentrations of pyrethrine.

(C) EFFECT, IF ANY, OF DIFFERENT INDIVIDUALS TREATING THE CLOTH PIECES WITH INSECTICIDE.

Tests were performed to determine the effect, if any, of variations due to treatment of cloth pieces with insecticide by different workers on mortality rates of bed-bugs. For this purpose, cloth pieces were treated by different persons using the same insecticidal concentration. Two of the three technicians had no previous experience of this work. The W.H.O. instructions for impregnation of the cloth pieces were explained to each of them. Each one of them was then asked to impregnate two pieces of cloth separately with two concentrations of pyrethrins. Tests were carried out separately on cloth pieces treated by each of the three technicians.

TABLE II.

Results of susceptibility of bed-bugs (collected from mice colony) to D.D.T. using the W.H.O. test-kit for body lice. Mortality was recorded at the end of 24 hours' continuous exposure.

Serial number of tests.	Concentrations of D.D.T. (per cent): 5·0		1·0		0·2		0·04		Control:	
	Number of bed-bugs exposed.	Number of bed-bugs dead and moribund.	Number of bed-bugs exposed.	Number of bed-bugs dead and moribund.	Number of bed-bugs exposed.	Number of bed-bugs dead and moribund.	Number of bed-bugs exposed.	Number of bed-bugs dead and moribund.	Number or bed-bugs exposed.	Number of bed-bugs dead and moribund.
1	10	7	10	9	10	7	10	9	10	Nil
2	10	9	10	10	10	9	10	4		
3	10	10	10	8	10	10	10	2	20	1
4	10	10	10	8	10	6	10	5		
5	20	20	20	18	20	16	19	13	20	1
6	20	18	20	11	20	6	20	10	20	4
7	10	10	10	8	11	8	10	1		
8	..	..	10	5	12	5	10	2	5	1
9	9	9	10	6	11	5	10	3	10	1
10	10	7	10	8	..	..	9	1	..	..
Total	109	100	120	89	114	72	118	50	85	8
Observed per cent mortality	91·7		74·2		63·2		42·4		9·4	
Corrected per cent mortality	90·8		71·5		59·4		36·4			

TABLE III.

Results of susceptibility of bed-bugs (reared in the insectary) to DDT using the W.H.O. test-kit for body lice. Mortality was recorded at the end of 24 hours' continuous exposure.

Replicates.	Concentrations of DDT (per cent): 5·0		1·0		0·2		0·04		Control:	
	Number of bed-bugs exposed.	Number of bed-bugs dead and moribund.	Number of bed-bugs exposed.	Number of bed-bugs dead and moribund.	Number of bed-bugs exposed.	Number of bed-bugs dead and moribund.	Number of bed-bugs exposed.	Number of bed-bugs dead and moribund.	Number of bed-bugs exposed.	Number of bed-bugs dead and moribund.
1	10	7	10	5	10	6	10	Nil	10	Nil
2	19	17	20	17	21	12	20	4	10	2
Total	29	24	30	22	31	18	30	4	20	2
Observed per cent mortality	82·8		73·3		58·1		13·3		10	
Corrected per cent mortality	80·9		70·3		53·4		3·7			

TABLE IV.

Summary of statistical analysis of the susceptibility test data of bed-bugs.

Insecticide.	Exposure period.	Holding period.	Equation of the *lc. pr.* line.	Factor by which the dosages were multiplied.	Goodness of fit of the *lc. pr.* line to the data.	Regression Coefficient values.	Variance of '*b*'	Variance of '*m*'	LC_{50}* with 95 per cent fiducial limits.
DDT	24 hours.	Mortality was recorded at the end of exposure period	$Y=0{\cdot}783X+4{\cdot}0852$	× 100	χ^2 (47) = 1·14	0·78	0·006998	0·009455	0·228—**0·147**—0·095
Gamma-BHC	½ hour.	24 hours.	$Y=1{\cdot}6067X+4{\cdot}1912$	× 100	χ^2 (4) = 0·97	1·16	0·1177	0·003405	0·0414—**0·032**—0·0245
Pyrethrins	1 hour.	24 hours.	$Y=2{\cdot}4446X+4{\cdot}0078$	×1000	χ^2 (7) = 0·031	2·44	0·131381	0·001673	0·0028—**0·0025**—0·0023
	½ hour.	24 hours.	$Y=3{\cdot}1429X+3{\cdot}0713$	×1000	χ^2 (4) = 0·0	3·14	0·495466	0·000001	0·00413—**0·00411**—0·00409

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

It may be seen from the data obtained (Table V) that the differences of mortality figures of bed-bugs, obtained with the cloth pieces treated by different persons with a particular concentration of pyrethrins, were within the range of normal variation. It suggested that the impregnation of the cloth pieces by different persons would give comparable results with this technique.

(D) THE MEAN RESPONSE RATES (MLC VALUES).

The susceptibility data of bed-bugs with D.D.T., gamma B.H.C. and pyrethrins were analysed and the Lc_{50} values along with their fiducial limits were estimated. The values are given in Table IV. To determine the M.L.C. values for different insecticides it was possible with this technique to obtain the critical range of 24 hours' mortality in insects after half-an-hour and half-an-hour and one hour exposure periods with gamma B.H.C. and pyrethrins respectively (Tables VI and VII). In the case of D.D.T., however, this range of mortality in insects could be obtained after 24 hours of continuous exposure to the insecticide. The M.L.C. figures of D.D.T., gamma B.H.C. and pyrethrins were found to be $0{\cdot}147 \times 24$* hours, $0{\cdot}032 \times \frac{1}{2}$ hour* and $0{\cdot}0079 \times \frac{1}{2}$ hour* and $0{\cdot}0025 \times 1$* hour respectively.

(E) COMPARATIVE INSECTICIDE SUSCEPTIBILITY TESTS FOLLOWING THE W.H.O. PROVISIONAL TECHNIQUE FOR BED-BUGS.

With a view to know the insecticide susceptibility status of the bed-bugs used for the present studies, tests were also carried out with D.D.T. following the W.H.O. provisional technique. using test tubes and pre-impregnated filter papers†. Mortality in insects was recorded daily for 5 days. The results are depicted in Table VIII. It may be seen from the data obtained that the mortality rates in insects in control tests were very high (65·0 per cent on the fifth day). Mortality rate figures were almost the same with the two lower concentrations of D.D.T. and control. High control mortality rates with the oil-impregnated filter papers have also been observed by other workers (Mofidi, 1958 and Burden, 1958). Burden (1958 *loc. cit.*) observed control mortality rate as high as 67 per cent.

In order to find out the causes of high control mortality rates, about 30 bed-bugs were exposed for 24 hours on a clean cloth piece, a filter paper (11 cm. diameter) impregnated with ethylene dichloride (1 ml.) and two filter papers impregnated with B.O.C. white oil-ethylene dichloride mixture‡ in ratios of 1 : 2 (1 ml.) and 2 : 1 (1 ml.). At the end of the exposure period, mortality counts were made. It may be seen from the data (Table IX) that the mortality rate in insects with substratum (cloth piece or filter paper) without B.O.C. white oil was 3·38 per cent as against 41·67 per cent in the one with B.O.C. white oil. The difference was found to be highly significant. This suggested that B.O.C. white oil contributed towards a high mortality in bed-bugs.

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* Period of exposure to the insecticide in hours. In the case of gamma BHC this value was calculated for ½ hour exposure only though critical range of mortality in insects was also obtained with 15 minutes' exposure.

† The W.H.O. pre-impregnated filter papers with varying concentrations of insecticide were cut to the size of 5 cm.×1 cm. and folded length-wise into the shape of "Z" for use in the tests.

‡ The filter papers were used for tests after 10 minutes of impregnation.

TABLE V.

Detailed statistical analysis of the bed-bug mortality data when the insects were exposed to cloth pieces treated with different concentrations of pyrethrins by three persons.

z concentration	x log 1000z	Cloth treated by	n	r	P C=0	Empirical probit.	Y	nw	y	nwx	nwy		$nwxy$
0·0016	0·2	Singh	19	9	47·4	4·9	4·6	11·40988	4·9	2·28198	55·90841	162·50071	11·18168
		Negi	19	6	31·6	4·5		11·40988	4·5	2·28198	51·34446		10·26889
		Prakash	20	6	30·0	4·5		12.01040	4·6	2·40208	55·24784		11·04957
								34·83016					
0·008	0·9	Singh	20	17	85·0	6·0	6·1	8·09480	6·0	7·28532	48·56880	148·13484	43·71191
		Negi	20	17	85·0	6·0		8·09480	6·0	7·28532	48·56880		43·71192
		Prakash	20	18	90·0	6·3		8·09480	6·3	7·28532	50·99724		45·89752
								24·28440					

$S_{nw} = 59{\cdot}11456$ $\quad S_{nwx} = 28{\cdot}82200$ $\quad S_{nwy} = 310{\cdot}63565$ $\quad S_{nwxy} = 165{\cdot}82150$

$\frac{1}{S_{nw}} = 0{\cdot}01691630$ $\quad \bar{x} = 0{\cdot}4876$ $\quad \bar{y} = 5{\cdot}2548$

$$\begin{array}{r} 21{\cdot}06357 \\ 14{\cdot}02250 \\ \hline S_{xx} = 7{\cdot}04107 \end{array} \qquad \begin{array}{r} 165{\cdot}82150 \\ 151{\cdot}45401 \\ \hline S_{xy} = 14{\cdot}36749 \end{array} \qquad \begin{array}{r} 1663{\cdot}24955 \\ 1632{\cdot}33003 \\ \hline S_{yy} = 30{\cdot}91952 \\ 29{\cdot}31316 \\ \hline \chi^2\ 1{\cdot}60636 \end{array}$$

(Residual sum of squares is divided into two).

Analysis of variance.

Sources of variance.	D.F.	Sum of squares.	Mean square.	
Regression	1	29·31		Significantly high value of sums of squares for regression and variation of dosages suggested a significant correlation between the probit mortality and log concentration of pyrethrins. The lower values of sum of squares and mean square (which are not significant) suggested that the variations in the mortality rates, observed with cloth treated by three persons were not significant.
Deviation from linearity	0*	0·13*		
Between the dosages	1	29·44		
Between the treatment at a dose	4	1·48	0·37	
Total	5	30·92		

b=2·0405±0·38 (This value is the same as that estimated graphically), $y = 2{\cdot}0405x - 4{\cdot}2590$, m=0·363, LC_{50}=0·0023×1 with fiducial limits—0·0031—0·0017

*Since only two dosages were used the question of deviation from linearity does not arise. Therefore the value of sum of squares should be added with the similar values for between the treatment at a dose.

TABLE VI.

Results of susceptibility of bed-bugs to gamma-BHC using the W.H.O. test-kit for body lice. Mortality in insects recorded after 24 hours.

Hours of exposure.	Concentrations of insecticide (per cent): 0·02			0·1			0·5			2·5			Control			Remarks
	n	r	p	n	r	p	n	r	p	n	r	p	n	r	p	
1 hour.	30	19	63·3	39	33	84·6	40	40	100·0	40	40	100·0	20	Nil	0·0	Total of 3 replicates.
½ hour.	25	9	36	26	22	84·6	25	24	96·0	24	24	100·0	20	Nil	0·0	Total of 2 replicates.
¼ hour.	28	2	6·0	27	14	49·3	31	25	79·6	32	32	100·0	20	1	5·0	Total of 2 replicates.

n=number of bed-bugs exposed, r=number of bed-bugs dead, p=corrected mortality.

TABLE VII.

Results of susceptiblity of bed-bugs to pyrethrins using the W.H.O. test-kit for body-lice. Mortality in insects recorded after 24 hours.

Hours of exposure.	Concentrations of insecticide (per cent.) 0·0016			0·008			0·04			Control		
	n	r	p	n	r	p	n	r	p	n	r	p
1 hour	21	8	38·1	21	20	95·2	21	21	100·0	20	Nil	0·0
½ hour	21	2	9·5	21	17	80·9	21	21	100·0	20	Nil	0·0

TABLE VIII.

Results of susceptibility of bed-bugs to DDT using the W.H.O. provisionl technique for bed-bugs. Mortality in insects was recorded daily for five days.

Concentration of DDT	Replicates	1 Day: n	1 Day: r	2 Days: n	2 Days: r	3 Days: n	3 Days: r	4 Days: n	4 Days: r	5 Days: n	5 Days: r
4·0	1	11	4	11	7	11	8	11	8	11	9
	2	10	5	10	8	10	8	10	9	10	10
	3	10	3	10	2	10	3	10	6	10	8
	4	9	4	9	5	9	7	9	8	9	9
	5	10	2	10	6	10	7	10	7	10	9
Total: Observed mortality rate		49	17 34·7	49	28 57·1	49	33 67·3	49	38 77·5	49	45 91·8
1·0	1	10	1	10	2	10	4	10	5	10	6
	2	10	1	10	3	10	4	10	4	10	7
	3	10	Nil	10	3	10	3	10	4	10	6
	4	10	1	10	3	10	4	10	6	10	7
	5	10	1	10	1	10	3	10	7	10	8
Total: Observed mortality rate.		50	4 8·0	50	12 24·0	50	18 36·0	50	26 52·0	50	34 68·0

(*Contd.*)

TABLE VIII (*Concld.*).

Concentration of DDT	Replicates	1 DAY: n	1 DAY: r	2 DAYS: n	2 DAYS: r	3 DAYS: n	3 DAYS: r	4 DAYS: n	4 DAYS: r	5 DAYS: n	5 DAYS: r
0·25	1	10	Nil	10	Nil	10	2	10	8	10	8
	2	10	2	10	3	10	3	10	4	10	6
	3	11	Nil	11	2	11	2	11	4	11	5
	4	10	Nil	10	2	10	3	10	5	10	7
	5	10	2	10	3	10	6	10	8	10	9
Total:		51	4	51	9	51	16	51	29	51	35
Observed mortality rate.			7·8		17·6		31·4		56·9		68·6
Control	1	10	2	10	4	10	5	10	8	10	8
	2	10	2	10	2	10	2	10	2	10	6
	3	10	Nil	10	Nil	10	1	10	4	10	8
	4	10	Nil	10	3	10	3	10	4	10	6
	5	9	1	9	1	9	1	9	4	9	6
Total:		49	5	49	10	49	12	49	22	49	32
Observed mortality rate.			10·2		24·4		20·5		44·9		65·3

n = number of bed-bugs exposed. r = number of bed-bugs dead.

TABLE IX.

Effect of substratum and solvents on the bed-bugs. (Insects were exposed for 24 hours and mortality was recorded after the exposure period).

CLOTH (SUPPLIED WITH BODY LICE TEST-KIT):			FILTER PAPER (11 cm. dim.) IMPREGNATED WITH 1 ml. OF ETHYLENE DICHLORIDE*			FILTER PAPER (11 cm. dim.) IMPREGNATED WITH BOC WHITE OIL AND ETHYLENE DICHLORIDE* MIXTURE 1 : 2			FILTER PAPER (11 cm. dim.) IMPREGNATED WITH BOC WHITE OIL AND ETHYLENE DICHLORIDE* MIXTURE 2 : 1		
n	r	p	n	r	p	n	r	p	n	r	p
29	2	6·9	30	nil	0·0	30	13	43·3	30	12	40·0

*Filter papers were used for tests 10 minutes after impregnation

Mortality rates on substratum without oil: 3·38 per cent.
Mortality rates on substratum with oil: 41·67 per cent.

$$t = \sqrt{\frac{p_1q_1}{n_1} + \frac{p_2q_2}{n_2}} = \sqrt{\frac{0{\cdot}0338 \times 0{\cdot}9662}{59} + \frac{0{\cdot}4167 \times 0{\cdot}5833}{60}} = \frac{\overset{d}{0{\cdot}3829}}{0{\cdot}0678} = 5{\cdot}65$$

which is more than 1·96. Therefore the difference in mortality rates may be considered significant.

n = number of bed-bugs exposed, r = number of bed-bugs dead. p = observed mortality rate.

In another experiment, bed-bugs were exposed in sets of test tubes containing W.H.O. control filter papers (impregnated with oil), unimpregnated filter papers and pieces of clean cloth. Daily mortality counts in insects were made up to 5 days. The results of this experiment (Table X) also indicated a high mortality due to B.O.C. white oil. It was also observed that mortality rates in insects increased progressively with days during which the bed-bugs were kept confined in tubes.

TABLE X.

Mortality rates in bed-bugs exposed to the W.H.O. control filter papers (oil impregnated), filter papers (clean) and cloth pieces (clean).

Substratum	Replicates	n	1 Day :		2 Days :		3 Days :		4 Days :		5 Days :	
			Dead	p (per cent)	Dead	p (per cent)	Dead	p (per cent)	Dead	p (per cent)	Dead	p (per cent)
WHO Control filter papers	1	21	4		5		7		12		13	
	2	21	4		4		7		8		12	
	3	17	1	18·4	1	20·4	4	30·7	5	51·0	9	69·4
Filter papers (clean)	1	21	0		0		2		4		7	
	2	21	1		3		4		7		9	
	3	18	0	1·7	1	6·7	1	11·7	3	23·3	5	35·0
Cloth pieces (clean)	1	21	2		4		4		5		6	
	2	17	1		1		1		4		7	
	3	22	1	5·7	1	10·0	4	15·0	5	23·3	7	33·3

n=Number of bed-bugs exposed.
p=Mortality rates (in per cent) observed.

Since the W.H.O. provisional technique involves the use of oil-impregnated filter papers and 5 days' exposure time, it is felt that this technique may have some limitations for use in the field.

DISCUSSION.

In developing a suitable field technique for measuring the insecticide susceptibility of insects it is desirable to consider the following points :—

(*i*) The technique should be simple and requiring minimum of specialized training for its handling.

(*ii*) It should give consistent results with identical bio-material.

(*iii*) As far as possible the time required for the end point readings should be reasonably small.

(*iv*) It should be sensitive enough to detect small changes in the insecticide tolerance of the insect population.

(*v*) It should be capable of measuring quantitatively fairly high degree of insecticide resistance in insects.

(*vi*) It should be least affected in its performance when used by different individuals.

(*vii*) With the same technique it should be possible to determine the susceptibility of the insect population to the common insecticides in use.

The technique used in the present studies meets most of the above mentioned requirements. It is, therefore, suggested that similar investigations are undertaken by other workers in different parts of the world to determine if the technique could be accepted as a standard method in due course.

From further experience of the use of the technique for bed-bugs in the field, it may be possible to make some improvements in it to increase its suitability. In this connection the following modifications are suggested for further exploration :—

(*i*) The petridishes used in the technique may be made of plastic material instead of glass to make them more acceptable for field use. Each petridish may have a hole in the centre for easy introduction and removal of insects.

(*ii*) In view of the very steep *lc-pr* lines obtained with gamma-B.H.C. and pyrethrins instead of the present high and widely spaced concentrations of the insecticides, low and narrowly spaced concentrations of gamma-B.H.C. and pyrethrins may be tried. Use of these concentrations may provide more than two points of partial mortality rates in insects for the *lc-pr* lines.

(*iii*) In the present studies control tests were performed on clean cloth pieces as the inert diluent powder was not supplied with the W.H.O. body lice kit. It is desirable that for proper comparison, control tests are carried out on cloth pieces treated with the diluent powder. Half gram packets of the diluent powder (without any insecticide) should be supplied in the kit along with the packets containing different insecticidal concentrations.

It has also been suggested that the equipment and procedures employed in a particular technique should be such that it could be used for several groups of insects (Ramachandra Rao, 1958). The W.H.O. test kit for body lice, which has been used in the present investigations, has been found quite suitable for use in the case of bed-bugs also. It is possible that the same technique could be used in the case of other insects also and for which detailed studies are indicated. A single kit for different groups of insects would be a distinct advantage to a worker in the field who can assess their insecticide susceptibility by employing the same procedures.

SUMMARY.

Results obtained on the susceptibility of bed-bugs (*Cimex hemipterus*) to D.D.T., gamma-B.H.C. and pyrethrins with the W.H.O. test kit for body lice have been discussed.

Requirements of a suitable field techique for measuring the insecticide susceptibility of insects have been stated.

The W.H.O. test kit for body lice was found quite suitable for use in the case of bed-bugs and it has been suggested that detailed investigations be undertaken by other workers in different parts of the world in order to determine if the technique could be accepted as a standard method in due course.

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Review

AEDES AEGYPTI, the yellow fever mosquito — its life history, bionomics and structure. By S.R. Christophers. (Published by the Cambridge University Press. *Price* 75s nett. pp. 740.)

This book deals with an insect of topical interest. *Aedes aegypti* has been widely used in laboratory investigations. Because of its ubiquitous distribution and its status as a vector of yellow fever, information on the distribution, habits and control of this mosquito is of great importance in public health practice, in framing quarantine regulations, chiefly those relating to air traffic.

The author, Sir Rickard Christophers, is one of the world-famous authorities on mosquitoes and the diseases they transmit. He spent many years in India and is one of the pioneer workers on Indian anophelines. He was one of the members of the Committee which came to India in the beginning of this century, closely following upon Ross's discovery of malaria transmission by mosquitoes, to investigate the implications of this discovery in the context of malaria control. He ended his thirty years' stay in India by producing a classic on Indian anophelines, which forms Vol. IV of the "*Fauna of British India*" series. This, as well as his other publications, constitute a mine of information and serve as excellent reference books for the students of medical entomology and tropical diseases. His writings bear the impression of his long and deep study based upon on a life dedicated to research. The reader gets a rare opportunity of learning to avoid the pit-falls of hazy thinking and hasty conclusions.

The book "*Aedes aegypti*" gives an exhaustive picture of all aspects of *Aedes aegypti* and is an up to date compilation on its synonyms, systematics, bionomics and genetics. In the author's words "such an account of an important and much studied mosquito like the *Aedes aegypti* may be of interest and useful as a work of reference to those engaged in work on the mosquitoes in the laboratory or in many connections in which *Aedes aegypti* has a place". The book is divided into thirty-one chapters dealing with Historical Review, Systematics, Bionomics, Morphology, Internal Anatomy and Medical Importance of this Insect.

The opening chapter deals with the History of *Aedes aegypti*. A very interesting and illuminating presentation on the name 'Mosquito' is followed by a review of early writings. (Here the author's attention may be drawn to the Sanskrit work 'MASAKAH', which very closely resembles 'mosquito', but to which no reference has been made). The detailed discussions on the 'mosquito' as a 'Natural History Object' and a brief summary of the early writings on the structure, systematics and the note on *Aedes aegypti* as a carrier of disease, apart from being of absorbing

interest, give an insight into the valuable information that the reader may expect in this voluminous compilation.

The second chapter, dealing with systematics, opens with the section on Synonymy. *Aedes aegypti* described by Linnaeus (1762) probably excels all other diptera in that it has 24 synonyms, the latest being *Duttonia albo annulis* (Ludlow, 1911). There is a wealth of information on the systematic position, identification, varieties, strains, hybrids and mutants as well as geographical distribution of this mosquito.

The next chapter dealing with Bionomics, apart from giving the life-history and breeding places, contains an exhaustive review of the natural enemies and ecto- and endo-parasites. This comprehensive treatment covers mosquitoes in general, besides *Aedes aegypti*.

Sir Rickard has given recognition to the supreme importance of *Aedes aegypti* in the sphere of international health and has devoted a separate chapter to this aspect. The role of this mosquito in the transmission of dengue, and as a carrier of certain other arbor-virus diseases of man and animals like lymphocytic choriomeningitis, Murray Valley encephalities, West Nile virus and Venezuelan equine encephalitis have also been discussed. This chapter has also got sections on the different aspects of control of *Aedes aegypti*, with special reference to the role of synthetic residual insecticides.

The chapter on 'Techniques' provides a fund of information, and records many of the author's personal experiences in the insectarium. Numerous inventions, adaptations and modifications have brought the techniques to a pitch of efficiency. Much stress has been laid on simplicity and easy improvisation. The author's succinct remarks with reference to *Aedes aegypti* "if more attention were given than is often the case to the use of standard material much of the ambiguities so often recorded with this species would be avoided" deserve special consideration, particularly because they are equally well applicable to any laboratory-bred mosquito used for special investigations.

There are two chapters on the Egg and Eclosion and a chapter on the Embryology. They deal with the structure of the egg, and the external and internal changes following oviposition, as also the complicated issues connected with the hatching of the mosquito egg. The studies on the embryology have been carried out in detail by the author himself on the eggs of *Culex molestus*, as he has found that "for various reasons the eggs of *Aedes aegypti* are not the most suitable form on which to study mosquito embryology". In his characteristic vivid manner the author has given a detailed and fascinating narrative of *in-vivo* studies on the development of the mosquito embryo from the time the egg is laid till it hatches and the first-instar larva wriggles out.

The six chapters that follow contain a complete study of the mosquito larvae. Commencing with a general description, the author goes on to a detailed account of the different parts and the chetotaxy of the fourth-instar larva. The different larval

instars are dealt with in detail, including their recognition and identification, ecdysis and chetotaxy. The impact of the environment on the larva is discussed at length with special emphasis on factors like the physics and chemistry of the medium and the morphology and physiology of the organism. The chapter on the structure of the larva gives a detailed account of the anatomy of the different systems of the mosquito larva.

Two chapters deal with the pupal stage of *Aedes aegypti*.

The remaining fifteen chapters are devoted to the Imago. Detailed description is given of the adult mosquito, its external characters, chetotaxy, changes in weight following emergence and other aspects The chapter on flight contains such details as flight range, mechanism of flight and frequency of wing stroke. There are chapters on growth, the special sense organs, on viability and a number of other related subjects including the action of insecticides. The last chapter contains a brief account of recent work on hormones, reserves and other physiological processes in mosquitoes.

By far the most interesting portion of the description of the internal structure of the mosquitoes, and perhaps the only one of its kind, is that dealing with the brain of the mosquito.

The author's profound knowledge of the subject places no hindrance in the way of his style being lively. He has presented the cream of his collective knowledge gained through decades of study and research. The fascinating tale which the book unfolds, subjects to a searching scrutiny an insect which has gained a vital importance in the international sphere of public health. Every chapter is followed by a subjectwise bibliography that, no matter what the special interest of the reader is in this subject, he will find reference to it here. Profuse illustrations add enormously to the value of this monumental volume of over 700 pages. The book is an invaluable addition on Medical Entomology to any library.

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SPECIAL NUMBER OF *INDIAN JOURNAL OF MALARIOLOGY* ON *W. BANCROFTI* AND *B. MALAYI*.

INTRODUCTION.

WORK on different aspects of *W. bancrofti* and *B. malayi* has been in progress in different parts of the world during the last decade and considerable advances have been made since. It is desirable to review the results of the studies carried out and to get together the experiences gained by workers in different theatres of the world. A written SYMPOSIUM on the subject was considered a most suitable forum for such a purpose. Such a SYMPOSIUM, it was felt, would facilitate a proper understanding of the present position and also help in planning the future research and control programmes.

Many of the workers engaged in the projects on filariasis both in the laboratory and in the field, in India as well as abroad, were invited to participate in this written SYMPOSIUM. The response to such an invitation has been spontaneous and has been most satisfactory. This SYMPOSIUM tries to present a cross section of the recent advances on the various aspects of the subject and problems faced by the research and public health workers in their efforts. It is expected that this will stimulate and materially assist research and control projects.

The contributions have been grouped under various heads, such as epidemiology, transmission, control, parasitology, entomology, pathology, immunity, technique, etc.

LIE KIAN JOE and colleagues record their observations about the epidemiology of *W. bancrofti* infection in Djakarta. They observe that filariasis in Djakarta is mainly a house infection but not a familial infection.

JORDAN discusses at length in his paper on the epidemiology of *Wuchereria bancrofti* in Africa, the epidemiological features, the vectors and vector-parasite relationship in bancroftian filariasis.

MORISHITA gives a detailed discussion of the epidemiology of filariasis in Central Japan. He presents a review of the earlier records of filariasis in this area with which he compares his present findings.

WHARTON has reviewed and discussed the records of Brugian and Wuchererian infections in Malaya and the vectors of these infections. He ends his paper with a classified list of the infections and their insect vectors.

SINGH has presented a detailed record regarding the epidemiological surveys carried out in Uttar Pradesh, India, during the last decade. Filarial endemicity due to *W. bancrofti*, which was originally believed to be confined to some of the eastern districts of the State, has now been found to be a more widely prevalent problem. According to the latest available estimates, based on surveys carried out

in the State, about half the population of the State (over 33 millions) is believed to be exposed to the risk of filariasis.

"A note on the distribution of Wuchererian infection in the Sudan and adjacent countries" by KIRK, presents the epidemiology of *W. bancrofti* in Sudan and adjacent countries. The author discusses its absence in wide areas in Central and North Sudan where several species of mosquitoes, known to be vectors of this infection, are widely present.

The importance of animal reservoirs, being a source of human infection and acting as a possible zoonosis, has been attracting the attention of workers in the field during the recent past. LAING in his "Review of recent research on filariasis in Malaya" presents an analysis of the interesting findings recorded in that country. Starting from the findings of POYNTON and HODGKIN in the Kra monkeys, LAING goes on to a detailed discussion of the recent records of Brugian infection in certain domestic animals. There has been a progress in the field of research and control of filariasis during the last two decades since the termination of the Second World War. There is still much to be known, particularly with regard to the pathology, immunology and serology of filariasis. The recent observations made in Malaya on the transmission of Brugian filariasis to the domestic cats, have opened up a new field of research. This may help in solving the mystery regarding the aetiology of tropical eosinophilia and lead to a better understanding of the epidemiology and pathogenicity of filarial diseases in man.

The importance of studies on the infectivity of the human reservoir to the mosquito hosts has been recognized and multifarious views have been expressed as to the possible existence of a sub-infective level of microfilaraemia, below which the vector will be unable to pick up the infection. This subject has assumed great importance in recent times, particularly in view of the significant reduction in the microfilarial infestations which follows the administration of diethylcarbamazine—a procedure which may be used under certain conditions in filaria control programmes. MUIRHEAD-THOMSON discusses at length the factors which require to be studied critically in assessing the infectivity of the filaria carriers.

BEYE and JOAN GURIAN present a discussion of the dynamics of transmission of *W. bancrofti* and *B. malayi*. The paper is divided into three parts which deal with (*a*) the life cycle of the two parasites, (*b*) the epidemiological factors involved in Wuchererian infection and disease, and (*c*) the host-parasite-vector relationship and the dynamics and transmission in filaria infection. The authors work out a model of dynamics and suggest that this may help planning investigations and aid better and comparative understanding of the epidemiology.

SASA and colleagues recount their experience in the control of *B. malayi* and *W. bancrofti* infections in Japan. Use of diethylcarbamazine therapy for the control of the former has limitations because of severe reaction. Anti-mosquito measures, by use of DDT and the use of larvicidal fish over a period of 10 years (1950-60),

have been followed by very gratifying results with a fall in microfilaria rate from 31·5 to 7 per cent. The authors report a total disappearance of the acute clinical attacks. Comparative studies were made on the efficacy of different combinations of drug administration, anti-adult and antilarval measures for interception of transmission of *W. bancrofti*. The disadvantages of selective treatment of microfilaria carriers for the control of *W. bancrofti* have been discussed in detail. In conclusion, the authors recommend "treatment of all inhabitants rather than selected positives." In the opinion of these workers, environmental sanitation and elimination of man-made breeding places were the most efficient method for the control of *C. pipiens*.

RAMAKRISHNAN and colleagues have presented a critical review of the progress of the National Filaria Control Programme in India from 1955-1959. Filariasis, particularly due to *W. bancrofti*, is a major public health problem in this country and according to the latest available data about 64 million people are estimated to be residing in the filarious areas of the country. The programme, commenced in 1955, aimed at affording protection to about 13 million people distributed in different parts of the country, on an experimental basis. The methodology adopted included anti-adult operations by use of indoor residual sprays, mass administration of diethylcarbamazine and larval control operations on a limited scale. The review brings out the futility of dieldrin in intercepting transmission of bancroftian filariasis and also the limitations in the use of diethylcarbamazine on a mass scale. Anti-larval measures appeared to be the only practical approach. These measures, which have been so far restricted only to urban areas, are now being extended to rural areas on an experimental scale.

GONSALVES has presented a review of mass therapy and follow-up surveys carried out under one of the control units of the National Filaria Control Programme in India. Follow-up data, collected over a period of two years after mass drug administration, reveal that the drop in microfilarial rate, which followed the drug administration, showed a gradual rise during the follow-up. The 'microfilarial density' in the treated community was, however, considerably lower than in the untreated community.

The increasing interest evinced by the various international organisations towards the ultimate objective of the "control and eradication" of *W. bancrofti* and *B. malayi* has been detailed by HENRY K. BEYE in his article entitled "International organisations and filariasis control". The author stresses the importance of sound epidemiological basic information for individual areas before commencement of control measures.

JOHN F. KESSEL, who has been directing the control of filariasis in the Pacific Islands under the Pacific Disease Control Project, is one of the few who could speak with authority on the epidemiology and control of non-periodic bancroftian infection. The genesis of the non-periodic type of *W. bancrofti* is discussed by this author who points to the possibility of its having resulted due to a natural selection consequent upon prevalence of the day-biting aedine vectors. The

epidemiology, transmission and the details of the control projects in operation in Tahiti, have been presented in detail.

ABDULCADER and PADLEY present a review of the activities of the antifilariasis campaign in Ceylon ever since its inception. The difficulties in the control methods presently being experienced, especially in the administration of diethylcarbamazine, have been narrated. The details of the procedures, adopted in detecting cases and following them up with the use of card index system, are presented.

RAMAKRISHNAN *et al.* present the details of laboratory and field investigations on the evaluation of a number of organophosphorus compounds as larvicides and adulticides for the control of *Culex fatigans*. Of the six compounds tested, 'Baytex' appeared to show some promise for larvicidal and imagocidal measures.

The causes underlying the phenomenon of alternate increase and decrease in the number of microfilariae in the peripheral circulation—a fundamental part of the biology of some species of filarial worms—has been a matter of speculation by earlier workers. This subject of microfilarial periodicity has been widely studied during the last decade. HAWKING gives an interesting narration of the present knowledge of this subject and concludes that periodicity is a response to some stimulus provided by changes in the host. The exact nature of this stimulus remains to be identified.

ALLEN SCOTT presents a discussion of classification of the immunity in Wuchererian infection. It is known that there has been a considerable amount of speculation in literature regarding the possibility of immunity in filarial infections. There are few facts either for or against the existence of such immunity. The lack of evidence for its presence does not and should not argue against the existence of immunity. After weighing the various factors, the author concludes that "a certain amount of immunity does exist but both the degree and nature of this immunity are at present unknown."

The importance of proper identification of the causative organism in its various stages of development cannot be over-emphasised. In the investigation of vector-borne diseases, the question, "can the organisms under investigation be correctly identified in their intermediate hosts ?" poses itself. NELSON presents a summary of the methods used for the identification of filarial larvae in their vectors. The article includes details of techniques for immediate and delayed discussions.

In a brief preliminary note, BURTON reports his finding the stages of development of filarial larvae in the bed-bug in South India. The finding is of interest. What the epidemiological significance of the finding is going to be, only further investigations can reveal.

Studies on the bionomics of the vectors of *W. bancrofti* and *B. malayi* have been reported by PAL *et al.* The investigations, which were carried out at

Ernakulam in South India, deal with *C. fatigans*, the main vector of *W. bancrofti* and *Mansonioides* spp. which are responsible for the transmission of *B. malayi* in the same area.

Observations on the resting habits of *Culex fatigans* have been recorded by WATTAL and KALRA. The details presented include data collected over a period of nearly two years in a rural area in Uttar Pradesh, India, before and after D.D.T.-spraying and also from outdoor and indoor catching stations.

COORAY's paper on the tissue responses in filarial infection gives an analysis of 48 cases studied over a period of 13 years (1947 to 1960). The tissue responses, and the pathological changes in the cases where filarial worms or remnants of such worms were found, are described. All cases in which worms, living, dead or calcified, or where microfilariae were present, have been studied.

Great interest has been shown by workers in the aetiology of tropical eosinophilia. During recent years, the possible role of helminthic infections in the causation of this disease has attracted many workers' attention. GAULT and colleagues discuss their clinico-pathological investigations of material from cases of tropical eosinophilia. The investigations include material obtained from liver biopsies, lung biopsies and lymph nodes, and observed microfilariae in some cases.

The relationship between lymphangitis and filarial infection has provoked a great deal of discussion in the past. Numerous authors have supported the filarial aetiology of this disease while many have equally vehemently supported its microbial origin. MONTESTRUC *et al.* in a very interesting discussion of "Endemic lymphangitis in French Guiana and its relation to bancroftian filariasis" have presented an analysis of the arguments in favour of these two theories as also a combination of both. Arguments based on geographical, parasitological, clinical, biological as well as therapeutic grounds, have been presented. It would appear that both the pathogenic agents, namely filarial and streptococcal infections, either singly or in combination, can give rise to the identical clinical condition. Streptococci probably played an exciting and dominant role in this condition.

The industrial and medical problems relating to filariasis in British Guiana, have been presented by GIGLIOLI and BEADNELL. After discussing the epidemiology and the effect of environmental sanitation on the incidence of the disease, the authors present an interesting analysis and discuss the impact of filarial disease on the Workmen's Compensation Act. The intricacy of the problem, posed by microfilaraemia and clinical signs of filariasis in assessing the fitness of workers, and the possible role of therapy in this context, have been gone into in detail.

The only contribution on the subject of technique in the filaria laboratory comes from MENON in India. The author describes a simple method to make permanent stained preparations of the stages of development of filaria worm in the mosquito.

B. malayi infection is restricted in its distribution in India and by far the largest focus, with about two million at risk, is located in the coastal tracts of

Kerala State. JOSEPH and co-workers have reviewed their experience in the control of *B. malayi* through measures directed against the vectors both by insecticidal application and by Pistia clearance. The advantages and disadvantages of these measures by themselves, and in combination with the adjuvant administration of diethylcarbamazine, have been presented in detail.

Filariasis remains a challenge to many parts of the tropical world. Control of the disease, and the prevention of its further spread, offer fascinating problems of research both fundamental as well as applied. It is hoped that this SYMPOSIUM will stimulate further research and readjustments in our approach to the problem.

—*Editor.*

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WUCHERERIA BANCROFTI INFECTION IN DJAKARTA, INDONESIA. A STUDY OF SOME FACTORS INFLUENCING ITS TRANSMISSION.

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WUCHERERIA BANCROFTI has been known to occur in Djakarta, the capital of Indonesia, since a long time. In 1921, Flu examined the night blood of 895 native people, living in different areas of Djakarta and found 91 persons positive for microfilaria *bancrofti* (Flu, 1921). However, no one has seemed interested in extending Flu's work, probably because the most disfiguring and disabling feature of the disease, elephantiasis, is very rare in Djakarta.

Since 1956, surveys have been carried out in different areas of the city. Some of the results have been published (Lie Kian Joe *et al.*, 1958 ; 1959*a* ; 1959*b* ; 1960 ; Chow *et al.*, 1959). The purpose of the present communication is to report on studies of some factors influencing the transmission of the parasite.

DESCRIPTION OF THE AREA.

The site chosen for these observations was located in the Rawasari District. A description of the area and its population was given in a previous paper (Lie Kian Joe *et al.*, 1958). Periodic *W. bancrofti* is found endemic in this district, with an average microfilarial rate of 7·8 per cent. The frequency in different localities of the district varied from 1 per cent to 23 per cent. The youngest child born in the district and harbouring microfilariae was 3 years old. Elephantiasis was not found in this area, but hydrocele was common, being found in 14 out of 92 adult persons examined.

A locality, more or less isolated and situated in this Rawasari District, was chosen for the present series of observations. This locality is large with an area of one kilometre square. It is on the fringe of the Djakarta City and is of a rural type, consisting of 56 bamboo houses, which are more or less evenly scattered throughout the area. In the garden between the houses, fruit trees and other

plants are grown. Map 1 shows the location of the houses, and Figure 1 shows the type of the houses found in this area. The people living in these houses are not protected from mosquito bites. The locality is surrounded by grass-land and paddy fields and there is communication with a neighbouring inhabited area in one section only. There is in this area no electricity or running water. The water supply consists of wells, dug in the ground near the houses.

The observations were made from May, 1959 to the end of April, 1960. The monthly rainfall, average relative humidity and mean temperatures in this period are shown in Table I. The temperature ranged usually from 22°C. to 32°C. It was somewhat lower or higher from time to time.

TABLE I.

Climatological data of Djakarta from May, 1959 until April, 1960.

Month.	Average relative humidity (per cent).	Mean temp. (°C.).	Total rainfall (mm.).
May	83	27·5	154·8
June	82	27·2	63·6
July	82	26·4	106·6
August	73	26·3	1·0
September	73	27·7	13·1
October	74	28·0	92·0
November	80	27·5	140·9
December	82	26·8	195·1
January	87	25·7	440·9
February	88	25·8	542·8
March	82	26·2	161·8
April	85	27·0	286·5

PARASITOLOGICAL FINDINGS.

A blood survey was carried out in June, 1959. Night blood samples were collected from 196 out of 211 persons living in this locality. From each person 20 c.mm. of blood were taken. The group of persons from whom no blood samples were obtained, was composed of 13 infants below the age of one year, and two other persons one of whom refused to be bled while the other was an invalid having lost both arms.

There were 32 persons showing microfilariae *bancrofti* in the blood, i.e., a frequency of 16·3 per cent (Table II). The average number of microfilariae in 20 c.mm. of blood per positive person was 33·6 and the microfilarial density, that is the average number of microfilariae on the total population, was 5·5 (Table III). There was no significant difference in frequency between males (16·8 per cent.) and females (15·8 per cent). There were 41 persons who came from other places to live in this area. These new comers had lived in this locality for a period varying from 2 months to 5 years at the time of the blood survey. Among these new comers, two were positive. These two persons had lived in the area respectively for five years and two months. It is evident that the second person had acquired the infection in the place where he came from. The remaining 155 persons were born

MAP 1.

Location of houses.

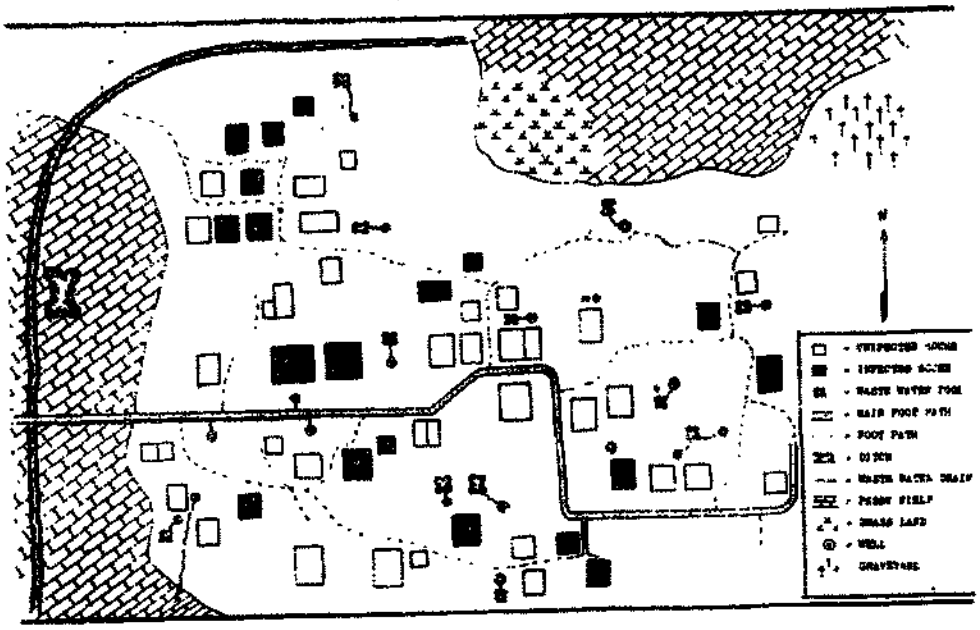
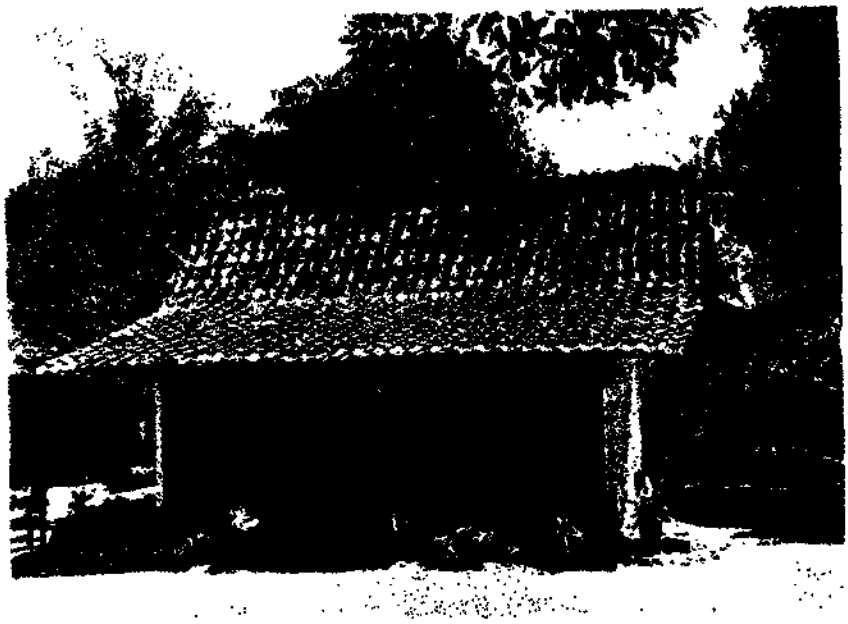


FIGURE 1.

A house in the area examined, with open veranda, the middle portion is a bed room, behind is the kitchen and surrounded by a garden.



in this locality and 30 among them were positive (19·4 per cent). The youngest person found infected was 3 years old.

TABLE II.

Results of blood survey according to age and sex.

Age (years).	Sex.	Persons examined.	Positive: Number.	Per cent.
1–5	M	26	..	..
	F	15	1	7
6–10	M	13	2	15
	F	13	2	15
11–30	M	37	9	24
	F	47	11	23
31–50	M	19	4	21
	F	15	1	7
50 and over	M	6	2	33
	F	5	..	..
Totals	M	101	17	17
	F	95	15	16
Totals	M+F	196	32	16·3

M = male. F = female.

TABLE III.

Results of blood survey according to number of microfilariae in 20 c.mm. of blood.

Microfilarial counts.	Number of persons examined.	Total microfilariae.
0	164	–
1—5	10	31
6—10	3	27
11—30	8	164
31—50	6	246
51—100	3	188
100 and over	2	420
Total persons positive	32	1076
Total persons examined	196	
Average infection		33·6
Microfilarial density		5·5

Attempts were made to carry out a second blood survey in April 1960, but without much success since many refused to be bled again. Only 160 blood samples could be obtained and microfilaria *bancrofti* was found in 21 samples. There were two persons who were positive in the first survey and who showed no microfilariae in the second survey. Two other persons were negative in the first survey but were positive at the second examination. It is likely that the helminths found in

the dissected mosquitoes were *W. bancrofti* larvae. The morphology of the larvae and the development in the thorax of the mosquitoes support this view. Moreover, it is well known that *Culex pipiens fatigans* is an anthropophilic species. In addition, there were not many domestic animals in this area : a few cats, two goats, no dogs, and a few chickens.

ENTOMOLOGICAL FINDINGS.

It is known from previous studies (Chow *et al.*, 1958 ; Lie Kian Joe *et al.*, 1959) that *Culex pipiens fatigans* is the only vector for *W. bancrofti* in Djakarta.

In this study, mosquitoes were regularly caught with a sucking tube in the houses in the morning hours and brought back carefully to the laboratory. They were dissected in the morning and in the afternoon of the day of capture. The method of dissection was described in a previous paper (Chow *et al.*, 1958). From May until December, 1959, the dissected mosquitoes were also stained with Giemsa in order to facilitate counting of the *Wuchereria* larvae present. Of each mosquito dissected, the stomach content, the stage of gonotrophic cycle, and in a number of mosquitoes the presence of follicular relics, were also determined. Each house in the locality was examined at least once a month, except in the month of April which was a fasting month.

MOSQUITOES HARBOURING FILARIAL LARVAE.

More than 99 per cent of the mosquitoes caught in the houses were *Culex pipiens fatigans*. They were found infected throughout the year. Of a total of 17,247 *C. p. fatigans* dissected during the year, 1,213 mosquitoes harboured only exsheathed microfilariae and 608 harboured all stages (Stage I to III, including infective stage) larvae. There were 374 mosquitoes containing Stage I larvae, 122 mosquitoes containing Stage II larvae and 92 mosquitoes containing Stage III larvae. Motile infective larvae were found in 20 mosquitoes. Table IV shows the monthly distribution of the positive mosquitoes. Mosquitoes harbouring different stages of larvae are included in the group of the most advanced larval stage.

The other species of mosquitoes caught were composed of 28 *Anopheles* and 62 *Culicine* mosquitoes. They were also dissected and were negative, except for one *Taeniorhynchus* (*Mansonioides*) *annulatus* with exsheathed microfilariae.

DENSITY OF FILARIAL LARVAE IN *C.P. FATIGANS*.

A total of 13,227 exsheathed microfilariae were found; 8,130 were found in the thorax and 4,998 in the abdomen. The average number of exsheathed larvae found in each positive mosquito was therefore 10·8.

The total number of Stage I larvae found was 2,612. The average number of Stage I larvae per positive mosquito was 2·1.

The total number of Stage II larvae found was 823. The average number of Stage II larvae per positive mosquito was 0·7.

TABLE IV.

Results of dissections of Culex pipiens fatigans *caught in the houses from May, 1959 to April, 1960.*

Month.	Number of mosquitoes.	Number positive for filarial larvae:					Total positive.
		Exsheathed microfilariae.	Stage I.	Stage II.	Stage III.	Infective stage.	
May	1,137	59	18	7	6	..	90
June	1,307	131	18	9	5	1	164
July	1,502	80	33	4	6	..	123
August	1,418	111	53	6	5	..	175
September	1,178	90	45	11	6	3	155
October	1,444	138	44	10	12	..	204
November	1,012	123	58	15	10	1	207
December	1,691	159	45	21	14	4	243
January	1,725	112	20	13	11	1	157
February	2,351	109	27	15	12	9	172
March	1,197	84	8	9	5	1	107
April	385	17	5	2	..	..	24
Total	17,247	1,213	374	122	92	20	1,821
Percentage		7·0	2·2	0·7	0·5	0·1	10·6

The total number of Stage III larvae, including the infective stage, was 585. The average number per positive was therefore 0·5. The number of active infective stage larvae only was 70, giving an average of 0·06 larva per positive mosquito.

FREQUENCY DISTRIBUTION OF FILARIAL LARVAE IN *C. P. FATIGANS.*

Among 1,213 mosquitoes harbouring only exsheathed microfilariae in the thorax, the frequency distribution of the number of microfilariae is shown in Table V. The exsheathed microfilaria found in the abdomen were not included in Table V since many of them would probably not reach the thorax. Experiments with the Djakarta strain of *C. p. fatigans* have shown that part of the ingested larvae remain in the stomach where they ultimately die.

TABLE V.

Frequency distribution of the number of microfilariae.

Number of exsheathed microfilariae.	Number of mosquitoes.
1	260
2	234
3	154
4	111
5	70
5–10	193
11–15	88
16–20	42
21–25	22
26–69	39

The frequency distribution of the number of larvae of all stages, including the infective stage larvae, among 608 mosquitoes, is shown in Table VI.

TABLE VI.

Frequency distribution of the number of Wuchereria *larvae (all stages).*

Number of larvae.	Number of mosquitoes.
1	100
2	94
3	74
4	56
5	61
6–10	105
11–15	63
16–20	25
21–25	11
26–30	19

Among 20 mosquitoes harbouring infective stage larvae, the frequency distribution of the number of larvae is shown in Table VII.

TABLE VII.

Frequency distribution of the number of infective stage larvae.

Number of infective larvae.	Number of mosquitoes.
1	6
2	3
3	2
4	2
5	1
8	2
9	1
12	1
13	1
15	1

The distributions of infective stage larvae in different parts of the mosquito body is shown in Table VIII.

TABLE VIII.

Distribution of infective stage larvae in different parts of the mosquito.

Site of the body.	Number of mosquitoes.
Thorax ...	13
Thorax and abdomen ...	4
Abdomen ...	1
Head and abdomen ...	1
Head, thorax and abdomen ...	1

BREEDING PLACES OF *C. P. FATIGANS.*

The main breeding places in this locality were the waste water pools, which were present near every well. They were suitable breeding places in which breeding was present throughout the year. In some of them breeding was very heavy,

yielding many egg rafts and hundreds of larvae per dipper of 10 cm. diameter. In some other pools breeding was variable, periods of heavy breeding being followed by moderate or scanty breeding. Again, in some pools breeding was never heavy. We do not know the factors responsible for these differences in breeding density. In addition to these permanent breeding places there were temporary ones such as discarded receptacles, chocked drains, road gullies, coconut shells and other water collections. Many of these temporary breeding places became dry during the dry season or were flushed away with heavy rainfall. These temporary breeding places were usually not numerous and were less important than the waste water pools. The wells, which had a water table of several meters below the ground surface, were free from breeding.

The density of *C.p. fatigans* in this locality was high throughout the year. We did not try to measure the density in this study, but the average number of mosquitoes caught each time in each house was 24. This number did not represent the actual average number of the mosquitoes in the houses, since no attempts were made to catch them all.

DISTRIBUTION OF INFECTED *C.P. FATIGANS.*

The 32 human carriers detected during the blood survey lived in 21 houses. These positive houses are indicated on Map 1. The remaining 35 houses were inhabited by negative persons.

C.p. fatigans, harbouring exsheathed microfilariae, were found both in the negative and positive houses. They were found much more frequent in the positive houses (Table IX). In the negative houses they were found only now and then and in small numbers, while in the positive houses they were found regularly and often in large numbers.

TABLE IX.

Results of dissections of C.p. fatigans *caught in positive and negative houses.*

Houses.	Number of mosquitoes.	Total positive.	NUMBER POSITIVE FOR FILARIAL LARVAE:				
			Exsheathed microfilariae.	Stage I.	Stage II.	Stage III.	Infective stage.
Positive houses.	8,418	1,390	1,063	216	60	43	8
Negative houses.	8,829	431	150	158	62	49	12
Total	17,247	1,821	1,213	374	122	92	20

There were found 216 *C.p. fatigans* harbouring Stage I larvae in the group of positive houses. It was a relatively small number when compared with the mosquitoes harbouring exsheathed microfilariae collected from the same group of houses. In the negative houses 158 *C.p. fatigans,* harbouring Stage I larvae, were found. This is a relatively large number in comparison with the group of

mosquitoes harbouring exsheathed microfilariae and collected from the same group of houses.

Mosquitoes harbouring Stage II, Stage III and infective stage larvae were small in numbers and they were found in about equal numbers in both groups of houses. These mosquitoes were found distributed at random among different houses throughout the area. They were found in equal numbers both in negative houses situated rather far from the positive houses as well as in houses where carriers lived.

PRESENCE OF FOLLICULAR RELICS.

Follicular relics (corpora lutea) were examined in 2,025 *C.p. fatigans* during the period from September, 1959 through April, 1960. There were 268 (13·2 per cent) mosquitoes with one follicular relic and 32 (1·6 per cent) with two follicular relics while one mosquito only was found to harbour three follicular relics. Most of the mosquitoes, occurring in this area during the period of study, were therefore nulliparous. Less than 15 per cent were parous and a few had laid eggs twice or more. The monthly frequency distribution of the mosquitoes, showing follicular relics, is shown in Table X.

TABLE X.

Follicular relics in C. p. fatigans *caught during September, 1959 to April, 1960.*

Month.	Number of mosquitoes examined.	Number of follicular relics.			
		0	1	2	3
September	245	229	14	2	..
October	313	284	25	4	..
November	181	161	16	4	..
December	302	254	48	..	..
January	320	250	62	8	..
February	222	175	37	9	1
March	308	266	39	3	..
April	134	105	27	2	..
Total	2,025	1,724	268	32	1

Out of 17 mosquitoes harbouring Stage II larvae, seven had one follicular relic and ten none. Out of ten mosquitoes harbouring Stage III larvae, four had one follicular relic, one had two and five had none. Out of three mosquitoes harbouring infective stage larvae, one had two follicular relics, one had one and the third one had none.

STAGES OF EGG DEVELOPMENT.

The changes in the ovarioles during oogenesis are divided into six arbitrary phases, *viz.*, Stage I the initial quiescent phase, Stage II starting with the appearance of granules of yolk, Stage III starting when the yolk granules become dense, Stage IV when the yolk granules occupy a large part of the somewhat elongated follicle, Stage V when the follicle is markedly elongated and Stage VI when the egg is fully

formed. In Table XI is shown the frequency distribution of these stages, divided in two groups : the group of mosquitoes harbouring filarial larvae and the group of uninfected mosquitoes. In the latter group, Stage I showed the largest number of mosquitoes, followed by a gradual decrease in subsequent stages with a rather sharp decline in Stage IV and V. This pattern shows that *C. p. fatigans* probably completes its gonotrophic cycle mainly indoors and that they leave the house when the egg development has reached Stage IV and V. Stage I in the group of the infected mosquitoes was relatively small in number, because all the mosquitoes in this group had taken blood. It is, however, difficult to explain why Stage II in this group of mosquitoes was smaller in number than Stage III. In Table XI is also shown the number of mosquitoes with or without visible blood in the abdomen.

TABLE XI.

Stages of the gonotrophic cycle of the dissected mosquitoes.

	Number of mosquitoes examined.	Blood in abdomen.	No blood in abdomen.	EGG DEVELOPMENT :					
				1	2	3	4	5	6
Infected mosquitoes	1,828	1,519	309	376	386	448	305	178	75
Uninfected mosquitoes	15,418	9,662	5,756	4,234	3,566	3,144	2,495	1,467	512
Totals	17,246	11,181	6,065	4,610	3,952	3,592	2,860	1,645	587

DISCUSSION.

It is striking that about 85 per cent of the mosquitoes dissected were nulliparous and 1·6 per cent only showed more than one follicular relic. It suggests that *C. p. fatigans*, as it occurs in the Rawasari District, is short-lived and that many mosquitoes die between the first and second gonotrophic cycle. Apparently, they do not die in large numbers shortly after feeding since many half gravid and gravid mosquitoes were found resting in the houses. It is, therefore, assumed that most deaths occur after the mosquitoes have left the houses to lay their eggs. Egg rafts and larvae were found in large numbers in the breeding places throughout the period of study. We assume that most of the mosquitoes do lay their eggs but they do not return to the houses, which form their only resting place. *C. p. fatigans* had never been found resting outside the houses despite a careful search made in the study area. It is, therefore, deduced that most of the mosquitoes die outside the houses after egg-laying, resulting in a low number of parous mosquitoes. Decrease in the number of the infected mosquitoes, with subsequent development of the filarial larvae, occurs most markedly when the larvae are in Stage I and Stage II and also when infective stage larvae are formed (Table IX). This pattern of reduction fits well with the high mortality of the mosquitoes occurring outside the houses. Most of the infected mosquitoes leave the houses to lay eggs when

the filarial larvae are in Stage I and II, and when they survive they lay the second batch of eggs when the larvae have become infective.

There are undoubtedly many factors responsible for this high mortality and it is difficult to say at present what these factors are. Fluctuations in humidity are probably one of the factors. Follicular relics were found most frequently in the months with heavy rainfall and high relative humidity. In the months of January, February and April, with an average relative humidity of more than 85 per cent, about 22 per cent of the dissected mosquitoes were parous. In the months of September and October, with an average relative humidity of less than 80 per cent, only 8 per cent of the mosquitoes were parous. The month of February, with the highest relative humidity, showed the highest number of mosquitoes with two or three follicular relics (Table X).

Another factor which may cause death of mosquitoes is the presence of *Wuchereria* larvae. Naturally, this factor, if operating in nature, would affect only the infected mosquitoes. It would also affect mosquitoes within rather than outside the houses. Therefore, it would not contribute much to the many deaths occurring among the mosquitoes leaving the houses to lay eggs.

From experiments, which will be published later, we know that the Djakarta strain of *C. p. fatigans* show an increased mortality with a large uptake of microfilariae. Many deaths are observed on the first day after blood meal which is associated with migration of the microfilariae from the midgut to the thorax and on the twelfth day which is associated with migration of the newly formed infective stage larvae. Migration of more than 25 microfilariae at a time, or of more than 12 infective stage larvae, is associated with a high mortality.

The uptake of microfilariae by the mosquito is dependent on the filarial density among the population; the higher the density the larger the uptake and consequently the higher the mortality.

Is the filarial density in the area examined high enough to cause deaths among the infected mosquitoes? We think it is so although we do not think that the mortality is great, since most of the mosquitoes harbour a few larvae only. More than 84 per cent of the mosquitoes, harbouring exsheathed microfilariae, had 10 or less worms in the thorax and more than 80 per cent had 10 or less developing and infective stage larvae (Tables V and VI).

A higher microfilarial density than is found among the population in the Rawasari District, is needed in order to kill a large part of the infected mosquitoes. In this connection it should be mentioned that not all microfilariae, taken up by *C. p. fatigans*, will migrate to the thorax. Experiments, which will be published later, have shown that a fairly large part of the ingested microfilariae may remain in the midgut where they ultimately die. They are probably caught by the clotting of the blood which has the effect of a barrier to protect the mosquito.

It should be added that not all strains of *C. p. fatigans* show an increased mortality with a large uptake of microfilariae. A strain of *C. p. fatigans*, kindly

sent to us from Kuantan, Malaya, by Dr. R.H. Wharton, shows a high resistance towards infection with the Djakarta strain of *W. bancrofti*.

It is important to know that the *C. p. fatigans*, caught in the Rawasari District, is short-lived. *C. p. fatigans*, caught in other areas of Djakarta, show the same feature; they all are short-lived. This is the main reason why infective stage larvae in *C. p. fatigans* is Djakarta are seldom found. Twelve days are required for the completion of the larval development in the mosquito in Djakarta and most of the *C. p. fatigans* do not survive that long. Out of 17,246 mosquitoes dissected in this study, only 20 (0·1 per cent) harboured infective stage larvae. Chow *et al.* (1958), working in the same district in 1956, found an incidence of 0·3 per cent, while Lie Kian Joe *et al.* (1959), working in 1957 in Mampang-Perapatan District which is the highest endemic part of Djakarta, found an incidence of 1 per cent.

Since the proportion of mosquitoes harbouring infective stage larvae is small, the chance of acquiring an infection in Djakarta is also small. In addition, the presence of infective stage larvae in a mosquito does not mean that it will infect man. From experiments in the laboratory, it is known that *C. p. fatigans* feed about every five days. Although it harbours infective stage larvae it may die before it feeds. This is likely in an area with such a high mortality rate among the mosquitoes. Moreover, the mosquito may lose the larvae before biting man. Loss of small numbers of infective stage larvae from *C. p. fatigans* has been observed by several workers and by us in our experiments. Even when a man is bitten by a mosquito harbouring infective stage larvae, it is not sure that infection will take place. It depends on the location of the larvae in the mosquito and the duration of the blood meal. When the larvae are situated in the abdomen or thorax and the feeding lasts for a short time due to interruption or other causes, infection is likely not to take place. Even when the larvae have been able to emerge from the proboscis, there is still a possibility that they cannot penetrate into the skin. Added to it that many of the infected *C. p. fatigans* harbour only one infective larva, it is evident that many of the persons living in these endemic areas do not show microfilariae in the blood until they have been resident for many years. The youngest child, showing microfilariae in the Rawasari District, is 3 years old, but it is evident from this and previous surveys (Lie Kian Joe *et al.*, 1958; 1959*a*) that many escape infection until a much later age. It is likely that some may never have shown microfilariae in the blood although they have been living for their life in this area. Despite this low transmission rate, there is in Djakarta a moderate endemicity. This is achieved because the adult *W. bancrofti* can live in the human body for many years. There were six persons from the area examined who showed microfilariae in the blood for at least four years. They were positive at the survey carried out in 1956 (Lie Kian Joe *et al.*, 1958) and again in this study in July, 1959 and April, 1960. Once a person is positive for microfilaria *W. bancrofti*, he remains positive for many years and under such circumstances a low transmission rate may ultimately result in a moderate endemicity.

Another important factor in the transmission of *W. bancrofti* in Djakarta is the density of the vector. The density of *C. p. fatigans* in Djakarta is extremely high. With such a short-lived vector, transmission is possible because of the high density. Endemicity in Djakarta is therefore maintained by the high density of the vector. Another important point is the question whether the vector is anthrophilic or zoophilic. We did not have opportunity to carry out precipitin tests. *C. p. fatigans* is, however, a well known anthropophilic species.

Elephantiasis is very seldom seen in Djakarta. In our surveys carried out in different areas of Djakarta, not a single case of elephantiasis was found. Whether the absence of elephantiasis is related to the low transmission rate, is not known. It would be interesting to study this point further.

From catching experiments, using human baits, we know that *C. p. fatigans* bite not only within or next to the houses, but also in the village garden fairly far from the houses. They do not occur outside the village area such as in the grass-field or paddy-field surrounding the village area. The whole village area is the operational field of *C. p. fatigans*. Infection may therefore be acquired in any place within the village area. However, we assume that infections occur most likely while the people are asleep, that is within the houses. Persons who are awake, get usually disturbed when bitten by a mosquito. Consequently, feeding is usually interrupted and infection is less likely to take place. We, therefore, think that filariasis in Djakarta is mainly a domestic infection.

The infected mosquitoes were found both in the negative and positive houses. Naturally, mosquitoes, harbouring exsheathed microfilariae, were found in much larger numbers in the positive houses. A few only were found in the negative houses. These were probably mosquitoes which had bitten carriers coming as visitors to these houses or mosquitoes which came into these houses for shelter after having bitten carriers in the garden. After egg laying, the mosquitoes which survive return to the houses. Do they return to the same houses where they came from? Apparently, they do not. Mosquitoes containing Stage II, III and infective-stage larvae were about evenly distributed in both the negative and positive houses. In other words, transmission occurs with the same intensity in the negative as well as in the positive houses. Filariasis in Djakarta is, therefore, not a family infection. Each person living in the endemic area has the same chance of acquiring an infection, no matter whether he lives in a positive or in a negative house.

Experiments, which will be published later, have shown that a colony of *C. p. fatigans* start laying eggs within four days after a full blood meal and many of the egg rafts are laid subsequently in the following days, but some mosquitoes may retain the eggs for a considerable period. Moreover, a small feed results in a retarded development of the eggs and the gonotrophic cycle may stop at Stage II or III. A second feed is needed for further development of the eggs. These phenomena, observed in the laboratory, occur probably also in nature. They occur probably not infrequently since out of 30 *C. p. fatigans*, harbouring Stage II, III and infective stage larvae, 16 showed no follicular relics.

SUMMARY.

Observations made on the human population and the vector mosquito in a small area of Djakarta where periodic *Wuchereria bancrofti* is endemic, are described. It was found that the microfilarial rate in man was 16·3 per cent, and that the average number of microfilariae in 20 c.mm. of blood per positive person was 33·6. A total of 17,247 *Culex pipiens fatigans*, the only vector in the area, were dissected; seven per cent harboured exsheathed larvae, 3·5 per cent harboured *Wuchereria* larvae (all stages included infective stage larvae), while 0·1 per cent only harboured infective stage larvae. The transmission rate of *W. bancrofti* in Djakarta is low. This is because *C. p. fatigans* in Djakarta is short-lived. About 85 per cent of the dissected *C. p. fatigans* were nulliparous and 1·6 per cent only showed more than one follicular relic (corpus luteum). It is deduced that many deaths occur among the mosquitoes which leave the houses for egg laying. Humidity is one of the factors influencing this mortality. With such a short-lived vector, transmission of *W. bancrofti* in Djakarta is possible only because of the high density of the mosquito. Filariasis in Djakarta is mainly a domestic infection. It is, however, not a family infection.

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EPIDEMIOLOGY OF *WUCHERERIA BANCROFTI* IN AFRICA.

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INTRODUCTION.

CLINICAL or parasitological evidence of Bancroftian filariasis has been reported from nearly every country between the North African coast and Southern Rhodesia, and Stoll (1947) has calculated that 22 million people are infected. In spite of this, detailed research into *Wuchereria bancrofti* infestation has been carried out in only a few countries and there are many gaps in our knowledge of the epidemiology of the conditions.

Owing to the vast area in Africa in which Bancroftiasis is endemic and the extremes of topography and climate which are found therein it is unlikely that epidemiological data from any single country is truly representative of the whole area. The general pattern of host-parasite relationships, as assessed by studies of microfilaraemia, appear to be similar in different areas, though differences in the effect of the infection, as judged by the incidence of clinical manifestations, are apparent from the literature.

Vector parasite relationships would appear to vary in different parts of the continent, but how much this is due to different strains of *W. bancrofti* and how much due to different vector strains, it is impossible to tell.

DISTRIBUTION OF *W. BANCROFTI.*

The distribution of Bancroftian filariasis in Africa has been described by Hawking (1957). Although the incidence of microfilaraemia in parts of many countries has been determined, in some territories there are only reports of clinical manifestations and sometimes isolated cases of filariasis are reported.

The incidence of microfilaraemia in different countries is indicated in Table I.

In addition to the reports given in tabulated form in Table I, Decrop and Stiers (1925) reported a case of filariasis in Tangier, as did Astolfi (1936) in Morocco and Luders (1937) in Abyssinia. Sharp (1928) found the infection to be of little importance or absent at Mamfe in the British Cameroons, and in Northern Rhodesia (Buckley, 1946), and Southern Rhodesia (Public Health Report, 1948) the infection is of little importance. Woodman and Bokhari (1941) found a low incidence in parts of the Sudan, and Hawking (1940) reported the infection in parts of Uganda.

TABLE I.

Incidence of Bancroftian microfilaraemia in different parts of Africa.

Country.	Locality.	Incidence (per cent.)	Authors.
Egypt	Rossetta	10·2	Khalil (1936).
	Near Cairo	26·5	Khalil (1935).
	Gharbiyah, Sharqiya, Giza	4 to 7	Baz (1946).
Belgian Congo	Banningville	1·51 to 12·8	Fain (1947).
	Coquilhatville	4·8	Chardonne and Peel (1949).
	Basoko District	47·4	Bellefontaine (1949).
Cameroons (French)	North	18	Languillon (1957).
French Equatorial Africa	Gabon	*Nil*	Gaillard (1932).
	Moyen-Chari	21·6	Bouillez (quoted by Hawking, 1957).
French West Africa	Senegal		
	Casamance	39·5	McFadzean (1954).
	Fatick, Kaolack	65·0	Dejou *et al.* (1950).
	French Guinea	14 to 36	Thiroux (quoted by Hawking, 1957).
	Upper Senegal & Niger Maradi	10	Pfister (1954).
	Upper Volta	7	Pfister (1954).
	Ivory Coast	8	Thiroux (quoted by Hawking, 1957).
	Bahomey	9 to 21	Thiroux (quoted by Hawking, 1957).
Liberia	Marshall Territory	16·4	Burch and Greenville (1955).
	Various	6·0	Young (1953).
		8·7	Diller (1947).
Portuguese Guinea	Costa de Baixo	49·2	Linto and de Almeida (1947).
Nigeria	Ilorin, Inkpa, Naraguta	14·0	Courtney (1923).
Sierre Leone	North Province	14·6	Blacklock (1930).
	Freetown	14·3	Wood (1923).
Gambia	West Kiang	35·0 Males 38·0 Females	McGregor *et al.* (1952).
		19·2 to 38·3	McFadzean (1954).
Tanganyika	Southern Province	0 to 73	Jordan (1954).
	Lake Province	0 to 40	Jordon (1956).
Kenya	Coast	35·3	Dunderdale (quoted by Hawking, 1957).
	Pate Island	36·6 Males 28·4 Females	Heisch *et al.* (1959).
Nyasaland	North	7·3 to 19·6	Oram (1958).
Zanzibar		26·3	Mansfield-Aders (1927).
Zanzibar	Weti Pemba	23·0	McCarthy (1930).
Mauritius	Port Louis	11·2	Kirk (1928).

It is difficult to determine which climatic and geographical characteristics are necessary for the successful propagation of the infection, but generally the parasite is more common in low-lying coastal or riverine locations in which comparatively hotter and more humid conditions are frequently found. In East Africa there was

a direct relationship between the mean maximum and minimum temperature and the infection rates in different areas (Jordan, 1955).

THE CAUSATIVE FILARIAE.

The periodicity of the microfilariae of *W. bancrofti* in Africa was investigated in Dar-es-Salaam, Tanganyika, by Engeland (quoted by Hawking, 1940). The nocturnal periodicity was confirmed by Carson (1925), and in West Africa, Poindexter (1950) has drawn attention to this periodicity.

Apart from "*Microfilariae bancrofti* var. *vauceli*" in Madagascar (Galliard and Brygoo, 1956), no other species of the genus *Wuchereria* have been reported in man though Heisch *et al.* (1959) have found *Wuchereria patei* in dogs, cats and genets in Kenya. The identification of the infective larvae in mosquitoes has been studied by Nelson (1959). This work serves to emphasise the importance of considering filarial infections in animals as a source of filarial larvae in mosquitoes when investigating the epidemiology of filariasis.

Owing to the large numbers of Indian immigrants in East Africa, and the prevalence of *Mansonioides* sp. in parts of these Territories, the introduction by them of *W. (Brugia) malayi* has been anticipated by Jordan (1955) though no evidence of this infection has yet been found.

MICROFILARAEMIA IN THE HUMAN CARRIER.

(i) IN DIFFERENT AGE-GROUPS.

Neves and Scalf (1954) reported microfilariae in the blood of babies 3 and 10 days old in Brazil. Although no such evidence of congenital transmission of microfilariae has been reported from Africa (Peel and Van Oye, 1950), Jordan (1952) reported microfilariae in the blood of babies of 3 months of age in an hyperendemic area in Tanganyika, and later showed that the incidence in childhood in an area was directly proportional to the incidence of infection in adults (Jordan, 1960).

McGregor *et al.* (1952), McFadzean (1954), Jordan (1960) and others have shown that the incidence of microfilariae increases with age, with a drop in the incidence sometimes occurring in the higher age-groups.

(ii) IN THE TWO SEXES.

Although males are frequently found to be infected more often than females, similar rates, or higher infection rates in females have been noted in a few areas. In the Pacific, it has been suggested (Jackowski and Otto, 1952) that males are more liable to mosquito bites as they go to their work outside the village. In many parts of Africa, the night-biting *Anopheles gambiae* and *Anopheles funestus* are the main vectors, and it is unlikely that one sex is exposed to infection any more than the other, though the differential biting habits of certain mosquitoes (Muirhead-Thomson, 1951) may lead to differences in the infection rate in the two sexes in some areas.

(iii) MICROFILARIAL DENSITY.

Little work has been done on the density of microfilariae in the blood in relation to intensity of infection in the individual or in the community. This is probably due to the difficulties arising from the 'microfilarial peak' and to the fact that individuals known to be infected—since they suffer from clinical manifestations—frequently have negative bloods. When, however, the mean microfilarial density of a village was calculated from bloods collected at standardised times, it was thought that day to day differences in counts in individuals would be eliminated and that such a mean value might give some idea of the intensity of infection in the village. Such an investigation on the results of many thousands of blood examinations has been reported by Jordan (1960) who found that in villages, situated in areas of different endemicity, there was close correlation and a direct relationship between the microfilaraemia rate and overall microfilarial density, and further that the genital filariasis rate was directly related to both. It was also found that in both sexes the mean microfilarial density in different age-groups followed closely the increase in incidence in different age-groups reported above.

(iv) EVIDENCE FOR AND AGAINST IMMUNITY.

Infection rates of 100 per cent in the higher age-groups have not been reported, though in hyperendemic areas in Tanganyika rates of over 70 per cent were found amongst males over the age of 56. Such a finding, based on the examination of a single thick drop of blood taken some hours before the peak of the microfilarial tide, suggests that if a larger quantity of blood had been repeatedly examined at a later hour an infection rate of nearer 100 per cent might have been obtained. In this case there would have been little evidence that immunity develops. The finding of higher mean microfilarial densities in the higher age-groups likewise suggests that immunity, if it occurs, can only be very incomplete.

On the other hand, the frequent finding of higher infection rates in males in areas where both sexes are equally exposed to infective bites, might indicate that females are partially immune, or more resistant to infection. Franks (1946) reported a higher incidence of infection in persons of Group A blood than in persons with other groups, and evidence of a similar nature was obtained in regard to *Acanthocheilonema perstans* in Tanganyika. Double filarial infections with *W. (Brugia) malayi* and *W. bancrofti* have been reported to be more common than would be expected statistically (Brug, 1931) and a similar finding with *W. bancrofti* and *A. perstans* was reported in Tanganyika (Jordan, 1955) in spite of different vectors. These findings support a view that certain people may be more susceptible to filarial infections than others—or that some people have a degree of immunity, which in view of the high rates of parasitaemia in older age-groups in hyperendemic areas, can probably be overcome.

In Africa and other areas where periodic *W. bancrofti* occur, the incidence of microfilaraemia in persons with Bancroftian elephantiasis is generally very low. If this is due to the blocked lymphatic vessels and glands, preventing the passage of

microfilariae to the general circulation (Acton and Rao, 1933 ; Napier, 1944), or to the death of adult worms during attacks of lymphadenitis, it would be expected that microfilariae developing as a result of fresh infections would be found. This, however, would seem not to be the case as there is no increase in the incidence of microfilaraemia amongst persons in the higher age-groups with elephantiasis. It has been suggested, therefore, that immunity to re-infection develops as a result of elephantiasis of the limbs (Jordan, 1960).

It is probable that no such immunity follows the development of filarial hydrocoele when the incidence of microfilaraemia is higher than in a corresponding group of persons without hydrocoele.

TRANSMISSION.

Althongh Bancroftian infection is endemic over such a vast area of Africa, transmission is by relatively few species of mosquito. In Egypt *Culex pipiens* would appear to be an important vector, while elsewhere *A. gambiae, A. funestus* and *Culex fatigans* are the main vectors.

These three mosquitoes have been incriminated in most countries where filariasis is of any importance and it is probable that they are the main vectors in other countries. The efficiency of these vectors varies—thus in the Congo, *C. fatigans* is not considered a good vector (Henrard *et al.*, 1946), while on Pate Island in Kenya, it is probably the only vector and is apparently very effective (Heisch *et al.*, 1959). It would appear, therefore, that the importance of these mosquitoes as vectors varies in different parts of the continent, though even a poor vector, if present in sufficient numbers, may be of more importance than a less prevalent efficient vector.

Data regarding the main vectors in different parts of the continent are shown in tabulated form, in Tables II, III and IV.

Differences in the infection rates of the same mosquito are apparent, but different methods of reporting infection rates make it difficult to compare the rates in various places.

Of many other mosquitoes found to contain filarial larvae, *Anopheles costalis* is probably the most important in the transmission of filariasis. In Freetown (Sierre Leone) 5·1 per cent were found to contain filarial larvae and 1 per cent had head and proboscis forms. In Kissy in the same country the corresponding figures were 14·6 per cent and 2·7 per cent (Gordon *et al.* 1932). Taylor (1930) found 1·6 per cent with mature larvae in the Northern Nigeria. Thoracic and proboscis larvae forms were also reported in these mosquitoes from Zanzibar (Mansfield-Aders, 1920).

Anopheles melas were reported infected in Liberia (Gelfand, 1955) who found 3·6 per cent with mature larvae.

Aedes aegypti is considered to be an efficient vector at Leopoldville, the cycle of development of larvae taking 14 days (Henrard *et al.*, 1946) though this species

TABLE II.

Countries in Africa in which A. gambiae *has been incriminated as a vector of* W. bancrofti

Country.	Location.	Rates per cent.	Larval stage.	Comments.	Author.
Gold Coast	Volta River	..	..	Probably principal vector.	Berner (1950).
Gold Coast	Nr. Accra	4·1	Mature larvae		Muirhead-Thomson (1954).
Portuguese Guinea	Bissau	0·51	Proboscis forms	Precipitin tests—anti-human serum in 55 per cent.	Ferriera *et al.* (1948).
Portuguese Guinea	Costa de Baixo			Main vector.	Pinto and de Almeida (1947).
Liberia	Marshall Territory	19·5 1·5	All stages Mature larvae		Gelfand (1955).
French W. Africa	Nr. Dakar	13·0 – 54·1	All stages		Kartman (1946).
Belgian Congo	Leopoldville			Efficient vector.	Henrard *et al.* (1946).
Tanganyika	Dar-es-Salaam	4·1	All stages	Salt water gambiae. No proboscis examination.	
		3·8	All stages	Fresh water gambiae. No proboscis examination.	Muirhead-Thomson (1950).
Tanganyika	Ukara Island. Lake Victoria.	2·0 0·6	All stages. Infective larvae.		Smith (1955).

was not incriminated on Pate Island (Kenya) by Heish and his co-workers (1959), and in the laboratory in Liberia and in Tanganyika, Gelfand (1955) and Jordan (unpublished) have found it to be a poor vector.

Many other mosquitoes have been found with filarial larvae (Taylor, 1930; Gelfand, 1955; Smith, 1955), but since they have not been found to contain mature forms they are probably of very little importance in transmission.

VECTOR-PARASITE RELATIONSHIPS.

With the introduction of dimethylcarbamazine and residual insecticides and the possibility of effective control or eradication of Bancroftian infection, it has become of some importance to have a knowledge of vector-parasite relationships as they affect the epidemiology of the infection.

In the case of *C. fatigans* some microfilariae, ingested in a blood meal, may be excreted or calcified and fail to develop into infective larvae. This loss of microfilariae is proportionately greater when mosquitoes feed on persons with high capillary concentrations of microfilariae. This probably protects the mosquito from severe lethal effects which large numbers of infective larvae might have; and possibly as a result of this, blood feeds from persons with heavy concentrations of microfilariae do not result in the development of more infective larvae nor consistently greater numbers of infected mosquitoes than following feeds on persons with

TABLE III.

Countries in Africa in which A. funestus *has been incriminated as a vector of* W. bancrofti.

Country.	Location.	Rates, per cent.	Larval stage.	Comments.	Author.
Gold Coast	Volta River	..	..	Probably principal vector.	Berner (1950)
Gold Coast	Nr. Accra	1·7	Mature larvae.	..	Muirhead-Thomson (1954)
Sierre Leone	Freetown	25·0 0·0	All stages. Head and proboscis forms.	8 dissections only.	Gordon *et al.* (1932)
Sierre Leone	Kissy	8·0 1·2	All stages. Head and proboscis forms.	.. ..	.. ..
Nigeria	Gadau	4·1 0·9	All stages. Head and proboscis forms.	.. ..	Taylor (1930)
Belgian Congo	Province of Leopoldville	..	..	Principal vector.	Henrard *et al.* (1946)
French W. Africa	Nr. Dakar	2·4 to 15·6	All stages.	..	Kartman (1946)
Zanzibar	..	..	Thoracic and proboscis forms.	..	Mansfield-Aders (1927)
Tanganyika	Ukara Island Lake Victoria	3·0 1·0	All stages. Infective larvae.	..	Smith (1955)

TABLE IV.

Countries in Africa in which C. fatigans *has been incriminated as a vector of* W. bancrofti.

Country.	Location.	Rates per cent.	Larval stage.	Comments.	Author.
Belgian Congo	Province of Leopoldville	..	..	Not good vector. Many dead and degenerated filarial worms.	Henrard *et al.* (1946).
Portuguese Guinea	Bissau	5·7	Microfilariae.	(No infected larvae).	Ferreira *et al.* (1948).
Mauritius	Port Louis	..	..	Local vector	de Charmoy, quoted by Kirk (1928).
Zanzibar	..	20·3	Proboscis forms.	Engorged females kept for 12 days.	Mansfield-Aders (1927).
	Pemba	5·3	Microfilariae.	Common carrier of filariae.	McCarthy (1930).
Tanganyika	Dar-es-Salaam	22·3 0·6	Filarial larvae. Mature larvae.		Hawking (1940)
Kenya	Mombasa			Probable vector.	Wiseman *et al.* (1939).
	Pate Island	24·7 7·1	Infected. Infective larvae.		Heisch *et al.* (1959).

low concentrations of larvae (Jordan, unpublished). Infective larvae were obtained following feeds on carriers with less than 1 microfilariae per 20 c.mm. (Jordan, 1959).

Gelfand (1955) in Liberia has shown, however, that when *A. gambiae* and *Anopheles melas* feed on persons with increasing numbers of microfilariae in their blood, the incidence of infection in the insect increases. He found 17·2 per cent of 29 *A. gambiae* with infective larvae following a feed on a carrier with 13 microfilariae per 20 c.mm and Jordan (1959) obtained a similar infection rate following a feed on blood containing less than 3 microfilariae per 20 c.mm., though Hicks (1932) in Sierre Leone was unable to infect mosquitoes from carriers with less than 20 larvae per 20 c.mm. The small numbers of infective larvae found in the mosquitoes in Gelfand's series suggests that there is a considerable loss of microfilariae during development similar to that which occurs in *C. fatigans*, and the result of field studies by Gelfand (1955) and Smith (1955) in Tanganyika, tend to confirm this since many more 'infected' mosquitoes were found than 'infective' (i.e., with infective larvae).

Laboratory experiments with *A. funestus* have not yet been reported in detail; no doubt this is due to the difficulty in colonising this mosquito.

Reference is frequently made to 'efficient' vectors or to 'poor' vectors, but at the present time there is no clear indication of how vector efficiency is defined and different workers probably have different ideas as to what constitutes efficiency.

Kartman (1953-1954) and Wharton (1957) have produced formulae to determine the efficiency of vectors in the laboratory, but neither of these formulae as described are entirely satisfactory for comparing the efficiency of one vector with that of another in different parts of the world. The introduction of a satisfactory formula would be a most useful adjunct to the comparative study of filarial vectors.

SUMMARY.

1. Bancroftian filariasis is widespread in Africa though considerable differences in incidence are reported.

2. The microfilariae are typical of periodic *W. bancrofti* and no other species of *Wuchereria* has been found infecting man.

3. The infection is more prevalent in adults than children, and males are generally infected more frequently than females. The density of microfilariae has also been shown to be greater in adults and males.

4. There is evidence that some groups of people are more resistant to infection than others.

5. Transmission is mainly by *A. gambiae*, *A. funestus* and *C. fatigans*, but vector-parasite relationships appear to vary in different parts of the continent.

6. It is suggested that the development of a formula to determine the efficiency of vectors would be a useful adjunct to the comparative study of vectors.

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AN EPIDEMIOLOGICAL ASPECT OF FILARIASIS IN CENTRAL JAPAN.

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THE first accurate case of filariasis in Japan was recognized by Baelz in 1876 as he found microfilariae in blood of a patient in Tokyo, although, on account of its peculiar appearance, elephantiasis was paid attention by the peoples, ignorant of its cause, since earlier time in some places of this country. Nowadays, it is well known that the disease occurs endemically in several places of the country except Hokkaido, the north-most island, and is most prevalent in Kyushu, the south-most island, including many adjacent small islands.

Central Japan, as designated in this paper, does not mean a special geographical division, but the designation is used for convenience to indicate the location of the region dealt with, which involves eleven prefectures situated about in the middle of Japan considered as a whole (Map 1).

In regard to filariasis in the region, there are some old records by previous investigators. Kawamura (1913), having had examined 3,004 persons mainly in the coastal area of Wakayama Prefecture, found microfilariae in 8·5 per cent and elephantiasis of various sites and stages in 2·97 per cent among them. Asano (1913) reported positive finding of blood for microfilariae in 2·4—8·3 per cent among 376 persons examined in Toyama Prefecture and Fuwa (1914) found the same parasite in 2 out of 173 persons in Ishikawa Prefecture. Besides these, there are two records of survey for this disease carried out by Takeuchi (1912) in Shiga Prefecture and by Yoshinaga (1912) in Hyogo Prefecture, with negative result respectively. For a long time since then, no survey has been undertaken on filariasis in the region concerned.

Recently, the surveys have been conducted by Tsubosaka *et al.* (1954) in Fukui Prefecture, by Masuda and Yamashiro (1954) in Hyogo Prefecture and by Morisita (T) *et al.* (1955) in Gifu Prefecture, by which some new endemic foci of this disease have been brought to light, and furthermore, Nogi *et al.* (1951) and Ozaki (1952) reported some clinical cases caused by filarial worm, in certain areas of Wakayama Prefecture where this disease has not ever been known to occur.

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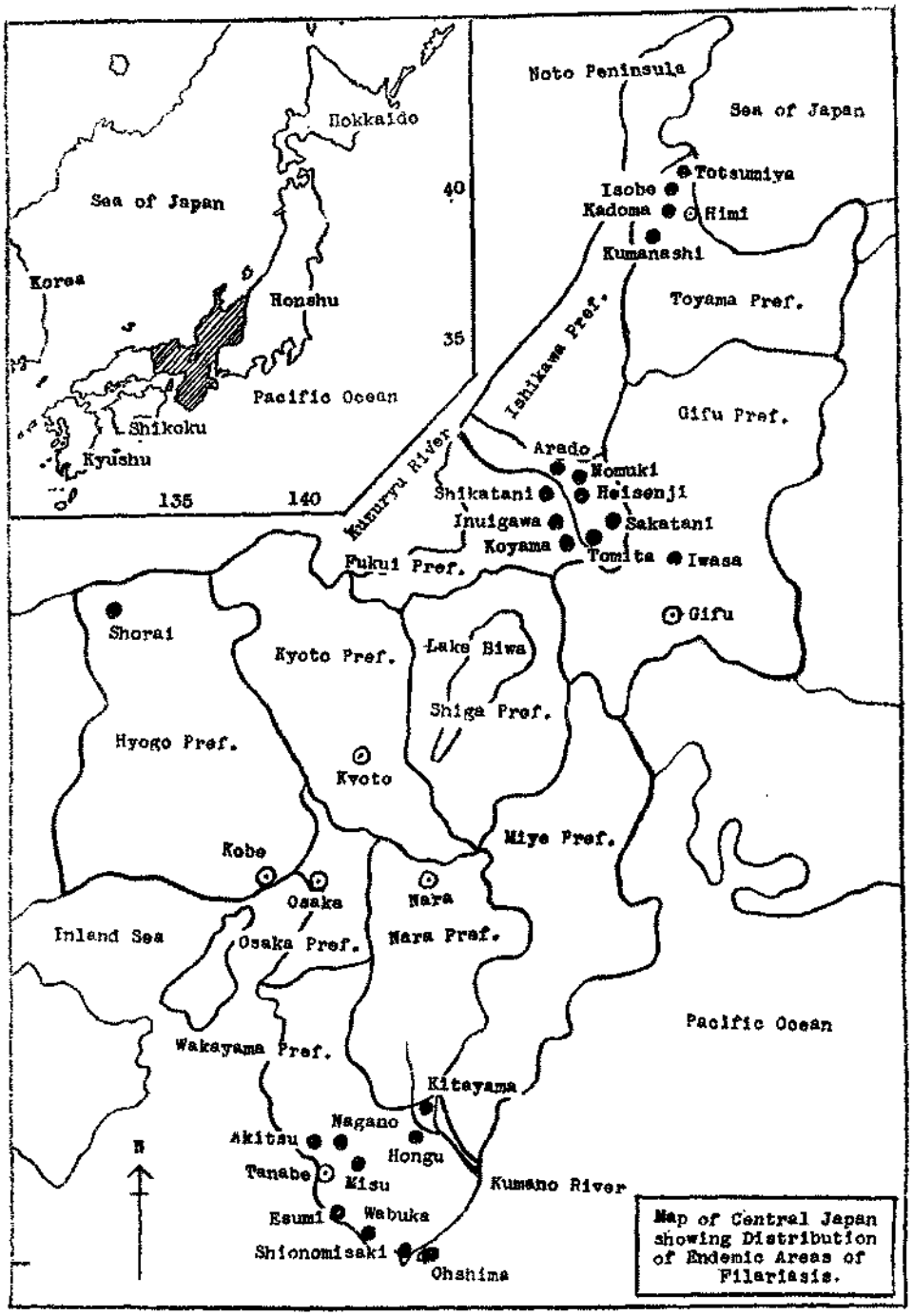
MAP 1.
Distribution of endemic areas of filariasis in Central Japan.



During the period from 1953 to 1957, the writer and his collaborators have undertaken surveys on filariasis in various places in this region in order to clear up its present conditions. Thus, in this paper, the results obtained by the writer as well as other investigators are presented for a general consideration on conditions of filariasis, past and present, in the region concerned.

Before proceeding further, it is necessary to note that filariasis in this region is entirely due to *Wuchereria bancrofti* (Cobbold).

METHODS.

For examination of blood for microfilariae, thick films were made with blood taken from ear lap between 8·30 p.m. and midnight. The films were dried, dehaemoglobinized in water, fixed with methyl alcohol, and stained with Giemsa solution. For this purpose, the people were asked to gather at the public assembly halls of the villages surveyed, and before taking blood, they were given a brief knowledge on filariasis and procedure for examining blood. They were also inquired of clinical manifestations in the present or past, such as peculiar fever, scrotal swelling, hydrocoele, lymphangitis, chyluria or haematchyluria, elephantiasis, and other symptoms suspected of filarial nature. This method, of course, may not be always accurate to know the truth of incidence of clinical cases. However, it is thought to be useful to get its general aspect, when their informations are carefully investigated to exclude doubtful cases. For searching clinical cases, at times the persons having any kind of clinical signs suspected of filarial nature, were called and seen personally.

GENERAL FEATURES OF GEOGRAPHICAL AND METEOROLOGICAL CONDITIONS OF THE REGION.

The region concerned is situated in the western part of Honshu, the main island of Japan, occupying about the centre of the country considered as a whole, with the Sea of Japan on the north and the Pacific Ocean on the south (Map 1). Viewing generally, it is rather mountainous and the plains open mostly in the coastal areas or along the big rivers. The region involves Lake Biwa, the largest lake in Japan, sorrounding which a basin is formed. There are many rivers, some of which run northward and enter into the Sea of Japan, while others run southward, entering into the Pacific Ocean or the Inland Sea connected with the latter.

The meteorological conditions are fairly different between the northern, the southern and the central parts of the region. In the northern part, influenced by seasonal wind blowing from Siberian continent, there are seen much rain and snow during the winter, with annual precipitation of about 2,000 to 2,500 mm., and thus, the annual mean temperature is relatively low, being 12·9° to 13·8°C.

In the southern part which forms a big peninsula projecting into the Pacific Ocean, the climate may be markedly influenced by the warm current "Kuroshio" coming from the East-Chinese Sea, being warmer than other parts with annual mean temperature of 15° to 16·4°C., and furthermore, typhoons prevailing during

summer bring heavy rain so that the annual precipitation amounts from 2,470 to 4,151 mm.

TABLE I.

Temperature and precipitation in different parts of Central Japan.

Part.	Prefecture.	Mean temperature (C.) Monthly. Min.	Max.	Annual.	Annual precipitation. (mm.)
Northern	Toyama	0·6	25·6	12·9	2,284
	Ishikawa	2·2	25·6	13·2	2,526
	Fukui	2·2	26·1	13·5	2,395
	Kyoto (north)	3·1	25·9	13·8	1,998
	Hyogo (north)	1·8	25·9	13·3	2,056
Central	Gifu	2·9	26·4	14·3	1,943
	Shiga	2·8	26·0	13·6	1,660
	Kyoto (south)	2·6	26·3	15·1	1,544
	Nara	3·3	26·3	14·3	1,408
	Osaka	4·1	27·3	15·1	1,332
	Hyogo (south)	4·2	27·0	15·1	1,330
	Miye (north)	3·9	26·3	14·2	1,754
Southern	Miye (south)	5·1	25·4	15·0	4,154
	Wakayama	4·1	26·0	16·4	2,470

In the Central part, the rain is comparatively less, with the annual precipitation of 1,332 to 1,943 mm., and the annual mean temperature ranges from 13·6° to 15·1°C. Thus the climate in this part shows a character roughly intermediate between the two other parts.

THE INCIDENCE OF FILARIASIS.

1. FEATURES IN WAKAYAMA PREFECTURE.

Wakayama Prefecture is situated on the top of the peninsula projecting into the Pacific Ocean. Filariasis in this prefecture was reported for the first time by Kawamura (1913) who obtained the incidence figure as already mentioned, and stated that the disease was prevalent especially in the coastal area north to Shiono-misaki. Since then, no report on the disease has heen available. Recently, Nogi *et al.* (1951) reported two cases of chyluria with positive blood finding for micro-filariae from coastal area in the vicinity of Tanabe City where its occurrence has hitherto been not recorded, and Ozaki (1952) called attention to some cases which he found in a mountainous area along the upper reaches of Kumano River where filariasis has also never been presumed to occur.

In 1954 and 1955, the writer and his collaborators carried out a survey for filariasis in different places of this prefecture, the results of which are given below in four paragraphs according to topographical condition.

(i) *Mountainous area.*—The area is located amongst mountains in the upper reaches of Kumano River, about 25 to 35 kilometers apart from the sea. Two

villages, Hongu and Kitayama, were selected for survey where recently some cases became known to occur. Contrary to the expectation to find the infection to a certain extent, 75 persons in Hongu and 185 persons in Kitayama examined were all negative for microfilariae, although in Hongu and its neighbouring villages six cases of chyluria or hydrocoele or elephantiasis, and in Kitayama a case of hydrocoele accompanied by chyluria were encountered.

(*ii*) *Interior plain.*—The area surveyed consists of three villages, Misu, Nagano and Akitsu, located at the foot of low mountains about 10 to 15 kilometers away from the sea. It includes the place from where in the recent time Nogi *et al.* (1951) reported some cases of filariasis. 427 persons examined, however, were all negative for microfilariae as well as for clinical signs.

(*ii*) *Coastal area.*—The area surveyed involves mainly the places where Kawamura (1913) carried out an extensive survey about 45 years ago, thus three villages, Shionomisaki, Wabuka, and Esumi, were subjected to investigation. As is seen from Table II, only 5 out of 219 persons examined in Wabuka were found to be positive for microfilariae, whereas all were negative among those examined in Shionomisaki and Esumi. The positive cases consist of one male aged 9 years and four females aged 13, 17, 17, and 25 years respectively, without any kind of clinical manifestation. Besides these, one case of chyluria in Wabuka, and one case of chyluria and three cases of hydrocoele in Esumi were noted. They were all over 55 years of age and negative for microfilariae.

TABLE II.

Results of blood examination for microfilariae in Wakayama Prefecture.

Areas.	Villages.	Number examined.	Number positive.
Mountainous	Hongu	75 (40 males, 35 females).	0
	Kitayama	185 (100 males, 85 females).	0
Interior plain	Misu	119 (54 males, 65 females).	0
	Nagano	128 (76 males, 52 females).	0
	Akitsu	180 (111 males, 69 females).	0
Coastal	Shionomisaki	154 (40 males, 114 females).	0
	Wabuka	219 (77 males, 142 females).	5 (1 male, 4 females).
	Esumi	419 (179 males, 240 females).	0
Island	Ohshima	556 (256 males, 300 females).	0
Total		2,035 (933 males, 1,102 females).	5

The result thus obtained, as compared with that of Kawamura, seems to indicate that filariasis in this area may have become markedly lower in its

incidence, although the transmission, though not so active, is supposed still to occur as the infection was demonstrated even in children under 13 yeas of age.

(*iv*) *Island.*—Ohshima, an island lying just in front of Shionomisaki, was surveyed for the first time with an expectation to prove the occurrence of this disease. The survey, however, resulted entirely negative, as among 556 persons examined no one did show microfilariae or clinical sign of filarial nature.

2. FEATURES IN FUKUI PREFECTURE.

Fukui Prefecture occupies a northern part of the region, between the Sea of Japan on the west and the mountainous area on the east. Kuzuryu River, arising in the eastern mountains, runs westward and enters into the Sea of Japan, forming a plain along its lower reaches.

No record on filariasis has been available in this Prefecture until the recent time when Tsubosaka *et al.* (1954), having had found a number of clinical cases of this disease, carried out an extensive survey in Katsuyama area located along the upper reaches of Kuzuryu River, and discovered microfilaraemia in 4·01 per cent among 4,115 persons examined, and furthermore, he stated that a symptom "*Shimomushi*" as named by local inhabitants of this area, should be nothing but the lower abdominal ache caused by filarial infection, a fact which reveals that the disease might have been present since old time in this area.

During 1955 to 1956, the writer and his collaborators carried out a survey in the same area as Tsubosaka *et al.* (1954) as well as in Ohno area, neighbouring to the former, and obtained the results as follows :—

(*i*) *Katsuyama area.*—In four villages, Heisenji, Shikatani, Nomuki and Arado, placed under the present survey, 932 persons altogether were examined for microfilariae and clinical signs. The result of blood examination for the parasite is shown in Table III.

TABLE III.

Result of blood examination for microfilariae in Katsuyama area, Fukui Prefecture.

Villages.	Number examined.	Number positive.	Infection rate (per cent).
Heisenji	114 (70 males, 44 females)	1 (male).	0·9
Shikatani	208 (115 males, 93 females)	11 (7 males, 4 females).	5·0
Nomuki	289 (140 males, 149 females)	22 (12 males, 10 females).	7·6
Arado	321 (201 males, 120 females)	16 (9 males, 7 females).	5·0
Total	932 (526 males, 406 females)	50 (29 males, 21 females).	5·4

As seen from Table III, the microfilariae rate was 5·4 per cent as far as four villages are collectively concerned, with the highest incidence of 7·6 per cent in

Nomuki and the lowest of 0·9 per cent in Heisenji. The age incidence of microfilariae, as shown in Table IV, was higher among the groups aged from 20 to 49 years, and the lowest age showing the parasite was 10 years.

TABLE IV.

Age incidence of infection in the populations in four villages of Katsuyama area.

Age-group (years).	Number examined.	Number positive.	Infection rates (per cent).
0– 9	67	0	0
10–19	523	22	4·2
20–29	123	10	8·1
30–39	95	7	7·4
40–49	70	6	7·9
50–59	29	1	3·4
over 60	18	4	2·2
uncertain	1	0	0
Total	932	50	5·4

Regarding the sex incidence, no notable difference was recognized according to sex, as seen in Table V.

TABLE V.

Sex incidence of infection in Katsuyama area.

Sex.	Number examined.	Number positive.	Infection rates (per cent).
Males	526	29	5·5
Females	405	21	5·2
Total	932	50	5·4

In the survey of clinical cases, 66 among 932 persons reported one or more kinds of symptoms of the present or past; fever, 27*; chyluria or haematchyluria, 25; lymph scrotum, 3; hydrocoele, 10; lymph varix, 14; "*Shimomushi*" which is thought to be related to lymphangitis of spermatic cord and epididymitis caused by filarial worm, 4. The number of the cases was the largest in Arado, followed by Nomuki. No case of elephantiasis was reported or observed. In eight among those cases mentioned above, microfilariae were positive in blood.

(ii) *Ohno area.*—This area is located also along Kuzuryu River, but upper in position than Katsuyama, and bounded on the north by the latter area.

Four villages, Sakatani, Tomita, Inuigawa, and Koyama were surveyed, with the results as shown in Table VI. The microfilariae rate among 788 persons examined in four villages, was 1·5 per cent, a rate lower than that in Katsuyama area. No remarkable difference in the incidence rate between four villages was observed, although it was somewhat higher in Koyama than in others.

* The people having more than two symptoms are calculated repeatedly in each category.

TABLE VI.

Result of blood examination for microfilariae in Ohno area, Fukui Prefecture.

Villages.	Number examined.	Number positive.	Infection rates (per cent).
Sakatani	68 (46 males, 22 females)	1 (male)	1·2
Tomita	178 (121 males, 57 females)	2 (1 male, 1 female)	1·1
Inuigawa	281 (148 males, 133 females)	3 (2 males, 1 female)	1·1
Koyama	261 (148 males, 113 females)	6 (3 males, 3 females)	2·3
Total	788 (463 males, 325 females)	12 (7 males, 5 females)	1·5

As is seen in Table VII, no special tendency in age incidence of infection was observed, although the infection was seen in those from 10 to 59 years of age. There was also demonstrated no difference in the incidence according to sex. These figures, however, are considered to have no statistical significance because of its low incidence in this area.

TABLE VII.

Age incidence of infection in the populations in four villages of Ohno area, Fukui Prefecture.

Age-group. (years)	Number examined.	Number positive.	Infection rates (per cent).
0– 9	88	0	0
10–19	354	6	1·7
20–29	148	1	0·7
30–39	87	3	3·5
40–49	67	1	1·5
50–59	27	1	3·7
Over 60	13	0	0
uncertain	4	0	0
Total	788	12	1·5

Thirty among 788 persons surveyed, reported one or more kinds of clinical signs of the present or past : fever, 12 ; chyluria, 9 ; lymph scrotum, 5 ; hydrocoele, 2 ; lymphangitis of spermatic cord, 6 ; lymph varix, 3, and only in one of these cases the blood was positive for microfilariae.

TABLE VIII.

Sex incidence of infection in Ohno area, Fukui Prefecture.

Sex.	Number examined.	Number positive.	Infection rates (per cent).
Males	463	7	1·5
Females	325	5	1·5
Total	788	12	1·5

3. FEATURES IN TOYAMA PREFECTURE.

Toyama Prefecture is situated in the extreme northern part of this region with the Sea of Japan on the north and back mountains of Honsyu on the south. In certain area near the base of Noto Peninsula which projects into the Sea of Japan, the occurrence of filariasis was reported by Yoshimura (1913) who found microfilariae in one out of 234 persons examined in Himi and its neighbouring villages, and by Asano (1913) who obtained microfilariae rate of 1·8 per cent among 610 persons examined in four villages, Uha, Isobe, Yatsushiro, and Kumanashi.

A survey was carried out by the writer and his collaborators in 1957, just 44 years after they did, involving nearly the same places as in the previous survey. The result is shown in Table IX. As is seen from Table IX, the microfilariae rate was 1·0 per cent among 585 persons examined in four villages, with the highest rate of 2·7 per cent in Kadoma where Asano recorded 8·3 per cent. The positive cases

TABLE IX.

Result of blood examination for microfilariae in Toyama Prefecture.

Villages.	Number examined.	Number positive.	Infection rate (per cent).
Kumanashi	176 (96 males, 80 females)	1 (male).	0·6
Kadoma	182 (81 males, 101 females)	5 (1 male, 4 females).	2·7
Totsumiya	106 (55 males, 51 females)	0	0
Isobe	121 (62 males, 59 females)	0	0
Total	585 (294 males, 291 females)	6 (2 males, 4 females).	1·0

consist of two males of age 26 and 29 years and four females of age 7, 9, 30 and 30 years respectively. As to the sex incidence, 2 out of 294 males and 4 out of 291 females were found to be positive for microfilariae, although the significance of such difference should be retained as uncertain on account of its low incidence. From these figures it can be said that the incidence of filariasis in this area is very low at present as compared with previous condition, although new infections are suspected still to occur to a certain degree, as those positive for microfilariae involved some children under the age of 10 years.

During the survey, thirteen persons reported one or more clinical symptoms they have or had, fever, 5 ; chyluria, 2 ; lymphangitis of spermatic cord, 3 ; hydrocoele, 1 ; lymph varix, 4, although they were all negative for microfilariae.

4. RECORDS ON FILARIASIS IN OTHER PREFECTURES.

(i) *Ishikawa Prefecture.*—The prefecture lies west to Toyama Prefecture with its northern half forming Noto Peninsula. The only record of filariasis in this Prefecture is that of Fuwa (1914) who found microfilariae in 2 out of 173 persons examined in certain rural area. Since then, no record is available about this disease.

(*ii*) *Hyogo Prefecture.*—This prefecture extends between the Sea of Japan on the north and the Inland Sea on the south so that the climate is quite different in both sides. In regard to filariasis, Yoshinaga (1912) reported a negative result in blood examination for microfilariae of 1,764 persons in Ikuno Silber Mine and Sasayama situated in the middle part of the Prefecture. Recently, Masuda and Tashiro (1954 : 1955) discovered Shorai area, Mikata District, not far from the northern coast, as a new endemic focus of this disease where they found microfilariae in 3·0 per cent among 2,108 persons examined. They noted also that seventeen had fever history, probably of filarial nature, and one suffered from swelling gland, though slight.

(*iii*) *Gifu Prefecture.*—This prefecture is situated near the east border of this region, without facing the sea on all sides. During five years from 1949 to 1954, thirty-one cases of chyluria were seen at Gifu Medical School Hospital, and they were all inhabitants of this prefecture. Morisita (T) *et al.* (1955), having had found microfilariae in scrotal fluid of a hydrocoele case coming from Iwasa, a village not far from Gifu City, undertook a survey in that village and found microfilariae in one out of 74 persons examined.

(*iv*) *Osaka Prefecture.*—So far, no record on filariasis is available in this prefecture. However, the writer was informed some years ago about a case of chyluria with positive finding of microfilariae in the urine. It is notable that the patient had never lived outside the Osaka Prefecture. Furthermore, recently the writer has come to learn that some cases of elephantiasis are seen in the south-most area of the prefecture.

(*v*) *Miye Prefecture.*—This prefecture occupies an eastern part of the big peninsula projecting into the Pacific Ocean. Some cases with clinical manifestations, possibly due to filarial worm, have been encountered in certain areas bordering to the areas of Wakayama Prefecture, where filariasis is known to occur.

(*vi*) In other prefectures such as Nara, Kyoto and Shiga, there is no record relating to filariasis, with an exception in Shiga Prefecture where Takeuchi (1912) carried out a survey in the coastal area of Lake Biwa, but with negative result.

SUMMARY.

(1) Basing on the previous and recent records, including the writer's own, a general aspect on filariasis in Central Japan, as defined in this paper, is given.

(2) In Central Japan there are not a few endemic foci of filariasis due to *Wuchereria bancrofti* ; its incidence is not so high, so far as the present condition is concerned.

(3) The occurrence of the disease in the coastal area of Wakayama Prefecture, and in the basal area of Noto Peninsula of Toyama Prefecture, has been reconfirmed by the recent investigations, which, on the other hand, have brought to light the presence of new endemic foci in the mountainous area of Wakayama Prefecture, in the area along upper reaches of Kuzuryu River of Fukui Prefecture,

in the northern area of Hyogo Prefecture, and in a village near Gifu City of Gifu Prefecture.

(4) It is noteworthy that filariasis occurs, in a similar way, not only in the southern part but also in the northern part of this region, although the climate is fairly different between both parts.

(5) The comparison of the present conditions with those previously recorded, so far available, reveals that filariasis may have remarkably lowered in its incidence, although new transmission is suspected still to occur, to a certain degree, as the infections have been recognized even in lower age-group.

(6) The data concerning clinical manifestations are more or less obscure, but at least it is asserted that elephantiasis has markedly decreased in its incidence in a similar manner as in other endemic areas in Japan where previously it was quite prevalent.

(7) In this paper no mention has been made of the vector of filariasis. However, it might be indisputable that *Culex pipiens pallens* should be the most important species, since it is very common in this region, and proved to be a suitable vector of *Wuchereria bancrofti* in other places of Japan.

Collaborators in the surveys planned by the writer are Dr. T. Nakabayashi, Dr. S. Takada, Dr. S. Haebara, and Mr. Nishimura (Department of Parasitology, Research Institute for Microbial Diseases, Osaka University).

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THE NAMES OF THE MOSQUITO HOSTS AND PARASITES OF MALAYAN FILARIASIS.

BY

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[June 24, 1960.]

MALAYAN filariasis refers to infection with the parasite which used to be known as *Wuchereria malayi*. It is widespread throughout Asia and is transmitted through much of its range by mosquitoes of the sub-genus *Mansonioides*. Unfortunately there has been some disagreement regarding the name of the genus to which these mosquitoes belong and changes have also been made in the specific name of one of the main vectors. At the same time there have been changes in the names of the parasites; new species have been described and more recently *W. malayi* has been placed in a new genus, *Brugia*. There are thus grounds for considerable confusion regarding the terminology to be used to describe both the mosquitoes and the parasites of Malayan filariasis. Changes in terminology, disagreeable as they may be, are sometimes inevitable and the object of these notes is to clarify the position. They have been written mainly for the epidemiologist, and technical taxonomic discussions have been avoided as far as possible.

PARASITE TERMINOLOGY.

Wuchereria bancrofti (Cobbold) is the cosmopolitan filarial parasite of man and is distributed throughout most of the tropical areas of the world. Until recently the genus *Wuchereria* contained one other parasite, *W. malayi*, which was first described from Sumatra by Brug (1927) and is widely distributed in Asia. Recent investigations in Malaya showed that two forms of *W. malayi* were present, largely geographically isolated from one another, and differing both in the periodicity of their microfilariae (thus resembling the periodic and non-periodic forms of *W. bancrofti*) and their vectors (Wilson *et al.*, 1958). The semi-periodic form of *W. malayi* occurs in fresh water swamp forest and is found in a wide range of animal hosts as well as in man. Also in the swamp forest is another filarial parasite, originally named *W. pahangi* Buckley and Edeson (1956), after the State of Pahang in which it was found, and occurring in an even wider range of animals. Both parasites are transmitted by *Mansonioides* mosquitoes. A fourth species of *Wuchereria*, *W. patei* has since been described from Africa, named from the island of Pate where infections were found in cats, dogs and genet cats by Buckley, Nelson and Heisch (1958). The latest development has been the transfer of *malayi*, *pahangi* and *patei* to the new genus *Brugia* Buckley (1960), leaving only *bancrofti* in the now monotypic genus *Wuchereria*.

The situation in Malaya is, therefore, that we have three species of parasite; *Brugia malayi* (occurring in two forms, the semi-periodic and the periodic), *B. pahangi* and *W. bancrofti*. *B. pahangi*, which is essentially an animal parasite, occurs in the swamp forest areas of Pahang and Perak along with the semi-periodic form of *B. malayi*, and the larvae of the two species are indistinguishable in their mosquito hosts. *W. bancrofti* has only recently been found to be endemic in Malaya but Danaraj, Schacher and Colless (1958) have found active transmission by *Culex pipiens fatigans* in the urban environment of Singapore, while Wharton (1960) has shown that *Anopheles letifer* is the vector in a rural area among indigenous Malays.

MOSQUITO TERMINOLOGY.

The names *Mansonia* Blanchard and *Taeniorhynchus* Lynch Arribalzaga have both been widely used to denote the genus of mosquitoes which includes the four sub-genera *Mansonioides*, *Coquillettidia*, *Rhynchotaenia* and *Mansonia*. There has been so much confusion that in some instances the name *Mansonioides* has been used alone when referring to these important filarial vectors (Bertram and Samarawickrema, 1958). In Malaya we have been fortunate in that from the time Edwards (1930) accepted *Mansonia* we have continued its use despite the decision by the International Committee on Zoological Nomenclature in favour of *Taeniorhynchus* (Edwards, 1941) which was followed especially by workers in Africa and Ceylon. A recent decision, which should be final, reverses the earlier one and *Taeniorhynchus* has now been suppressed in favour of *Mansonia*, and this is the name used by Stone, Knight and Starke (1959) in their catalogue of the mosquitoes of the world.

Another problem has been the name of the Malayan vector, *Mansonia longipalpis* (Van der Wulp). This species was originally known as *M. annulipes* (Walker), but was changed later to *M. longipalpis*, and now Stone has shown that the correct name is *M. dives* (Schiner). A male specimen, with slightly different terminalia, was described by Mrs. Bone-Wepster as *annulipes* var. *A* and subsequently raised to specific status by Edwards (1930) under the name *M. bonneae*. At this stage two very similar species, *M. longipalpis* and *M. bonneae*, were recognised, but only the males could be separated, and bulk collections were usually referred to as *M. longipalpis*. Edwards eventually found a small character which he thought would separate the females and informed Hodgkin in Malaya just before World War II. This was the position when Wharton (1957) compared the efficiency of the two species as vectors of *B. malayi* in a swamp forest area of Malaya. *M. longipalpis* and *M. bonneae* were fed on human carriers and it was found that there was no difference in their susceptibility to infection. They were both found infected in nature, seemed to have similar habits and were presumed to play similar roles in the transmission of filariasis. For routine trapping and dissections the two species were therefore combined as *M. longipalpis* and this is the name which has been used in the Institute's publications up to the present time.

With the discovery of the essentially animal parasite *B. pahangi* in areas of endemic human filariasis, observations were made on its larval development in mosquitoes, and it was found that *M. longipalpis* was not a good host (Edeson, Wharton and Laing, 1960). Mosquitoes, examined 10·5 days after feeding on an infected animal, contained large numbers of immature larvae but only 2 per cent were Stage III (infective). When the mosquitoes were identified as *M. dives* (=*M. longipalpis*) and *M. bonneae* it was noticed that the few larvae of *B. pahangi*, which developed beyond Stage I, were almost all in *M. dives*. The difference was clearer in mosquitoes kept for 13-14 days when about 20 per cent of the larvae were Stage III in *M. dives*, while less than 1 per cent had reached this stage in *M. bonneae* (Wharton, unpublished information). The development of periodic (as opposed to semi-periodic) infections of *B. malayi* in *M. dives* and *M. bonneae* follows a similar pattern to that of *B. pahangi*—though neither mosquito can be regarded as a really efficient host, quite large numbers of larvae reach Stage III in *M. dives*, but practically none do so in *M. bonneae*. There are, therefore, differences in the biology of the two species of mosquito and it is now necessary to make species identifications where possible. This has been made easier by the gradual accumulation of bred material, both from larvae and from individual females and a number of characters are available which usually allow a definite identification to be made. The simplest character for separation of females is the presence of a few white scales among the supra-alar bristles in front of the wing-roots in *M. dives*. These scales are absent in *M. bonneae* and separation of the two species by this character was used to show the biological differences in filarial larval development. Specimens are now recorded as *M. dives* or *M. bonneae*, with bulk collections recorded as *M. dives/bonneae*. Both species are vectors of semi-periodic *B. malayi*, but from laboratory observations, only *M. dives* would be a possible vector of *B. pahangi* and it has, in fact, been proved to carry infective larvae in nature (Edeson, Wharton and Laing, 1960).

Another small change has been the alteration of *M. annulatus* to *M. annulata* to make the specific name agree in gender with the generic name (Macdonald, 1957).

The names now used to describe the filarial parasites and their principal vectors in nature in Malaya are summarised below.

Parasites	Vectors
Brugia malayi	
Semi-periodic form	*Mansonia bonneae, M. dives, M. annulata, M. uniformis.*
Periodic form	*Anopheles barbirostris, M. uniformis, M. indiana, M. annulifera.*
Brugia pahangi	*M. dives, M. annulata.*
Wuchereria bancrofti	
Urban	*Culex pipiens fatigans.*
Rural	*Anopheles letifer.*

It should be mentioned that further changes in the names of some of the mosquitoes may be necessary in the future. This applies particularly to *Anopheles barbirostris* and *A. letifer*, as the vector forms appear to differ from the type forms. *C. p. fatigans* is the *C. p. quinquefasciatus* of American authors.

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GEOGRAPHICAL DISTRIBUTION OF FILARIASIS IN UTTAR PRADESH.

BY

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[August 2, 1960.]

THE present State of Uttar Pradesh, originally known as United Provinces of Agra and Oudh, lies between the north latitude 23° 52′ (Mirzapur) and 31° 18′ (Tehri-Garhwal) and east longitude 77° 3′ (Muzaffarnagar) and 84° 39′ (Ballia). Its boundary in the north runs with that of Nepal and Tibet in the Himalayas ; on the west and south-west are the States of Himachal Pradesh, Punjab, Delhi and Rajasthan ; on the south the State of Madhya Pradesh and on the east the State of Bihar.

The total area of the State is 1,13,409 square miles, having a population of 6,32,15,742 according to the 1951 census. The State was divided into 51 revenue districts but from April 1960, 3 more border districts have been created by certain adjustments, making a total of 54. The total number of villages is 1,11,722 and the number of towns is 486.

PHYSIOGRAPHICAL DIVISIONS.

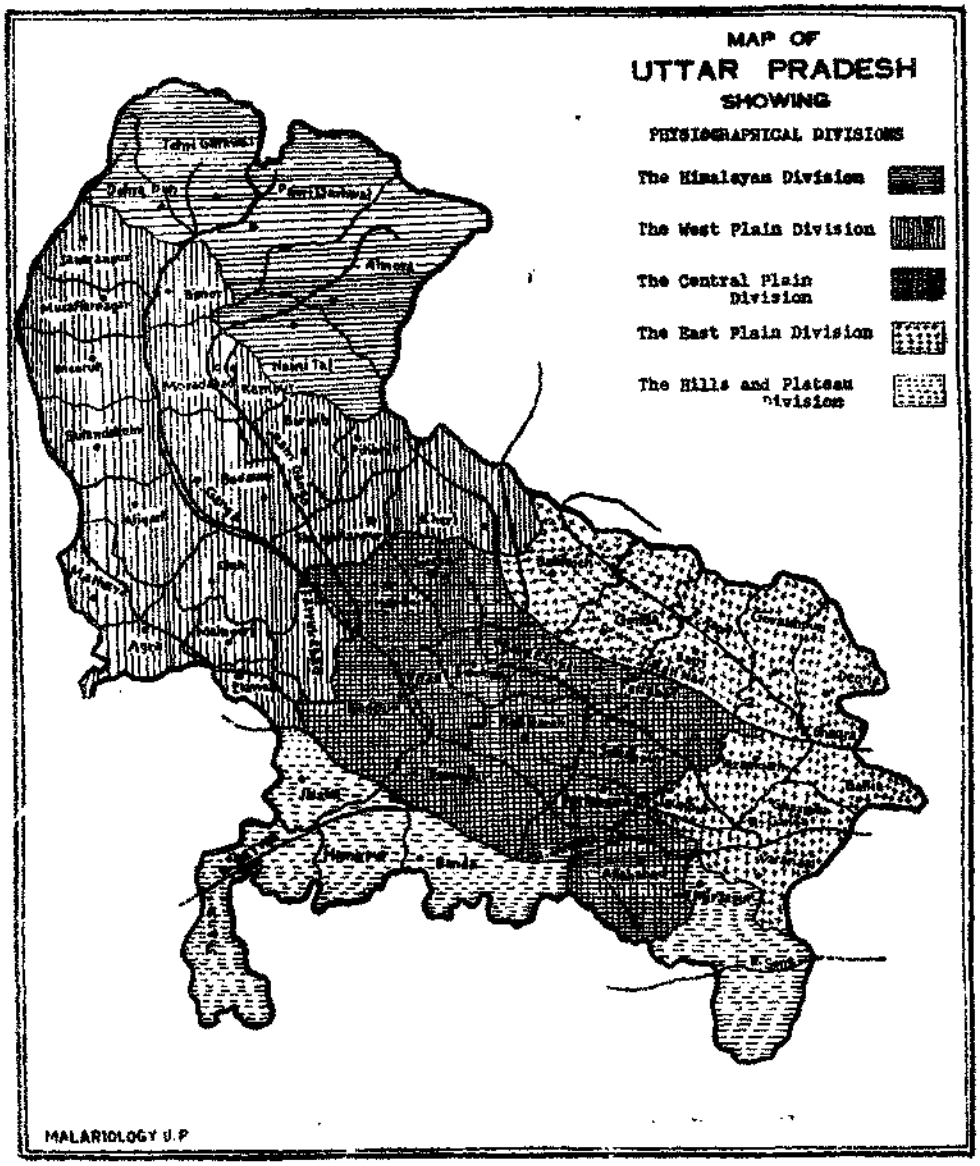
Physiographically, the State comprises three natural divisions. The first is formed by the Himalayas along the north ; the second is the alluvial plain region of the rivers Yamuna and the Ganga and the third is the plateau region lying to the south of the plains, covering the trans-Yamuna tract.

Up to the 1941 census, the State was divided into eight natural divisions, namely :—

1. The Himalaya West.
2. The Sub-Himalaya West.
3. The Sub-Himalaya East.
4. The Indo-Gangetic Plain East.
5. The Indo-Gangetic Plain Central.
6. The Indo-Gangetic Plain West.
7. The Central Indian Plateau.
8. The East Satpuras.

During the census of India in 1951, certain modifications were made in the natural divisions when the State was divided into five divisions (Map 1), namely :—

MAP I.


MAP OF
UTTAR PRADESH
SHOWING
PHYSIOGRAPHICAL DIVISIONS
The Himalayan Division
The West Plain Division
The Central Plain Division
The East Plain Division
The Hills and Plateau Division
MALARIOLOGY U.P

1. The Himalayan Division.
2. The East Plain Division.
3. The West Plain Division.
4. The Central Plain Division.
5. The Hills and Plateau Division.

HISTORY OF THE DISEASE.

Filariasis has been known in certain eastern districts of Uttar Pradesh for a sufficiently long period. In the District Gazetteers of United Provinces of Agra and Oudh for the districts of Gorakhpur and Benares, published as early as 1909, it has been mentioned that hydrocoele and elephantiasis are prevalent in these areas. Megaw and Gupta (1927) published a filaria Map of India, showing the prevalence of the filariasis in the eastern districts of Uttar Pradesh.

Napier (1946) in his work "*The Principles and Practice of Tropical Medicine*" mentioned that the moderately infected regions for filariasis consisted of United Provinces also, besides other areas in India.

Raghavan (1955) published another filaria Map of India showing all eastern districts of Uttar Pradesh as known to be filarious.

In spite of all this knowledge of the history of the prevalence of filariasis in the State, not much attention was focussed either by the public or by the State Health Authorities to study and control this important public health problem. The chief cause for this apathy towards the disease was the chronicity and un-dynamic character of filariasis and the social stigma attached to it. Moreover, there are neither epidemics nor any mortality in filariasis as in some of the diseases like malaria, cholera, small-pox, etc.

With the launching of the National Filaria Control Programme during the year 1955-56, a new era dawned in the history of filariasis in India and in Uttar Pradesh, large scale surveys for delimitation of the filarial problem, the control measures by the use of diethylcarbamazine, anti-larval and anti-adult measures against the vector mosquitoes, the health education and the publicity have been started under this National Programme. The Control Programme, more appropriately a pilot project, has paved way for several new avenues for further studies in various spheres. All these activities under this programme have drawn the attention of the administrators, and the public has become more conscious about the prevalence of the disease and has started demanding control measures. In endemic areas, the medical profession has also become more conscious of the disease.

DISTRIBUTION OF FILARIASIS.

The prevalence and distribution of filariasis in Uttar Pradesh has been studied under the National Filaria Control Programme launched during 1955-56

when Filaria Survey and the Filaria Control Units were established. As the problem is much more extensive than originally estimated, the delimitation operations in the entire State would involve many more years to come.

In the present paper, attempt has been made to give the details of those areas and districts where the delimitation work has been undertaken uptil now. As has already been mentioned, the State of Uttar Pradesh is divided into five geographical divisions, out of which survey has been carried out only in three divisions, namely, The East Plain, The Central Plain, and the Hills and Plateau.

THE EAST PLAIN DIVISION.

It is formed of the two former natural divisions of the Sub-Himalaya East and the Indo-Gangetic Plain East. It consists of 10 districts where the soil is very fertile but the area is sometimes subjected to drought. The monsoon sets in by the middle of June and continues till the end of September. The mean annual rainfall varies from 40 to 50 inches.

The delimitation of filarial problem has been carried out in all the 10 districts. The details of infection, average infestation, disease and endemicity rates are depicted in Table I.

TABLE I.

Infection, average infestation, disease and endemicity rates in the East Plain Division.

Area.	Infection rate, per cent.	Average infestation per 20 c.mm. of blood.	Disease rate, per cent.	Endemicity rate, per cent.
Azamgarh	8·5	6·8	11·8	19·7
Ballia	12·05	28·19	34·47	43·81
Bahraich	5·65	7·4	9·09	14·04
Basti	9·7	18·25	16·02	24·4
Deoria	7·75	11·0	13·61	19·58
Gonda	8·75	9·15	5·06	13·52
Gorakhpur	13·1	17·8	11·9	23·3
Ghazipur	7·9	9·7	8·68	16·12
Jaunpur	10·5	8·3	11·20	19·50
Varanasi	7·81	9·22	26·62	23·9

On a perusal of Table I, it is evident that the East Plain division has varying degree of filarial endemicity, ranging from moderate to hyper.

THE CENTRAL PLAIN DIVISION.

The division lies to the west of the East Plain division in the central portion of the great alluvial plain of the River Ganga. It is made up of 12 districts which were originally in Indo-Gangetic Plain Central. The mean maximum temperature of the division is 105°F. to 107°F., reaching up to 115°F. to 119°F. The annual rainfall varies between 32 to 40 inches and occurs during June to September.

The filaria endemicity has been worked out in five districts. The details of the infection, average infestation, disease and the endemicity rates are depicted in Table II.

TABLE II.

Infection, average infestation, diseases and endemicity rates in the Central Plain Division.

Area.	Infection rate, per cent.	Average infestation per 20 c.mm. of blood.	Disease rate, per cent.	Endemicity rate, per cent.
Barabanki	10·04	19·87	3·62	13·28
Faizabad	12·04	9·5	8·3	19·3
Hardoi	10·29	13·05	10·72	19·82
Kanpur	16·3	7·4	7·2	16·96
Lucknow	6·79	9·17	4·20	10·85

On perusal of Table II it is clear that the filarial endemicity in the Central Plain division varies from 10·85 per cent to 19·3 per cent., which is of moderate endemicity.

THE HILLS AND PLATEAU DIVISION.

This division is formed of the two former divisions, the Central India Plateau and the East Satpuras. It consists of five districts, four belonging to the Jhansi Division and one Mirzapur district. The districts of Jhansi Division are situated on the plateau and are broken up by low rocky hills covered by stunted trees and jungle. In Mirzapur, there are hill and valley, jungle and forest, ravine and crag with here and there hills encircled by alluvial basins.

The climatological features are high variability in rainfall which varies from 30 to 45 inches and rainy months are from June to September. There is excessive heat during summer, temperature rising to 117°F. In winter, the temperature falls up to 50°F.

The delimitation of filarial problem has been carried out in only one district, namely, Mirzapur. The details of infection, average infestation, disease and the endemicity rates are shown in Table III.

TABLE III.

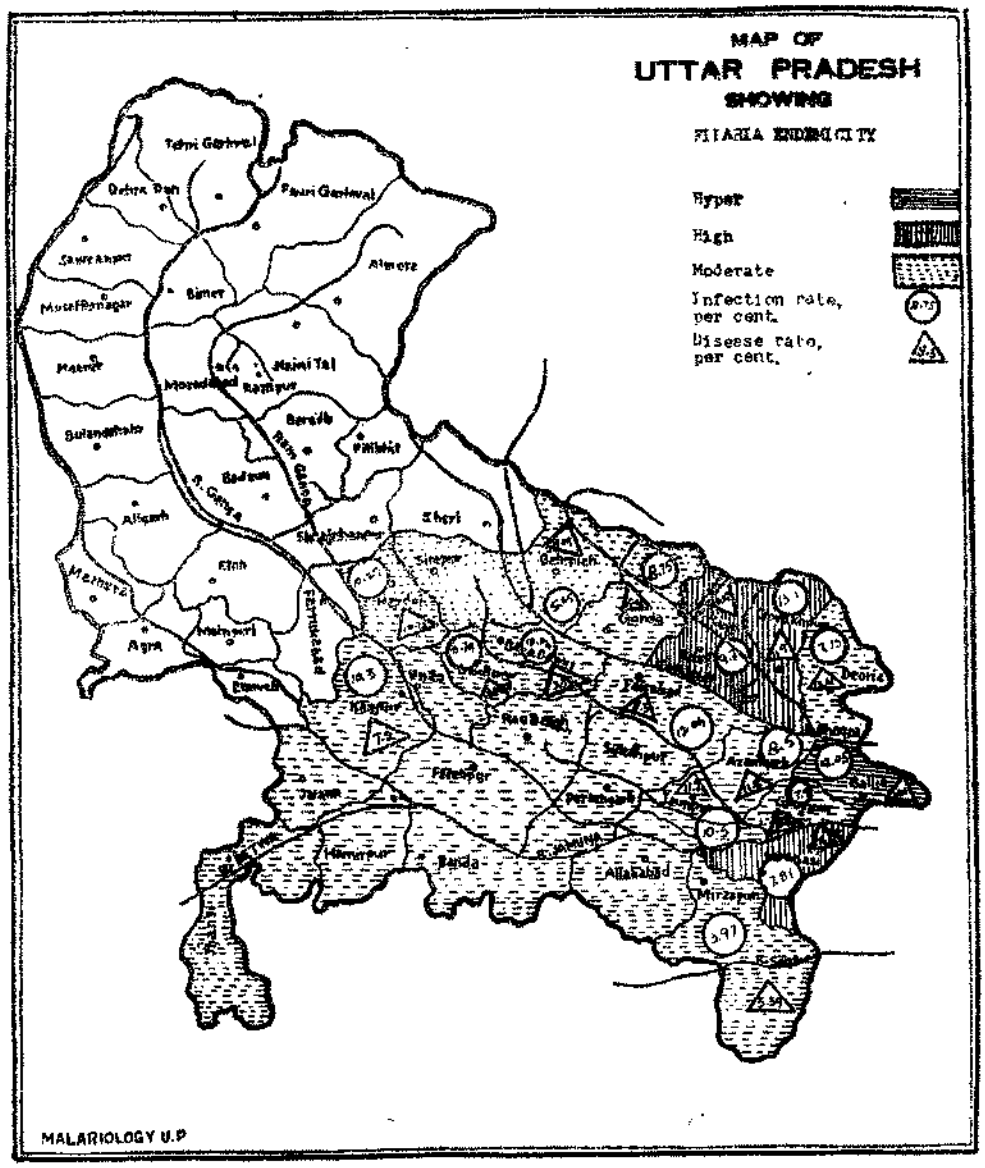
Infection, average infestation, diseases and endemicity rates in Mirzapur District.

Area.	Infection rate, per cent.	Average infestation per 20 c.mm. of blood.	Disease rate, per cent.	Endemicity rate, per cent.
Mirzapur	3·97	6·7	5·39	9·02

The Hills and Plateau Division is moderately endemic for filariasis.

In Map 2, the type of filarial endemicity, as prevalent in the geographical divisions of Uttar Pradesh, along with the infection and disease rates of the districts surveyed, has been shown.

MAP 2.



CONCLUSION.

The delimitation of filarial problem in three of the five geographical divisions of Uttar Pradesh is shown in Tables I, II and III and in Map 2. It can be safely concluded that all the three divisions have definite filarial endemicity, though of varying degrees.

The highest degree of endemicity has been observed in the areas included in the East Plain Division and the worst affected district is Ballia adjoining the State of Bihar. Though there is no definite data available to prove as to when and from where filariasis has been imported in this State, it seems likely that the disease has entered from the east and has taken its foot-hold there and has progressed towards the central and the western districts.

The areas included in the Central Plain Division also show moderate degree of endemicity. This division extends up to the districts of Hardoi and Sitapur. The survey conducted at Hardoi shows an endemicity rate of 19·82 per cent. It is definite that the disease has progressed up to Hardoi in the west. Since no survey operations have been carried out in the areas included in the West Plain Division, it is difficult to assess whether these areas are completely free from filariasis.

The Hills and Plateau division, consisting chiefly of the districts of Jhansi revenue division and Mirzapur district, adjoins the known filarious areas of Vindhya Pradesh. Moderate degree of endemicity (9·02 per cent.) has been observed in Mirzapur District which shows that the division has got definite filarial endemicity but it is not high as in the East and the Central Plain divisions.

In view of the above findings, it is observed that out of the 63·25 million population, more than 50 per cent residing in three of the five natural divisions* of Uttar Pradesh, where filarial surveys have been conducted, are living in the filarial endemic areas and are exposed to the risk of the disease.

It can safely be said that filariasis is an important public health problem of Uttar Pradesh and requires a high priority during the expansion of the National Filaria Control Programme.

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*A rapid filaria survey of Vrindaban in Mathura District (West Plain Division) was carried out by the Malaria Institute of India during the last week of July, 1960. Examination was made of night blood and mosquitoes were collected from a small locality where the residents consisted mostly of settlers from other areas of the country, endemic for filaria. Four out of 40 blood smears and 3 out of 93 specimens of *C. fatigans* were positive. Two of the positive mosquitoes showed infective larvae.

—EDITOR.

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A NOTE ON THE DISTRIBUTION OF *WUCHERERIA* INFECTION IN THE SUDAN AND ADJACENT COUNTRIES.

BY

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[May 25, 1960.]

It is known that *Wuchereria bancrofti* is absent from many dry parts of the world where vectors are abundant (Buxton, 1933). It does not appear to be very common in Africa except in lower Egypt, various coastal regions and islands and certain areas in the vicinity of the great lakes. The parasite has been recorded in North and West Africa, the Sudan, Uganda, Kenya, Tanganyika and Nyasaland; in some of these areas only comparatively recently. Henrard, Peel and Wanson (1946) did not find it in the part of the Belgian Congo adjoining the Sudan but Hawking (1940) recorded it from parts of Uganda near the Sudan.

DISTRIBUTION OF *W. BANCROFTI* IN THE SUDAN.

Among early workers in the Sudan, Balfour (1904*a*, 1904*b*) commented on the rarity of filariasis despite the presence of man-biting species of *Taeniorhynchus* and *Culex* in the Sudan and expected that the infection would be found in parts of the Southern Sudan infested with *T. uniformis* (Theo.). Waterfield (1918) commented on the rarity of filariasis in Suakin. Ensor (1909) reported that "*Filaria nocturna*" occurred at Maridi and was common in other southern areas, but his statement has not been confirmed. Woodman and Bokhari (1941) refer to an old record of the occurrence of *W. bancrofti* in a slide made by Sir Robert Archibald on the Abyssinian border, but this was not published and the particulars were lost.

Cruickshank (1936), discussing the distribution of tropical diseases in the Southern Sudan, reported that *W. bancrofti* had not been identified, but Woodman (1936) thought that it occurred near Li Rangu though it had not been identified in spite of systematic searching. Later, in the course of studies of *Loa loa* in the Zande country during which they examined over 1,500 blood slides containing sheathed microfilariae, Woodman and Bokhari (1941) discovered *W. bancrofti* in twelve cases between the years 1937 and 1938. The microfilariae had the typical morphological appearance of *W. bancrofti* larvae and the identification was confirmed without hesitation by Professor R.T. Leiper to whom the slides were sent. Three cases of diurnal or non-periodic infection were observed; the remainder showed nocturnal periodicity. Commonly there was a mixed infection with *Loa loa*.

In a limited area where infections with *Loa loa*, *Acanthocheilonema perstans* and *Onchocerca volvulus* are well known it is notable that cases of *W. bancrofti*

infection were found only after prolonged and intensive search. Only the microfilariae have been seen. There is no record of the adult *W. bancrofti* having been found in the tissues of any Sudan case. Apparently no cases of *W. bancrofti* infection have been reported from the Li Rangu area since the twelve cases observed by Woodman and Bokhari in 1937 and 1938, although a few positive blood slides have been found in the laboratories of other hospitals between this area and the point of entry of the River Nile from Uganda at Nimule.

These findings are difficult to explain, especially the extremely sporadic nature of the *W. bancrofti* infections. So also are the findings of Woodman and Bokhari, summarized later by Woodman (1955). These workers could find no correlation between elephantiasis and *W. bancrofti* infection. None of their cases of *W. bancrofti* infection showed any signs of elephantiasis and they failed to find this parasite in cases of elephantiasis and allied conditions which are prevalent in the areas in which Woodman and Bokhari worked. *Onchocerca volvulus* and *Loa loa* are common there and Woodman (1955) considered the possibility that these parasites might be responsible for elephantiasis in the absence of *W. bancrofti* infection.

Findlay, Kirk and MacCallum (1941) reported *W. bancrofti* in a Nuba who died in Malakal in 1935. Although the existence of occasional cases of elephantiasis in the Nuba mountains had been known for years this was the first identification of the causal filaria. In 1944 Dr. Mohi Din Mahdi found that *W. bancrofti* was not uncommon in the Nuba mountains in the area round Kadugli. The diagnosis was confirmed in the Stack Laboratories, Khartoum, and blood films taken at intervals, throughout the 24 hours, revealed the classical nocturnal periodicity of the species. Reporting these findings, Horgan (1945) pointed out that although *W. bancrofti* had previously been regarded as extremely rare in the Sudan, the Kadugli observations showed the need for surveys in other areas where clinical filariasis was known or suspected, particularly along the Abyssinian border where cases of elephantiasis and hydrocoele were frequently reported by administrative officers. This is a very primitive area and knowledge about medical conditions in it is still deficient. Onchocerciasis has been found there (Bloss, 1949) and there are old records of Sir Robert Archibald which indicate that *A. perstans* and possibly also *W. bancrofti* are present, but nothing is known about the prevalence of the infections in this area.

ABSENCE OF *W. BANCROFTI* INFECTION IN SOME AREAS.

Culex fatigans is said to be the principal vector of bancroftian filariasis in Egypt. In East Africa *Anopheles gambiae* and *A. funestus* are regarded as the chief vectors in the region of the great lakes. Heisch *et al.* (1956) suggest that *Aedes pembaensis, A. aegypti* and *Culex fatigans* may be important vectors on the East African coast where the epidemiological pattern of bancroftian filariasis is different from that on the islands of Lake Victoria. No studies have been made to define

the vectors of *W. bancrofti* in the Sudan. It is known that this parasite can be transmitted by many different mosquitoes, including species of *Anopheles, Culex* and *Aedes*. In all places in the Sudan where *W. bancrofti* infection has been found, several species of mosquitoes, proved vectors in other places, were known to be present.

Much interest attaches to the apparent absence of *W. bancrofti* infection in wide areas of the Central and Northern Sudan where several species of mosquito, known to be vectors elsewhere, are prevalent. In view of the scanty and erratic distribution of *W. bancrofti* in the Southern Sudan and its occurrence in the north in Egypt it may be rash to state that this infection is completely absent from wide areas of the Sudan. But places like Atbara, Khartoum and the irrigated cotton growing area of the Sudan Gezira are served by a well organized system of hospitals and dispensaries in which many thousands of blood films are examined annually. The fact that no instances of *bancrofti* infection have come to light among the local inhabitants, indicates that this condition is extremely rare or probably absent. During the East African campaigns of the Second World War, large numbers of African troops from other parts of the continent entered the Sudan. Knowing that suitable vectors were present the Public Health authorities became concerned about the possible introduction of *W. bancrofti* infection and insisted that blood films should be taken from these immigrants and examined by the Laboratories staff. The work was tedious and time consuming at a time when we had other things to worry about but it revealed a large number of infections. As far as the writer knows, no special precautions were taken with immigrants found to be infected. Diethylcarbamazine, which might have been used for temporary sterilization of the peripheral blood, was not then available and reliance was placed on the ordinary anti-mosquito measures of malaria control. These measures did not completely control even malaria. In 1942 *Anopheles gambiae*, the principal malaria vector of the Northern Sudan and an important vector of *W. bancrofti*, spread northwards from the Sudan into Egypt, producing calamitous epidemics of malaria in that country. Yet subsequent records indicate that *W. bancrofti* infection did not become established in the Northern and Central Sudan although we had a susceptible population, a source of infection in the infected immigrants and no shortage of potential vectors.

DISCUSSION.

The scanty and erratic distribution of bancroftian filariasis in the Southern Sudan suggests the possibility of some animal reservoir host. No such reservoir host has been discovered so far in the case of *W. bancrofti* although it has recently been found that animal reservoirs are involved in the life histories of *W.* (*Brugia*) *malayi* and *Loa loa*. The faunal and avian filaria reported in the Sudan have been listed by Kirk (1957). One of those, the filaria described by L.J. Davis in the blood of an ox from the Nuba mountains, was said to resemble *W. bancrofti* morphologically, but the adult was not seen, only the microfilariae in the blood.

In East Africa, Nelson and Heisch (1957) have reported the finding of *malayi-like* microfilariae in the blood of cats and dogs in Patta Island off the Kenya Coast, but the adults of this microfilaria have been recovered and have proved to be hitherto undescribed species (Buckley, 1958).

Buxton (1933) suggested that low atmospheric humidity imposes limits on the transmission of *W. bancrofti* infection which is essentially a disease of the damp tropics. It is known that many efficient vectors of the worm exist, among them *Culex fatigans*, several species of *Anopheles* and members of other genera. Recalling how abundant these mosquitoes may be in human dwellings in nearly all warm countries, Buxton (1933) notes that filarial infection is absent from large parts of the world where vectors are abundant and that in countries in which it occurs its distribution is not uniform. He quotes the work of Acton and Rao on the distribution of filarial infection in India which indicates how closely the presence of the infection coincides with the area of high rainfall. The problem is a complex one, however, influenced by factors such as temperature, altitude, local variations in ecoclimate, force and direction of the prevailing wind, density of the human population at risk and the application or otherwise of control measures (Jordan, 1955).

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A REVIEW OF RECENT RESEARCH ON FILARIASIS IN MALAYA.

BY

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[July 10, 1960.]

INTRODUCTION.

RECENT filariasis research in Malaya dates from the work of Poynton and Hodgkin who, in extensive surveys of endemic areas in 1937 and 1938, gathered a mass of information concerning the epidemiology, clinical manifestations and transmission of the disease (Poynton and Hodgkin, 1938 ; Institute for Medical Research, Federated Malay States, 1937-1940). They showed that it was almost entirely due to *Brugia* (formerly *Wuchereria*) *malayi* (Buckley, 1958 : 1960), and *W. bancrofti* was confined to infections amongst immigrants. It was a disease endemic in the swamp-forest regions of Pahang, Perak, Selangor and Johore States, and in the coastal rice-field areas of north-west Malaya. Investigations in Perak and Pahang showed that transmission was mainly by mosquitoes of the genus *Mansonia* (*Mansonioides*), particularly *M. longipalpis* (Wharton, 1960) (now known to be two species *M. dives* and *M. bonneae*) and *M. uniformis*. They also found two species of microfilariae in long-tailed macaque monkeys (*Macaca irus*) (Poynton and Hodgkin, 1939) ; one closely resembling the microfilariae of *B. malayi*, and one which was probably the recently described monkey parasite *Dirofilaria magnilarvatum* (Price, 1959).

Clinically the disease was manifested by periodic bouts of lymphangitis with occasional abscesses, and elephantiasis of the legs in chronic sufferers varied from 2 to 10 per cent of the population in endemic areas. In heavily infected areas in Lower Perak and East Pahang microfilaria carriers were commonest in children of school age and in young adults, and rates were higher in males than in females. Periodicity of the microfilariae was nocturnal, though not so marked as that shown by *W. bancrofti*. No effective method of treatment was available, and any attempts at control were stopped by the war. Investigations were resumed in 1947 when efforts were made to identify the mosquitoes responsible for transmission in the rice-field areas of Kedah, Province Wellesley and Penang. *Anopheles* mosquitoes, *A. barbirostris* and *A. lesteri* were identified as filaria carriers with the *Mansonia* mosquitoes *M. uniformis*, *M. indiana* and *M. annulifera* also important (Wilson and Reid, 1953). The finding that *A. barbirostris* was a vector was surprising since Hodgkin had shown that they were poor hosts under laboratory conditions (Institute for Medical Research, Federated Malay States, 1937-1940). Knowledge

of the larval breeding places of the *Mansonia* mosquitoes was extended and an attempt was made to reduce transmission in Kedah by removing water-hyacinth (*Eichornia crassipes*), one of the main host plants (Reid, 1950). Prospects of control were further enhanced by the wartime discoveries of the residual insecticides and the promising filaricidal drug, diethylcarbamazine. Experiments with the insecticides DDT and BHC showed that *Mansonioides* mosquitoes were moderately susceptible (Wharton, 1955 ; Reid and Wharton, 1956) and preliminary hospital trials with daily doses of hetrazan, a citrate salt of diethylcarbamazine, showed that microfilariae were quickly cleared from the blood (Wilson, 1950). A drawback of drug treatment, which it was thought would detract from its use as a method of control by mass treatment of rural populations, was the marked reaction in microfilaria carriers after taking the drug, this reaction taking the form of fever and prostration for periods up to 5 days (Wilson, 1950 *loc. cit*). In 1951, a small scale trial of mass treatment with daily doses in an endemic area on Penang Island ran into difficulties, some people refusing to take further doses of the drug because of the reaction experienced (Turner and Sodhy, 1959). Attempts to prevent these reactions by the administration of antihistamines were unsuccessful.

A discovery of epidemiological importance was the finding by Polunin of endemic filariasis with elephantiasis amongst aborigines living far away from known endemic foci, in mountain valleys at heights of over 1500 feet (Polunin, 1951). This was the last major contribution to the knowledge of filariasis in Malaya prior to the setting up in East Pahang of the Kuantan Branch Laboratory of the Institute for Medical Research, in 1953. Financed jointly by the Malayan Government and the Colonial Development and Welfare Funds, this laboratory, with one medical officer and one entomologist, has since been engaged in full-time filariasis research, and has produced a large amount of information published in some 28 papers. It is a review of this work that now follows, in an attempt to give a concise account of what has been achieved, and what our present concept of the disease is in Malaya.

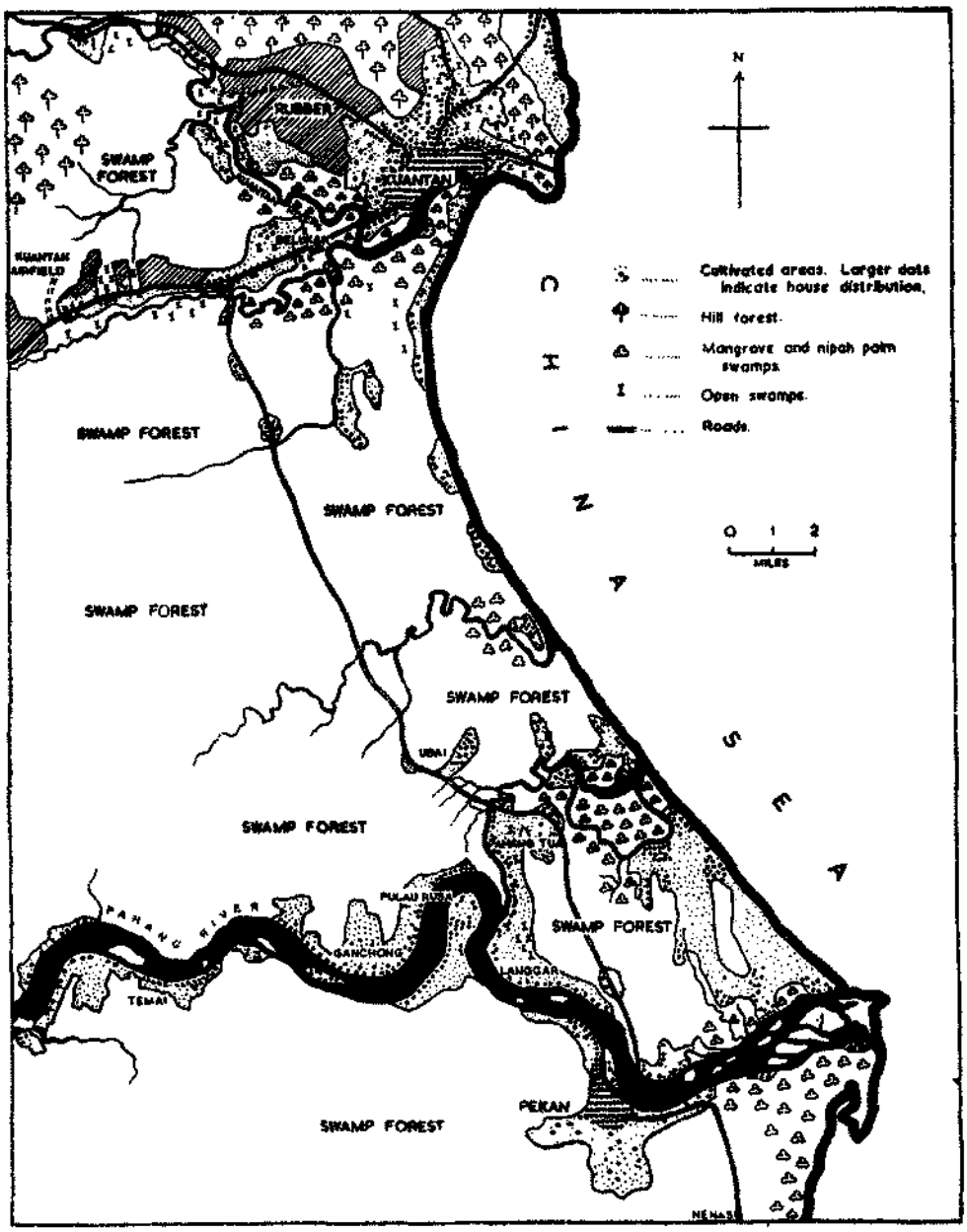
EPIDEMIOLOGICAL INVESTIGATIONS.

THE ENDEMIC AREAS.

Intensive studies have been confined to one area in the State of Pahang. Kuantan, the State capital, lies 20 miles north of the heavily infected area of Pahang Tua on the lower reaches of the Pahang River (Map I). A road leading to the royal town of Pekan runs through the area, some of which is good agricultural land, undeveloped because of the presence of filariasis, for the appearance of elephantoid deformities amongst the people discourages new settlers. About 4500 people, almost all Malays, live in the area comprising the *mukims* (sub-districts) of Pahang Tua, Langgar, Pulau Rusa, and Ganchong. Their houses are situated mostly near the banks of the river or its delta tributaries, but are widely scattered so that the first house in a *kampong* (village) is usually a mile or more from the last. The houses

MAP I.

Filariasis studies in an area in the State of Pahang. Kuantan, the State capital, lies 20 miles north of the heavily infected area of Pahang Tua on the lower reaches of the Pahang River.



are raised a few feet off the ground and made of wood with *attap* (palm thatch) roofs. A few cattle and buffaloes, goats or sheep, and fowls, are kept in each village. The people earn sufficient from rubber small-holdings, coconuts, rattan, and the sale of fruit in season to keep themselves in a simple way of life, where the heaviest form of work is the seasonal planting and harvesting of rice.

Some observations were also made in endemic areas further inland on the Pahang River where microfilaria rates of *B. malayi* were low and there was little filarial disease but *W. bancrofti* infections were present (Wharton, 1960*b*). With the discovery of different forms of *B. malayi* (Wilson *et al.*, 1958) special studies were made in other areas but the aim at the beginning of these investigations was to make a thorough study of one area in East Pahang.

INITIAL SURVEYS.

By the end of 1953 a complete house-to-house census, and clinical and blood survey had been made. Registers of the people were compiled by houses, and so arranged that all details of a person and his movements, results of blood surveys, treatments, etc., could be recorded. Blood surveys were done starting at 7 p.m., 20 c.mm. blood films being taken from the finger (or toe of infants), dried overnight, and stained in dilute Giemsa the following day*. Ninety per cent of the population of all age-groups were examined ; of these, 40 per cent had microfilaria (*B. malayi*) in their blood ; 6 per cent had elephantiasis, and less than 1 per cent had both, making a total filarial infection rate of 47 per cent. (Wilson, 1958).

The youngest microfilaria carrier was an infant aged 3½ months. Seven per cent of children below the age of one year, 57 per cent of children aged 4-5 years, and over 60 per cent aged 6-13 years, were microfilaria carriers. There was little difference in sexes up to the age of 10 years ; thereafter males had a higher infection rate, higher mean microfilaria levels, and males had a higher elephantiasis rate (11 per cent) as opposed to females (3 per cent.) (Wilson, 1958 *loc. cit.*).

FORMS OF *B. MALAYI*.

While these basic surveys were being undertaken, microfilaria carriers from an endemic area, nearer Kuantan, were persuaded to come to hospital. From these persons it was found that the periodicity of the microfilariae was much less marked than in a similar series of patients examined about the same time on Penang Island in north-west Malaya (Turner and Edeson, 1957) ; a nocturnal rise in microfilaria counts did occur but was less marked, and microfilariae could always be found in the peripheral blood by day. In addition, a feature of Giemsa-stained blood films from patients in north-west Malaya was the large number of

* The use of dilute Giemsa stain has been a routine practice in malaria and filaria blood surveys in Malaya for many years. Where necessary, infection rates of both diseases can be calculated from the same blood films, and the constant colour contrast between the microfilariae of *B. malayi* and *W. bancrofti* make for easy and swift species identification, in areas where both may occur (Wilson, 1956).

exsheathed microfilariae, with the empty sheaths usually lying nearby; measurements of formalin-fixed microfilaria showed that they had a greater mean length than those from near Kuantan, but there were no anatomical differences to be seen in stained films. However, the contrast in terrain of the two kinds of endemic area (open, settled rice-fields in the north-west, and swamp-forest near Kuantan), with their differences in microfilarial behaviour and appearance, clearly indicated the presence of two forms of *B. malayi*, which have been named semi-periodic and periodic (Wilson *et al.*, 1958). The semi-periodic form seems characteristic of the swamp forest areas with *M. dives/bonneae*, *annulata*, and *uniformis* as natural vectors, and the periodic form of the open rice-fields with *A. barbirostris*, the *A. hyrcanus* group and *M. uniformis*, *indiana* and *annulifera* as natural vectors. The anomaly of Hodgkin's failure to infect *A. barbirostris* is thus explained, the carriers used in the experiments being infected with the semi-periodic form. Subsequent research has revealed (*see below*) that the semi-periodic form is adapted to a number of vertebrate hosts other than man, an important fact in the consideration of control measures.

MOSQUITO INFECTIONS.

Early collections and dissections in the Kuantan area showed that species of *Mansonioides* predominated in the swamp forest, and had an overall infection rate of 1·5 per cent (Reid, 1954). The *M. dives/bonneae* group had a higher infection rate than *M. uniformis* and about 0·5 per cent harboured full-grown infective larvae. In the first two years of investigation 28,000 mosquitoes were dissected. *M. dives* and *M. bonneae* were both confirmed as experimental vectors, and *M. annulata* and *M. uniformis* were of importance (Reid, 1955). Catching, on the bare legs of men for 1½ hours after dusk, has proved a satisfactory method to determine mosquito infection rates and the intensity of transmission. Magoon traps, baited with goats, were used for long term observations on mosquito populations while window-trap huts were used in experiments designed to test the susceptibility of *Mansonioides* to insecticides. Results showed that dieldrin was superior to DDT or BHC, but that a dose of 100 mg. per square foot was needed to give a satisfactory kill for 6-7 months.

HOSPITAL TRIALS.

About this time trials were started to find a suitable regime for mass treatment of a rural population with diethylcarbamazine (Edeson and Wharton, 1958*a*). It was already known that 100-110 mg. per kg. of body weight over a period of 16-21 days effectively destroyed all, or nearly all, microfilariae, but that such a regime was unsuitable for use in the field (Turner and Sodhy, 1959). Doses* of 4-6 mg. per kg., given monthly for six months, were found to be the most effective, all patients so treated being negative 7 months after their final dose.

* Throughout this paper doses refer to diethylcarbamazine acid citrate (hetrazan, banocide, carbilazine), which is made up in 50 mg. tablets, each containing approximately 51 per cent base.

Similar doses given weekly had almost as good an effect but none of the dosage schedules tested succeeded in eliminating the reaction, while at the same time retaining the effect on the microfilariae. The drug had no effect on the enlarged legs of elephantiasis patients who benefited equally by rest in bed or bandaging, but reverted again on going home.

CLINICAL FEATURES.

Enlarged lymph nodes have been shown to be equally prevalent in a non-endemic area (Edeson, 1955) and are therefore unreliable as early signs of infection. Turner (1959) stresses the importance of early recognition of episodic adeno-lymphangitis which, if left untreated, can lead to the onset of elephantiasis. These episodes recur at intervals, often provoked by exercise, and are commonest in the lower limbs. Elephantiasis of either one or both legs is therefore the commonest sequel to filarial infection with *B. malayi* ; arms, breasts or genitals being rarely affected. No significant differences have been found in the clinical features of the two forms of *B. malayi* in this country.

PILOT CONTROL EXPERIMENTS.

Following the hospital drug trials and the observations on the effect of the residual insecticides on the vector mosquitoes, it was decided to start pilot control experiments. Weekly and monthly doses of 5 mg. per kg. diethylcarbamazine were compared and dieldrin was sprayed at the heavy dose of 100 mg. per square foot at six months intervals (Wharton, Edeson, Wilson and Reid, 1958).

Results are summarised in Table I.

TABLE I.

Pilot Control Experiments—showing the effects of control. Records compiled from the same people examined at each survey (Edeson, Wharton and Buckley, 1945).

Area.	Method of control.	Survey number and time.	Number of persons examined.	Mf. rate per cent.	Mean number of mf. per 20 c. mm. (all films)*
Sawah	Mass treatment with diethylcarbamazine : weekly doses of 0·5 mg. × 1, 1 mg. × 1, 5 mg. × 6 (per kg. body weight)	1. Before treatment.	78	49	19·7
		2. One month after end of treatment.	78	10	0·26
		3. One year after end of treatment.	78	14	0·68
Tanah Puteh	Mass treatment with diethylcarbamazine : monthly doses of 5 mg. × 6 (per kg. body weight).	1. Before treatment.	119	35	11·2
		2. One month after end of treatment.	119	6	0·16
		3. One year after end of treatment.	119	7	0·17
Lubas	Spraying of houses with dieldrin at 100 mg. per square foot.	1. Before spraying	251	39	12·6
		2. One year after spraying.	251	45	15·2
		3. Two years after spraying.	251	41	12·6

In Sawah, 70 per cent of the population completed the course of treatment; the two small initial doses did not help in alleviating the reaction. In Tanah Puteh, only 37 per cent of the population completed the full course of 6 monthly doses. More people were found to turn up weekly than monthly, and over a period of 6 months some had moved elsewhere. There was little difference between the effect of weekly or monthly doses on microfilaria rates. However, what was a cause of concern was that neither treatment had any effect on infection rates in mosquitoes caught in the areas—in spite of a 99 per cent reduction of the human microfilarial reservoir. In addition, subsequent annual surveys showed a steady increase in human infection rates, so that 3 years after treatment the infection rates in Sawah were little lower than in an untreated comparison area (Ganchong) where transmission was of similar intensity. As the hospital trials had shown the efficacy of the dosage regimes used, it seemed likely that reinfection was occurring, and as the *Kampong* (village) was well isolated from others an animal reservoir of infection was considered a possible cause of the reinfection.

In Ubai, spraying with dieldrin over a period of two years had no effect on microfilarial rates or densities, and although large numbers of *M. dives bonneae* were killed in houses, there was no observable effect on transmission (Wharton, Edeson, Wilson and Reid, 1958). This, as in the drug-treated areas, may have been due to the small area under control. but the presence of an animal reservoir could also be responsible. However, it was decided to continue spraying at the same heavy dose (100 mg. per square foot), and to continue observations for possible long-term effects (*see below*).

FILARIAL INFECTIONS IN ANIMALS.

Another investigation was in progress into filarial infections in animals. Poynton and Hodgkin's finding of *malayi*-like sheathed microfilariae in long-tailed macaque monkeys (*M. irus*) in 1938 (Poynton and Hodgkin, 1939) indicated that man might not be the only definitive host of *B. malayi*. The investigation therefore commenced with trapping of macaque monkeys and among the first examined was found one infected with *malayi*-like microfilariae—a lucky finding in view of the very low infection rate of this species that the subsequent prolonged survey revealed (Laing, Edeson and Wharton, 1960). In addition, the chance finding of *malayi*-like microfilariae in a dog led to the examination of other animals. As a result *malayi* like microfilariae were found in domestic dogs and cats, slow loris (*Nycticebus coucang*) and a leaf monkey (*Presbytis* sp.). A search for adult worms in some of these animals resulted in the recovery for the first time from animals of adult specimens of *Brugia* (Buckley and Edeson, 1956). What is more, examination showed that there were two species; those found in a long-tailed macaque (*M. irus*) and 3 cats resembled the earlier descriptions of adult worms recovered from man (Rao and Maplestone, 1940; Bonne *et al.*, 1941). Those from a cat, a dog, and a slow loris were found to be a hitherto unknown species, and were

named *B. pahangi*. The adult worms are not as long as those of *B. malayi*, and there are morphological differences in the spicules of the male, and internal anatomy of the female. The microfilariae are difficult to distinguish from those of *B. malayi*, though they are significantly longer, and there are minor morphological differences between the two species. But when only a few microfilariae are available, identification by microfilariae alone can be open to doubt.

With regard to the developing larvae of *B. pahangi*, experimental feedings with *M. dives/bonneae, uniformis* and *annulata* showed that for practical purposes the larvae were indistinguishable from those of semi-periodic *B. malayi* but *M. dives/bonneae* were much less efficient hosts of *B. pahangi* (Edeson, Wharton and Laing, 1960). Another important characteristic of *B. pahangi* was its ability to develop very readily in *Armigeres obturbans*. By contrast when this mosquito was fed on carriers of semi-periodic *B. malayi*, the majority of the microfilariae failed to develop, being chitinised in the thorax. This striking difference, together with the difference in *M. dives/bonneae*, was found to be a useful confirmatory test in species diagnosis, but the identical appearance of the developing larvae of the two species made interpretation of dissection results from wild-caught mosquitoes extremely difficult.

It was, therefore, very necessary to continue animal investigations in an attempt to find out whether the parasite resembling *B. malayi* in animals was in fact the same species as in man, and if so, what animals were sufficiently heavily infected to act as reservoir hosts. Such investigations would also reveal the hosts of *B. pahangi* and some idea of the importance of this parasite could be obtained.

Results of extensive surveys of all the commoner forest and domestic animals in the endemic area have shown that the dusky leaf monkey (*P. obscurus*) (Plate I) is an important host in East Pahang, with a natural infection rate of 70 per cent. Subsequent investigations, which are still continuing, leave little doubt that the parasite in man and the parasite in the leaf monkey are one and the same species and share a common vector. Leaf monkeys, including specimens of the other two species occurring in Malaya (*P. melalophos* and *P. cristatus*), have been found infected in other endemic areas of semi-periodic *B. malayi*, and there can be little doubt that they form an animal reservoir of semi-periodic *B. malayi* from which nearby human populations can be infected. Other animals found infected include the domestic cat which, however, is more frequently infected with *B. pahangi* (Laing, Edeson and Wharton, 1960). Precipitin tests to discover the feeding habits of *Mansonioides* vectors found resting outdoors by day near houses showed that though the great majority fed on cattle, more fed on man than on cats (Edeson and Wharton, *in press*). So it seems probable that cats are more often infected from man than man from cats.

Other animals found naturally infected with semi-periodic *B. malayi* were the long-tailed macaque monkey (*M. irus*) which transmission experiments (*see below*) showed to be a comparatively unreceptive host, the wild cat (*Felis bengalensis*) (relatively uncommon), and the pangolin (*M. javanicus*) (also relatively uncommon).

PLATE I.

Dusky leaf monkeys (*Presbytis obscura*), the principal animal reservoir host of semi-periodic *B. malayi*—at the Filariasis Research Laboratory, Kuantan.

B. pahangi was found to have an even greater diversity of hosts; though commonest in Carnivores [found in the tiger, domestic cat, two species of wild cats, three species of civet cats, the dog, and an otter (*Lutra sumatrana*)], it has also been found in the slow loris (*N. coucang*), moon-rat (*Echinosorex gymnurus*), giant squirrel (*Ratufa bicolor*) (Laing, Edeson and Wharton, 1960 *loc. cit.*) and more recently in the dusky leaf monkey (*P. obscurus*).

Limited animal surveys have been done in the periodic *B. malayi* endemic area of Kedah (north-west Malaya), and 1 out of 106 cats, examined by night, was found infected (Laing, Edeson and Wharton, 1960 *loc. cit.*). *B. pahangi* was not encountered. Recently 5 leaf monkeys examined (including post-mortem examination of lymph glands and concentration tests on heart blood) showed no evidence of infection. The periodic form of *B. malayi*, therefore, does not appear to be associated with reservoir hosts other than man. Further confirmation of this was obtained in transmission experiments (*see below*) in which infections of the semi-periodic form were readily established in a variety of animals, but infections with the periodic form, for the most part, did not develop well, and those successfully transmitted were either light or transient.

LARGE SCALE CONTROL.

Experiments in the pilot control area had shown that mass drug treatment with diethylcarbamazine was a practical proposition, although a single course of treatment was unlikely to result in permanent control. It was now decided to undertake a large scale experiment with drugs in the neighbouring area of Pahang Tua and Langgar with a population of 2000 people, using a weekly dosage regime, and with the residual insecticide dieldrin plus drugs in Pulau Rusa with a population of 1500. About this time also a filariasis campaign, run on similar lines, was started in Kedah by the State Medical Department, and organised and carried out by a laboratory technician trained in filariasis work first under Dr. T. Wilson and then in Kuantan. In both areas mass treatments were successfully accomplished [in East Pahang 81 per cent, and in Kedah 85 per cent, received the full course of treatment (5 mg. per kg. body weight weekly × 6)]. Between 5 and 10 per cent of both populations were left untreated on account of infancy (under 4 months of age only), pregnancy or ill health. Table II compares the results of treatment on human infection rates.

In Pahang Tua the microfilaria rate fell from 31 to 5 per cent and the mean number of microfilariae in 20 c.mm. (all films) from 7·1 to 0·3. Retreatment of known microfilaria carriers, those insufficiently treated previously, and those excluded previously for one reason or another, was carried out 2 years later. The regime was similar and although the number of people involved was about one sixth of the original, the actual work was considerable. The result was merely to reduce the microfilaria rate from 5 to 3 per cent and the mean number of microfilaria in 20 c.mm. (all films) from 0·5 to 0·3, four months afterwards (Reid and

TABLE II.

Comparing the effect of mass treatment with diethylcarbamazine 5 mg./kg. weekly for 6 weeks on the microfilaria rates and densities of B. malayi *in Pahang Tua, and in Kedah.*

Area.	Control period.	Blood surveys: Number of persons examined.	Mf. rate per cent.	Mean number of mf. per 20 c. mm. (all films).
Pahang Tua.	Before treatment.	1,632	31	7·1
	One year after treatment.	1,712	5	0·5
Semi-periodic *B. malayi*	Two years after treatment (and 4 months after re-treatment of microfilaria carriers and others).	1,009	3	0·3
Kedah.	Before treatment.	887	23	3·36
	7—12 months after treatment.	888	2	0·22
Periodic *B. malayi*	Two years after treatment (and 10 months after re-treatment of microfilaria carriers).	815	0·5	0·02

Laing, 1958). Similar results were obtained in Pulau Rusa but in neither area did control succeed in eliminating infected mosquitoes. The rate of transmission appeared to be reduced by about half but *M. dives/bonneae* with infective stage larvae continued to enter both the controlled areas.

The effect of treatment in Kedah was much better; microfilaria rates fell from 23 to 2 per cent 7-12 months after treatment and dropped again to 0·5 per cent two years after treatment, with a parallel drop in microfilarial density (Mohamed Yusof B. Hassan, 1959). Only microfilaria carriers found at the post-treatment survey were re-treated. These results indicate that with periodic *B. malayi* a single mass treatment, properly undertaken and with subsequent surveillance and treatment of remaining carriers, is probably capable of stopping further transmission of the disease. Semi-periodic *B. malayi* which seems to be associated with a considerable animal reservoir, and has much wider areas of endemicity, will probably require repeated mass treatment. Re-infections are certainly taking place but it is not yet possible to say how often re-treatment will be necessary.

Associated with these large scale control experiments, observations on the parasite rate in Ubai were continued where spraying has now been in progress for six years. Microfilaria infection rates have fallen from 42 per cent to 24 per cent and the mean number of microfilariae in all films from 15 to 4. However, a similar drop occurred naturally over this period in a comparison area where no control has been tried; moreover, mosquito infection rates have remained unaltered, and new infections in people born since the onset of spraying have

occurred every year. Under these conditions, control by residual insecticide spraying must be considered ineffective (Reid and Laing, *in press*).

No toxic effects were noted either amongst the inhabitants or their animals as a result of the dieldrin sprays and apart from the usual effect of the killing of bedbugs, cockroaches, etc., a much appreciated result was the virtual eradication of head-lice infestation with *Pediculus humanus capitis* in school children. In the unsprayed area of Pahang Tua, a small survey showed that 90 per cent of girls and 60 per cent of boys were infested, whereas in Pulau Rusa and Ubai no active infestations were found (Wharton and Omar, 1959). A dieldrin-resistant strain of *Culex fatigans* was found after 2-3 years (Wharton, 1958), but no resistance was encountered in *Mansonia* mosquitoes.

EXPERIMENTAL TRANSMISSION OF *BRUGIA* INFECTIONS.

TRANSMISSION OF *B. MALAYI* (SEMI-PERIODIC FORM).

A large number of experiments have been carried out in attempts to infect various species of animals with semi-periodic *B. malayi* from man (Edeson and Wharton, 1957 : 1958*b*). Infective larvae, from mosquitoes fed 10-11 days before on a microfilaria carrier, were inoculated subcutaneously into the prospective animal host. The animals were kept in mosquito-proofed rooms and their blood examined weekly for microfilariae. As a result, successful transmission (the appearance of microfilariae in blood films) was recorded in the long-tailed macaque monkey (*M. irus*), rhesus monkey (*M. rhesus*), slow loris (*N. coucang*), a civet cat (*Paradoxurus hermaphroditus*) and the domestic cat. Adult worms corresponding to the description of *B. malayi* were also recovered from slow loris, a rhesus monkey and domestic cats. The long-tailed macaque monkey was difficult to infect compared to the domestic cat which was quite the most receptive host, and in which infections appeared to be stable with high microfilaria counts. A special experiment, designed to exclude any possibility of contamination by naturally acquired infections and to supply absolute proof of transmission, was done using cats from a completely non-endemic area, Kuala Lumpur, and laboratory-bred mosquitoes only (*M. uniformis*) (Edeson and Wharton, 1958*b*). Unfortunately several of the cats died of distemper early after inoculation, but of the 5 which survived, all became positive for microfilariae 80 to 96 days after inoculation, and adult worms were obtained from two. As an extra precaution two of these cats were flown back to Kuala Lumpur directly after inoculation. Following on from these experiments, strains of *B. malayi* from man were passaged via mosquitoes through a monkey, a cat, and a slow loris, and through a cat, into a monkey, and into other cats. Prepatent periods in all these experiments were commonly between 75 and 95 days.

TRANSMISSION OF *B. PAHANGI*.

Similar experiments have also been done with *B. pahangi* (Edeson, Wharton and Laing, 1960), successful transmission being achieved in slow loris, civets, and

domestic cats. Attempts to infect macaque and rhesus monkeys were quite unsuccessful. Again domestic cats were the most receptive of hosts, and development (pre-patent periods 59-83 days) was quicker than that of *B. malayi*.

MIGRATION AND RATE OF GROWTH OF *BRUGIA* IN EXPERIMENTALLY INFECTED CATS.

Information on the migration and rate of growth of both species was obtained from cats killed at varying times during the pre-patent period, and from those cats dying of distemper. It was found that infective larvae migrated to lymph vessels within 16 hours, and that they were usually found near the site of inoculation, though some migrated to the internal lymph vessels. Two moults occurred with *B. malayi* at 9 - 10 days and 35 - 40 days. Moults of *B. pahangi* were not seen. Females of *B. malayi* increased in size up to 288 days (55 mm. mean length) and males only up to 64 days (25 mm. mean length) (Edeson and Buckley, 1959).

TRANSMISSION BY MOSQUITO BITE.

Transmission was also achieved with both species by mosquito bite; in the case of *B. malayi* with laboratory bred *M. uniformis* fed on a human carrier, 10 days prior to feeding on two uninfected cats (Wharton, Edeson and Laing, 1958). In the case of *B. pahangi* with *A. obturbans* fed on an infected cat, only one mosquito fed on the latter. Pre-patent periods were 76, 106, and 69 days respectively.

MIXED INFECTIONS.

In a further experiment transmission of both species to the same cat was achieved, microfilariae of both *B. malayi* and *B. pahangi* being present in subsequent blood films (Reid and Edeson, 1959). Later, such mixed infections were found to be quite common in several different kinds of animals in nature (Laing, Edeson and Wharton, 1960).

OTHER LABORATORY HOSTS.

More recently transmission experiments with semi-periodic *B. malayi* have been carried out with other laboratory animals—guinea-pigs, white mice, white rats, golden hamsters and dogs (Reid and Laing, *in press*). Transmission was successful in 2 of 6 hamsters and 2 of 2 dogs. Development to maturity also occurred in 2 of 6 white rats though microfilaraemia was only demonstrated in one, after death. These experiments indicate further the extraordinary adaptability of the parasite, and with repeated passage a strain might be developed that could be maintained in hamsters or rats.

TRANSMISSION OF *B. MALAYI* (PERIODIC FORM).

Transmission was achieved in some cats, but infections were light and transient. In a dusky leaf monkey (*P. obscurus*) a heavy microfilaria level was achieved, and in a rhesus the infection has persisted for 2 years but at a low level. The characteristic marked nocturnal periodicity was not attenuated by transmission

to another host (Reid and Laing, *in press*). Further experiments on these lines might well confirm that leaf monkeys particularly are receptive to periodic *B. malayi*. The apparent absence of infections in nature is probably due then to vector habits.

Attempts to transmit *W. bancrofti* to cats and monkeys have been totally unsuccessful (Reid and Laing, *in press*).

LONGEVITY OF EXPERIMENTAL INFECTIONS.

The longevity of microfilaraemia following a single inoculation has been extremely variable with semi-periodic *B. malayi*. One cat is still positive 4 years after inoculation ; others have been positive for periods of a few weeks or months only. Similar results have been achieved with *B. pahangi*. Investigations are continuing.

THE ACTION OF DIETHYLCARBAMAZINE IN CATS.

Another investigation made possible by transmission experiments was a study of the effect of diethylcarbamazine on *Brugia* parasites in cats (Edeson and Laing, 1959). Microfilaria levels were reduced slowly in spite of large doses of the drug given orally and intraperitoneally, and those microfilariae remaining after treatment were capable of developing normally in *Mansonioides* mosquitoes. In some cats microfilariae continued to circulate for 6-10 months after treatment. Both adult *B. malayi* and *B. pahangi* were killed by the drug and death was followed by calcification.

TRANSMISSION—ANIMAL TO MAN.

Attempts were made to transmit *B. malayi* and *B. pahangi* from animals to man. In an early experiment (Buckley, 1958*b*) a volunteer was inoculated with *Brugia* sp. (*malayi* ?) from *M. irus*, and a syndrome very similar to tropical pulmonary eosinophilia developed 14 weeks after inoculation which subsided after treatment with diethylcarbamazine. The same volunteer was then inoculated with *B. pahangi* and after an interval of 10 weeks a high eosinophilia with pulmonary symptoms developed again, likewise responding to diethylcarbamazine. On the other hand no microfilariae were apparent in blood films, but the onset of eosinophilia in each case corresponded to the end of the pre-patent period. The concept that filarial infections are involved in the aetiology of tropical pulmonary eosinophilia, was thus strongly supported.

Another experiment (Edeson *et al.*, 1960) in which two volunteers were inoculated with semi-periodic *B. malayi* of human origin passaged through a cat, and a further two volunteers who were inoculated with *B. pahangi*, resulted in similar manifestations in all volunteers. Adeno-lymphangitis in the inoculated limb occurred during the pre-patent period and eosinophilia, but without much leucocytosis, developed at the end of the pre-patent period. Pulmonary manifestations were absent, and there was no microfilaraemia except a very light one in

a volunteer inoculated with *B. pahangi*. These microfilariae developed in *A. obturbans*, and a cat inoculated with infective larvae was successfully infected. *B. pahangi* is thus capable of developing in man, though a long-continued search for microfilariae in blood films from human populations infected with *B. malayi*, exposed equally to risk of infection by *B. pahangi*, has failed to discover the latter.

DISCUSSION.

When these investigations began in 1953, filariasis due to *B. malayi* was thought to be a purely human disease like that of *W. bancrofti*. The finding of *malayi*-like sheathed microfilariae in a simian host 15 years previously still awaited investigation. This has now been done with the result that the disease appears to be partly a zoonosis, with at least two forms—the semi-periodic with a reservoir in leaf monkeys as well as in man and capable of maintaining itself in other animal hosts, and the periodic, perhaps the true parasite of man, which has no animal reservoir. The forms of *B. malayi* are adapted to different environments and different vectors, resembling the two forms of *W. bancrofti* in this respect, and whereas it would appear probable that transmission of the periodic form can be interrupted by mass treatment with diethylcarbamazine, the semi-periodic form will continue to maintain itself in animals unless some means of eliminating the vector mosquito is found. In this respect residual insecticide spraying with dieldrin has proved a failure, and unless elimination of the breeding places by draining the huge areas of swamp land is undertaken, control will have to rely on repeated mass treatments at intervals yet to be decided.

Comparatively little has been said about the work on the biology of the *Mansonia* (*Mansonioides*) mosquitoes which are responsible for the transmission of both *B. malayi* and *B. pahangi*. All the evidence points to *M. dives/bonneae* and *M. annulata* as the major vectors, capable of maintaining both parasites in a forest and canopy environment and also bringing the infection to populated areas. From the entomological point of view a means of distinguishing between the morphologically identical infective-stage larvae of *B. malayi* and *B. pahangi* would lead to a more intelligent appreciation of mosquito infection rates.

Filariasis due to *W. bancrofti* in Malaya is still rather an unknown quantity. It exists in Singapore and Penang and with the speedy growth of urban development in Malaya to-day, it may well constitute a public health problem in years to come, particularly as its vector *Culex fatigans* is widely distributed, common in urban areas, and difficult to control. The distribution of rural *W. bancrofti* carried by *A. letifer* appears at present to be very restricted although our knowledge of it is far from complete. It is unlikely to be responsible for much clinical filariasis in rural areas.

There is still much to be done, particularly with regard to the pathology, immunology, and serology of filariasis. The easy transmission and maintenance of *Brugia* parasites in domestic cats opens a door to these spheres of research which

may help to solve the mystery of tropical pulmonary eosinophilia, as well as leading to a better understanding of the epidemiology and pathogenicity of filarial disease in man.

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EXPERIMENTAL STUDIES ON THE INFECTIVE RESERVOIR OF *W. BANCROFTI* IN THE HUMAN POPULATION.

BY

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[May 21, 1960.]

MANY disconnected observations have been carried out on the infectivity to mosquitoes of different microfilariae carriers, mainly in an attempt to relate microfilarial density in the blood to the degree or intensity of infection produced in the mosquito. In very few localities, however, have these methods been applied on a sufficiently representative scale to give any real indication as to what constitutes the main reservoir of infection in the human population, or to what extent each human age-group contributes towards this reservoir.

The problem of defining more accurately this infective reservoir in the human population is one that has never received due attention, but it is one which urgently requires investigation, particularly in relation to the development of mass therapy and chemoprophylaxis.

Work on this subject was carried out on a limited scale in Ghana a few years ago in a study of the infectivity of *W. bancrofti* carriers to *Anopheles gambiae* (Muirhead-Thomson, 1954*a*). On the basis of that experience and in the light of recent developments elsewhere it is suggested that the following factors are among those which require critical study in areas with different degrees of human microfilaraemia. Although the comments refer in particular to nocturnal *W. bancrofti* many of them may prove to have wider application.

1. Present indications are that the infectivity of the human population to mosquitoes can only be studied satisfactorily by employing xenodiagnostic methods in which batches of laboratory-reared vector mosquitoes, such as *A. gambiae* and *Culex fatigans*, are allowed to feed at night,—e.g., 9-10 p.m.—on human subjects selected at random from all age-groups. The gorged mosquitoes are then kept alive in the laboratory, and after the appropriate number of days they are examined for mature infective filaria larvae. The need for this objective method originally suggested by Highby (1946) and first applied practically in Ghana has been emphasized by Jordan's (1959) recent observations in East Africa to the effect that a person with demonstrable microfilariae in 40 c.mm. (i.e. a person who may still fail to reveal microfilariae in the standard 20 c.mm. blood sample) can still infect, and produce infective larvae in a small proportion of feeding mosquitoes—in this case *Culex fatigans*.

Similarly Wharton (1957), in studies on filariasis in Malaya, was able to show that when *Mansonia longipalpis* was allowed to feed on a *Brugia malayi* carrier showing 0·025 microfilariae per c.mm. (i.e. 3 microfilariae in 120 c.mm. of blood), 12 per cent

of the mosquito batch became infected. In this connection Edeson (1959) has produced very pertinent figures about the high proportion of low-density microfilaria carriers which can remain undetected in a routine survey based on the examination of 20 c.mm. of blood. Symes (1960) has also reported recently that with *W. bancrofti* in Fiji, *Aedes pseudoscutellaris*, *Aedes fijiensis* and *Culex fatigans* can all become infective after feeding on blood with such a low microfilarial density that they may not be detected in 20 c.mm samples. In the case of *A. pseudoscutellaris* infection may still occur after feeding on people whose microfilarial density is too low to be detected by ordinary survey techniques.

Jordan's observations also stressed the need for using large batches of mosquitoes in order to detect these low-grade infectors. In Ghana, for example, the infectivity of subjects selected at random was based on the infection rate of 20—30 *A. gambiae* surviving 12 days or more after the infective feed (Muirhead-Thomson, 1954*a*). With such a sample, however, there is a distinct possibility of missing low grade infectors, and it would now appear advisable to base final conclusions on several batches of this size, possibly taken at intervals over a few days on each subject. Although in theory the use of a single exposure to a very large batch of mosquitoes would seem the least laborious method of testing infectivity, in practice the unfavourable reactions of the patient to such mass biting would suggest the advantage of using several smaller batches.

In areas where more than one vector is involved, e.g., *Anopheles gambiae* and *Culex fatigans* in parts of Africa, it would be particularly instructive to run parallel xenodiagnostic tests with both species on the same subject. Different vectors are liable to differ not only in their capacity to acquire infection, but, what is more important in this case, in their capacity to develop these infections, to the infective stage. Parallel exposures of this kind would undoubtedly provide valuable additional data of this kind, and might also help to determine to what extent the importance of the low-grade infector varies according to the identity of the dominant mosquito vector.

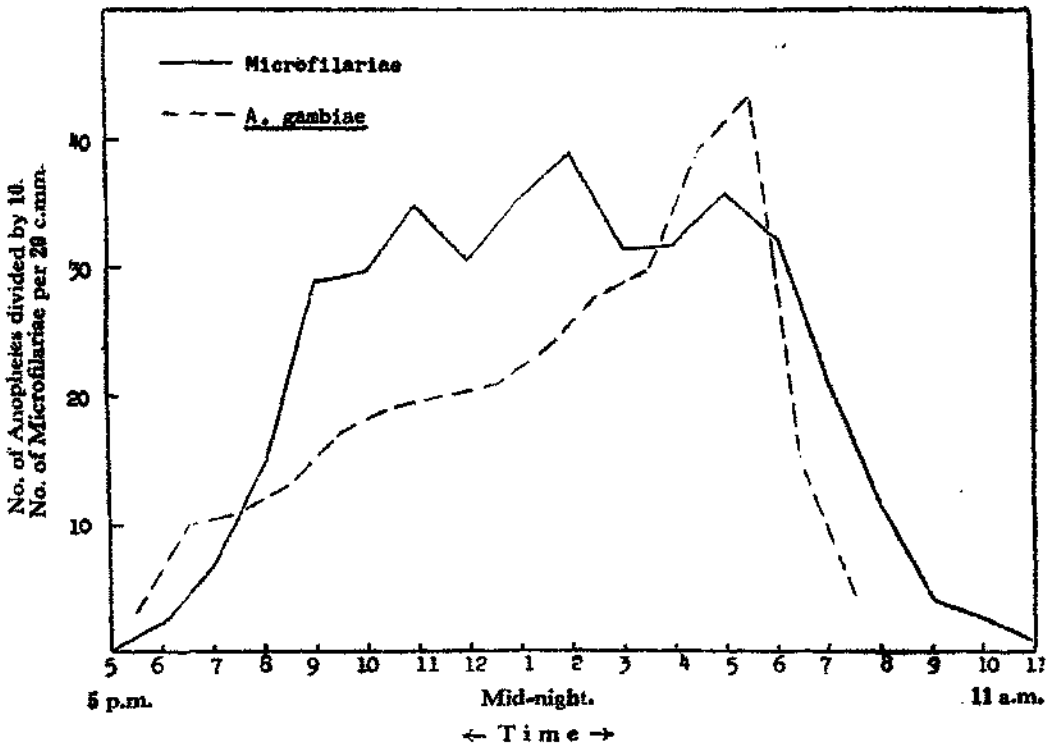
2. On the basis of this random xenodiagnostic survey it would be most instructive to select one or two good infectors and one or two poor infectors and to study their infectivity at intervals throughout the night. Again, parallel experiments with two vectors would be suggested. Some tentative observations of this kind have been carried out in Ghana (Muirhead-Thomson, 1954*a*) but on a scale which was too small, however, to produce valid conclusions. The experience gained in that work, however, showed that it had great possibilities for further development, and that the kind of information produced was clearly essential for a fuller understanding of the human reservoir of infection.

3. On the basis of these experiments described in the two sections above, and on the basis of the well-established facts about the nocturnal periodicity of *W. bancrofti* microfilariae (e.g. in W. Africa, Poindexter, 1950; Burch and Grenville, 1955; Kershaw, 1950) the next step is to correlate these findings with the nocturnal

biting cycle of the vector species. For example, Graph 1 depicting the biting activity of *A. gambiae* typical of many part of Africa shows a tendency to reach a peak in the later part of the night, at a time when microfilarial density is still high. This pattern of biting, however, is by no means universal among potential vectors of *W. bancrofti*. In the case of *Culex fatigans* (*quinquefasciatus*) the nocturnal biting cycle appears to follow different patterns in different countries. A trend similar to that of *A. gambiae*, with an increased intensity of biting in the latter part of the night, has been reported for *C. fatigans* in British Guiana (Giglioli, 1948). In Belem, Brazil, where *C. fatigans* is almost the sole vector of *W. bancrofti*, Deane (1951) has reported a peak of biting activity at midnight. At the other extreme it was pointed out several years ago in Porto Rico (O'Connor and Hulse, 1935) that this species fed most vigorously in the evening and early part of the night; those which had not fed by 10 p.m. rarely doing so later that night. A similar biting peak early in the evening was also reported for *C. fatigans* in West Africa by Kerr (1933).

GRAPH 1.

Nocturnal periodicity of W. Bancrofti *microfilariae (mean of 11 subjects, 20 c.mm. blood samples* compared with biting cycle of main vector,* Anopheles gambiae.†

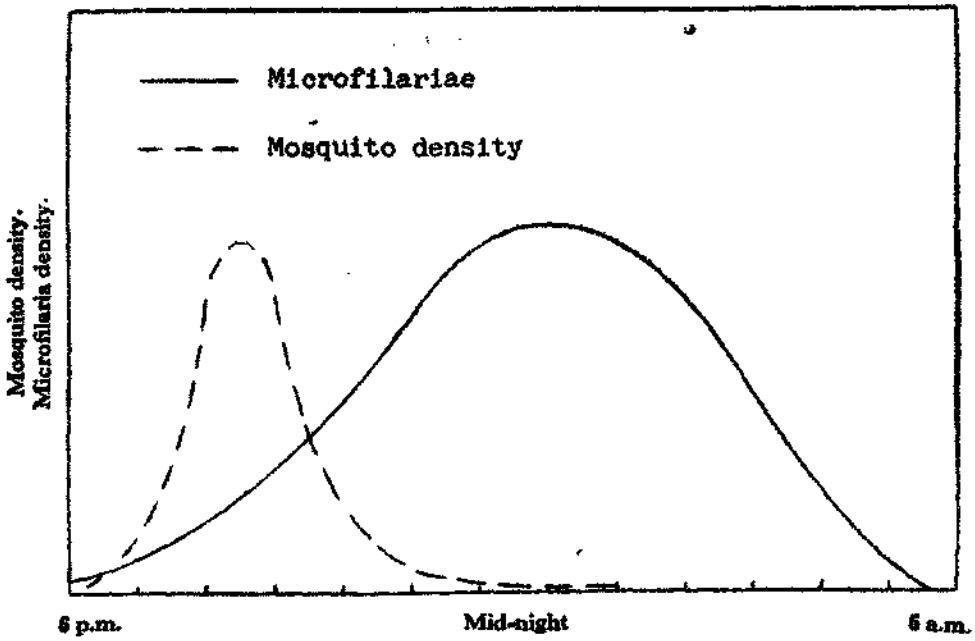


* Burch and Grenville (1955), Liberia.
† Haddow *et al.* (1947), Uganda.

Such a pattern of biting (Graph 2) would appear to offer greatly reduced chances of such mosquitoes becoming infected, especially from low-grade microfilaria carriers, and this divergence has, in fact, been put forward as a possible explanation in Porto Rico of the fact that *C. fatigans* has a remarkably low natural infection rate in spite of the high incidence of microfilaraemia in the human population.

GRAPH 2.

Normal curve of nocturnal periodicity of W. bancrofti *microfilaria compared to theoretical biting cycle of potential mosquito vector, with a biting peak in the early evening.*



There is a great deal of information in the literature about nocturnal biting cycles of various species of Anopheles, and of various species of culicines which have been studied in the course of work on yellow-fever and other mosquito-borne viral diseases (Haddow, 1954). The biting cycles of potential culicine vectors of nocturnal *W. bancrofti* and *B. malayi*, however, have not been investigated in such detail. Raghavan *et al.* (1958), in their studies on *B. malayi* infection in Travancore, found that both *Mansonia uniformis*—the main vector—and *Culex vishnui* had a peak of biting in the first quarter or first third of the night. There is clearly need for further intensive work of this kind in order to elucidate to what extent the vector efficiency of various suspect species is determined by the relation between the peak of biting activity and the peak of microfilarial infection in the human carrier.

4. Another factor which would have to be taken into account in order to judge the extent which each human age-group provides infective meals for biting

mosquitoes is that the available evidence suggests that mosquitoes do not bite all age-groups or all individuals indiscriminately (Muirhead-Thomson, 1951, 1954*b*; Thomas, 1951; Ribbands, 1949). The available direct observations on *Anopheles*, for example, indicate that older children and adults are bitten more than very young children and infants. In addition, men appear to be bitten more than women. To what extent these observations apply to the culicine vectors of filaria, is not known, and such work would obviously have to be repeated and confirmed in the study areas selected.

It is possible that the application of all these findings might provide a much clearer indication as to which individuals, or type of individual, show a high potential infectivity combined with a high degree of attraction to the mosquito vector. It seems quite likely that only a small proportion of such individuals in the community would be sufficient to maintain an effective and permanent reservoir of infection.

In considering the human reservoir of *W. bancrofti* infection it should be borne in mind that the infective individual occupies a place of unusual importance in that, unlike in the case of malaria, his contribution is not transient, but may maintain a steady high level of infectivity for months or years. It seems worth a great deal of trouble, therefore, to find these individuals and to devote particular attention to them, not only from the point of view of improving the efficiency of mass chemotherapy, but also as a potential source of persistent infection when mass chemotherapy or prophylaxis has failed to interrupt transmission.

SUMMARY.

Recent findings about the infective role of low-grade microfilaria carriers of *W bancrofti*, together with the increasing extent of mass chemotherapy and chemoprophylaxis in control of filariasis, have made it imperative to study more critically the infective reservoir in the human population. Various factors are briefly described which must be taken into consideration in assessing the infective contribution made by each microfilaria carrier in the human population, and in determining the relative importance of different potential vectors when exposed to carriers showing differing degree of microfilaraemia.

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THE EPIDEMIOLOGY AND DYNAMICS OF TRANSMISSION OF *WUCHERERIA BANCROFTI* AND *BRUGIA MALAYI.*

BY

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AND

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[May 15, 1960.]

INTRODUCTION.

THE purpose of this paper is to discuss the epidemiology of *Wuchereria bancrofti* and *Brugia malayi* in terms of the dynamics of transmission. Part I includes a brief review of data related to the perpetuation of the life cycles of the two parasites ; Part II consists of a brief description of the epidemiologic factors involved in *Wuchereria* infection and disease ; while Part III concerns the dynamics of transmission and a suggested working model expressing the interrelationship of host, parasite and vector.

Other filarial worms, in common with *W. bancrofti* and *B. malayi*, have evolved patterns of relationship with their definitive and vector hosts. Although considerable knowledge is available about some of these such as *Dirofilaria immitis, Litomosoides carinii* and *Conispiculum guindiensis,* the spectrum of filarial infections in nature is meagre. Because of ignorance concerning *W. bancrofti* and *B. malayi,* information on non-human filarial worms has been utilized when necessary.

The major problem for the filarial parasite is the maintenance of life and perpetuation of the species, the major problem of the definitive hosts and vectors is the prevention of disease. Variations in individual parasites, hosts, and vectors, however, as well as different circumstances related to the numbers of parasites and their locations within the hosts, result in a diversity of manifestations as well as patterns of prevalence.

PART I. CRUCIAL FACTORS IN THE LIFE CYCLE OF *WUCHERERIA BANCROFTI* AND *BRUGIA MALAYI.*

The production of microfilariae (m/f) by the adult worms is a crucial event in the perpetuation of the species. Hawking (1954) estimated that an adult *Litomosoides carinii* produces 12,000 - 22,000 (average 15,000) microfilariae per 24 hours. Studies by Raghavan *et al.* (1956) indicate that an adult female *Conispiculum*

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guindiensis gives birth to some 19,517 (range 1,350 to 87,660) microfilariae per 48 hours. Their longevity is important ; Rao (1933) concluded the life span of microfilariae of *W. bancrofti* to be as long as seventy days, although Knott (1935) in transfusion experiments observed their persistence no longer than 14 days. It is of interest to relate these figures to estimates of the microfilariae reservoir and of the number of adult female worms in an endemic area.

Beye *et al.* (1952) and Kessel (1953) reported that carriers in Tahiti had microfilaria densities of 79 - 128 m/f per 20 c.mm. (aperiodic *W. bancrofti*) and assuming a random distribution of m/f in the circulating blood volume, an estimated 2×10^7 m/f would be circulating. Using Knott's microfilariae survival time, such a carrier should harbour 150 adult female worms ; if Rao's are used, such a carrier would harbour 30 adult female worms. The fact that a carrier harbours from 30 to 150 adult worms is interesting, the important fact being, however, that the adult females must produce m/f in sufficient quantities and m/f must remain viable for a sufficient length of time to insure the transfer of microfilariae to a suitable mosquito vector. Microfilarial periodicity observed in periodic *W. bancrofti*, and to a lesser degree in *B. malayi*, is associated with parasite survival even though its mechanism has not been completely elucidated.

The ingestion of microfilaria by a suitable vector mosquito is another crucial factor. Stage and Yates (1936) report that *Aedes vexans* and *A. aldrichi* are capable of imbibing as much as 1·99 c.mm. of blood at a meal. Wharton (1957) with *W. (Brugia) malayi* reports that 2·5 c.mm. of blood is taken up by a biting *Mansonia longipalpis*. Byrd and St. Amant (1959) and others indicate that there may be some mechanism whereby mosquitoes selectively take up microfilariae.

The probability of the carrier host being bitten by a suitable female vector mosquito, is individualized in accordance with the bionomics of the particular vectors in any given area, and their population in relation to human or other hosts.

Once microfilariae have been ingested other variables may operate. Kartman (1953*a*), noting that microfilariae survived longer in some species than others, suggested the presence of some lethal factor in the midgut of the inhospitable host. Microfilariae of *D. immitis* were also mechanically inhibited from migrating from the midgut by rapid formation of blood clots in *Aedes aegypti* and *A. albopictus*. It has also been noted by Kartman (1953*b*) that microfilariae may be lost in the dejecta of the mosquito.

In a review on the arthropod as a host to the nematode, Lavoipierre (1958) categorizes the events in the mosquito vector as those associated with : (*a*) ingestion of microfilariae, (*b*) development of filariae larvae and their migration within the mosquito, and (*c*) the emergence of the infective forms. Host injury, and probably host reaction, occurs at each stage, and although there are many variations by species, and changes in atmosphere influencing the rate of development of the contained parasite, etc., the density of infection is an important factor.

The influence of these variable responses are reflected in the efficiency of specific vectors to serve as transmitting agents.

Rosen (1955) obtained an infection rate of 5·3 per cent in *Aedes polynesiensis* fed on a carrier with an average density of infection of 0·4 microfilariae per 20 c.mm. (non-periodic *W. bancrofti*) and 11·6 per cent infection rate when fed on a carrier of 0·6 per 20 c.mm. Wharton (1957) records an infection rate of 12 per cent in *Mansonia longipalpis* when fed on *B. malayi* carrier with 0·4 microfilariae per 20 c.mm. Jordon (1959) observed that 2·6 per cent of *Culex quinquefasciatus* fed on a carrier of periodic *W. bancrofti*, with a mean microfilarial density of 0·5 per 20 c.mm. of blood, harboured late developmental forms of *W. bancrofti*. On the other hand, Bahr (1912) reported that much denser human infections were necessary.

The effect of heavy infection on the vector mosquito has been observed by many investigators. Bahr (1912) commented that mosquitoes fed on small infections lived generally till the twenty-first day, but out of a batch of 200 fed on a patient with 71 m/f per 15 c.mm. of blood, only 17 survived till that time.

Bruyning (1953), Byrd and St. Amant (1959) and others have reported an increased mosquito vector mortality after feeding on human carriers with heavy microfilaremia. Wharton found no difference between the survival of *Mansonia longipalpis* fed on *B. malayi* carrier with 2·3 microfilaria per c.mm. (46 per 20 c.mm.) and the survival of mosquitoes fed on uninfected persons. Mosquitoes which fed on carriers with 10·7 microfilaria per c.mm. (214 per 20 c.mm.), survived normally during the first week or so after infection, but died rapidly from the 8th day onwards. Wharton and also Krishnaswami *et al.* (1959), using *Dirofilaria repens* and *Aedes aegypti*, showed an association between microfilaria carrier density and the number of filaria larvae in the mosquito.

In fact, there is probably general agreement of a direct relationship between the intensity of infection in the vector mosquito and the microfilariae density in the human carrier.

The optimum level of infection undoubtedly varies from one mosquito species to another, and the vector carrier efficiency represents a balance of many variables associated with the bionomics of the mosquito vector, including biting habits, and life span. For example, Hu (1935) found viable infective stage larvae in *C. pipiens* var. *pallens* as long as twenty-nine days after exposure and proved that repeated infections in the same mosquito may occur.

Another set of factors operates during the transfer of the infective stage filarial larvae from the infected mosquito to host.

Lavoipierre (1958) summarizes the general view that the normal route of emergence of filariae is either from the mouth parts or from the base where they are united to the head by membranes. The significant observation of Pratt and Newton (1946) that infected *C. quinquefasciatus*, allowed to feed on sugar solution

without a blood meal, lost nearly all their filariae larvae some eight days after the larvae had become mature, emphasizes another type of parasite mortality in terms of species perpetuation.

Mosquito vector preferences for blood may also result in blind alleys for the parasite. Horsfall (1955) writes that *Culex pipiens fatigans* (*quinquefasciatus*) certainly requires blood in its diet and the host of choice seems to be any that is available. He cites the following : Hammon *et al.* (1945) examined the blood of 266 freshly engorged females collected from houses in California. Birds had been chosen by 88 per cent and the rest had fed on horse, cow or dog. In Guam, 352 females were caught in dwellings and 87 per cent had fed solely on man (Reeves and Rudnik, 1951). In the Belgian Congo, 77 per cent had fed on man in one case and 50 per cent in another (Wanson and Nicolay, 1937). In Indo China, Toumanoff (1935) found that all of 96 mosquitoes collected in dwellings contained human blood, and that 61 per cent of 72 caught in stables had fed on man, and 11 on buffalo. In Dakar, Mathis (1935) stated that the preferences were for birds as sources of blood.

An educated guess by Dalmat (1959) is that this species probably has a blood meal about every 6 days.

After the young filaria is within the host, the probabilities against its survival may still be formidable. O'Connor and Hulse (1935) present evidence that on penetrating the skin many parasites develop locally near the point of entrance.

Edeson and Buckley (1959) injected infective larvae of *B. malayi* into cats and the recovered worms were usually related to the site of inoculation. It is of interest that the migration into the lymphatic nodes was rapid, often less than 16 hours. Females continued to increase in size up to 288 days. Of a total of 3,124 infective larvae injected, 408 (13 per cent) *B. malayi* were recovered.

Romiti (1935), on the basis of his extensive surgical experience in British Guiana, believes that *W. bancrofti* in that country selectively goes to the lymphatic plexuses of the spermatic cord in males and the broad ligament in females.

Finally, another set of factors exists in the necessity of the female parasite being fertilized by a male before she begins the production of microfilariae. Webber (1954), using *L. carinii* transplanted into cotton rats, concluded that repeated insemination of the female worm was not necessary for continued microfilariae production.

Table I recapitulates some of the crucial conditions and associated factors involved in the life cycles of these two parasites.

It is interesting to consider quantitative measurements of transmission and the events contributing toward completion of the life cycle, through the consideration of the probabilities reflecting the experience of the host, parasite and vector.

It seems quite clear, however, (1) that the factors involving transmission and the prevalence of infection in an area, is a complex of quantitative relationships, in

a dynamic everchanging situation, (2) that presently available information only partially contributes to an understanding of these relationships and (3) that the complex in one area is undoubtedly different from that in another area and generalizations are dangerous.

Before accepting these conclusions, however, it may be of value to review briefly some of epidemiologic factors concerning *Wuchereria* and *Brugia* infections and disease.

TABLE I.

Listing of crucial conditions in the life cycle of W. *Bancrofti and indication of associated factors.*

Event.	Associated factors and variables.	Components on the problem of biological survival of parasite.
I. Viable microfilariae in peripheral blood.	1. Number of m/f produced by adult filarial worms. 2. Variation in m/f production. 3. Life span of microfilariae*. 4. Absolute numbers of m/f in host. 5. Periodicity of m/f in peripheral blood*. 6. Host factors*.	If m/f counts range from 0 to 70 per 20 mm ^{3}, and other factors in life cycle remain optimum, the probability of this component of biological survival is good.
II. Vector mosquito biting host and ingesting microfilariae.	1. Amount of host blood ingested by mosquito*. 2. Microfilarial density*. 3. Number of m/f ingested by mosquito. 4. Biting habits of specific mosquito vector*. 5. Mosquito vector population and densities.	For parasite survival, and other factors in transmission being optimum, only one m/f needs be ingested by a suitable vector mosquito, and only one mosquito bite is necessary.
III. Development of parasites within mosquito.	1. Number of m/f ingested by mosquito*. 2. Mosquito host factors. 3. Vector efficiency*. 4. Mosquito bionomics and life span. 5. Differences between infected and non-infected mosquitoes.	This is a question of the life expectancy of wild vector mosquitoes, their vector differences and the problem of whether or not infected mosquitoes have a different life span than non-infestations.
IV. Infective vector mosquito biting host and transferring infective filariae larvae.	1. Number of infective filariae larvae in mosquito*. 2. Mortality of infective larvae in transfer from mosquito to host. 3. Biting habits of specific mosquito vector*. 4. Biting mosquito density in population.*	If other factors in transmission are optimum, and there is no mortality of infective larvae in transfer from mosquito to host, only one infective filarial larvae is necessary. For parasite survival, however, both a male and a female must be in the same host.
V. Development in host-site selection and reproduction.	1. Host factors. 2. Parasite factors related to growth and development. 3. Trorhism? male and female worms for fertilization. 4. Fertilization.	For perpetuation, one female and male adult filarial worm must migrate and mate.

*Available knowledge

PART II. EPIDEMIOLOGIC CHARACTERISTICS OF *WUCHERERIA BANCROFTI* AND *BRUGIA MALAYI.*

(A) THE PARASITES.

Wuchereria bancrofti.—The more universally distributed of the two parasites, is to be found throughout the world where combinations of carriers and vectors are suitable for transmission. Stoll (1947) estimated there to be 189 million persons infected, but with additional surveys, especially in India, this estimate may be conservative. Generally, the parasite is to be found in the area of the world bounded by 41° North to about 28° South Latitude in the Eastern Hemisphere and about 30° South in the Western Hemisphere (Craig and Faust).

Two types of *W. bancrofti* exist, the periodic and the so-called non-periodic—the latter being found in the Philippines, Fiji, Ellice Island, The Cook and the Society Islands and perhaps the Andamans as observed in a preliminary survey by Basu (1958). Although *W. bancrofti* collected from various areas of the world show no morphological differences, observations such as Rosen's (1953), in which he indicated greater infectivity of the Tahitian strains of *C. fatigans* when fed on a carrier from Martinique infected with the nocturnal type of *W. bancrofti* than when fed on a carrier with the non-periodic type, suggest physiological differences.

Certainly, clinical manifestations of the nocturnal type vary in British Guiana and Surinam from those observed in India, as well as from manifestations of the aperiodic type observed in Tahiti. The host range of *W. bancrofti* has been limited to man, as far as we know.

The description of *W. bancrofti* var. *vaucelli* by Galliard and Byroo (1955) from the south-east coast of Madagascar, however, opens up possibilities of this parasite having a wider host range than man.

Brugia malayi.—Since this parasite was first described by Brug in 1927, it has also been found in Indonesia, Thailand, Borneo, Tonkin, Kerala, Orissa, Assam, Ceylon, China, and among Tonkinese in the New Hebrides and Koreans in Hawaii.

The work of Buckley, Wilson, Wharton, Edeson, Turner and others in the Malay group, in unravelling the problem of *B. malayi* and its vectors, is of the greatest epidemiologic significance. In Malaya there are two strains of *B. malayi*, the Kedah/Penang strain and the Pahang strain. According to Wilson (1958) these differ markedly in respect to nocturnal periodicity, animal reservoirs, and incubation period ; the Kedah/Penang strain exhibiting marked nocturnal periodicity, as yet no known animal reservoirs, but transmitted experimentally to cats, and an incubation period of 13 months. The Pahang strain, on the other hand, does not have a marked nocturnal periodicity, animal reservoirs are known in cats and monkeys, and the filarial worms are transmitted to these and other animals by inoculation to cats by mosquito bites ; the youngest infection is 3½ months.

Physiologic and immunologic differences between these *B. malayi* strains may exist. The differences in the degree of periodicity of these two Malayi strains is

even more marked in the periodic and aperiodic types of *W. bancrofti*. Even in the aperiodic type, however, there may be a periodic tendency as indicated by the data of Eyles *et al.* (1947) and Edgar and Beye (1952).

(*B*) VECTORS.

Wuchereria bancrofti.—The major natural biological vectors include representatives from *Culex*, *Aedes*, *Anopheles* and *Mansonioides*.

Craig and Faust (1957) contains a list prepared by Dr. Lincicome of 58 species in which complete development has been demonstrated to occur in nature ; Raghavan (1956) lists 45 species or varieties of *Anopheles* alone that can serve as vector by laboratory or by natural infections. Vectors of major importance in the Pacific are *Culex quinquefasciatus* (fatigans), *C. pipiens*, *Aedes* (Stegomyia) *scutellaris* subgroup ; (*A. polynesiensis* and *A. pseudoscutellaris* in Fiji, *A. tongae* in Nuie, a species of *A. tongae* in Tonga, a species related to *A. polynesiensis* in Rotuna and *A. polynesiensis* in Tahiti), *Anopheles farauti* in the Melanesian Islands, *Aedes* (Ochlerotatus) *vigilax* in the Loyalty Islands and New Caledonia, *Anopheles darlingi* in British Guiana, and many others.

The bionomics of these various vectors have a great influence on the prevalence of the infection in the human population, and determine to a considerable extent the epidemiologic characteristic of *Wuchereria bancrofti* infection within a given area.

For example, *A. polynesiensis* is a relatively wild mosquito, and as shown by Buxton (1928) and many others, has a flight range of approximately 100 meters. On islands such as Fiji, the Solomons, and the Cook Islands, transmission can take place in the villages where carriers are concentrated or in the bush as indicated by Jachowski (1952) when the inhabitants are working on their plantations. *C. quinquefasciatus* is more of an urban mosquito, breeding in stagnant water with sewage, and in areas such as certain cities of India, British Guiana, and Surinam, infections with *W. bancrofti* are considered to be urban in character. *Aedes poicilius*, one vector in the Philippine Islands, breeds in the leaf axils of the abaca plant as demonstrated by Cabrera and Tubangui (1951). Workers in the abaca industry have for many years experienced a high prevalence of hydroceles, attributed to their occupation of stripping the abaca fiber. There are good probabilities that this is related more to filarial infection and plantation transmission.

Brugia malayi.—As far as is known, the vectors of these parasites are less universally distributed. The major ones in nature include *Mansonia annulifera*, *M. annulata*, *M. annulipes*, *M. indiana*, *M. indica*, *M. longipalpis*, and *M. uniformis*.

Each of these *Mansonia* species are associated with aquatic plants, especially *Pistia stratiotes*, and *Eichhornia speciosa*. Burton (1959) observed the attachment of *Mansonioides* larvae to 12 species of plants growing in tanks or ponds in Kerala and

these associations condition the epidemiologic characteristics of *B. malayi* infections. It is of interest that Giglioli (1948*b*) in British Guiana observed the biting habits of *M. titilans* to be nocturnal.

In areas such as Thailand and China, *Anopheles barbirostris* and *A. hyrcanus* var. *sinensis* are implicated in the transmission of *B. malayi*.

(C) HOST FACTORS.

The separation of host from exposure factors by using statistical epidemiologic techniques on the distribution of microfilaria carriers is hazardous. Variables in any consideration of the distribution of prevalence in an area include age, race, sex, occupation, and many intangibles associated with population density, dress, and work habits, contact with mosquito vectors and others. A brief review of these factors for both *W. bancrofti* and *B. malayi* may be informative and indicative of some participation of the host in the host, vector, vector-parasite complex.

Age.—The age specific infection rates for both *W. bancrofti* and *B. malayi* increase rapidly until approximately the 20-30 age-group and then increase only gradually or level off through the older age-groups in most areas of the world. Infections with *W. bancrofti* can begin as early as 14 months, while infections with *B. malayi* (Pahangi) have been reported by Wilson as early as 3½ months.

Congenital filariasis apparently does not occur, or if so, is quite rare. Peel and Van Oye (1950) did not find any parasites in 107 infants of 104 mothers infected with various filariae in a survey in Leopoldville. In a review by Raghavan (1958), the conclusion is made that this subject still remains open for investigation. Correlation between age of the carrier and microfilaria density is questionable. Brygoo (1951) writes that Murray in Samoa notes that the density increases to reach 30 per 20 c.mm. for the age-group 50-54 and then decreases from that age to a density of 11 per 20 c.mm. for the age-group 75-100. Beye *et al.* in Tahiti (1952) observed that adult carriers averaged higher microfilarial densities than children, with the highest number of microfilariae being in the 40-49 age-group. The greatest density of microfilariae was in a 42-year old male who had 1,094 microfilariae in 20 c.mm. of blood (subsequently a carrier was found with more than 2,000). Buxton, in his observations in Samoa, felt that from the age of 11 years onward there is no consistent rise or fall in the number of microfilariae per positive count. Iyengar, in studies at Trivandrum (1938), felt that the "average infestation is lowest in the first age-group and rises in the second and third age-group. In the later age-groups, the infestation does not exhibit a constant rise or fall." Singh *et al.* (1956) observed no significant variation in microfilaria density in Travancore.

The indication of a tendency toward levelling in the percentage of carriers in an endemic population older than the 20-30 age-group, suggests that host immune factors may be operating.

The association of age and clinical manifestations of filariasis, especially elephantiasis, is different in that specific disease rates progressively increase with age.

Sex.—The association of infection and disease rates with sex is more variable than the association with age.

In India, (Singh, Raghavan); in American Samoa, (Jachowski); in Tahiti (Galliard, Beye, Kessel) and in other places, the infection and disease rates are generally higher in males. Interestingly enough, in each of these places, slightly higher infection rates are observed in females up to 10-15 years of age. Whether or not this represents a sex difference, or differences due to frequency of contact with infective mosquitoes, is not known.

On the other hand, in British Guiana, females not only have a slightly higher infection rate according to Giglioli (1948) and Adams (1959) but a three to four times higher disease rate.

Fros (1953) and Wolff (1948) report higher infection and disease rates among females in Paramaribo, Surinam, while Iyengar (1938) observed slight or no differences in infection and disease rates in Trivandrum as part of his studies in Travancore.

Whether or not these observations in Surinam and British Guiana reflect a sex difference in response to a possible different strain of *W. bancrofti* in these two countries, will have to wait further clarification.

Race.—The fact that endemic areas of *W. bancrofti* and *B. malayi* are distributed to affect every race, suggests that race may not be of epidemiologic significance.

On the other hand, in British Guiana and Surinam where people of many races, Chinese, Hindustani, Negro, Indonesian, live in almost identical environments there are considerable differences in infection and disease rates. To quote Giglioli (1948*a*) "Our figures indicate consistently high rates among the Negroes and Portuguese, low rates among the East Indians and still lower rates among the Chinese. Of 213 Chinese examined, only 3·7 per cent were found positive. In Georgetown City, the rate was as low as 1·6 per cent and no cases of elephantiasis were recorded. With the East Indian the problem is straightforward, as it is difficult to prove comparable exposure to infection. Many have immigrated from the mildly infected sugar estates or small rural settlements to the towns. Our most significant findings were obtained in Buxton and Friendship where a well established East Indian population lives, scattered in between the prevailing Negro element. We examined 274 East Indians of all ages, 70 per cent of whom were born and bred in the village, and found only 9·8 per cent infected against 25·2 per cent among Negroes. Among 187 East Indians actually born in Buxton the rate was 9·7 per cent. Exposure conditions and living standards at Buxton are entirely comparable for the two races and we are forced to admit that the East Indian race is definitely less prone to infection than the Negro." Adams (1959) observed similar differences. Fros *et al.* (1953) in surveys in Paramaribo involving 50,861 persons representing more than 60 per cent of the population, reported the following prevalence figures for microfilaremia : Creole, 32·5 per cent of 35,485 examinations ; Hindustani, 13·5 per cent

of 10,698 examinations ; Indonesians, 7·3 per cent of 1,479 examinations ; Chinese, 7·0 per cent of 1,512 examinations ; Europeans, 8·9 per cent of 1,473 examinations ; Indians, 14·2 per cent of 214 examinations.

It is difficult to explain how corrections for age and sex distribution among the samples surveyed by Giglioli or Fros, could account for these differences and one must agree that for the type of *W. bancrofti* found in British Guiana, and Surinam, there may be some degree of racial difference. At the same time detailed epidemiologic studies in these two countries may disclose sufficient differences in exposure to account for the variations in prevalence.

Other Host Factors.—To our knowledge no one has investigated the relation between abnormal hemoglobins and filariasis. Franks (1946), however, did note that microfilaremia was more common among those inhabitants in Okinawa whose serum did not show antibodies for blood Group *B*.

No studies have assayed a possible dietary role in infection.

Socio-Economic Factors Created by the Host.—Iyengar has emphasized the role of the mosquito vector in conditioning the epidemiology of *W. bancrofti* and *B. malayi*. It is now our purpose to illustrate how man-made practices contribute to the prevalence of these parasites.

The importance of rat-eaten coconuts as breeding sources of *A. polynesiensis* in Tahiti has been pointed out by Beye *et al.* (1952), Rosen (1954), and Bonnett (1958). Edgar and Bambridge (1951) observed that 28 per cent of coconuts were damaged in plots where the trees did not have rat bands, whereas in plots with banded trees less than one per cent of the nuts were damaged.

The demonstration of bush transmission by Jachowski in American Samoa, is associated with the practice of locating gardens, etc., in clearings surrounded by bush and other suitable resting places for *A. polynesiensis*.

The well-known association of *Pistia stratiotes*, soakage pits, *Mansonia* species of mosquitoes, and the coir industry in Kerala, conditions the rural epidemiologic characteristics of *B. malayi* in that area.

The propensity for *C. quinquefasciatus* to breed in collections of water, containing human sewage, results in *W. bancrofti* being generally an urban infection. Increase of human populations in cities where open or inadequate sewers still exist, are closely associated with *C. quinquefasciatus* population densities, and transmission of *W. bancrofti* is well pointed out by Menon (1958) in Trivandrum. In British Guiana, suburbanization and the use of pit latrines is a special situation. Here high water tables provide favourable conditions for *C. quinquefasciatus* breeding.

Van der Kuyp (1955) reports that in Paramaribo, Surinam, the costs of mosquito nets were subsidized, and their use actively pursued as part of the Filariasis Campaign since 1950. As of 1959, the majority of the inhabitants of the city use mosquito nets. Infection rates have fallen from 17 per cent to 8 per cent

and although the influence of the use of these nets has not been evaluated, it might be considerable.

The possible effects on exposure to infection of types of clothing worn day or night, housing construction, and work habits have not been studied in detail.

The relation of crowding within houses and of population density to infection is fairly well documented. Giglioli (1948*a*) found 48 per cent of households examined to include infected individuals. Of the total of 1,275 positive households, almost 40 per cent had more than one member infected; families with three, four, five and six infected members were recorded. He points out that the strictly domestic habits of *C. quinquefasciatus* obviously favour transmission within the limited circle of a house or a group of houses. Similar observations have been made by Iyengar and others.

(*D*) INFECTION AND DISEASE.

The relationships between infections with *W. bancrofti* and *B. malayi* and disease manifestations such as lymphangitis, lymphadenitis, funiculitis, elephantiasis, hydrocele, chyluria, and lymph varicies, have never been clearcut, although now there is a little question that these two filarial parasites constitute the underlying pathology. The greatly decreased incidence of clinical disease in Tahiti is epidemiological evidence of such a relationship. March *et al.* (1959), and Kessel (1955), write that there have been no new cases of elephantiasis developed in Tahiti.

Although host factors may operate to produce very different clinical manifestations in separate areas, the evidence remains obscure. Studies by MacDonald and Scott (1958) demonstrate some degree of immune response to *Litomosoides carinii* infection by the cotton rat. Many observers have noted a decrease in carrier rates among persons with elephantiasis, and use this as evidence of the operation of a generalized host response to infection with the parasites. Wartman (1944), O'Connor and Hulse (1932) have demonstrated the local responses to infection. Bozicevich and Hutter (1944), Beye (1956) and others, have shown that there is a host response as judged by changes in skin reactivity and the complement fixation tests.

As previously discussed, Romiti (1935) observed that *W. bancrofti* in British Guiana selectively prefer the spermatic cord and associated lymphatics in the human male and the broad ligaments and associated lymphatics in the human female.

He considers these to be the natural sites for adult filarial worms. The differences then between the internal milieu in these regions, and other parts of the body, may be significant. According to Romiti, worms in other areas of the body are the result of hyperfilariation.

Once the lymphatic system and other tissues of the host have been preconditioned by the living or dead adult worm, other aetiological factors may operate.

Micro-organisms such as hemolytic streptococci or staphylococci may well operate either directly or through triggering already hypersensitive tissue, and accelerate lymph stasis and its sequelle. In addition, other antigens in common with toxic products of filarial worms may sometimes act in this hypersensitive tissue and aid the production of the disease. Observations of Clearkin (1944) and Beye (1956) that the frequency and severity of filarial attacks can be decreased by desensitizing with extract of *Dirofilaria immitis*, or the occasional provoking of filaria-like lymphangitis on intradermal injection of this extract, suggest a common antigen in these related filarial worms. Extending this observation further, there is little reason to exclude the possibility of the patient developing heterosensitivity. Attacks of filarial lymphangitis in some individuals, within a few hours after their return to an endemic area, indicate that such a mechanism may operate.

The general observation that the verrucous, hyper-proliferating skin changes seen in some patients with elephantiasis, are decreasing in prevelence in British Guiana and Surinam, is explained by many as resulting from general higher standards of medical care, including use of antibiotics and to better living standards, as, for example, the wearing of shoes. Legs which twenty years ago would progress to elephantiasis, may do so at the present time, but perhaps at a slower rate, and less frequently with pronounced secondary skin changes.

The epidemiology of those aspects of filarial disease which are distinct from *Wuchereria* infection is little understood, although there is little question as to the underlying aetiology of the filarial worms.

PART III. DYNAMICS OF TRANSMISSION.

To return to a consideration of the possibility for utilizing quantitative measurements in an expression of the intensity and degree of transmission, we believe that the conclusions expressed in Part I are substantially sound.

However, despite the many variables which have been indicated to influence transmission, there is probably general agreement that the major factor influencing infection and disease is the amount of exposure to filarial worms, i.e., the number of filarial worms that the human host receives during his life time.

If this over-simplified assumption is more or less correct, quantitative expressions of intensity of transmission may be feasible, and with increasing knowledge of the many variables, may become useful tools.

It is important, moreover, that attempts toward characterizing the dynamics of transmission be made for at least two reasons: (*1*) various control programs need methods for approximate evaluations of results at intervals more frequent than 5-10 years, and (2) the attempts toward quantitatively defining this expression will help promote our understanding of basic epidemiology of filarial infection and disease. Another reason is that a quantitative description of the intensity of transmission might be related to a "critical transmission level." In areas where the intensity of transmission is below this critical level, clinical manifestations of

filarial infection may not become manifest in the population, and below another critical level the parasite may not perpetuate its biological life. This concept is expressed by the World Health Organization Study Group meeting in Kuala Lumpur (1957) in 1955 as follows : "Although the ultimate aim of control should be to eradicate filarial infection, the immediate aim will often be to reduce transmission of infection to a level where clinical manifestations of the disease cease to occur and to prevent any subsequent recrudescence. Any marked and continued reduction in the reservoir of infections and the intensity of transmissions will probably be followed by a decrease in the clinical manifestations of the disease, even if the percentage of infected persons is little affected." The spontaneous decrease of filarial disease in Charleston, South Carolina, the Barbados and in Brisbane, Australia, and the decreases so far noted in Tahiti by March and Kessel (1959) and in other cases, indicate that such a critical level may operate.

Attempts to characterize transmission have been made by Jachowski and Otto (1952), Beye (1953, 1957), Kartman (1954), Kessel (1957), Bonnett (1956) and Wharton (1959). Individual measurements which have generally been used to evaluate transmission include: (*1*) percentage of microfilariae carriers in a community, (*2*) microfilariae density per 20 c.mm. or 60 c.mm. of peripheral blood, (*3*) frequency distribution of microfilariae density, (*4*) mosquito density, as measured by the number of mosquitoes captured in biting or resting stations per unit time, (*5*) number, stage, and percentage of filarial parasites in captured mosquitoes, (*6*) number, stage, and percentage of filarial parasites in captured mosquitoes and kept in captivity for a standard period of time, (*7*) prevalence of clinical manifestations at certain intervals, (*8*) incidence of clinical disease, (*9*) results of skin tests with filarial antigens, usually *Dicrofilaria immitis*, and (*10*) changes in carrier rates among the younger age-group, for example, under 5 years, at stated intervals such as 5 years.

None of these measurements alone fully describes the intensity of transmission that is occurring in any given place at any given period of time. Also since little is known of filarial infections in vertebrates other than man, even the results of mosquito dissections may not be clearly interpreted.

The most reliable criterion of transmission remains the incidence of new cases of clinical disease, probably filarial elephantiasis. The use of this criterion, however, is impracticable because of the length of time before infection is reflected in disease. Another reliable criterion would be the occurrence of infection in one of the younger age-groups. Since continuous and repeated re-infection is probably necessary for the production of clinical manifestations this, however, would not necessarily be a good indication of ultimate disease rates.

Another criterion is the *index of experimental infection*, developed by Kartman (1954), which depends on the survival of the infected mosquito, the survival of the m/f in the mosquito, and the m/f density in the mosquito.

For our purposes we shall define and utilize a *transmission index*, and a *generation index*.

The transmission index relates the intensity of transmission to human contact with infective vector mosquitoes at an instant in time. This can be formulated as :

Average number of mosquito bites per person	×	Number of infective larvae per mosquito	×	Proportion of mosquitoes infected.

However, any such measure of conditions at a given instant may give an erroneous picture of the true state of the infection in the population, since a short-term effect may completely alter the index. A measure which combines all such separate indices gives a better picture of the long-term condition of the infection, and the long-term effects of any control measures. Such a measure is the *generation index*, (G). This is an expression of the number of fertile female worms to which one fertile female worm gives rise in the course of her life-time. A $G \leqslant 1$ could be said to express the "critical transmission" level, below which the infection cannot continue in the population. Likewise, the generation index is related to the incidence of clinical manifestations.

In the face of incomplete knowledge of the factors involved and the undoubted existence of a variety of complicated underlying relationships, the definition of simple manageable indices of disease transmission requires that corresponding simplifying assumptions be made. It is anticipated that indices so defined will, nevertheless, prove useful. Some of the indices derived will not represent absolute quantities, but rather relative values—the population with a low index is in a better condition than that with a higher index. As more knowledge regarding the transmission process is obtained, the index will become more refined and come closer to some absolute measure.

While the presence of mature worms, fertilized or not, can produce clinical manifestations of filarial disease, the perpetuation of the infection requires that the worms be fertilized. To keep things simpler, an expression for the expected number of mature worms resulting from one fertile worm will be developed, and then the additional factors, necessary for such mature female worms to be fertilized, will be discussed.

The expected number of mature worms, arising from one fertilized worm, represents the total number of microfilariae produced by one worm throughout its reproductive life, multiplied by the probability that a newly extruded microfilaria will reach maturity. Thus, while the chances of one given microfilaria reaching maturity may be very small, the large number of these embryos produced by a fertile worm, can result in a considerable number of mature offspring per fertile worm. Consequently a large number of fertile worms can thus produce a great incidence of infection.

After a m/f has been extruded from the adult female and is in the blood of the host, it must be ingested by a suitable vector mosquito. The probability that any m/f is ingested by a mosquito, is proportional to the portion of the host's blood

which is ingested by vector mosquitoes during the life-span of the m/f in the bloodstream, with

$$\frac{\text{Average m/f density in blood ingested by mosquito}}{\text{Average m/f density in blood of host}}$$

as the proportionality factor. This proportionality factor depends on whether the distribution of m/f in the host's blood is random, or whether there is preferential uptake of m/f by the vector, or whether the periodicity of the m/f is the same as that of the mosquito. It has been found to be greater than one (Byrd and St. Amant, 1959) and less than one (Kershaw, 1953). The probability that a given m/f be ingested by a mosquito is then :

$$\frac{\text{Average m/f density in blood ingested by mosquito}}{\text{Average m/f density in blood of host}} \times \frac{\text{Volume of blood ingested per bite}}{\text{Total blood volume of carrier}} \times \text{Number of times carrier is bitten during life-span of m/f.}$$

The number of times the carrier is bitten during the life-span of the microfilaria, is a function of the human population density, per cent of carriers, and vector mosquito density.

The amount of blood ingested per bite may differ between species, but is on the average about 3 c.mm., and the human blood volume about 5×10^6 c.mm. The proportion of host's blood ingested per bite is then $\frac{3 \text{ c.mm.}}{5 \times 10^6 \text{ c.mm.}} = 6 \times 10^{-7}$. Assuming an infected person to be bitten on the average of 100 times per day, a life-span of 20 days, and letting the average density m/f in ingested blood equal the average m/f density in host's blood, the probability of one microfilaria successfully getting out of the human carrier's bloodstream and into a vector mosquito is 13×10^{-4}.

The ingested m/f needs to remain in the mosquito long enough to develop into the mature larva. The probability that it attains the infective stage is dependent upon both the survival of the parasite within the mosquito and on the survival of the insect host itself. The parasite may not survive within the host, because of some defence mechanism within the mosquito, or the larva may not be in position to infect, because of being eliminated before reaching maturity, or inability to migrate to the head or proboscis. These factors differ in the various species, and contribute in sum toward the vector efficiency of a particular strain or species of mosquito. Given that the mosquito survives, the probability that a m/f survives within the host to become an infective filarial larva may be estimated from

$$\frac{\text{Number of infective stage larvae within surviving mosquito}}{\text{Number m/f ingested by surviving mosquito}}$$

as indicated by Kartman (1953*a*). The survival of the infected mosquito is closely

associated with m/f density. However, the matter of interest here is not only the survival of the mosquito, but rather how this affects the survival of the parasite. Thus, while fewer mosquitoes infested with large number of m/f will survive, and the percentage of parasites surviving will be less in the population with the higher m/f density, the total number of parasites surviving under such circumstances may still be much larger than under conditions of low density. The frequency distribution of the m/f densities, rather than simply their average in a region is the important point. Suppose, in areas *A* and *B* the following conditions obtain (assuming all carriers being bitten by an equal number of mosquitoes, say *b*; and assuming survival of all m/f ingested by surviving mosquitoes, and the average m/f density in the blood ingested by the mosquito being equal to that in the host's blood), using some of the data on the mosquito survival and m/f density observed by Wharton (1957) in Malay, we see :

M/f density per c.mm. ...	0—0·3	0·3—2	2—5	5—10	10—13	13—17	
M/f density per 3 c.mm. ...	0—1	1—6	6—16	16—30	30—40	40—50	
Proportion of mosquitoes surviving 15 days after ingesting blood with such densities. ...	0·71	0·65	0·52	0·30	0·085	0	
Area *A*							Total
Number of carriers with such densities ...	55	10	10	10	10	5	100
Number m/f ingested by all mosquitoes ...	27·5*b*	35*b*	110*b*	230*b*	350*b*	225*b*	999·5*b*
Number mosquitoes surviving ...	39*b*	6·5*b*	5·2*b*	3·0*b*	0·85*b*	0	54·45*b*
Number m/f surviving ...	19·5*b*	22·75*b*	57·2*b*	69·0*b*	29·75*b*	0	198·2*b*
Area *B*							
Number of carriers with such densities ...	10	10	10	10	55	5	100
Number m/f ingested by all mosquitoes ...	5*b*	35*b*	110*b*	230*b*	1925*b*	225*b*	2530*b*
Number mosquitoes surviving ...	7·1*b*	6·5*b*	5·2*b*	3·0*b*	4·7*b*	0	26·5*b*
Number m/f surviving ...	3·55*b*	22·7*b*	57·2*b*	69·0*b*	163·6*b*	0	316·1*b*

Thus, in Area *A*, with an average m/f density of 9·8 per 3 c.mm. (65 per 20 c.mm.), 45·5 per cent of the mosquitoes succumb due to the m/f infection, while in Area *B*, with an average m/f density of 25·3 per 3 c.mm. (169 per 20 c.mm.), more than double that in Area *A*, 73·5 per cent of the mosquitoes die. In Area *B*, however, 316*b* m/f reach maturity, or 1·6 times the number in Area *A*. It is assumed that the crowding of m/f in the infected mosquito does not affect its development into larvae, or change its chances of reaching the infective stage within the mosquito.

The probability that the ingested m/f mature into an infective stage larva is then :

$$\frac{\text{Number infective stage larva within surviving mosquito}}{\text{Number m/f ingested by surviving mosquito}} \times \frac{\Sigma\ (\text{m/f density}) \times (\text{number of carriers}) \times (\text{proportion mosquitoes surviving})}{\Sigma\ (\text{m/f density}) \times (\text{number of carriers})}.$$

The summations in the second expression are taken over the various m/f densities, each multiplied by the number of persons having such density, and, in the numerator, by the proportion of mosquitoes surviving after feeding on persons with such densities. Thus for Area *A* above, using one as the probability of m/f reaching maturity within the surviving mosquito, the probability of the m/f reaching the infective stage within a mosquito is $1 \times \frac{198 \cdot 2b}{977 \cdot 5b} = 0 \cdot 20$. It is seen that this probability is independent of the number of mosquitoes biting each carrier, providing that there is no selection due to microfilaria density.

Final steps in the asexual portion of the parasite life cycle are for the larva to be deposited on the skin of the human host, gain entry, and to develop into a mature worm. The probability that the larva be deposited by the mosquito on the skin of the human host, rather than be lost from the proboscis of the insect, depends on the likelihood that when the larva is in the proboscis, the mosquito bites a human before it feeds on plant juice, bites some other animal, or engages in any activity which causes the larva to escape from the proboscis. Let p be the probability that the larva exits when the mosquito feeds or bites ; then $(1-p)$ is the probability that the mosquito retains the larva under such activity. If s is the number of chances the mosquito has to lose the larva by feeding on plant juice (or similar activity) before it bites a potential human carrier, then $(1-p)^s$ is the probability that the larva is available for transfer to the human host when the mosquito bites, not having been lost on any one of the s feeding occasions and $p(1-p)^s$ is the probability that the larva is transferred when the human is bitten. Setting p equal to 0·22* and assuming s equal to 10, the probability that the mature

* The probability of $p=0 \cdot 22$ was estimated from the data of Pratt and Newton (1946) on loss of mature larvae, assuming $s=3$, and using the relation :

Proportion of larvae lost $= N = (1-p)^s$

Days after ingesting m/f. ...	16·5	18·5	20·5	22·5	24·5	26·5	28·5	30·5	
Average number of mature larvae per mosquito ...	48·7	42·1	30·0	20·2	13·6	11·6	10·2	8·8	177·4
Average loss of mature larvae per mosquito in 2 days ...	5·6	13·1	9·8	6·6	2·0	1·4	1·4		39·9

Average per cent larvae lost in 2 days $= \frac{39 \cdot 9}{177 \cdot 4} = 22 \cdot 5$ per cent.

$(1-p)^s = 0 \cdot 225$; $1-p = 0 \cdot 78$; $p = 0 \cdot 22$.

larva be transferred from the proboscis on to the skin of a human is $0{\cdot}22(1-0{\cdot}22)^{10} = 0{\cdot}018$.

Whether the larva does enter the human host, once it is deposited on his skin, may be uncertain, but since at this time the conditions affecting this factor are unknown, this factor is simply set equal to a constant, k, which is at the most one. Once the larva has entered the human host, its survival to maturity is by no means assured, having been found in cats to be of the order of 0·13 (Edeson and Buckley, 1959). The probability that the mature larva enter the human host and develop into a worm is then :

$$k \times \text{Probability of survival of mature larva in human host}$$

and in the present example is $0{\cdot}13k$, which is less than 0·13.

The probability, P, that a particular m/f develop into a mature worm is the product of the separate probabilities developed this far :

$$P = \frac{\text{Average m/f density in blood ingested by mosquito}}{\text{Average m/f density in blood of host}} \times \frac{\text{Volume of blood ingested per bite}}{\text{Total blood volume of carrier}} \times \text{Number of times carrier is bitten during life-span of m/f}$$

$$\times \frac{\text{Number of infective stage larva within surviving mosquito}}{\text{Number m/f ingested by surviving mosquito}} \times \frac{\Sigma\,(\text{m/f density}) \times (\text{number of carriers}) \times (\text{proportion surviving mosquitoes})}{\Sigma\,(\text{m/f density}) \times (\text{number of carriers})}$$

$$\times \left\{\begin{matrix}\text{Probability that} \\ \text{larva is retained} \\ \text{during feeding}\end{matrix}\right\}^{\text{Number of feedings other than human}} \times \text{Probability that larva is lost during feeding}$$

$$\times\ k \times \text{Probability of survival of mature larva in human host.}$$

Since k is at present unknown but is less than one, inserting the values, that have been calculated above for the various probabilities, results in

$$P = 12 \times 10^{-4} \times 0{\cdot}20 \times 0{\cdot}018 \times 0{\cdot}13k = 5{\cdot}6 \times 10^{-7}k < 5{\cdot}6 \times 10^{-7}$$

The average expected number E, of mature offspring per fertilized worm is then this probability P, times the total number of m/f produced by the worm throughout its reproductive life. Thus, while the probability per m/f is very small

the large number of m/f produced by each worm may result in a considerable number of mature offspring per fertilized worm. If each fertilized worm produces $2{\cdot}5\times10^7$* m/f during its life-time, the expected number of mature offspring per fertilized female worm is $3\times10^{-7}k\times3{\cdot}5\times10^7=10{\cdot}5k<10{\cdot}5$.

Whereas the expected number of mature offspring from one fertile worm is affected by the total number of fertile worms only indirectly (through the survival of mosquitoes as related to the m/f density), the expected number of fertilized worms is affected directly by the number of worms giving rise to offspring, since it depends on the probability that the mature female encounters a mature male worm.

The total number of fertilized female worms in the population is a function of the m/f density, and the number of carriers in the population. If each of N fertilized female worms gives rise to an expected number of female worms proportional to the total number of offspring, say fE, and an expected number of male worms, mE, $(f+m=1)$, then NfE mature female worms and NmE mature male worms appear in the population. These are distributed over cH sites, where c is the number of sites per person, and H the size of the human population. Assuming Poisson distribution of the mature worms among the cH sites, it can be shown that the expected number of fertilized female offspring per fertile female worm is the generation index $G=fE\ (1-e^{-\bar{N}mE/c})$, where $\bar{N}$ is the average number of fertile female worms per person.

The effect of the various factors on the pattern of the change of the worm population can be seen by examination of this expression. In the early stages of infection in a population when N, and therefore $\bar{N}$, is still small, $(1-e^{-\bar{N}mE/c})$ can be approximated by $\bar{N}mE/c$, so that G can be approximated by $mf\bar{N}E^2/c$. As the worm population (and therefore $\bar{N}$) gets larger, the generation index increases at an even faster rate. This is characteristic of the growth of a sexually reproducing population, with mating dependent on chance encounter. At such early stages the generation index is sensitive both to factors which affect the total worm population and to factors which affect the number of mature, though not fertilized, offspring to which a single fertile female worm gives rise. Changes in the latter affect the generation index doubly. Where the worm density is large ($\bar{N}$ large) the term $e^{-m\bar{N}E/c}$ becomes negligible and the generation index becomes $G=fE$, the rate of increase in the worm population is at a maximum with each female being certain of becoming fertilized. Population growth is exponential, as it is in an asexually reproducing population. Now the generation index is affected only by changes in the number of mature worms produced by each fertile female, not by changes in population density, unless such changes be of sufficient magnitude to

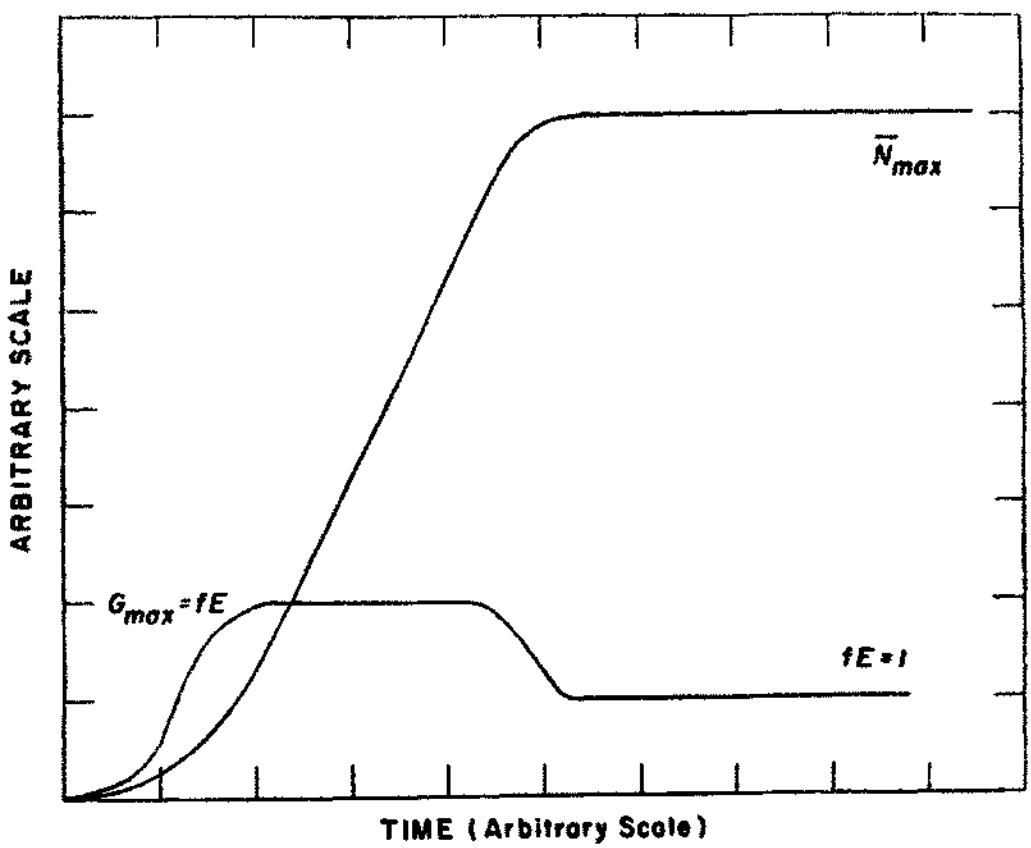
* Using Raghavan's (1956) data of 19,517 m/f per adult female *Conispiculum guindiensis* in each 48 hour period, and a fertile life-span of 10 years for each such female, a total of $3{\cdot}5\times10^{-7}$ m/f per fertile life-time per female worm is obtained.

return the population to the earlier condition, where $e^{-m\bar{N}E/c}$ is of appreciable magnitude. Although this model implies a continuing increase in the worm population, the actual population size is limited by the size of the human population, and by saturation of the individual carrier. At some maximum $\bar{N}$, fE is reduced to one, so that each worm is merely replaced.

Graph 1 is an illustration of the relationship of the changes in G and $\bar{N}$, (fE assumed to be constant), until $\bar{N}$ reaches such magnitude that fE is depressed to one.

GRAPH 1.

PATTERN OF CHANGE IN G AND $\bar{N}$, ASSUMING E CONSTANT UNTIL $\bar{N}_{max}$ IS REACHED



Implications relative to an attack on the filariasis problem may be gathered from the above. In a region, where either the percentage of carriers or the m/f

density in the carriers is small (both conditions indicative of low $\bar{N}$), measures which reduce the number of mature offspring from each fertilized worm are doubly effective. In a more seriously infected region, where the parasite population has become stable, a measure aimed at decreasing fE to below unity will gradually decrease the worm population. Measures to substantially decrease $\bar{N}$ at the same time so that conditions corresponding to $G = (1-e^{-\bar{N}mE/c})$ or even $G = mf\bar{N}E^2/c$ obtain, will greatly enhance the effect of reduction in the number of mature offspring from a single fertilized female. The decrease in the generation index will then be accelerated through successive generations. The numerical limits indicated refer of course, to the theoretical parameters; at present the pattern of increase and decrease can only be investigated by observing relative changes in the values of the parameters.

The other indices discussed above may be useful to obtain an interim picture of conditions or as a clue to the reasons for changes in the generation index. The generation index is more stable, and thus worsening conditions may not be detected until the situation has deteriorated so far, that improvement is costly and time consuming. Inspection of the "instantaneous" indices may give warning at a much earlier time.

Frequent estimates of the filarial reservoir will serve as a good check on the "infectious situation". With this estimate, and a knowledge of E, the generation index can be found (or at least changes in it can be detected). The components entering into an estimate of the filarial reservoir, i.e., the m/f density, and the percentage of carriers, in the population contribute to the estimate of E. In addition, mosquito density, population density, and frequency distribution of the m/f densities, are factors which could change and whose alteration would change E. From these factors the transmission index described above can be also calculated. Other factors entering into the estimate of E, are (1) the ratio of m/f density in ingested blood to m/f density in blood of host, (2) proportion of human blood volume ingested by the mosquito, (3) proportion of parasites surviving in the surviving mosquitoes, (4) proportion of larvae lost to non-human recipients. These need probably be determined once for a given situation, or in some cases only once for a given species.

Thus a program of: (1) determining species-constant factors for a situation; (2) periodic determinations of the factors entering into the calculation of the transmission index; and (3) frequent determination of the filarial reservoir, should give a reasonable check on the continuing effectiveness of control measures, without adding greatly to the measurements which are already being obtained in many situations.

SUMMARY.

Epidemiological data on the perpetuation of the life cycles of *W. bancrofti* and *B. malayi*, and field information on the distribution of these parasites and associated

diseases, have been presented. On the basis of this information a model of the dynamics of transmission has been developed.

This model is used to suggest necessary lines of investigation for a better understanding of the epidemiology of these parasites.

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EXPERIMENTAL STUDIES ON FILARIASIS CONTROL IN JAPAN.

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THE aim of this paper is to review recent investigation made by the authors and their associates in the control of Wuchereriasis in Japan, with special reference to the mode of action of diethylcarbamazine in the mass treatment of microfilarial carriers. In this country, both *Wuchereria bancrofti* and *Wuchereria malayi** are endemic. In contrast to the distribution of the latter species being restricted to a small island of Hachijo-Koshima south of Tokyo, the former is widespread in three of the four main islands of Japan and in a number of adjacent islands in the south-west, and at least one million people are estimated to be infected, involving several thousands of clinical filariasis, such as those suffering from fever attacks, chyluria, elephantiasis or scrotal swellings. The mode of distribution of human filariasis in Japan was reviewed by Sasa and Hayashi (1953) and more recently by Sasa (1959).

Laboratory and field studies in the control of filariasis were started recently by several groups of Japanese workers. The use of diethylcarbamazine in the mass-treatment of bancroftian and Malayan filariasis in this country has been practised since 1950 on an experimental basis and with more or less successful results. The Filariasis Control Research Committee was organized in 1958 by specialists of the University of Tokyo, Nagasaki and Kagoshima, under the sponsorship of the Ministry of Education, the Japanese Government. Pilot experiments for the real control of filariasis have been the main project of the committee, and several reports were already made by the members who worked in different fields. Their results suggest that extreme reduction in the microfilarial rates or in the clinical filariasis in an area may be attained when the anti-parasitic and anti-vector measures are adequately applied under systematic schedules.

I. FIELD EXPERIMENTS ON MALAYAN FILARIASIS.

The occurrence of filariasis due to *Wuchereria malayi* (Brug, 1927) in Japan was first reported by Hayashi, Sasa, Kano and Sato (1951) from a small island of

* *W. malayi* = *B. malayi* (*Brugia* gen. nov. Buckley, 1960).—Editor

Hachijo-Koshima in the Izu Archipelago located in the Pacific, south of Tokyo. In spite of the extensive survey made by various workers before and after this report, the species has never been recognized from other localities in Japan. Although the filarial infection is more or less commonly distributed among natives of the islands of the same archipelago adjacent to Hachijo-Koshima, results of blood examination have shown that they are all due to *Wuchereria bancrofti* (Cobbold, 1877), including those in the mainland of Hachijo which is located at a distance of only 4 kilometers from Koshima.

Studies on the epidemiology and control of Malayan filariasis in Hachijo-Koshima have repeatedly been made by the same group of workers. Hayashi *et al.* (1951) and Sasa *et al.* (1952) reported that out of 85 native persons examined, 29 (34 per cent) were microfilariae positive and 44 (52 per cent) exhibited clinical symptoms, with a total of 55 (65 per cent) persons being affected by the parasite. The clinical manifestations involved acute fever attacks, elephantiasis of legs or hands, swellings of lymph nodules, but neither chyluria nor scrotal swellings could be detected.

Among six species of mosquitoes collected in this island, *Aedes* (*Finlaya*) *togoi* (Theobald, 1907) was demonstrated to act as the vector through experimental studies and field observations. The species represent one of the most common mosquitoes all over Japan, and it was found to breed abundantly in water tanks and rock pools of this island. None of the members of genera *Mansonia* and *Anopheles*, which represent the main vectors of Malayan filariasis in South Asia, have been collected from this island.

The first administration of diethylcarbamazine citrate (Supatonin, Tanabe Seiyaku Co., Ltd.) was made in May, 1950, to 26 cases, of which 19 were microfilariae positive and the rest with elephantiasis but negative for microfilariae. The treatments started with about 2 mg. of diethylcarbamazine per kilogram of body weight, three times a day for varying periods. In most cases thus treated, especially in those with high microfilarial counts, severe reactions such as fever attacks, headache, vomiting and swellings of lymph nodules were encountered in the first few days of the course. However, the reactions soon subsided even though administration was continued. Some of the cases refused to continue the treatment because of the unpleasant side-reactions, and therefore the total doses per person varied from a maximum of 46·7 mg./kg. to a minimum of only 1·8 mg./kg. of body weight. In the blood examination made 10 days after the initial dose, all the 6 cases who had received more than 26·7 mg./kg. turned out to be negative, and 3 out of 10 cases whose doses were less than 12 mg./kg. and all of 4 cases who refused to take the drug still remained positive.

The second course of administration was made in 1950 to a part of the cases with daily doses of 2 to 4 mg./kg. for a period of 1 to 3 weeks. In the blood examination made 4 months after the initial treatment, all the 6 cases whose doses were higher than 60 mg./kg. were negative, and 5 out of 12 cases with dosages lower than 50 mg./kg. still harboured the microfilaria in 20 c.mm. blood samples.

The blood surveys as well as the drug administrations were repeatedly made during a period of 10 years from 1950 to 1960, together with anti-mosquito measures applied under various systems as discussed later. The authors and their co-workers have visited the island 12 times since 1948, but have not yet succeeded in persuading all of the microfilarial carriers to accept the drug administration. Though the microfilarial rate dropped from 31·5 per cent (29/92) in May, 1950 to 16·2 per cent (12/74) in September, 1950 and had kept the similar level until 1952, it increased to 33·3 per cent (22/66) in the blood survey made in August, 1956, while no control measures were applied for 4 years. It again dropped to 11·1 per cent (7/63) in July, 1958 as the drug administration was reinforced.

It should be noted here that although diethylcarbamazine has been introduced as the effective chemotherapeutic against Malayan filariasis (probably more effective than in the treatment of bancroftian or other types), the side-reactions caused after the initial few doses might act as to discourage the patients to accept the full course of treatment, especially when it was not announced prior to the first dosage.

In the island of Hachijo-Koshima where no swamps, wells or streams could be found, the main breeding places of mosquito larvae (especially of the vector species, *Aedes togoi*) were represented by rain-water tanks and by rock pools in the beach. Among various procedures tested, the use of fish (gold fish or *Orizias latipes*) was found to be most effective to eradicate the mosquito breeding from the tanks. To the rock pools, 5 per cent DDT powder was applied with a helicoptor or an engine duster with successful results. Residual spray of wettable DDT emulsion at the rate of 2 gm. per square meter to side walls of houses was found to reduce mosquito population for a period of several months as was applied in August, 1956. Because of the extreme difficulty in reaching the island, the investigators could not make continuous survey of mosquitoes and the parasites except for the field trips once or twice a year for a period of one to several weeks. Results of these control measures, as estimated from the blood examination, were reported by Sasa, Hayashi *et al.* (1957, 1959) and are depicted in Table I.

TABLE I.

Positive rates of Wuchereria malayi *microfilaria at Hachijo-Koshima in relation to control measures.*

	May 1950.	Sept. 1950.	Aug. 1951.	May 1952.	Sept. 1952.	Aug. 1956.	Nov. 1956.	July 1958.	Oct. 1958.	May 1960.
Number examined.	92	74	67	67	49	66	66	73	63	43
Number positive.	29	12	14	11	11	22	19	17	7	3
Rate per cent.	31·5	16·2	20·8	16·4	22·4	33·3	28·8	23·5	11·1	7·0
Drug administration.	+	–	–	–	–	–	–	+	+	–
Larval control.	+	–	–	–	–	–	–	+	–	–
Residual spray.	–	–	–	–	–	–	–	+	+	–

II. FIELD EXPERIMENTS ON BANCROFTIAN FILARIASIS.

Pilot experiments in the control of bancroftian filariasis in Japan under a systematic planning started in 1958 when the Filariasis Research Committee was organized by members of the Universities of Tokyo, Nagasaki and Kagoshima. They selected about twenty villages as the experimental field, and various systems of anti-filarial and anti-mosquito measures were compared so as to establish the most effective and economic methods to be applied as a public health measure. The results obtained in the first year were reviewed by Sasa *et al.* (1959), and additional information has been accumulated.

The villages selected as the experimental field by us were located in Ehime Prefecture in West Shikoku and in Amami Island south-west of Kyushu. The latter represented one of the endemic foci of bancroftian filariasis in Japan where the prevalence was the highest, while the incidence in the former district was much lower. Omori and others of the University of Nagasaki established their field in the Goto Islands of Nagasaki Prefecture, whose results are also cited in this paper. Abe and others of the University of Kagoshima made experiments in villages of Kagoshima Prefecture.

In each of the experimental fields, preliminary surveys of microfilarial carriers, clinical filariasis, and of mosquitoes were made prior to the application of control measures. As an anti-filarial measure, mass-administration of diethylcarbamazine (Supatonin) was proceeded under various systems to the microfilariae positives or to the whole population, and its effects were observed by periodic blood survey. Blood examination was made between 9 p.m. and 1 a.m., either by the routine three drops thick smear method (about 30 c.mm.) or by the quantitative method of 60 c.mm. (20 c.mm. ×3) blood samples. Anti-mosquito measures were directed both to adults and to larvae. Residual spraying of DDT or dieldrin was found to be effective to the adults of *Culex pipiens pallens*, the main vector of bancroftian filariasis in Japan, and the destruction of its breeding places by environmental sanitation was proved to be most efficient for the control of its larvae.

(A) ANTI-FILARIAL MEASURES.

1. Effect of diethylcarbamazine administration on the microfilarial density.

Although it had been a well recognized fact that the drug was effective on various types of human or animal filariasis, it obviously required more investigations to elucidate its mode of effects. In the previous experiences of the authors or of other workers, it often failed to act as the perfect filaricide, and only partial effects on both microfilariae and adults could be attained. The present experiments were designed to compare various systems of the drug administration in the mass-treatment of bancroftian filariasis.

Two kinds of diethylcarbamazine administration and their combination were compared in its effect on microfilarial counts; (1) the short-period system, in

which the drug was given daily for a period of 5 to 10 days; (2) the repeated application of short-period system; (3) the long interval system, in which the drug was given at weekly to monthly intervals for about 10 times; (4) the combined system, in which the initial doses were given daily for about 5 days, and the remaining doses were administered at long intervals. The drug used was diethylcarbamazine dihydrogen citrate in 50 mg. tablets of Supatonin (Tanabe Seiyaku Co., Ltd.). Periodical blood surveys were made before, during and after the treatments, and microfilarial counts in each of the three 20 c.mm. blood samples (total 60 c.mm.) were recorded. The results are shown in Table II.

(1) *The short-period system.*—In the treatment of any disease, it is obviously desirable to complete the treatment within a short period rather than after months or years of duration, and the short-period system has thoroughly been studied in different areas. The dosages applied were (*a*) 2 mg./kg. for 10 days, total 20 mg./kg. (Daikuma of Amami, Noshima of Nagasaki); (*b*) 2 mg./kg. for 5 days followed by 4 mg./kg. for 5 days, total 30 mg. (Ariya and Nakakachi of Amami, Misaki of Ehime); and (*c*) 1 mg./kg. a day, 2 mg./kg. a day, and 6 mg./kg. daily for the following 10 days, total 63 mg. (Shionashi of Ehime, Sato of Amami). The doses were given once a day in the evening under the supervision of doctors or health officers. Smaller doses were administered during the first few days of the treatment so as to avoid severe side-reactions. In a part of the areas, additional treatment under the short-period system was repeated at intervals of several months.

It is observed from *A* to *F* of Table II that marked reduction in the microfilarial counts took place in all of the experimental fields at the end of 10 days' administration. The counts on an average were usually reduced to about 5 per cent of the pre-therapy level and a part of the carriers (especially those with low counts) turned out to be negative for microfilariae. However, the complete eradication of microfilarial carriers could never be attained even when the largest doses of 63 mg./kg. were administered (*D*. Shionashi).

In the villages *B* (Noshima) and *E* (Nakakachi), blood examination was made during the course of 10 days' treatment. It should be noted that the reductions in the microfilarial counts to the lowest level took place within the first few days while the small doses of 2 mg./kg. were administered, and that the additional administrations of larger or the same doses, in the latter half of the course, did not act as to reduce them further to lower levels.

In the follow-up examinations, made one to four months after end of the treatment, it was demonstrated that the microfilarial counts tended to increase to the level of about 10 per cent of the original, or about twice of that right after end of the course. Such recoveries in the microfilarial densities were more obviously seen as the doses were smaller. The sole exception was seen in village *D* (Shionashi), where the largest doses of 63 mg./kg. were administered and the counts tended to drop further without additional treatment.

TABLE II.

Effects of diethylcarbamazine treatments on the microfilarial carriers as observed by the mean microfilarial count in 60 c.mm. blood and by the negative rate.

Explanation of the Table.

Column 1. The name of village (district), microfilarial rate (number of positives/number examined), and the total dose administered to each microfilarial carrier.

Column 2. Time when each survey was made, as counted by days (weeks or years) after start of the treatment.

Column 3. Dose of diethylcarbamazine administered during the period from the previous survey and the present. 2 mg./kg.×10, for example, means daily administration of 2 mg./kg. fo 10 times.

Column 4. Mean microfilarial count in 60 c.mm. blood sample at each survey, and its percentage to that before the treatment in parenthesis.

Column 5. Percentage of persons who were negative at each survey (number of negatives/number of persons examined, who were all positive before the treatment).

Village (District) Density.	Days after start of treatments.	Doses during the period.	MICROFILARIAL COUNT: Mean.	(Rate).	PERCENTAGE NEGATIVE: Per cent.	(Number negative/ Number examined.)
(1)	(2)	(3)	(4)		(5)	
I. The short-period system (daily administration).						
A Daikuma (Amami)	Before treatment.		152·07	(100·0)	0	(0/67)
Mf. rate 11·8 per cent (102/886).	10 days	2 mg.×10	11·96	(7·86)	17·9	(12/67)
20 mg./kg.	36 days	None	15·63	(10·28)	27·4	(17/67)
	122 days	None	17·64	(11·60)	23·9	(16/67)
B. Noshima (Nagasaki)	Before treatment.		9·86	(100·0)	0	(0/14)
Mf. rate 5·26 per cent (14/266).	3 days	2 mg.×3	0·43	(4·36)	64·4	(9/14)
20 mg./kg.	6 days	2 mg.×3	0·57	(5·78)	71·5	(10/14)
	10 days	2 mg.×4	0·43	(4·36)	71·5	(10/14)
	33 days	None	0·80	(8·12)	60·0	(6/10)
	80 days	None	1·15	(11·68)	36·3	(4/11)
C. Ariya (Amami)	Before treatment.		68·67	(100·0)	0	(0/21)
Mf. rate 18·8 per cent (36/192).	10 days	4 mg.×5	2·76	(4·02)	57·1	(12/21)
30 mg./kg.	39 days	None	7·14	(10·40)	42·8	(9/21)
	142 days	None	12·38	(18·03)	33·3	(7/21)
D. Shio-ashi (Ehime)	Before treatment.		37·3	(100·0)	0	(0/18)
Mf. rate 1·82 per cent. (18/998)	10 days	1 mg.×1, 2 mg.×1, 6 mg.×10	3·06	(8·21)	72·2	(13/18)
63 mg./kg.	45 days	None	2·67	(7·16)	66·7	(12/18)
	80 days	None	2·28	(6·12)	77·8	(14/18)
	108 days	None	0·72	(1·93)	83·3	(15/18)

TABLE II.—*Contd.*

Village (District) Density.	Days after start of treatment.	Doses during the period.	Microfilarial count: Mean.	Microfilarial count: (Rate).	Percentage negative: Per cent.	Percentage negative: (Number negative/ Number examined.)
(1)	(2)	(3)	(4)		(5)	
II. Repeated application of the short-period system.						
E. Nakakachi (Amami) 1958 Mf. rate 16·2 per cent (32/198). 50 mg./kg.	Before treatment.		55·83	(100·0)	0	(0/23)
	5 days	2 mg.×5	1·57	(2·80)	47·8	(11/23)
	13 days	4 mg.×5	1·96	(3·50)	56·5	(13/23)
	39 days	None	4·43	(7·94)	26·2	(6/23)
	124 days	None	4·26	(7·63)	47·8	(11/23)
	8 months.	4 mg.×5	1·04	(1·86)	63·6	(14/22)
	1 year.	None	0·43	(0·77)	82·1	(23/28)
F. Misaki (Ehime) 1958 Mf. rate 1·61 per cent (33/2050) 70 mg./kg.	Before treatment.		27·00	(100·0)	0	(0/30)
	13 days	2 mg.×5 4 mg.×5	3·43	(12·7)	33·3	(10/30)
	59 days	4 mg.×5	1·93	(7·15)	50·0	(15/30)
	135 days	4 mg.×5	1·36	(5·03)	56·0	(14/25)
	1 year.	None	0·53	(1·96)	75·0	(24/32)
III. The Long-Interval System (weekly or monthly administration). (After Omori *et al.*, 1959)						
G. Yaburoki (Nagasaki) Mf. rate 20·6 per cent. (34/165). Monthly, 4 mg./kg.	Before treatment.		46·67	(100·0)	0	(0/30)
	25 days	2 mg.×1	24·83	53·20)	0	(0/30)
	62 days	2 mg.×2	23·22	(49·75)	3·86	(1/26)
H. Noshima (Nagasaki) 1958 Mf. rate 6·90 per cent (20/290) Weekly, 20 mg./kg.	Before treatment.		7·90	(100·0)	0	(0/20)
	2 weeks	2 mg.×2	7·80	(99·0)	10·0	(2/20)
	6 weeks	2 mg.×4	0·67	(8·43)	66·7	(12/18)
	9 weeks	2 mg.×4	0·31	(3·90)	92·4	(12/13)
	14 weeks	None	0·71	(8·95)	61·6	(8/13)
IV. The Combined System (daily administration for a short period followed by administration once a day at long intervals).						
I. Aminoko (Amami) 1958 Mf. rate 30·2 per cent (73/241) 30 mg./kg.	Before treatment.		184·79	(100·00)	0	(0/47)
	29 days	2 mg.×5 daily	9·40	(5·09)	14·9	(7/47)
	4 Months	2 mg.×10 every 10 days	1·45	(0·78)	61·7	(29/47)
	7 Months	None	6·50	(3·52)	46·0	(23/50)
	1 year	None	3·31	(1·79)	55·8	(29/52)

TABLE II.—*Contd.*

Village (District) Density.	Days after start of treatment.	Doses during the period	Microfilarial count: Mean.	Microfilarial count: (Rate).	Percentage negative: Per cent.	Percentage negative: (Number negative/ Number examined.)
(1)	(2)	(3)	(4)		(5)	
J. Misaki (Ehime)	Before treatment.		33·4	(100·0)	0	(0/30)
1959	10 days	1 mg.×1, 2 mg.×1, 6 mg.×3	5·97	(17·9)	44·5	(12/27)
Mf. rate 0·72 per cent (32/4443)	45 days	6 mg.×5 weekly.	2·32	(6·95)	83·3	(25/30)
63 mg./kg.	80 days	6 mg.×2 monthly.	3·46	(1·38)	83·3	(25/30)
	108 days	None	0·036	(0·11)	96·7	(29/30)
K. Sato (Amami)	Before treatment.		23·05	(100·0)	0	(0/44)
1959	5 days	1 mg.×1, 2 mg.×1, 6 mg.×3	6·08	(26·38)	36·4	(16/44)
Mf. rate 20·1 per cent (50/249)	45 days	6 mg.×5 weekly.	0·48	(2·07)	77·3	(34/44)
63 mg./kg.	79 days	6 mg.×2 monthly.	0·44	(1·90)	72·1	(33/43)
	106 days	None	1·25	(5·42)	65·9	(29/44)

(2) *Repeated application of the short-period system.*—At Nakakachi (*E*) in Amami and at Misaki [Futanatsu and Matsu (*F*)] in Ehime, additional doses of 4 mg./kg. daily for 5 days were administered once or twice a year following the initial course of the short-period treatment. In contrast to the above discussed fact that daily administration of 2 mg./kg. (at *B*, Noshima) or 4 mg./kg. (at *E*. Nakakachi) in the latter half of the initial course of 10 days' treatment were not effective to reduce the microfilarial counts to further lower levels, the same doses were found to be effective if applied at several months' interval. At Nakakachi where the mean count recovered to 7·63 per cent of the original at the time of examination made on the 124th day, it was again reduced to 1·84 per cent after the second course of the treatment, and it further dropped to 0·77 per cent with the negative rate of 82·1 per cent at the survey made after one year while no additional doses were administered. Such effects were also observed at Misaki (*F*) following the second and the third course of the drug administration.

(3) *The long-interval system.*—It was reported by Kessel *et al.* (1953-57) in Tahiti for non-periodic type of bancroftian filariasis, and by Edeson and Wharton (1958) and Wharton *et al.* (1958) in Malaya for Malayan filariasis that monthly or weekly administration of diethylcarbamazine to the whole population of an endemic

area produced excellent results in reducing the microfilarial carriers. Experiments on similar lines were made by Omori *et al.* (1959) in Nagasaki and Abe *et al.* (unpublished) in Kagoshima as a part of the joint activities of the Filariasis Control Research Committee of Japan.

By weekly doses of 2 mg./kg. applied at Noshima (*H*), the mean microfilarial counts per 20 c.mm. tended to be reduced at each dose, and at the end of 10 weeks' course (total dose 20 mg./kg.), 12 out of 13 carriers turned out to be negative, with the reduction of the mean count from 7·90 to 0·31 (3·90 per cent). At the survey made 4 weeks after treatment, however, the count had risen to 0·71 and 4 out of 12 persons became positive again. In the monthly administration system at Yaburoki (*G*), where the final results had not yet been observed, the reduction in microfilarial counts took place much slower as compared to the daily or weekly system.

The more or less disappointing results observed in the long-interval system were probably due to the doses being too small for this purpose. On the other hand, such system could not be attempted by our members because of the difficulty in staying continuously in an endemic area for a long period.

(4) *The combined system (daily administration for short period followed by administration at long intervals).*—The drug administration method, defined here as the combined system, was intended to introduce the advantage of both the short-period system and the long-interval system into the mass-treatment of filariasis. In Aminoko (*I*) of Amami, microfilarial carriers were treated with daily doses of 2 mg./kg. for 5 days, and then the same dose was given at 10 days' interval for 10 times, with the total of 30 mg./kg. At the end of the initial course of the treatment for a short period, the mean microfilarial count was reduced from 184·79 to 9·40 (5·09 per cent) and 7 out of 47 (14·9 per cent) carriers turned out to be negative. At the survey made 4 months after the start when the additional long-interval administrations were completed, the mean count was reduced to as low as 0·78 per cent of that before the treatment, though complete eradication of microfilariae could not be attained. At the blood examinations made 7 months and one year after the start, the mean counts as well as the positive rates were found to increase slightly as the administrations were suspended.

In the next year, experiments on a similar line were repeated by applying larger total doses. At Misaki (*J*) of Ehime and at Sato (*K*) of Amami, the carriers were treated with daily doses of 1 mg./kg. on the first day, 2 mg./kg. on the second day and 6 mg./kg. for the following 3 days, with the initial total dose of 21 mg./kg. They were then administered weekly doses of 6 mg./kg. for 5 weeks, followed by monthly doses of 6 mg./kg. for two months, with a total of 63 mg./kg. At periodical surveys made during the course of the treatment, progressive reductions in the mean microfilarial counts as well as in the positive rates were observed at both villages. At Misaki (*J*), where the most satisfactory result was obtained, the mean microfilarial count in 60 c.mm. blood samples was reduced from 33·4 (before treatment) to 0·46 (1·38 per cent) on the 80th day (after full course of treatment). At the final survey made on 108th day, the mean microfilarial count was further

reduced to 0·033 (one microfilaria in a single sample), and 29 out of 30 cases (all positive for microfilariae before the treatment) turned out to be negative.

At Sato village (*K*) of Amami, where the prevalence of filariasis was much higher and the same system of treatment was applied, the mean microfilarial count was reduced from 23·05 to 6·08 (26·38 per cent) at the end of the first course of daily administration, to 0·48 (2·07 per cent) at the end of additional weekly administration, to 0·44 (1·90 per cent) at the end of full course. Slight increase in the mean count as well as in the positive rate was seen at the survey made on the 106th day or about one month after end of the treatment.

DISCUSSION.

Although diethylcarbamazine is obviously an effective anti-filarial drug, it requires further investigations to elucidate its mode of action as well as to establish the most effective and efficient method of its administration. Through field observation, made with microfilarial carriers of *Wuchereria bancrofti* described above, peculiar aspects in its effects were demonstrated. In the daily administration system, an acute reduction in microfilarial density took place within a few days' doses, but additional doses did not reduce it to further lower levels. However, the same additional doses were found to be effective if administered at several months' intervals. Through treatment with weekly or monthly doses, a step-by-step reduction in the counts was observed, though it occurred much slower than in the daily system. By combining the above two systems, marked reduction was induced with the daily doses for a short period and additional effect was seen at each dose given at long intervals. In administration of the same dose, better effects in long terms seem to be expected by dividing it at long intervals than by giving it continuously for a short period, though it is recommended to start the treatment with daily administration of small doses for a few days so as to introduce prompt recovery from the infection.

The drug had been known to be more effective on microfilariae than on adult filaria. Recovery in the microfilarial counts was often observed after a course of treatment, especially when the doses were insufficient. In areas where larger doses were applied under adequate system, such as in Shionashi (*D*), Nakakachi (*E*) and Misaki (*F* and *J*), spontaneous reduction in the counts occurred after completion of the course. The dose to be applied is another important factor to expect better results.

Basic studies in the mode of effects of diethylcarbamazine and of certain arsenicals have been made in the authors' laboratory with the cotton rat filaria, *Litomosoides carinii*, the results of which were partly reported by Sato (1959, 1960). Diethylcarbamazine, administered with various doses from 20 mg./kg. to 200 mg./kg. (nearly lethal dose) daily for periods ranging from 10 days to a month, reduced the microfilarial counts to the lowest within several days of the course, and no further effects could be seen with the additional daily doses. After the treatment ended, the microfilarial counts in most animals tended to increase towards certain

levels, and survival of adult worms was demonstrated at autopsy. Although a part of the adult worms were killed by the drug even in the group of the lowest dosage (30 mg./kg. daily for 10 times), considerable numbers of the parasite were demonstrated to survive and produce microfilariae in animals treated with the maximum dose of daily administration of 200 mg./kg. for 30 days until the host mostly died of intoxication with the drug. In animals infected with small numbers of the worm, however, complete cure was often seen with the drug administration for a short period.

In animals treated with weekly to monthly doses for long periods, step-by-step reduction in microfilarial counts took place as the administrations were repeated, but complete eradication of adult worms could not be achieved except in those with slight infections. In the group treated with the combined system, more rapid reduction was attained by the initial daily doses and further effects were seen by additional doses at long intervals, same as experienced with human filariasis.

Our strain of cotton rat filaria seemed to be more resistant to the drug than is the human *Wuchereria*, and the number of worms infected was usually much higher. However, the results presented above are mostly comparable to that observed in the field with human *Wuchereriasis*, and more detailed analysis in its effects could be made with the laboratory experiments. It was again demonstrated here that both the dosage and the interval of the drug administration acted as the important factors to induce best effects in the filariasis control, and that there existed certain limits in the effects of diethylcarbamazine on the worms, especially on the adults, which should be covered with other control measures than the use of chemotherapeutics.

2. Comparative efficacy of diethylcarbamazine treatment to the selected positives and to the whole population.

At the present status of our researches, the aim has mainly been directed to find out the most efficient method of administration to treat individual microfilarial carriers, but a few observations were made to compare the effects of selected treatment directed only to the microfilariae positives and that directed to all the inhabitants of a village. At 4 villages of Amami Island and at 2 villages of Ehime Prefecture, blood survey of the whole population was repeatedly made at various intervals by the routine three droplets thick smear method (non-quantitative, about 30 c.mm.) at various intervals from two weeks to a year. The number of positives, detected from the group identified as negative in the previous examination (new positives), in each village is shown in Table III.

At Nakakachi in Amami, for example, blood surveys of all inhabitants were made 4 times. At the first examination before the treatment, 32 cases out of 193 (16·2 per cent) were positive. At the second examination made 13 days later, 4 cases out of 163 persons (2·45 per cent) who were negative at the previous examination, showed microfilariae in their blood samples. As the result of

TABLE III.

Effects of diethylcarbamazine treatments of microfilarial carriers on the positive rate of the villages.

Village, number.	Dose during the period.	Period between the previous and the present examination.	Positive rate of inhabitants at the previous examination.	Rate of new positives.	Positive rate of carriers after treatment.	Positive rate at the present examination.
(1)	(2)	(3)	(4)	(5)	(6)	(7)
Nakakachi 1–2	30 mg./kg.	13 days	32/198 (16·2 per cent)	4/163 (2·45 per cent)	18/30 (60·0 per cent)	22/195 (11·3 per cent)
Nakakachi 2–3	20 mg./kg.	1 year	22/195 (11·3 per cent)	7/126 (5·56 per cent)	5/28 (17·9 per cent)	12/154 (7·8 per cent)
Nakakachi 3–4	30 mg./kg.	4 months	12/154 (7·8 per cent)	4/ 93 (4·30 per cent)	2/11 (18·2 per cent)	6/104 (5·8 per cent)
Aminoko 1–2	30 mg./kg.	1 year	72/241 (30·2 per cent)	6/ 97 (6·19 per cent)	23/52 (44·2 per cent)	29/149 (19·5 per cent)
Aminoko 2–3	30 mg./kg.	4 months	29/149 (19·5 per cent)	5/170 (2·94 per cent)	17/29 (58·7 per cent)	22/199 (11·1 per cent)
Sato A 1–2	63 mg./kg.	4 months	32/163 (19·6 per cent)	10/126 (7·93 per cent)	8/27 (29·6 per cent)	18/153 (11·8 per cent)
Sato B 1–2	63 mg./kg.	4 months	18/ 86 (20·9 per cent)	1/ 60 (1·67 per cent)	7/17 (41·2 per cent)	8/ 77 (10·4 per cent)
Misaki 1–2	70 mg./kg.	1 year	33/2050 (1·62 per cent)	12/2046 (0·59 per cent)	8/32 (25·0 per cent)	20/2063 (0·76 per cent)

Explanation of the Table.

Column 1. Name of village, and the examination number concerned; 1–2 means that the observation deals with the difference between the first (previous) and the second (present) examination of the whole population.

Column 2. The total dose of diethylcarbamazine administered during the period between the previous and the present examination.

Column 3. Period between the present and the previous blood surveys of the whole population.

Column 4. Positive rate of microfilarial carriers as detected at the previous survey.

Column 5. Number of the new positives detected at the present survey divided by the number of persons recorded as negative at the previous survey, and its percentage. "New positives" at the present survey were all negative at the previous examination.

Column 6. Number of positives at the present survey (not including the new positives) divided by the number of positives at the previous survey.

Column 7. Number of all positives at the present survey divided by the number of persons examined.

diethylcarbamazine administrations restricted to the positive group (not to the total inhabitants), 18 out of 30 microfilarial carriers turned out negative at the second examination, and the positive rate of this village dropped to 11·3 per cent (22/195, including 4 new positives). At the third examination made one year later, 7 new positives were detected among 126 previously negative group, and at the fourth survey additional 4 new positives appeared. After repeating the blood surveys and the drug administration to the positives for a period of about 16 months, the positive rate of this village dropped from 16·2 per cent before the treatment to 5·8 per cent.

Similar results were obtained at other villages thus treated, as are shown in Table III. At every blood survey repeated, considerable numbers of new positives were detected from the negative group at the previous survey. The only exception was Sato-*B* in Amami, where the drug was administered to the whole population with the total doses of 63 mg./kg. to the positives and 33 mg./kg. to the negatives. The number of new positives at the second examination after end of the treatment was only one out of 60 (1·67 per cent). In all the villages listed here, residual spraying of either DDT or dieldrin was applied to every house once or twice a year together with larval control measures.

The appearance of new positives is obviously a serious problem in the filariasis control project under such selective administration system. It apparently is due either to the technical failures or to new infections during the period. The efficiency in the detection of microfilariae by the routine three drop sampling method should also be taken into consideration, since positive or negative may be a matter of chance, especially when the microfilarial density is low.

In the filariasis control project of an area, it is therefore recommended, as the principle, to treat the whole population rather than only the selected positives. The mass administration system is obviously more effective and less expensive in areas of high prevalence. However, in areas of extremely low endemicity often encountered in central Japan, the selective administration system may be the choice, mainly from economic and social standpoints.

B. ANTI-MOSQUITO MEASURES.

As was reviewed by Omori (1959) on the studies made by the Japanese workers, the vector of bancroftian filariasis in Japan is mainly represented by *Culex pipiens pallens* and its closely allied forms; other species of mosquitoes are of minor importance though some may act as intermediate hosts under experimental conditions. The species is widely distributed in Japan and is one of the most common human biters.

Results of our field and laboratory studies on the control of mosquitoes as the vector of bancroftian filariasis were reported by Ikeshoji *et al.* (1959), Sasa *et al.* (1959) and Omori *et al.* (1959), and further studies are now in progress. In all the areas concerned, residual spraying of either DDT (2 gm./m^2) or dieldrin (0·2 gm./m^2) was applied to all houses once or twice a year, with more or less satisfactory

results. The measures were effective to reduce mosquitoes and other household pest insects for several months of the summer season, except for the recovery of housefly (*Musca vicina*) populations noted after about one month in all the villages, and it sometimes reached a level higher than before the spraying. The observation on the development of insecticide resistance in mosquitoes and flies in these areas was made by both the contact methods (such as Busvine-Nash's method) and the topical application method. Although considerable increase in the insecticide resistance (both to DDT and lindane) was noted in *Culex pipiens pallens* of Tokyo during the past 10 years, this was not a practical problem in these experimental fields due to the periods of observation being much shorter.

It was also experienced by us that *Culex pipiens pallens* was the species most easily controlled in its larval stage by environmental sanitation measures. It usually breeds in artificial poluted waters, such as sewage, cesspools or discarded containers, but not in swamps or ricefields. In the experimental areas where education of the inhabitants by health officers was adequately made, most of these breeding places were drained or removed, or were treated with insecticides. The successful results in larval control measures in all of the experimental fields apparently disturbed our observation on the effects of residual sprayings on the mosquito population.

SUMMARY.

1. In Japan, both bancroftian and Malayan filariasis are endemic. The former is widely distributed and represents one of the most serious health hazards to people in south-west regions, while the latter occurs restricted to the small island Hachijo-Koshima south of Tokyo.

2. Experiments in the control of Malayan filariasis, prevalent among some one hundred inhabitants of this island, began in 1950 when the disease was discovered by the authors and co-workers. The experiments were started with mass-administration of diethylcarbamazine to the microfilarial carriers, and with mosquito control measures. Although the drug was found to be very effective to remove the parasite and to cure clinical attacks when adequate doses were taken, it caused severe side-reactions which discouraged a part of the carriers to continue the treatment. *Aedes togoi* was the main vector of Malayan filariasis in this island. The anti-mosquito measures, e.g., the use of fish in water reservoirs, application of DDT powder to rockpools and residual spraying with DDT emulsion, all proved to be effective. As a result of repeated application of these control measures during some 10 years' period, the microfilarial rate was reduced from 31·5 per cent (29/92) in 1950 before the treatment to 7·0 per cent (3/43) in 1960, and acute clinical attacks almost disappeared from the inhabitants.

3. Pilot experiments in the control of *bancroftian* filariasis under systematic planning started in 1958 at some twenty villages in south-west Japan. Both anti-filarial measures with mass administration of diethylcarbamazine and antimosquito

measures with residual insecticide spraying and larval control procedures, were applied under different systems to compare their efficacy.

(*a*) Two essentially different modes of diethylcarbamazine treatment were tested. In the short-period system with doses of 2 to 6 mg./kg. daily for 5 to 10 days, prompt reduction in the microfilarial counts as well as recovery from clinical attacks were observed, but the counts tended to increase after the treatment ended, especially when the doses were small. By repeating the treatment once or twice a year, remarkable reduction in the counts and positive rates could be induced. In the long-interval system with weekly to monthly dose of 2 to 6 mg./kg., step-by-step reduction in the counts were observed, and better long-term effects were obtained than in the short-period system. It was recommended that either the repeated application of the short-period system, or the combination of short-period method followed by additional doses at long intervals, would be the choice for the mass-treatment of microfilarial carriers. In areas where sufficient doses were applied under adequate systems, spontaneous reductions, in microfilarial counts were seen after end of the treatment.

(*b*) In the selective treatment applied only to the microfilarial positives, considerable numbers of new positive cases, missed in the first examination, were always detected at the second examination. As a rule, treatment of all inhabitants rather than the selected positives was recommended so as to approach eradication of the disease from an area within a short period. However, it was also pointed out that in areas of extremely low endemicity, such as often encountered in central Japan, the whole treatment system is difficult to be practised due to economic and social reasons.

(*c*) Residual spraying with DDT or dieldrin seemed to be the effective measure for the control of the vector of bancroftian filariasis, *Culex pipiens pallens*. However, the species was found to be more efficiently controlled by the inhabitants with environmental sanitation measures, or the removal of breeding places which were restricted to artificial poluted waters.

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NATIONAL FILARIA CONTROL PROGRAMME IN INDIA : A REVIEW (1955-59).

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INTRODUCTION.

FILARIASIS is prevalent in many parts of the world and continues to be a challenge both from the point of view of treatment and control. The disease is widely distributed in India; the only States which are free from the infection/disease being Punjab, Himachal Pradesh, Jammu-Kashmir and Rajasthan. According to present estimates, it is expected that more than 40 million people live in the filarious areas exposed to the risk of contracting the disease. The lack of effective treatment of the carriers or people having disease and of insecticide sufficiently potent to control the vector of *W. bancrofti*, *Culex fatigans*, have made attempts at filaria control a formidable problem. Measures were, however, undertaken from time to time in limited areas, more with a view to controlling the nuisance mosquitoes, majority of which happen to be *Culex fatigans*, than as a filaria control measure. Some State Governments encouraged anti-mosquito measures organised by local bodies by contributing part of the expenditure involved. But such an approach was not very effective either in reducing the mosquito nuisance or in controlling filariasis transmission. The status of filariasis as a public health problem and its control in India were discussed in detail by Jaswant Singh and Raghavan (1953).

The World War II produced certain discoveries beneficial to mankind. Among them could be classed the synthetic insecticides and a filaricidal drug, Diethylcarbamazine. The advent of powerful synthetic residual insecticides and the anti-filaria drug raised hopes of controlling filariasis in different parts of the world. Large scale field studies in India and in other parts of the world gave encouraging results. Diethylcarbamazine brings down the density of microfilaria by a significant degree and the effect of single course of treatment lasts for varying intervals between one and two years.

In India, a pilot programme of filariasis control was carried out in the Orissa State, jointly sponsored by the State Government and the Indian Council of Medical Research. A dosage schedule of Diethylcarbamazine (2 mg. per kilo twice daily), administered over a period of 5 consecutive days, showed conclusive proof of the utility of the drug in the control programme. The microfilarial densities showed an immediate drop to almost zero level and continued to remain so for periods as long as 20-24 months. (Annual Reports of the Indian Council of Medical Research, 1955).

Synthetic insecticides, chiefly of the chlorinated hydrocarbon series, have proved their efficacy in the control of insect-borne diseases in general and of malaria in particular. The field studies with the available insecticides during the early fifties showed that D.D.T. was of restricted value in the control of *Culex fatigans.* This mosquito was more tolerant than the vectors of malaria and a dosage of 200 mg. of D.D.T. per sq. ft. was found to be having a residual effect lasting over a period of about eight to ten weeks. It was not considered practicable for routine large scale use in the control of filariasis, one reason being the fear of precipitating resistance in mosquitoes due to application of high dosage. The introduction of newer insecticides like Dieldrin appeared to make the picture brighter. Field trials were carried out to assess the efficacy of Dieldrin and B.H.C. in the endemic filarial areas of the Kerala State and following such trials, Jaswant Singh *et al.* (1956) reported that Dieldrin at 50 mg. per sq. ft. or B.H.C. at 44 mg. gamma isomer per sq. ft. were equally effective in controlling the incidence of *Culex fatigans* in the sprayed areas, the effect lasting over a period of 12 to 14 weeks. The structures sprayed in these areas were of non-absorbent materials, mostly consisting of plaited coconut leaves.

THE NATIONAL FILARIA CONTROL PROGRAMME

With the availability of insecticides and drugs for control operations, the desirability of undertaking control of filariasis in the country became apparent. The Government of India, with the co-operation of the United States Technical Co-operation Mission, accepted a programme for tackling the problem of bancroftian filariasis. This programme, called the National Filaria Control Programme, was launched during 1955-56, following an Indo-U.S. Agreement signed in March, 1955 (Raghavan, 1955).

The programme was to be run by the State Governments. The Govt. of India, with the aid from the U.S.T.C.M., made available the requisite quantities of insecticides, drugs, larvicides, spraying equipment and transport; the respective State Governments were responsible for the operational cost for the execution of the programme in the field.

The programme had as its objectives : (*a*) to carry out filariasis surveys in the different States of the country where the problem was known to exist in order to determine the extent of prevalence, types of infection and their vectors; (*b*) large scale pilot studies to evaluate the known methods of filariasis control in selected areas in the States where the problem existed; and (*c*) to train professional and sub-professional staff required for the programme.

The programme was phased so that during the first two years, extensive surveys could be carried out by 22 survey units allotted to the different States. Thirteen control units were allotted during 1955-56 and have since been increased to 46 during the two subsequent years; 44 of these have been established. The location of the units in the country is shown in Map 1.

The programme, however, took into its fold only the problem of *W. bancrofti* which is by far the more extensive problem in the country. Control of *B. malayi* was excluded during the present planning for the following reasons:

(*a*) Knowledge was meagre regarding the methods of control of the infection which has got a restricted distribution in the country;

(*b*) The vector has peculiar breeding habits and the methods of control needed to be studied;

(*c*) Workers in other parts had observed severe reactions following administration of Diethylcarbamazine to persons with *malayi* infection. Studies on this aspect of *malayi* control have since been instituted in one of the highly endemic areas in India and the results of these investigations are under analysis for the guidance in planning *malayi* control operations.

ADMINISTRATION AND ORGANISATION.

The Indo-U.S. operational agreement on the filaria control programme in India was signed in New Delhi on the 25th March, 1955. The programme was scheduled to commence during the financial year 1955-56. The number of survey and control units allotted to the participating units so far are presented in Table 1.

TABLE 1.

Allotment of Units under the National Filaria Control Programme.

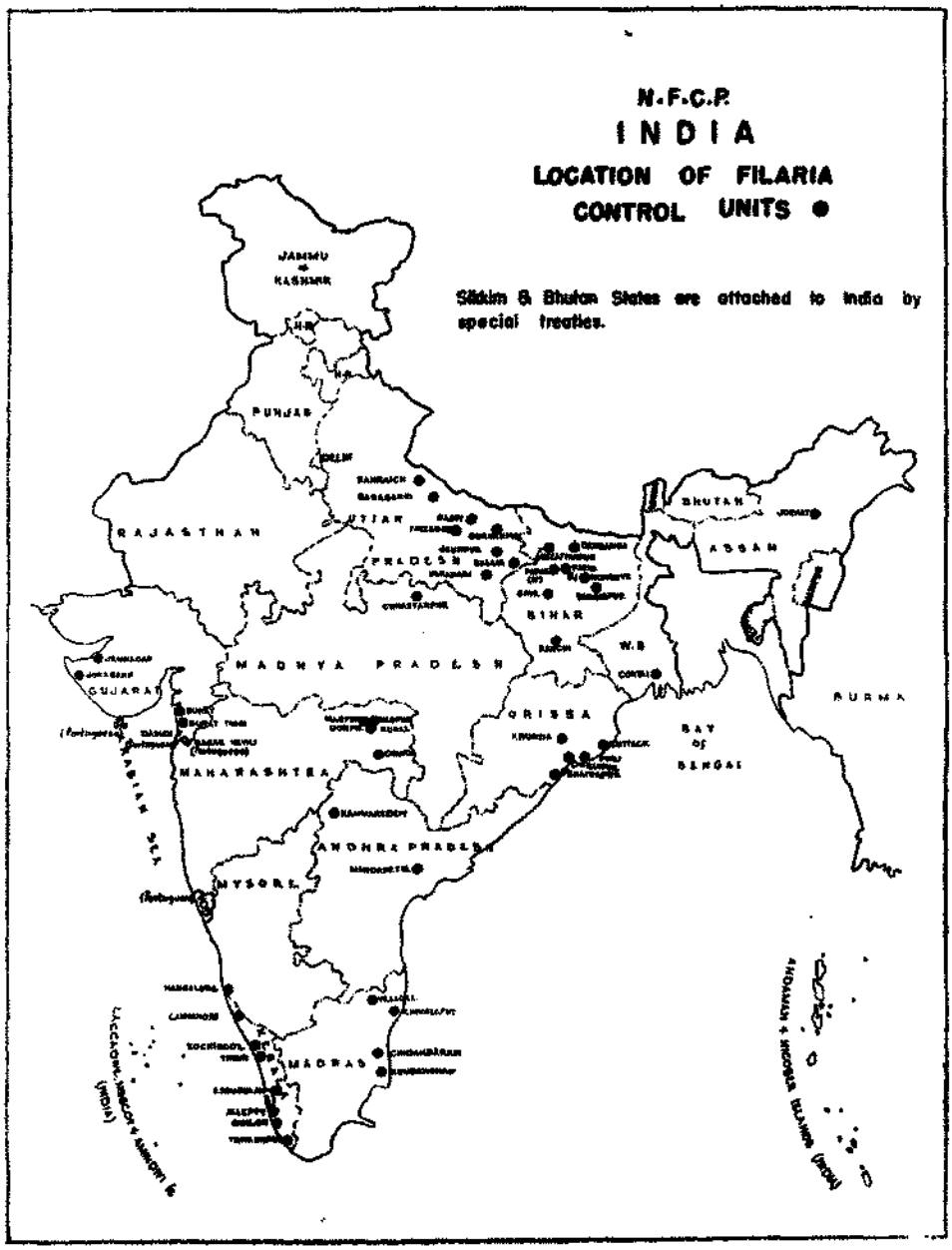
Serial number.	Name of the State.	1955–56	1956–57	1957–58	1958–59	Total units allotted.
1	Andhra	2	..	..	..	2
2	Assam	..	..	1	..	1
3	Bihar	2	2	4	..	8
4	Bombay	3	1	4	..	8
5	Kerala	1–3/5	2	3	..	6–3/5
6	Madhya Pradesh	1	..	..	..	1
7	Madras	..	2	2	..	4
8	Mysore	2/5	..	..	3/5*	1
9	Orissa	1	4	..	..	5
10	Pondicherry	..	..	..	1	1
11	Uttar Pradesh	1	2	5	..	8
12	West Bengal	1	..	..	..	1
Total :		13	13	19	1–3/5	46–3/5†

* Transferred from the second surplus unit of West Bengal.

† The total number of units allotted is 47. The remaining 2/5 unit is to be accounted for against the Islands Administration.

MAP 1.

National Filaria Control Programme in India, Location of Filaria Control Units



The programme was to be jointly financed by the States and the Central Governments, the latter being partly met from the aid from the U.S.T.C.M. The breakdown of the annual expenditure on the project is presented in Appendix I. The aid from the U.S.T.C.M. has been discontinued with effect from 1958-59.

(*i*) *Staff of the Central Organization.*—Expansion of the staff at the Malaria Institute of India was recognised as a necessary step for the execution of the programme for (*a*) guiding and co-ordinating the activities, and (*b*) for training the medical and para-medical staff to develop the programme and improving the methodology. To maintain proper liaison with the States, it was proposed to open three regional centres at Ernakulam (Kerala), Nagpur (Maharashtra) and Allahabad (Uttar Pradesh). The Centre at Ernakulam was started in August, 1955. The staff sanctioned for the Malaria Institute of India is shown in Appendix II.

(*ii*) *Staff of the State Governments.*—The staff patterns recommended for the survey and control units are shown in Appendix III. The provision of two Medical Officers per unit was to ensure adequate man-power for the investigations so indispensable in a programme which is essentially a large scale experiment. Every phase of the activity had to be investigated and the data collected had to be thorough and complete in all aspects. The knowledge of epidemiological and clinical aspects of filariasis was considered necessary at all phases of a unit's activities, commencing from survey and ending with assessment.

(*iii*) *Supplies.*—Only one microscope and one jeep were made available to each survey unit as aid by the Govt. of India/U.S.T.C.M. The State Governments had to make arrangements for the other requisite supplies. A list of supplies made available to the units is depicted in Appendix IV. The supplies of Diethylcarbamazine were made on the assumption that 65 per cent adult doses would be adequate to cover the population under protection. Twenty of the 32 hand-compression-sprayers were designed for larval control operations and were included in the supplies only from 1958-59.

The estimates of insecticides, as well as the staff pattern recommended per unit, were based on those of the National Malaria Control Programme on the assumption that the average surface area per house was 1,000 sq. ft. The average coverage per pump per day, however, was put down at 40 houses for calculating the staff requirements for field operations.

THE UNITS UNDER THE NATIONAL FILARIA CONTROL PROGRAMME.

The activities of filaria control programme in the country can be divided into three major heads, namely, those of the survey units, those of the control units and training.

FILARIA SURVEY UNITS.

While it was known that the filariasis problem in the country was considerable (Jaswant Singh and Raghavan, 1953 *loc. cit.*), a dependable estimate of the population

at risk was not available. Almost the entire coastal belt in the country is filarious. It is also known that both *W. bancrofti* and *B. malayi* are prevalent in the country; the latter having a very limited distribution, mainly in the Kerala State. While certain areas were recognised as filarious due to the presence of large numbers of people with visible elephantoid swellings, the presence of filariasis in certain other areas was not noticeable due to the absence or insignificant incidence of filarial disease. It was essential to collect all possible data regarding the prevalence of filariasis, to get a proper appraisal of the problem and institution of control measures. Enquiries and circular letters issued to the State Governments and data collected from hospitals and dispensaries and malaria units functioning in different parts of the country, gave incomplete idea of the affected areas; details remained to be collected.

The filariasis survey units had the exclusive assignment of getting a correct appraisal of the extent of filariasis and epidemiological data regarding the incidence of filariasis in the participating States. The collection of such data was absolutely necessary so that no filarious areas would be missed from the control programme which was expected to be extended at a future date. The unit necessarily was to be mobile, collecting epidemiological and entomological data.

ACTIVITIES OF THE SURVEY UNIT.

The survey units were expected to complete the delimitation of the problem during the first two years. No further aid or additional unit on this account was to be given from the Govt. of India. Twenty-two survey units were allotted to the various participating States during the year 1955-56 as mentioned earlier. If the survey was not completed within the specified period it was expected that the State Government would continue maintaining the survey units till the delimitation was completed. It was expected that the completion of surveys would coincide with the allotment of new control units so that the staff could be absorbed on the control units.

A total population of 21·5 millions have been covered during the sample surveys carried out so far under the programme. The surveys revealed a number of new filarial foci in areas where filariasis was not suspected to be prevalent. The findings have led to wider understanding of the extent of filarial endemicity in India. While Jaswant Singh and Raghavan (1953 *loc. cit.*) estimated that the problem was prevalent among a total population of about 25 million people, data now available show that a population of at least 40 to 50 millions live in the filarial tracts (Table II and Map 2) and are exposed to the risk of contracting the infection/disease. Details of surveys carried out in the participating States are presented in Table III.

ACTIVITIES OF THE CONTROL UNITS.

The object of the control units, as mentioned earlier, is to evaluate the known methods of filaria control in the different areas of the country and, based on the experience gained, to evolve suitable measures for the control of bancroftian filariasis.

MAP 2.

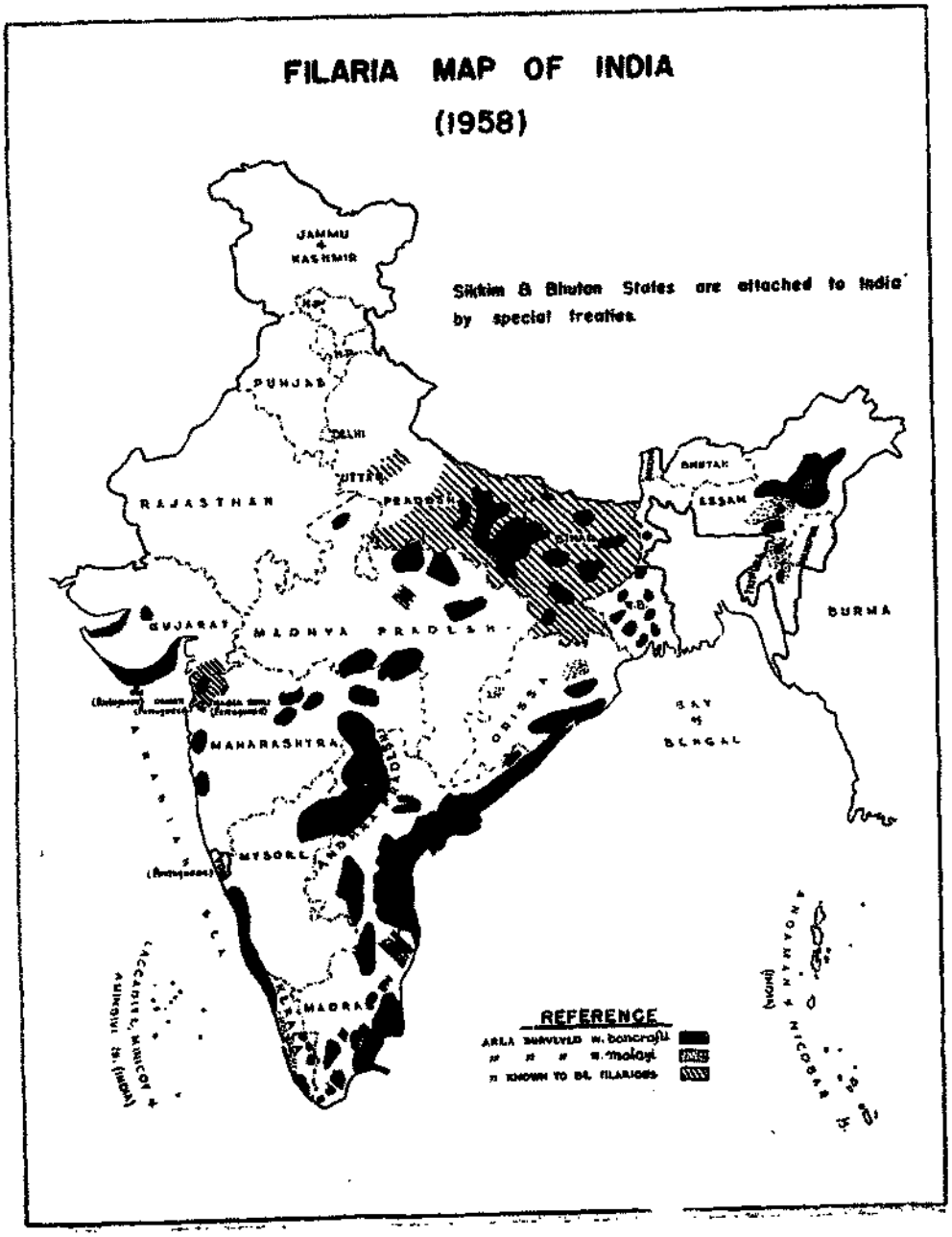

FILARIA MAP OF INDIA
(1958)
JAMMU & KASHMIR
Sikkim & Bhutan States are attached to India by special treaties.
PUNJAB
DELHI
RAJASTHAN
BHUTAN
ASSAM
GUJARAT
MADHYA PRADESH
BURMA
ORISSA
BAY OF BENGAL
MAHARASHTRA
MYSORE
MADRAS
REFERENCE
AREA SURVEYED W. bancrofti
" " " W. malayi
" KNOWN TO BE FILARIASIS

TABLE II.

Estimated population at risk to filariasis in different States of India.

Serial number.	Name of the State.	Estimated population at risk (in millions).
1	Andhra ...	4·00
2	Assam ...	1·00
3	Bihar ...	5·00
4	Bombay ...	3·00
5	Kerala ...	4·00
6	Madras ...	3·00
7	Madhya Pradesh ...	1·00
8	Mysore ...	0·50
9	Orissa ...	6·00
10	Uttar Pradesh ...	10·00
11	West Bengal ...	2·00
12	Andaman ...	0·50
	Total :	40·00

TABLE III.

Data on the filarial indices in the areas surveyed under the National Filaria Control Programme.

State.	Districts.	Infection rate.	Disease rate.	Endemicity rate.
Andhra	West Godavari ...	2·50	0·20	2·69
	Guntur ...	0·60	0·50	1·06
	Hyderabad ...	7·33	3·90	11·10
	Nizamabad ...	14·97	6·03	20·63
	Karimnagar ...	14·70	6·30	20·70
	East Godavari ..	11·72	4·36	15·10
Bihar	Gaya ...	12·15	22·00	32·06
	Bhagalpur ...	13·89	24·95	28·87
	Darbhanga ...	9·44	7·14	13·19
	Ranchi ...	4·33	4·26	6·71
	Monghyr ...	6·80	11·20	16·60
	Patna .	14·20	21·00	31·50
	Muzaffarpur ...	8·96	14·21	21·88
Bombay	Chanda ...	10·62	6·33	15·96
	Nagpur ...	7·38	1·21	8·10
	Surat ...	33·90	1·70	35·60
	Sorath & Halar ...	2·70	0·50	3·20
Kerala	Trivandrum ...	6·42	2·40	7·00
	Trichur ...	5·02	3·44	6·36
	Kozhikode ...	5·64	6·36	11·94
	Cannanore ...	13·32	9·04	22·12
	Quilon ...	2·16	3·30	5·27
	Kottayam ...	2·04	1·24	3·26

(Contd.)

TABLE III (*Concld.*).

State.	Districts.	Infection rate.	Disease rate.	Endemicity rate.
Madras	Chingleput ...	11·20	8·01	19·20
	Ramanathapuram ..	0·52	0·22	0·74
	Madurai ...	0·14	0·02	0·16
	Tiruchinapalli ...	1·67	1·09	2·76
	Kanya Kumari ...	3·00	2·23	5·23
	Tanjore ...	9·56	6·37	15·59
	Salem .	2·20	0·30	2·50
	Tirunelvely ...	..	0·16	0·16
	Nilgiris ...	..	..	..
	Coimbatore ...	..	..	..
	North Arcot ...	9·63	6·20	15·60
	South Arcot ..	8·38	2·62	10·88
Uttar Pradesh*	Mirzapur ...	9·20	10·40	18·90
	Fatehpur .	6·40	12·30	18·70
	Deoria ...	8·50	13·20	21·10
	Azamgarh .	10·90	10·90	21·00
	Varanasi ..	10·80	10·10	20·30
	Ghazipur ..	8·10	10·50	17·80
	Jaunpur ...	11·48	8·48	16·36
	Faizabad ...	10·90	6·61	16·47
	Barabanki ...	8·60	1·96	10·49
	Basti ...	7·03	14·48	20·83
	Ballia ..	9·80	7·10	15·90
Madhya Pradesh	Panna & Satna ...	4·41	1·14	5·55
	Chhattarpur ...	5·89	1·24	7·13
	Tikamgarh ...	1·11	0·29	1·40
	Shahdol ...	0·09	..	0·09
	Rewa ...	0·93	..	0·93
	Saugor ...	1·52	0·39	0·91
	Damoh ..	1·23	0·29	1·52
	Jabalpur ..	0·36	0·07	0·43
	Raipur ...	1·01	0·06	1·07
	Raigarh .	0·007	0·02	0·027
	Durg ..	0·02	0·007	0·027
Orissa*	Chatrapur ...	5·90	5·60	11·50
	Khurda ..	4·51	5·00	8·96
	Puri ..	10·30	14·90	28·30

* The disease rates in these States include cases with acute manifestations like filarial fever, lymphangitis, etc.

It was with this object that planning was restricted to covering a limited population in the different affected States. The activities of the control unit and the protection afforded were to cover a population of 3 lakhs per unit; but it was laid down that this population should, wherever possible, cover both urban* and rural* areas roughly in the proportion of 2 to 1. This pattern has not always been strictly adhered to due to variations in the local conditions and non-availability of strictly "urban"

*A population of 40,000 and over was called urban, while those below this figure were called rural. A revision of these standards appears necessary.

or "rural" areas as desired in the plan. Certain units have been covering totally urban areas while there are instances where almost the entire area covered by a unit has been rural. The activities of a control unit under the National Filaria Control Programme are as follows :

(*a*) Preliminary surveys.

(*b*) Control operations: drug administration and mosquito control operations.

(*c*) Follow-up investigations for assessment to evaluate the results.

A rapid random sample survey, covering a population of about 3 to 10 per cent of the total population to be protected, was to be carried out to determine the indices of endemicity in the community prior to the institution of control measures. An index area, called the special study area, was to be selected with a population of about 10 per cent of the total population, i.e., 30,000 under each unit, and an intensive survey of this special study area was required to be carried out before, and annually, following the control operations. A large sample of 10 to 15 per cent of residents, including about 500 children between 1 to 5 years of age and another 500 between 5 to 10 years, was to be examined during these surveys to enable proper appraisal of the results. Such stratification was considered essential as in a disease like filariasis, the benefits of interruption of transmission could best be measured among the younger age-groups, particularly those born since the commencement of the control activities.

For purposes of comparison, it was required that each unit should select a comparison area with a population of about thirty thousands outside the control zone. The epidemiological and the filariogenic conditions in the two areas should be, as far as possible, identical to make the evaluation reliable. No control measures, whatsoever, were to be carried out in these comparison areas. The epidemiological and entomological data, similar to those collected from the special study area, had necessarily to be collected from the comparison area also, both initially and annually during the follow-up period.

The principle underlying the programme of mass drug administration is that the individuals who harbour the microfilariae, are the reservoirs of the infection. and the measure adopted to bring about a reduction of the reservoir factor will, to that extent, contribute towards reduction of transmission of the disease. The mass treatment acted as an adjuvant to vector control to reduce transmission of filariasis. Diethylcarbamazine has the reputation for effectively suppressing the microfilariae for long periods.

WHOM TO TREAT.

It would appear logical to select those persons in the community who have microfilariae in the peripheral blood, and give them a course of treatment. While on theoretical grounds this is correct, from the practical point of view this procedure is not feasible for the following reasons :—

1. This would necessitate examination of night blood of every member of the community.

2. One examination is often not enough and repeated blood examinations may be necessary to detect the infection or to declare a person negative.

3. Persons harbouring immature stages of the worms cannot be detected by this procedure, but will become positive for microfilariae at a later date.

4. The microfilaria carriers in many instances are free from any symptoms, and do not readily subject themselves to an examination. Paradoxically enough, a majority of the individuals with elephantoid swellings are negative for microfilariae, and will consequently have to be omitted from the schedule. Omission of the latter category of persons is not in the interest of public co-operation in the programme.

The course of drug administration under the programme has, therefore, to be given to all the individuals in the community—hence the name "mass therapy" to this part of the programme.

THE ORGANISATION OF MASS DRUG ADMINISTRATION.

Mass treatment under the National Filaria Control Programme is stretched over a period of 5 days, the dosage schedule varying with the age-group*. The drug is to be given after food, and never on an empty stomach. The drug administration is carried out by a number of drug distributing teams, each team covering a population of about 300 in one week. Forty-five such teams should be able to cover the entire population within 6 months.

A census of the residents in the area is first prepared by a planned house-to-house visit and this is followed by the drug administration over a period of 5 successive days. Details of the procedure adopted are given below :—

(*a*) *Census taking.*—As in any programme aiming at reaching every member of the community, it is essential to make a list of all the residents in the area at the outset. A good plan appeared to be to carry out the census in each selected locality of 300 residents, on the first day of the week, and commence drug administration in the area on the following day. The nominal roll must contain details regarding street and door number of the house, and a list of names of residents, their age, sex and remarks regarding illness, if any, pregnancy etc. The Filaria Officer or the Research Officer scans the list thus prepared and marks the dosage to be administered to each person, and scores out with red pencil the names of those who are to be exempted from the drug administration, viz., pregnant women, children below two years and debilitated and chronically ill persons.

(*b*) *The drug administration.*—The course of drug administration extends over a period of 5 consecutive days. The drug distributing team which should preferably include a local resident, whose presence will help in securing public co-operation, goes from door to door and administers the tablets to the residents. The drug is swallowed in the presence of the visiting team by washing it down with some water. For ease of distribution 100 mg. tablets, with a groove

*Adults (above 18 years) are given a daily dose of 200 mg.; adolescents (12-18 years) a dose of 150 mg.; children between 6-12 years a dose of 100 mg. and 2-6 years 50 mg. per day. The drug is withheld from the age-groups below 2 years.

in the middle, have been supplied. It should be ensured that the drug is swallowed in the presence of the distributor and not handed over for use later. Handing over was, however, resorted to where they were satisfied about the drug being put to proper use. In all such cases the unit officer had to satisfy himself that the drug was actually consumed. Such verification is essential as otherwise, not only is there a chance of the drug being put to improper use, but also the follow-up data and assessment are likely to be misleading.

PUBLIC CO-OPERATION.

Experience has shown that a lot of preliminary education is essential regarding the scope and objective of the mass drug administration. Individual non-co-operation to mass treatment is mainly due to the unpleasant reactions which sometimes follow the administration of the drug. Varying number of cases show reactions within about 24 to 48 hours of taking the drug. The death of the microfilariae in the system is believed to be one of the factors responsible for such reactions which range from slight indisposition, headache and feverish feeling to prostration and high fever. Occasionally cases of severe body pains, abdominal cramps, gastro-intestinal upset accompanied by choleraic symptoms, have also been reported.

The drug has a powerful action on intestinal round worms ; while this may be beneficial in many cases, rare instances are known, particularly where the round worm infestation is heavy, of intestinal obstruction necessitating hospitalisation.

The individuals who are the reservoirs of the infections are often free from any manifestations and are apparently healthy. It is this group of persons which obviously has to be given the course of treatment, as the sole object of the mass treatment programme is to reduce the reservoir of infection in the community. Occurrence of reactions in these persons leads to public non-co-operation.

The unpleasant reactions, though transitory in a majority of cases, sometimes take a severe course. This has frequently led to certain adverse propaganda ; the gullibility of the lay public has been exploited by mischievous rumours like "the programme is for family planning" and "these are birth control tablets". Deaths occurring in the area during the course of the programme of drug administration, be it due to co-existing infectious diseases or other causes, are glibly attributed to the drug.

PROGRESS OF THE MASS DRUG ADMINISTRATION PROGRAMME.

Mass administration of Diethylcarbamazine has been in progress under one or more units in all the participating States. So far, 25 units have completed this part of the programme and given the drug to 42·01 lakh persons (Table IV) against the present total targeted population of 138 lakhs under the 46 units sanctioned. Coverage has varied ; the number of persons receiving treatment among the population under the different units range from 31·9 to 99·5 per cent (average 61·89 per cent) of the target. These do not represent persons who took

the full 5 days course (Table VI) who account for 38·3 to 97·6 per cent of those treated. Details regarding the percentage coverage under the different units are presented in Table VII. The details in respect of this aspect of the programme in the units in Bombay State have been discussed by Patel and Paranjpey (1958).

TABLE IV.

Percentage population covered by mass drug administration with Diethylcarbamazine in the National Filaria Control Programme and percentage of treated persons showing reaction.

State.	Unit.	Number of persons eligible for treatment	Number of persons treated.	Percentage of eligible persons treated.	Percentage of treated persons who showed reaction.
Uttar Pradesh	Basti	3,05,821	2,82,979	92·5	..
	Faizabad	3,00,093	1,99,153	66·4	28·8
	Varanasi	3,00,011	1,93,491	64·5	10·3
	Ballia	2,74,295	1,90,267	69·4	16·8
	Gorakhpur	3,10,950	1,68,976	54·3	23·8
	Barabanki	2,77,304	2,27,492	82·0	10·2
Bombay	Nagpur (Rural)	2,83,237	1,59,903	56·4	37·4
	Junagadh	87,576*	31,516	36·0	13·6
	Surat	1,67,717	73,245	43·7	23·9
Bihar	Monghyr	2,78,979	2,04,803	73·4	10·6
	Patna West	1,84,884	1,26,078	68·2	15·3
	Bhagalpur	2,57,454	1,86,062	72·3	34·7
	Darbhanga	3,05,413	1,94,938	63·8	9·5
	Gaya	3,23,661	2,19,872	67·9	9·6
	Ranchi	3,12,629	1,27,270	40·7	4·4
	Muzaffarpur	2,63,042	2,00,804	76·3	4·1
Kerala	Ernakulam	2,50,000	1,17,112	46·8	22·7
	Trivandrum	2,38,847	1,91,621	80·2	17·5
Madhya Pradesh	Chattarpur	2,38,860	2,37,683	99·5	7·3
Mysore	Mangalore	1,17,095	80,967	69·1	17·0
Madras	Kumbakonam	3,86,174	2,36,670	61·3	25·4
Orissa	Cuttack	2,83,042	1,17,054	41·4	..
	Khurda	3,88,651	1,24,388	32·1	..
	Chatrapur	3,02,156	96,270	31·9	..
Andhra Pradesh	Mandapetta	3,51,090	2,12,843	60·6	..
Total	... ...	67,88,981	42,01,957	61·89	

*Does not represent the entire area.

STAFF EMPLOYED.

It has been recommended that 90 paid workers per Filaria Control Unit should be employed for drug administration. They were to form 45 drug distributing units and each unit was expected to cover about 300 residents in one day. Enlistment of voluntary help was suggested to ensure local co-operation. Most units had appointed 80 to 105 persons. Basti unit had 34 men, Kumbakonam (Madras) had 45 men and Chattarpur (Madhya Pradesh) unit had 73 men. Regular help from voluntary workers has been recorded only in two units, viz., Ernakulam (Kerala) and Chattarpur(Madhya Pradesh) (Table VII).

TABLE V.

The percentage incidence of reactions on the different days of mass drug administration.

State.	Unit	Number of persons treated.	Percentage of treated persons showing reaction.	Percentage incidence of reaction on different days of drug administration:				
				1st day.	2nd day.	3rd day.	4th day.	5th day.
Uttar Pradesh	Barabanki ...	2,27,492	10·2	17·0	21·8	24·4	21·4	15·4
	Basti ...	2,82,979		0·005	25·1	32·0	25·1	17·8
	Faizabad ...	1,99,153	28·8	20·7	25·6	24·6	18·3	10·7
	Ballia ...	1,90,267	16·8	3·4	27·3	31·6	24·5	13·2
	Gorakhpur ...	1,68,976	23·8	6·3	21·3	27·7	25·7	19·0
	Varanasi ...	1,93,491	10·3	7·6	29·5	31·1	23·1	8·5
Bihar	Monghyr ...	2,04,803	10·6	16·0	36·5	26·7	19·6	1·2
	Patna West ...	1,26,078	15·3	2·5	27·3	30·5	24·9	14·8
	Bhagalpur ...	1,86,062	34·7	..	27·0	29·4	24·4	19·2
	Darbhanga ...	1,94,938	9·5	9·2	22·2	25·9	23·6	19·1
	Gaya ...	2,19,872	9·6	0·9	36·6	29·9	21·2	11·4
	Ranchi ...	1,27,270	4·4	..	30·2	36·9	21·5	11·4
	Muzaffarpur ...	2,00,804	4·1	91·5	15·8	2·5	0·2	..
Kerala	Ernakulam ...	1,17,112	22·7	27·6	31·4	25·0	15·1	0·9
	Trivandrum ...	1,91,621	17·5	4·8	24·0	29·7	24·9	16·6
Bombay	Nagpur ...	1,59,903	37·4	7·3	25·8	26·3	23·3	18·0
	Junagadh ...	31,516*	13·6	3·0	21·0	32·0	24·5	19·5
	Surat ...	73,245	23·9	0·8	25·1	29·4	24·3	19·7
Madhya Pradesh	Chattarpur ...	2,37,683	7·3	7·8	32·4	29·5	18·4	11·9
Mysore	Mangalore ...	80,967	17·0	3·3	33·9	30·6	21·6	10·6
Madras	Kumbakonam ...	2,36,670	25·4	2·1	28·5	30·4	24·3	14·7
		36,50,902	16·5	8·5	26·7	27·9	23·0	13·9

*Does not represent the whole unit area.

TABLE VI.

The percentage of persons treated for different number of days (1-5 days) during the mass drug administration programme. (Up to March, 1959).

State.	Unit.	Number of persons treated.	PERCENTAGE OF PERSONS TREATED FOR :				
			1 day.	2 days.	3 days.	4 days.	5 days.
Kerala	Ernakulam	1,17,112	9·1	5·8	5·7	11·8	68·1
	Trivandrum	1,91,621	7·6	6·1	6·3	9·5	70·5
	Quilon	67,479	2·4	2·8	2·3	3·3	89·1
Bombay	Nagpur (Rural)	1,59,903	14·1	14·0	15·3	18·3	38·3
	Porbander	31,516	9·3	6·0	4·4	6·1	74·2
	Surat	73,245	15·6	12·6	13·0	19·0	39·8
Madhya Pradesh	Chattarpur	2,37,683	2·8	1·6	1·9	2·3	91·2
Mysore	Mangalore	80,967	3·4	2·4	3·0	6·0	85·1
Madras	Kumbakonam	2,36,670	7·6	6·8	11·8	19·6	54·2
Bihar	Monghyr	2,04,803	6·2	6·0	7·8	5·3	74·7
	Patna West	1,26,078	3·0	1·9	1·9	4·5	88·7
	Bhagalpur	1,86,062	1·4	1·2	1·5	2·1	93·8
	Gaya	2,19,872	2·3	2·3	3·4	5·0	87·0
	Ranchi	1,27,270	7·6	8·8	11·3	17·2	55·0
	Muzaffarpur	2,00,804	2·6	2·9	3·1	4·6	86·8
	Darbhanga	1,94,938	14·7	13·7	13·4	17·3	40·1
Orissa	Cuttack	1,17,054	2·6	3·0	2·8	3·8	87·8
	Rampur	1,25,258	2·1	2·0	3·0	6·6	86·4
	Khurda	87,705	5·0	5·6	9·2	16·3	63·9
	Puri	70,339	10·6	10·6	12·5	15·4	50·9
	Chatrapur	95,271	8·4	9·6	13·1	15·3	52·6
Uttar Pradesh	Basti	2,82,979	0·4	0·4	0·6	1·0	97·6
	Faizabad	1,99,153	1·3	1·6	2·3	2·7	92·1
	Ballia	1,90,267	1·4	1·5	2·3	6·1	86·7
24 units (Approximately 70 lakhs population)		36·34 lakhs	5·3	4·9	5·9	8·4	75·5

DURATION OF MASS THERAPY

Twelve of the 24 units, in which the full complement of recommended staff was appointed, completed the mass therapy within the targeted time of 6 months and some of them in 4 months. All these units are either purely urban like Surat, Mangalore, Ernakulam, Trivandrum and Patna or have a major urban component like most of the units in Bihar.

The units in Uttar Pradesh, the Nagpur rural unit, the Chattarpur (Madhya Pradesh) unit and the Mandapetta (Andhra Pradesh) unit, all of which cover mainly rural and semi-urban areas, were able to complete the programme in 7 to 10 months. The targeted population under the Mandapetta unit, however, is higher, i.e., 3·5 lakhs, instead of 3 lakhs scheduled to be protected by a control unit. The Khurda unit (Orissa) having a similar distribution of the areas took 15 months for the completion of the programme, while the units at Cuttack (Orissa) and Kumbakonam (Madras) took 13 and 18 months respectively.

The percentage of the targeted population, completing the treatment in the different units, does not appear to show any correlation with the time taken for

completion of the programme (Table VII). The highest coverage is recorded to have been obtained in those units which completed the mass therapy in 7 to 8 months. The Mandapetta unit obtained a coverage of 60·6 per cent in 10 months, whereas in spite of having taken much longer periods the Cuttack and Khurda units were able to cover only 41·4 and 32·1 per cent respectively.

TABLE VII.

The staff employed and the time taken for completing mass drug administration by different units.

State.	Unit.	Total number of persons suitable for drug.	Number of persons administered drug.	Percentage administered drug.	Duration of mass therapy (in months).	STAFF EMPLOYED.	
						Paid.	Volunteers.
Andhra Pradesh	Mandapetta	3,51,090	2,12,843	60·6	10	305	..
Uttar Pradesh	Basti	3,05,821	2,82,979	92·5	8	90	..
	Faizabad	3,00,093	1,99,153	66·4	8	90	..
	Varanasi	3,00,011	1,93,491	64·5	9	90	..
	Gorakhpur	3,10,950	1,68,976	54·3	9	90	..
	Barabanki	2,77,304	2,27,492	82·0	7	90	..
	Ballia	2,74,295	1,90,267	69·4	14	90	..
Bombay	Nagpur (Rural)	2,83,237	1,59,903	56·4	8	100 to 94	..
	Junagadh	87,576	31,516	36·0	2*	80	..
	Surat	1,67,717	73,245	43·7	5	65 to 69	..
Bihar	Monghyr	2,78,979	2,04,803	73·4	6	97	..
	Patna West	1,84,884	1,26,078	68·2	6	105	..
	Bhagalpur	2,57,454	1,86,062	72·3	6	90	..
	Darbhanga	3,05,413	1,94,938	63·6	6	90	..
	Gaya	3,23,661	2,19,872	67·9	6	100	..
	Ranchi	3,12,629	1,27,270	40·7	6	94	..
	Muzaffarpur	2,63,042	2,00,804	76·3	5	90	..
Kerala	Ernakulam	2,50,000	1,17,112	46·8	4	100	30
	Trivandrum	2,38,847	1,91,621	80·2	5	100	..
Madhya Pradesh	Chattarpur	2,38,860	2,37,683	99·5	7	73	16
Mysore	Mangalore	1,17,095	80,967	69·1	6	..	..
Madras	Kumbakonam	3,86,174	2,36,670	61·3	13	45	..
Orissa	Cuttack	2,83,042	1,17,054	41·4	13	80	..
	Khurda	3,88,651	1,24,888	32·1	15	80	..
	Chattarpur	3,02,156	96,270	31·9	6	100	..

*Mass therapy was completed only in part of the area, i.e., Porbandar City and a few rural areas.

TABLE VIII.

Incidence of reactions in relation to infection rate and percentage coverage during the mass therapy with Diethylcarbamazine.

Name of Unit.	Infection rate.	Percentage covered by mass therapy.	Percentage showing reaction.
Ernakulam	7·6 (Malaria Inst. of India)	46·84	22·7
Mangalore	15·0 (Malaria Inst. of India)	69·14	17·0
Surat	10·3	43·67	23·9
Kumbakonam	9·5 (Tanjore Dist.)	61·28	25·4
Muzaffarpur	9·1	76·34	4·1
Patna West	16·4	63·20	15·3

The progress of mass therapy under some of the units needing attention is discussed below.

(*a*) *Kamareddy Unit* (*Andhra Pradesh*).—This is one of the 13 units allotted in 1955-56 and started functioning during the following year. Mass therapy was commenced in July 1958, but did not make much progress. The drug administration was stopped after covering a population of 30,000. The work is reported to have been started again recently.

(*b*) *Junagadh Unit* (*Bombay*).—Mass therapy was inaugurated in November, 1956 in the City of Porbandar. The programme came to a standstill after covering a population of about 87 thousands in this town and a few other small areas.

(*c*) *Chanda Unit* (*Bombay*).—This unit was established in October, 1958. The drug administration programme could not be commenced during the financial year as the unit was busy with surveys and other essential preliminary activities. The sanction of staff for mass therapy, therefore, lapsed and the work could not be commenced during the following year as necessary sanction, having lapsed once, could not easily be obtained*.

(*d*) The drug administration programme has not been started under the units at Jamnagar (August, 1958†), Surat and Thana District (January, 1959†), Cannanore (January, 1959†) and Bahraich (November, 1959†).

TABLE IX.

Assessment surveys : Microfilaria rates and average microfilaria counts before and after mass therapy.

Unit.	Percentage coverage.	Number of persons examined :		Microfilaria rate :			Average infection (Number mf. per 20 cmm.)			Year of post-therapy survey.
		Pre-therapy	Post-therapy	Pre-therapy	Post-therapy	Variation per cent	Pre-therapy	Post-therapy	Variation per cent.	
Khurda	32·1	2,687	1,238	16·4	10·6	−35·4	33·56	11·37	−64·6	1958–59
Ranchi	40·7	10,961	2,847	4·5	2·6	−42·2	10·00	7·28	−27·2	1958–59
Cuttak	41·4	4,427	1,448	13·3	5·9	−55·6	..	14·26	..	1958–59
Surat	43·7	4,500	4,500	10·3	10·22	− 0·8	8·5	5·7	−32·9	1957–58
		..	4,500	..	9·7	− 5·4	..	4·7	−44·7	1958–59
Nagpur (Rural)	56·4	4,547	2,794	14·9	6·2	−58·4	5·68	78·3	10 times	1957–38
		..	5,863	..	9·3	−37·6	..	11·4	100	1958–59
Mandapetta	60·6	30,284	3,202	11·7	7·21	−38·4	5·39	..	..	1957–58
Darbhanga	63·8	18,173	6,096	5·7	1·87	−67·2	16·94	17·6	+ 3·9	1958–59
Faizabad	66·4	4,417	2,139	13·1	10·9	−16·8	13·89	12·37	−10·9	1958–59
Gaya	67·9	8,656	452	10·8	6·19	−42·7	21·48	26·42	+23·0	1958–59
Patna	68·2	22,067	7,462	16·4	11·9	−27·4	30·51	17·47	−42·7	1958–59
Ballia	69·4	2,927	3,727	32·4	4·85	−85·0	19·54	5·44	−72·1	1957–58
Muzaffarpur	76·3	14,365	7,532	9·1	8·27	− 9·1	30·27	4·66	−84·6	1958–59
Monghyr	74·4	13,925	1,205	7·2	2·2	−69·4	15·53	9·47	−39·0	1958–59
Barabanki	82·6	7,351	3,030	10·18	7·3	−28·3	13·37	6·7	−49·9	1958–59
Chhatarpur (Madhya Pradesh)	99·5	7,787	2,326	6·16	4·3	−30·2	6·89	9·32	+35·3	1958–59

*It is understood that the sanction for staff has since been obtained.

†Date of commencement of the unit.

MOSQUITO CONTROL OPERATIONS.

The mosquito control operations under National Filaria Control Programme envisaged both anti-larval and anti-adult measures; the former were restricted at the outset to urban areas only. Indoor residual spraying with Dieldrin at 50 mg. per sq. ft. was recommended to be carried out thrice a year in all the rural areas at intervals of 10 to 12 weeks, while in the urban areas the residual spraying operations were restricted to a single round during the peak mosquito season. During the period 1955-59, 862·01 tons of 50 per cent Dieldrin water wettable powder have been supplied to the different participating States. Out of these 481·91 tons have been expended, leaving a balance of 380·10 tons at the end of the year 1958-59.

NUMBER OF HOUSES SPRAYED.

Up to March 1959, 31,72,302 houses have received the treatment with insecticides of which 21,16,725 have received a single round* and 9,15,005 two rounds and 1,40,574 three rounds. The average number of houses sprayed per pump per day has ranged from 11·4 to 51. The average surface area sprayed per pound of insecticide has also shown wide variations, ranging from 2,492 sq. ft. to 4,420 sq. ft.

SPRAYING ROUNDS: COVERAGE AND TIME TAKEN.

The details of the number of rounds of insecticidal spraying operations, the time taken and the percentage of houses sprayed in each round are presented in Table X. It will be observed that the spraying operations have not been maintained according to schedule in most cases. No area appears to have received three rounds of spray during the first two years of operation of the programme. The three rounds of spraying, required under the programme for rural areas, have been applied only during the year 1958-59 and that only by 6 units (*viz.* the units at Mandapetta and Kamareddy in Andhra Pradesh, Darbhanga in Bihar, Trivandrum in Kerala, Mangalore in Mysore and Ballia in Uttar Pradesh).

The durations of spraying rounds (Table X) show vide variations from unit to unit. In urban units this has varied from one month (in Surat and Mangalore) to 9 months (in Kozhikode) while in rural and urban-cum-rural units the duration has varied from 3 to 10 months. The normal duration allowed for each round (2½ to 3 months) should be adequate for completion of the spraying, ensuring at the same time a high coverage of the houses.

The proportion of the targeted number of houses actually sprayed, also leaves much to be desired. The percentage has varied from 6·5 to 97 (average 53·3 per cent) during the first round, 1 to 98 per cent (average 38·9 per cent) in the second round, and 3 to 37 per cent (average 24·9 per cent) during the third round.

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*These include houses in urban areas which are scheduled to receive only one round of spray.

TABLE X.

Time taken for completion and the percentage coverage during residual insecticidal spraying rounds.

State.	Unit.	Year.	Spray round: I		II		III	
			Duration (months).	Per cent coverage.	Duration (months).	Per cent coverage.	Duration (months).	Per cent coverage.
Andhra	Mandapetta Rural	1956–57	4½	44·8	..	..	..	..
		1957–58	10	89·0	1	15·2	..	..
		1958–59	4½	76·5	3½	67·0	2½	74·5
	Kamareddy Rural	1956–57	2	20·9	..	..	..	15·9
		1957–58	8	75·7	..	..	..	..
		1958–59	5	45·7	4	26·8	2	..
Bihar	Patna West Urban	1958–59	5	14·8	..	..	..	..
	Patna East Rural	1956–57	5	39·3	3	4·1	..	..
		1957–58	7	49·9	1	5·1	..	..
		1958–59	3	9·6	4	17·5	..	..
	Gaya Rural	1958–59	3	46·4	3	54·5	..	..
	Ranchi Rural	1958–59	6	67·7	..	..	..	..
	Darbhanga Rural	1958–59	1¾	52·0	2	43·1	2	32·0
	Monghyr Rural	1958–59	3	65·8	4	63·3	..	..
	Bhagalpur Rural	1958–59	4	77·1	2	70·5	..	..
	Muzaffarpur Urban	1955–56	3	46·5	..	..	..	..
	,, Urban	1956–57	3	15·5	2	38·5	..	..
	,, Urban	1957–58	5	96·4	2	29·0	..	..
	,, Rural	1957–58	5	30·4	..	..	..	..
	,, Urban	1958–59	3	65·2	3	..	..	..
	,, Rural	1958–59	3	28·0		98·0	..	..
Bombay	Nagpur Corporation Urban	1958–59	5	69·9	..	..	..	..
	Surat B.M.* Urban	1957–58	1	74·1	1	76·2	..	..
	Junagadh Rural	1957–58	3	79·8	2	45·7	..	..
		1958–59	2	41·7	..	..	..	..
	Jamnagar Rural	1958–59	3	49·4	..	..	..	17·9
Kerala	Ernakulam Urban	1958–59	3	22·8	..	..	..	..
	Kozhikode Urban	1956–57	9	23·3	..	..	..	..
		1957–58	4	6·5	6	22·7	..	..
		1958–59	5	10·6	1	1·0	..	..
	Trivandrum Urban	1957–58	11	84·4	1	5·2	..	..
	,, Urban	1958–59	3	..	3	21·4	6	5·0
	,, Rural	1958–59	3	32·3		19·7		11·8
Mysore	Mangalore Urban	1956–57	12 days	30·1	..	..	..	..
		1957–58	1	19·6	..	..	..	..
		1958–59	1	29·8	..	..	..	..
Madras	Kumbakonam Urban	1957–58	2	94·4	..	..	..	..
	,, Rural	1957–58	2	65·8	..	..	..	..
	,, Urban	1958–59	4	67·1	..	..	..	..
	,, Rural	1958–59	4	97·9	..	..	..	..
Orissa	Cuttack Rural	1957–58	4	84·1	4	95·7	..	..
	,, Rural	1958–59	6	93·6	..	..	..	..
	,, Urban	1958–59	6	88·5	..	..	..	..
	Chatrapur Rural	1957–58	7	92·4	3	43·7	..	..
	Ranpur Rural	1958–59	4	77·3	..	..	..	..
	Ranpur Rural	1957–58	3	52·8	6	53·8	..	..
		1958–59	6	97·0	..	..	..	..
Uttar Pradesh	Ballia Rural	1956–57	4½	20·8	..	..	..	..
		1957–58	4	36·2	2	8·5	..	..
		1958–59	1	11·6	4	47·6	4	36·9

The percentage has been calculated on the assumption that the number of houses under each unit are 60,000, in the absence of any other information from the unit.

* B.M. = Burrough Municipality.

ANTILARVAL MEASURES.

Larval control operations have been recognised as an essential part of the control of *Culex fatigans* under the programme, particularly in urban areas. While larviciding operations have been in progress under the National Filaria Control Programme in a number of urban areas, similar work in rural areas is in progress under the units in Nagpur District and Chanda in Bombay State and Mandapetta Unit in Andhra Pradesh.

In the early stages of the programme, it was considered that application of oil, though effective for larval control, would be difficult from the point of view of procurement, storage, transportation and application. 50 per cent water wettable powder of BHC (6·5 per cent gamma) was, therefore, used as a larvicide under the programme. B.H.C. formulation has been used since the beginning of the programme in some units (Trivandrum, Kozhikode), while in some of the other units like Surat and Patna, oil was preferred. In these latter areas, B.H.C. was used on a very limited scale under restricted conditions.

Those units which have been using B.H.C. on a large scale complained about the lack of control with the formulation ; this was particularly so in the presence of highly polluted water which favoured breeding of *Culex fatigans*. The demand for oil was pressed for by many units and in some, they started using small supplies of oil obtained from local resources in preference to the supply of B.H.C. made available under the programme. The need, for substituting B.H.C. with a more effective larvicide, was considered in 1957-58 and larvicidal oil is now being supplied under the programme since 1958-59.

ASSESSMENT.

Evaluation of the results of control operations is to be made by both epidemiological and entomological observations. The data available so far are discussed below :

(*i*) *Epidemiological assessment.*—The assessment surveys are restricted to the special study and the comparison areas. As in the pre-control surveys, about 10 to 15 per cent of the population has to be examined during the assessment surveys which are to be carried out regularly every year. The first follow-up survey is to commence during the identical month in which the initial survey was made one year after the institution of mass therapy.

The epidemiological data from 15 units are presented in Table IX. The variations in the different indices collected, *viz.*, the microfilaria and disease rates, and the average microfilarial infestation, are discussed below :

(*a*) *Microfilaria rates.*—The microfilaria rates have recorded a fall in all the units from which the data are available. While in some units the fall is marked, as in Ballia unit (from 32·4 to 4·85 per cent), others have recorded a less spectacular fall. The stability of the decline observed in the microfilaria rate, and further decline, if any, which is to be expected in the face of effective vector control, can be indicated only by subsequent annual follow-up surveys. Data on second year's follow-up

surveys are available from the Ballia, Nagpur (rural) and Surat units. The former two units record a slight rise in the microfilaria rate at the end of two years following mass drug administration, though the rates in both cases still remain below the pre-control level. In the Surat unit, however, a further fall is noticed during the second follow-up survey as compared with the first.

(*b*) *Disease rates.*—The sample surveys included study of this index also. An analysis of the data shows considerable fall in the disease rate in most of the units, while in a few there has been a rise in the rate. Such variations in the course of just within 1 to 2 years appear inexplicable and are likely to be due to sampling errors. The indices are not, therefore, presented or discussed.

(*c*) *Average microfilarial infestation.*—Details of microfilarial counts during the initial as well as the follow-up surveys are available from thirteen units. A fall in the average infestation is noticeable in a majority (nine) of the cases; the fall being marked in most of them. In four units (Nagpur, Dharbanga, Gaya, and Chattarpur (Madhya Pradesh), this index has shown a rise. The difference in the average count is not marked in Dharbanga where the pre- and post-therapy counts are 16·94 and 17·6 respectively, while in Gaya the corresponding figures are 21·8 and 26·4. In the Nagpur rural area, however, a large rise has been recorded from 5·68 during the initial survey to 78·3 during the first follow-up, and 11·4 in the second year's follow-up survey. Seeing that the microfilaria rate in this latter unit has recorded a fall of nearly 60 per cent during the first year, the high rise in the average count in the positive cases is inexplicable in the absence of details regarding the counts in the individual cases. In the unit at Chattarpur (Madhya Pradesh), the mean microfilaria count during the follow-up survey was found to be 9·32 as compared to the initial pre-control count of 6·9 mf. per 20 c.mm.

ENTOMOLOGICAL ASSESSMENT.

The following data were collected for evaluation of the vector control operations :

(*i*) Vector density,

(*ii*) 24-hours survival rates of *C. fatigans*,

(*iii*) Infection and infectivity rates in *C. fatigans*.

Vector densities and survival rates.—The effect on the density of *C. fatigans* and the survival rates, following spraying operations, are shown in Table XI. An analysis of the data shows that there has been no appreciable reduction in the vector density even during the week immediately following the application of spray. Except in one unit (Bhagalpur) where the reduction in density has been maintained at more than 50 per cent during the observation period of 4 weeks, the fall in vector density has varied between 4 and 33 per cent during the first week in some units, while in others there has been actually a rise in density.

TABLE XI.

Weekly vector densities and 24-hours survival rates in sprayed stations following application of Dieldrin at 50 mg. per sq. ft.

Unit.	Insecticide used.	Week following application of spray: 1st week: Per cent *variation in density.	1st week: Per cent survival.	2nd week: Per cent *variation in density.	2nd week: Per cent survival.	3rd week: Per cent *variation in density.	3rd week: Per cent survival.	4th week: Per cent variation in density.	4th week: Per cent survival.	Remarks.
Jamnagar	Dieldrin	− 4	70	+135	64	− 14	67	+ 50	17	
Muzaffarpur†	do	− 4	84·5	− 4	84·4	− 4	84·9	− 4	85·7	
Bhagalpur†	do	−80	0	− 71	23·9	− 59	55·6	− 53	57·9	
Mangalore†	do	−19	86·7	+ 13·6	..	+ 92	..	− 14	100·0	
Katol	do	1 +14	35·7	..	..	..	66·7	..	75·0	Observations in 5 villages.
		2 +14	60·6	− 41·3	43·3	..	52·7	..	65·0	
		3 +39	58·7	+100	87·8	− 6·0	50·0	..	52·0	
		4 +73	65·3	− 27	56·0	..	40·0	..	60·0	
		5 −26	27·7	− 48·4	78·3	− 42·8	65·0	..	70·0	
Surat B.M.†	do	1958 ..	66	+ 6	63	+ 15·6	63	− 90	65	
	do	1959 −33	..	− 45	..	− 61	..	..	..	
Nagpur Corporation	do	1 +62·5	..	+136·5	..	+107·5	..	+475	..	
		2 0	..	+118	..	..	..	..	..	

* The variations in density are in comparison to the density prior to control.

† Year-round larval control operations.

TABLE XI—A.

Weekly vector densities and survival rates for unsprayed/comparison stations.

Unit.	1st week :		2nd week :		3rd week :		4th week :		Remarks.
	Per cent variation in density.	Per cent survival rate.	Per cent variation in density.	Per cent survival rate.	Per cent variation in density.	Per cent survival rate.	Per cent variation in density.	Per cent survival rate.	
Jamnagar	− 7	50	+28	30	+34	19	−109	60	
Muzaffarpur	0	33·4	− 1	32·8	− 1	31·6	− 2·7	31·6	
Bhagalpur	−18	50	− 5	57	+18	65	0	74	
Mangalore	− 6	100	+78	100	−64	100	+ 94	100	
Nagpur Rural	1 +76	54·0	..	..	−50	100	− 12	87	Observations in five villages.
	2 −29	72	..	..	−43	68	− 29	77	
	3 −24	55	+24	75	− 5	51	− 18	49	
	4 −90	78	− 1	58	−13	51	+ 7	94·7	
	5 − 3	56	−68	100	−74	83	− 80	67	
Surat	1958 No Data 1959 − 1	No Data 51	+13	57	0	43	− 24	43	
Nagpur	No Data	No Data							According to reports there is 100 per cent mortality in unsprayed stations.
Ballia	1958 +8°	..	−13	..	−28	..	− 39	..	

The survival rates have also been high among mosquitoes captured from sprayed stations and kept in the laboratory for 24 hours, except in the Bhagalpur unit where 100 per cent mortality was recorded during the first week, followed by 23·9, 55·6 and 57·9 per cent during the following weeks. Very low mortality has been recorded even during the first week following spraying in the other units.

Data on vector densities and their survival rates from the comparison areas are presented in Table XI-A. It is observed that in many cases a decline in the mosquito density has been recorded in the comparison area also. This is in many instances accompanied by a low survival in the laboratory among the collections from those areas. It was found in many cases that suitable laboratory conditions for keeping mosquitoes, for observation on their longevity, were not available, due to contamination with insecticides. The observations recorded are, therefore, not used for appraisal of the results.

Indices of mosquito transmission.—The results of mosquito dissections are presented in Table XII. Comparable data for more than one year are available from seven units. The total infection rate as well as the infectivity rate have been separately analysed and presented.

The two indices have shown varying changes in the different units during the period of observations. The total infection rate has shown a general fall in some units (Surat, Nagpur Rural and Muzaffarpur). A marked decline is noticeable in the Muzaffarpur Unit during 1958-59 as compared to the data for the corresponding months during the previous year. A similar fall in the index is recorded by the Nagpur (rural) unit during 1957-58 compared to the period of 1956-57. The records for 1958-59, appear to indicate that this fall is, in general, being maintained in the unit area. Similarly a sharp fall is noticed in the Surat Unit during 1957-58 compared to the previous year, and the fall is being maintained during the subsequent year.

The infectivity rates in these units, which will be an index of the longevity of the vector, are as follows : Not much variation is noticed either in the Muzaffarpur or the Nagpur Rural Unit. While during some months there has been no significant difference, during others a rise in infectivity rate has been recorded—this is comparatively more marked in the Nagpur Rural Unit. The observations recorded by the Surat Unit show a rise during the months January to April, but a reduction in transmission is noticeable during the remaining part of the year.

A general rise in the total infection, as well as the infectivity rates, has been recorded by the units at Patna, Gaya and Kozhikode. The mosquito transmission appears to be of a low degree in the area under the Ballia Unit, and the slight increase or decrease recorded in the mosquito transmission indices would not justify any conclusion at this stage.

TABLE XII.

Results of mosquito dissections showing total infection and infectivity rates in C. fatigans *for* W. bancrofti.

Unit.	Details.	April			May			June			July		
		1956	1957	1958	1956	1957	1958	1956	1957	1958	1956	1957	1958
Patna (Sept. 1958 to Feb. 1959)*	Number dissected.	..	..	317	..	113	305	..	128	148	83	247	249
	Per cent 4th stage infection.	..	..	0·0	..	0	0·6	..	0·7	0·6	10·0	2·4	1·2
	Per cent total infection.	..	..	6·6	..	7	10·5	..	5·4	9·4	20·0	12·5	12·4
Muzaffarpur (Oct. 1957 to Feb. 1958)*	Number dissected.	..	379	411	..	287	589	..	110	836	..	..	753
	Per cent 4th stage infection.	..	1·3	0·2	..	1·3	0·7	..	0	2·3	..	..	1·1
	Per cent total infection.	..	18·2	5·3	..	15·0	5·2	..	18·1	7·0	..	..	7·7
Gaya (Sept. 1958 to Feb. 1959)*	Number dissected.	..	517	176	..	276	166	..	75	255	..	123	389
	Per cent 4th stage infection.	..	0	0	..	0	1·8	..	0	8·6	..	0	6·1
	Per cent total infection.	..	23·7	15·3	..	2·1	14·4	..	4·6	26·0	..	1·6	36·5
Nagpur (Feb. 1957 to Sept. 1957)*	Number dissected.	..	1995	305	188	1149	230	425	1104	207	600	1935	423
	Per cent 4th stage infection.	..	0·02	3·9	0·3	0·2	0	0	0·7	0·4	0·3	0·3	1·1
	Per cent total infection.	..	4·3	13·1	28·5	5·2	6·9	21·2	3·1	4·8	13·1	6·6	9·9
Surat B.M. (Sept. 1956 to Jan. 1957)*	Number dissected.	..	244	133	..	146	181	..	107	200	..	365	..
	Per cent 4th stage infection.	..	2·8	0	..	4·1	0	..	3·7	0	..	0·5	..
	Per cent total infection.	..	14·4	8·0	..	10·2	11·0	..	10·2	9·0	..	4·1	..
Kozhikode (Sept. 1956 to Feb. 1957)*	Number dissected.	..	93	116	..	176	336	..	156	648	..	189	614
	Per cent 4th stage infection.	..	0·9	0	..	1·1	2·3	..	1·9	2·4	..	0·1	1·6
	Per cent total infection.	..	6·4	5·2	..	6·2	9·8	..	8·3	12·8	..	0·5	12·2
Ballia (June 1956 to Aug. 1957)*	Number dissected.	..	520	937	..	644	1200	..	319	726	..	608	958
	Per cent 4th stage infection.	..	0	0·6	..	0·1	0·1	..	0·6	0	..	0·4	0·7
	Per cent total infection.	..	2·6	2·1	..	0·7	1·7	..	1·8	0·4	..	1·6	1·7

*Period of Mass Therapy Programme.

(*Contd.*)

TABLE XII. (Contd).

Unit.	Details.	August			September			October			November		
		1956	1957	1958	1956	1957	1958	1956	1957	1958	1956	1957	1958
Patna (Sept. 1958 to Feb. 1959)*	Number dissected.	95	280	321	74	421	228	95	253	..	76	505	259
	Per cent 4th stage infection.	3·1	4·6	4·6	2·7	0·4	4·3	3·1	0·3	..	0·1	0·3	0
	Per cent total infection.	28·4	20·3	28·0	10·8	13·3	2·2	19·5	9·1	..	1·1	8·9	10·0
Muzaffarpur (Oct. 19 7 to Feb. 1958)*	Number dissected.	..	47	639	..	368	670	..	380	382	..	480	231
	Per cent 4th stage infection.	..	0	1·2	..	3·8	0·9	..	5·2	0·8	..	5·8	0
	Per cent total infection.	..	17·0	8·1	..	13·4	7·6	..	16·8	6·5	..	12·9	5·6
Gaya (Sept. 1958 to Feb. 1959)*	Number dissected.	..	201	480	365	365	655	..	119	442	..	628	739
	Per cent 4th stage infection.	..	0	0·6	..	0	6·4	..	0	0·4	..	0	0·1
	Per cent total infection.	..	2·0	30·8	..	0·8	22·1	..	1·6	25·6	..	0	27·3
Nagpur Feb. 1957 to Sept. 1957*	Number dissected.	406	1638	274	315	738	383	336	794	454	176	734	253
	Per cent 4th stage infection.	0·5	0·4	2·2	0·9	0·3	2·6	1·5	5·0	1·5	0	0·4	0·4
	Per cent total infection.	12·1	7·5	8·0	15·0	9·3	6·0	12·2	10·4	8·0	10·8	7·9	5·9
Surat B.M. (Sept. 1956 to Jan. 1957)*	Number dissected.	..	301	307	..	421	379	94	196	235	152	262	147
	Per cent 4th stage infection.	..	2·3	3·9	..	6·8	0·8	1·0	6·1	0·8	4·6	3·0	0·6
	Per cent total infection.	..	10·2	9·4	..	14·7	7·7	40·4	14·2	8·0	33·5	12·5	8·8
Kozhikode (Sept. 1956 to Feb. 1957)*	Number dissected.	..	257	438	..	291	284	..	564	967	..	536	388
	Per cent 4th stage infection.	..	1·2	0	..	2·5	0·9	..	0·5	4·2	..	0·1	0·7
	Per cent total infection.	..	5·0	21·2	..	4·4	11·4	..	0·3	8·6	..	4·1	9·0
Ballia (June 1956 to Aug. 1957)*	Number dissected.	60	892	546	41	1342	866	98	1133	780	58	1016	528
	Per cent 4th stage infection.	..	1·1	1·1	8	1·6	9·9	0	2·3	0·7	0	2·2	0·9
	Per cent total infection.	3·3	3·2	2·3	0	2·4	1·4	0	3·5	1·9	0	4·2	2·4

*Period of Mass Therapy Programme.

TABLE XII. (*Contd*).

Unit.	Details.	December			January			February			March		
		1956	1957	1958	1956	1957	1958	1956	1957	1958	1956	1957	1958
Patna (Sept. 1958 to Feb. 1959)*	Number dissected.	80	285	361	224	568	287	348	557	321	..	114	297
	Per cent 4th stage infection.	1·2	0	0	0	0	0	0	0	0·6	..	2·4	0·3
	Per cent total infection.	10·0	3·5	17·7	7·5	5·6	11·1	8·9	5·7	8·7	..	10·3	0·5
Muzaffarpur (Oct. 1957 to Feb. 1958)*	Number dissected.	304	537	305	390	585	333	170	480	264	297	328	..
	Per cent 4th stage infection.	0	2·9	0	0	0·8	0	0	0·8	0·4	0	2·4	..
	Per cent total infection.	12·5	8·3	4·2	15·4	7·3	4·2	14·1	5·6	4·9	17·8	1·8	..
Gaya (Sept. 1958 to Feb. 1959)*	Number dissected.	..	678	807	..	684	739	..	680	651	301	274	675
	Per cent 4th stage infection.	..	0	1·1	..	0	0·3	..	0	0·6	0	0	0·3
	Per cent total infection.	..	0	24·3	..	0	21·0	..	0·9	17·5	3·9	2·2	9·4
Nagpur (Feb. 1957 to Sept. 1957)*	Number dissected.	192	334	503	296	656	288	916	448	222	2246	404	245
	Per cent 4th stage infection.	0·5	2·1	0	0	0	0	0·4	0	0·4	0·1	0·2	0·8
	Per cent total infection	8·2	5·9	3·5	10·0	7·0	4·5	5·3	8·2	6·3	3·8	6·9	6·5
Surat B. M. (Sept. 1956 to Jan. 1957)*	Number dissected.	180	246	282	216	213	341	123	211	303	220	183	147
	Per cent 4th stage infection.	1·1	1·2	1·7	0·5	0	0·6	1·6	0	2·6	0·9	1·1	2·0
	Per cent total infection.	15·5	8·9	9·2	14·8	7·6	7·6	21·1	2·8	8·6	14·5	6·9	9·5
Kozhikode (Sept. 1956 to Feb. 1957)*	Number dissected.	..	370	470	..	..	566	..	239	340	..	360	466
	Per cent 4th stage infection.	..	0·2	0·4	..	..	0	..	0·4	0·3	..	0·5	0
	Per cent total infection.	..	4·3	7·8	..	..	6·2	..	5·4	2·6	..	5·0	5·2
Ballia (June 1956 to Aug. 1957)*	Number dissected.	107	625	718	720	558	743	486	599	522	463	646	774
	Per cent 4th stage infection.	0	0·5	0·2	0	0·1	0	0·2	0·1	0·9	0	2·4	0·2
	Per cent total infection.	0·7	0·7	1·1	0·6	0·7	1·1	0·6	2·1	2·4	0·2	2·7	2·2

*Period of Mass Therapy Programme.

DISCUSSION.

The National Filaria Control Programme has been in operation since 1955-56. The allotment of the 46 units, currently functioning in the participating States, was staggered during the first three years, 1955-58. Due to inevitable delays in commencing the operations following recruitment and training, the different units have been functioning for periods ranging from one to three years. The follow-up data to the extent available have been analysed.

In a programme like the National Filaria Control Programme which is essentially a large scale experiment, an absolute and urgent need exists for proper execution of the schedule of activities and collection of data as required under the plan. This is highly essential as the results of the present programme have to form the basis for future planning and expansion of the programme. The staff pattern recommended and the reporting procedure laid down have duly taken the above mentioned requisites into consideration.

Appendix IV-*A* shows the position regarding the staffing of the various units. It is noticed that a number of units have been understaffed ; in many cases there has been no Research Officer or Entomologist at all at any time on the units' strength. There are a few instances where the Filaria Officer has been absent for different periods. In view of the great difficulty experienced by the participating States in getting medical graduates, and also the demand for this category of personnel on various other projects, the feasibility of appointing non-medical biologists as Research Officers on the Filaria units appears worthy of consideration. These non-medical biologists should be adequately trained to help the Filaria Officer in the organisation and execution of the different lines of activities of the control operations and also plan and carry out investigations related to the problems in the field. The importance of having the full complement of staff has been stressed repeatedly both by the visiting officers of the Institute and through the Ministry of Health.

Reporting under the National Filaria Control Programme has been planned so as to obtain all possible information on the field observations, to enable a proper appraisal of the achievements. The proformae have been altered whenever considered necessary and suggestions from the State Health Department have been asked for and received on the subject from time to time. A number of returns have never been submitted by some units and a list thereof is given in Appendix V. Incomplete returns have been received in many instances and have been intimated to the States/Units concerned from time to time.

As mentioned earlier, the object of the present phase of the National Filaria Control Programme is to study the efficacy of the known methods of control. The planning has envisaged a two pronged drive with measures directed against both the vector mosquito and the parasite. Mosquito control operations have to be the main pillar of the programme, and the mass drug administration is expected to exert an adjuvant effect by reducing the reservoir of the microfilariae in the

community and thereby reducing the quantum of transmission. The reduction in the seeds of transmission, followed by anti-mosquito measures maintained unremittingly over a number of years, should normally be expected to result in effective interception of transmission.

The mass drug administration has yielded gratifying results, producing varying degree of reduction in the microfilaria rate as well as the mean microfilarial density in the community. Though there are a few exceptions, there appears to be a general direct correlation between the percentages of population treated during the mass drug administration and the reduction in the microfilaria rate in the different units. The decline in the mean microfilaria counts in the community does not show any such correlation. The reduction in mean mf. counts at the end of one year, following drug administration, ranges from 10·9 per cent to 84·6 per cent. The data regarding those units which recorded a decline of more than 50 per cent in the count, are tabulated below :

Unit.	Percentage of population covered.	Per cent reduction in mean mf. count.
Khurda	32·1	64·6
Ballia	69·4	72·1
Muzaffarpur	76·3	84·6

It is observed that in some units which have reported a high coverage of population during the mass drug administration, the effect is not proportionately reflected in the follow-up surveys as detailed below :

Unit.	Per cent of population covered.	Change in mean mf. count.
Nagpur (rural)	56·4	Increase more than 10 times.
Dharbanga	63·8	Increase 3·9 per cent.
Faizabad	66·4	Decrease 10·9 per cent.
Gaya	67·9	Increase 23·0 per cent.
Monghyr	73·4	Decrease 39 per cent.
Barabanki	82·6	·do· 49·9 per cent.
Chatrapur (Orissa)	99·5	Increase 35·3 per cent.

The purpose of the mass drug administration is to bring about a reduction in the microfilaria load in the community with a view to reducing the reservoir of the infection. While the low degree of reduction in some of the units is disturbing, the positive increase, which is very marked in some of the units, is disconcerting and inexplicable. Can such results be due to migration of people to and from the areas—and sampling errors ? Or is it that there are strains of human infections in these areas which are relatively more tolerant to the effect of Diethylcarbamazine ? Before considering these possibilities, one has to investigate into the procedure adopted for the drug administration, and whether the drug was actually swallowed by the proportion of population reported to have been covered. Enquiries have revealed that a certain percentage of the tablets were handed over to be consumed

later in the day, after meals, but details are not available regarding the extent of such handing-overs, nor have there been any record of later verification that the drug so given had been actually consumed.

The occurrence of reactions, following drug administration, has been considered to be a result of mass destruction of the microfilariae in the system. One of the observations supporting such a hypothesis would be the high incidence of reactions, some of them severe and referable to the lymphatic system among the apparently healthy and normal people who are often presumed to be symptomless carriers. The incidence of the reaction in a community would, in that case, be in direct proportion to the microfilaria rate in the community as revealed by sample surveys. No such correlation, however, is found to be present (Table VIII). There appear to be other cause(s) for the occurrence of the reactions. Factors such as biochemistry of the blood, the presence of other helminthic or other infections and the possibility of individual idiosyncracy may have to be investigated with a view to evolving suitable measures for reducing or eliminating the reactions.

The total infection rates in the mosquitoes should normally be expected to register a fall following mass drug administration, due to the reduction in microfilarial load in the community resulting from the drug. A comparison of the infection rates in mosquitoes (Table XII) during the months following mass drug administration, with corresponding data for the previous years, shows that a fall is noticeable in the Muzzaffarpur, Nagpur (rural) and the Surat units.

The mass therapy programme was completed only in February, 1959, under the units of Patna West and Gaya. The mosquito infection rates during the months, following mass drug administration, in the units at Ballia and Kozhikode do not show any significant variation from the earlier indices and do not warrant any conclusions.

The present methods of assessment include annual epidemiological surveys as also weekly entomological observations in the index areas. The former have included recording of the microfilarial infection and filarial disease rates as also average microfilarial counts as determined by a sample survey. While the utility of sample surveys for measuring the filariousness of an area cannot be disputed, such surveys would appear to be of limited value for assessment of filaria control measures. Horizontal survey, covering a representative sample of the population, every year is liable to sampling errors which might make assessment difficult and the data unreliable. Such has been found to be the case in many instances in the present programme, as has been discussed earlier in this review. It is considered desirable to have a vertical examination of a select group of the population, examining the same individuals year after year. Restricting the surveys to a few institutions (schools, colleges, especially residential institutions) will help such vertical examination. Inclusion of adequate number of children of age-groups below 10 years, will considerably aid appraisal of the degree of interception of filaria transmission.

The clinical enquiries have not lent themselves to any analysis or conclusions. This type of investigation needs specially trained staff and regular house-to-house visits. It is considered that clinical enquiries can be suspended for the present and commenced only when a substantial reduction in the transmission is achieved.

The entomological assessment, as planned at present, should continue. The infection and infectivity rate as also the density of infection in the mosquitoes can be the main criteria for assessing the control measures. Meticulous care should, therefore, be exercised in collecting and recording the data.

The organisation of larval control operations under the National Filaria Control Programme has posed an important problem and needs particular consideration. Due to wide variation in the terrain and breeding conditions, it has not been possible to standardise the work load for the anti-larval crew or the extent of breeding surface per capita or to specify the requirements for anti-larval operations of a specific population even in urban areas. Though a pattern of staff has been suggested in the working notes, the States have been modifying the same to suit the extent of breeding areas to be treated. Generally it has been noted that sufficient personnel for anti-larval measures and supervision are not employed. The difficulty in getting a correct magnitude of the problem can be understood when one notes that with a population of nearly 2·0 lakhs in Surat (Bombay State) the extent of breeding surfaces is stated to be about 50 acres and about 30 mazdoors are employed; for a similar population in Patna (Bihar State) the breeding area extends over a few hundreds of acres and over 200 mazdoors are required. A common handicap noted is that no anti-larval operations are carried out beyond the boundaries of municipal or local bodies and prolific infiltration of mosquitoes occurs from uncontrolled areas in the neighbourhood.

THE FUTURE TRENDS IN THE FILARIA CONTROL PROGRAMME.

(1) The need for the maintenance of proper environmental sanitation for reducing the breeding of culicine mosquitoes cannot be over-emphasised. Provision of drainage for disposal of waste water will have to be given high priority as a measure for reducing transmission of filariasis. The ultimate answer for the control of the *C. fatigans* transmitted *W. bancrofti* infection appears to be provision of proper drainage of waste water. This is a long term project and involves heavy expenditure. Recurrent mosquito control operations can be adopted only as interim measures.

(2) *Mosquito control operations.*—Experience so far has indicated that none of the residual insecticides used so far in the programme have been able to give result in successful interception of transmission.

The efficacy of pyrethrum as a space spray is currently under evaluation. Preliminary results, following weekly and fortnightly application of this spray, indicate that the former is likely to prove suitable for controlling the transmission of *W. bancrofti* infection. Large scale use of pyrethrum under the programme

will, however, pose questions like (*a*) procurement of pyrethrum and/or synthesis of the pyrethrum, (*b*) a suitable formulation for use in rural areas as use of kerosene spray is beset with risk of fire and (*c*) a suitable type of manually operated equipment for space spraying.

The anti-larval operations also will have been intensified. No data are available on organisation of anti-larval work in rural areas as no large scale larval control operations have so far been attempted in rural areas in this country hitherto. It has, therefore, been decided to recommend to the units that larval control operations should be extended to rural areas. This extension has, however, to be restricted to certain selected areas only and worked out on a planned experimental basis. It is expected that such experiments will give us the necessary information, the staff pattern and the feasibility of larval control operations in strictly rural areas in India.

(3) Intensification of anti-larval measures will necessarily need provision of requisite field staff. The provision made for indoor insecticide spraying operations can be used to meet the requirements for anti-larval operations. Experience in the field has shown that special attention has to be paid in making necessary provision for drain cleaners whose duty it will be to clear and maintain the drains and other breeding places and to prepare them for the application of oil.

SUMMARY AND CONCLUSIONS.

A critical appraisal of the progress of the National Filaria Control Programme in India, which was commenced in the year 1955-56, is presented for the period 1955-59.

Forty-six control units and 22 survey units were allotted under the programme. The former allotment was distributed during the first 3 years of operation while the latter was allotted during the first year.

Indoor residual spraying was the exclusive mosquito control measure adopted in the rural areas. In the urban areas, year-round anti-larval measures were carried out with one round of indoor spraying. Dieldrin at 50 mg./sq. ft. was used for adult control while BHC was used as a larvicide. The larvicide was changed to oil during 1958-59.

Mass drug administration with Diethylcarbamazine was also used as an anti-parasitic measure. The drug was given at a dose of 4 mg./kilo once daily for 5 days.

Mass drug administration with Diethylcarbamazine has been completed by 25 units so far—covering a population of about 7·5 millions. The percentage of population, given the drug, varied from 31·9 to 99·5 per cent, covering a total of 42·01 lakh persons, i.e., about 56·0 per cent of the population targeted under these 25 units. Follow-up surveys have revealed a decline in the microfilaria rates as also in the mean microfilaria counts in some of the units.

The survey units have ceased to operate in most of the areas except in the States of Andhra Pradesh, Kerala, Madhya Pradesh and Uttar Pradesh. The surveys so far carried out under the programme reveal that about 40 million people in India live in the filarious areas in the country.

Dieldrin has not been found effective in intercepting transmission. The residual effect was found to last for very short periods and ranged from 2 to 4 weeks at best. High vector densities and high survival rates led to discontinuance of this insecticide under the programme. Formulations of BHC with high gamma content are currently being evaluated in the field for use in the programme.

Larva control operations appear to be the answer for control of filariasis due to *W. bancrofti*. The operations, which have so far been limited to urban areas, are now being extended as an experimental measure to rural areas under some units.

The units have, in many instances, not had the full complement of the staff recommended under the programme.

ACKNOWLEDGEMENT.

The compilation of the Review of the activities under the National Filaria Control Programme over a period of 4 years would not have been possible but for the keen interest of the authorities of the State Health Departments in organising the programme in the participating States. Thanks are due to the unit officers for their active co-operation in the execution of the work, and for collection and submission of the data presented in this review.

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APPENDIX I.

National Filaria Control Programme in India.

Statement showing the year-wise Central and the State Governments expenditure

(Figures are Rupees in lakhs.)

Year.	U.S.T.C.M.	Govt. of India.	State Governments expenditure.
1955-56	14·48	0·83	4·75
1956-57	32·38	15·48	9·48
1957-58	64·38	43·09	16·45
1958-59	0·18	29·16	29·14
1959-60	0·61	31·09	32·08
Total	112·03	119·65	91·85

APPENDIX II.

Central Organization of the National Filaria Control Programme in India.

Deputy Director	... 1	
Assistant Directors	... 4	(3 medical, 1 entomologist)
Deputy Assistant Director	... 1	
Research Officers	... 4	
Assistant Entomologist	... 1	
Technicians	... 3	
Laboratory Assistant	...	
Insect Collector	...	
Laboratory Attendant	...	

APPENDIX III.

Recommended staff pattern for the field units of the National Filaria Control Programme in India working in different States.

(a) *Control Unit :*

Filaria Officer	...	1
Research Officer	...	1
Entomological Assistant	...	1
Inspectors	...	2
Stenographer	...	1
Upper Division Clerk	...	1
Insect Collectors	...	4
Drivers-cum-Cleaners	...	2
Watchman	...	1

(b) *Survey Unit :*

Filaria Officer	...	1
Laboratory Officer	...	1
Insect Collector	...	1
Laboratory Attendants	...	2
Driver-cum-Mechanic	...	1
Watchman.	...	1

Appendix IV.

Supplies through Central/T.C.M. and to the field units.

CONTROL UNIT :

(i) Newly established :

Station wagon	...	1
Jeep with trailor	...	1
Microscope	...	1
Hand compression sprayers	...	32
Stirrup pumps	...	25
Insecticides	...	6 tons
Larvicidal oil	...	30,000 gallons
Diethylcarbamazine (100 mg. tablets)		1·95 million

(ii) Continuing unit :

Insecticide	...	12 tons
Larvicidal	...	30,000 gallons.

APPENDIX IV-A.

Status of vacant posts at the National Filaria Control Programme units functioning in the various participating States.

State/Unit.	Year of allotment.	Filaria Officer.	Research Officer.	Assistant Entomologist.	Inspector.	Remarks.
Andhra						
Kamareddy	1955–56	P	V	V	P (6 posts, all P)	Post sanctioned on April 27, 1959. Not filled for want of personnel.
Mandapetta	1955–56	P	P	P	P (2 posts both P)	
Assam						
Survey-cum-demonstration unit	1957–58	V (since inception)	P (Entomologist-cum-Research) Officer	V	V (since inception i.e. March 4, 1959)	No post sanctioned by Govt.
Bihar						
Patna East	1955–56	P	P	V (February 1959)	P	
Muzaffarpur	1955–56	P	P	P	P	
Ranchi	1956–57	P	V (February, 1959)	P	P	
Gaya	1956–57	P	P	P	P	
Patna West	1957–58	P	P	P	P	
Monghyr	1957–58	P	P	P	P	
Dharbhanga	1957–58	P	P	P	P	
Bhagalpur	1957–58	P	P	P	P	
Bombay						
Surat Municipality	1955–56	V (since inception)	V (since inception)	P	P8	
Nagpur (Rural) 1-1/3	1955–56	V (since Aug., 1958)	P (since inception)	V	P (one vacant)	
Junagadh	1955–56	P	V (since inception)	P	P	
Jamnagar	1956–57	P	V (since inception)	P	P	The Distt. Health Officer is holding additional charge of this unit. There is no full-time Filaria Officer.
Chanda	1957–58	P	V (since inception)	(since inception)	P	Unit established in May, 1958.
Nagpur Corp. 12/3	1957–58	P	P	P	P	
Surat Thana	1957–58	P	V (since inception)		P	Unit established in June, 1959.

P = Present. V = Vacant.

APPENDIX IV-A (*Concld.*)

State/Unit.	Year of allotment.	Filaria Officer.	Research Officer.	Assistant Entomologist.	Inspector.	Remarks.
Kerala						
Trivandrum	1955–56	P	P	V	P4	No post of Research Officer.
Kozhikode	1955–56	V	P	V	P4	
Quilon	1956–57	P	P	V	P4	
Ernakulam	1956–57	P	P	V	P4	
Alleppey ½ unit	1957–58	P	V	P	P2	
Tirur	1957–58	P	V	V	P4	
Cannanore	1957–58	P	V	P	P4	
Madhya Pradesh						
Chattarpur	1955–56	P	V (since inception)	V (since inception)	P	
Madras						
Chingleput	1956–57	P	P	V (since April 9, '59)	P	
Kumbakonam	1956–57	V (since May 9, 1959)	V (since May 9, 1959)	V (since April 11, '59)	P	
Vellore	1957–58	P	V (since inception)	V (since April 7, '59)	P	
Chidambaram	1957–58	P	V (since inception)	V (since inception)	P	
Orissa						
Khurda	1955–56	P	V (June, 1958)	V (March, 1955)	P	
Cuttack	1956–57	P	V (October, 1958)	V (August, 1958)	P	
Ranpur	1956–57	P	V (May, 1958)	V (March, 1957)	P	
Chattarpur	1956–57	P	V (March, 1957)	V (March, 1957)	P	
Puri	1956–57	P	V (April, 1957)	V (March, 1957)	P	
Uttar Pradesh						
Ballia	1955–56	P	P	P	P	
Basti	1956–57	P	P	P	P	
Barabanki	1956–57	P	V (since Apr. 5, 1959)	P	P	
Gorakhpur	1957–58	P	P	P	P	
Varanasi	1957–58	P	P	P	P	
Faizabad	1957–58	P	P	P	P	
Bahraich	1957–58	Units were reported to have been established since June 1, 1959.				
Jaunpur	1957–58					

P = Present. V = Vacant.

APPENDIX V.

Statement showing the delinquency in reporting from units of the National Filaria Control Programme.

1. **24 Hours Survival Report** (Form number C.A.F. 1)

ANDHRA PRADESH	:	1. Kamareddy	2. Mandapetta.					
BIHAR STATE	:	1. Gaya	2. Patna West.					
MADHYA PRADESH	:	1. Chattarapur						
ORISSA	:	1. Khurda	2. Puri	3. Ranpur	4. Chatrapur.			
MADRAS	:	1. Kumbakonam	2. Chingleput	3. Vellore	4. Chidambaram.			
KERALA	:	From none.						
UTTAR PRADESH	:	From none.						
WEST BENGAL	:	Contai.						

2. **Larval collection Report** (Form number E.L. 2)

ANDHRA PRADESH	:	1. Kamareddy.						
MADRAS	:	From none.						
KERALA	:	1. Ernakulam	2. Tirpur	3. Alleppy	4. Cannanore.			
UTTAR PRADESH	:	1. Bahraice	2. Jaunpur.					
WEST BENGAL	:	Contai.						

3. **Mateorological Reports** (Form number G. 10)

ANDHRA PRADESH	:	1. Kamareddy.	
BIHAR	:	1. Muzaffarpur	2. Patna West.
BOMBAY	:	1. Jamnagar	2. Chanda.
MADRAS	:	From none.	
KERALA	:	From none except Trivandrum.	
UTTAR PRADESH	:	From none.	
WEST BENGAL	:	Contai.	
ANDAMAN	:		

4. **Persons treated in Public Health Dispensaries** (Form number G. 5)

ANDHRA PRADESH	:	Both the units.
BIHAR	:	All the units except Bhagalpur.
BOMBAY	:	Chanda (Lul).
ORISSA	:	Khurda and Puri.
KERALA	:	All the units.
UTTAR PRADESH	:	Except Faizabad and Gorakhpur.
MYSORE	:	Mangalore.

5. **Clinical Enquiries Reports** (C.L.E. 2)

ANDHRA PRADESH	:	Kamareddy.	
BIHAR	:	Patna West.	
ANDAMAN	:	Nancowry.	
MADRAS	:	1. Kumbakonam	2. Vellore.
WEST BENGAL	:	Contai.	

6. **Larval Control Reports** (C.L. 4)

ANDHRA	:	Mandapetta.
BIHAR	:	All units except Patna West.
ORISSA	:	Khuraoda, Puri, Ranpur and Chatrapur.
MADRAS	:	All units except Kumbakonam.
KERALA	:	All units except Kumbakonam.
UTTAR PRADESH	:	All units..
WEST BENGAL	:	Contai.

7. **Entomological Reports** (Form number E.A. 3 and E.A. 7)

BIHAR	Patna West.

Indian Journal of Malariology, 14, 4, December 1960.

MASS THERAPY WITH DIETHYLCARBAMAZINE CARRIED OUT IN MANGALORE TOWN UNDER THE NATIONAL FILARIA CONTROL PROGRAMME, INDIA.

BY

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[March 21, 1960.]

INTRODUCTION.

MANGALORE Town (Map I), the headquarters of South Kanara District in Mysore State, has a population of 1,17,095 (1951 census) and an area of 8 square miles. The town is divided into 25 wards for administrative convenience.

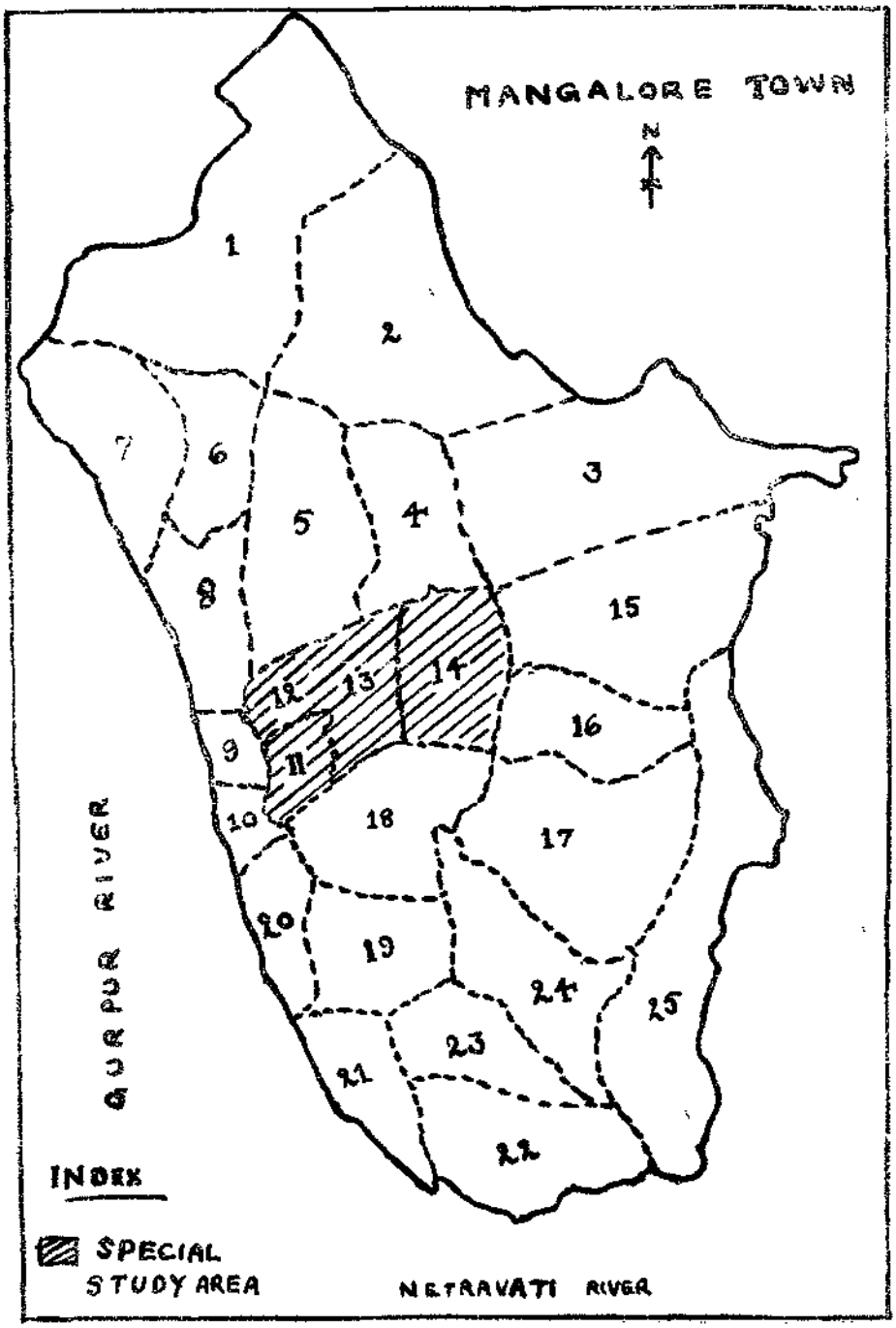
A filaria survey of the town was carried out during March—April, 1954 (Krishnaswami, 1955) and the results of that survey are shown in Table I. Since the town was not surveyed immediately before the start of control operations, the only pre-control data available for evaluating the results are those of the filaria survey referred to above.

TABLE I.

Mangalore Town—Filariasis survey (March—April, 1954). Infection, disease and endemicity rate per cent (Ward-wise.).

Ward number.	Population.	Number of houses surveyed.	Disease rate.	Infection rate.	Endemicity rate.
1	7,285	1,315	6·8	16·5	23·0
2	4,962	1,050	6·8	17·0	23·8
3	9,094	1,768	5·3	11·1	16·2
4	3,284	558	4·7	12·7	17·4
5	5,784	997	17·0	10·6	27·0
6	3,846	716	8·6	13·4	22·0
7	4,542	990	8·4	17·6	26·0
8	7,703	1,165	7·3	19·3	23·4
9	1,976	424	16·2	19·6	38·6
10	2,119	311	11·6	16·9	27·7
11	4,445	778	13·0	20·7	33·7
12	2,404	573	15·7	16·4	35·7
13	4,098	736	10·8	16·5	26·7
14	2,688	579	7·7	9·2	16·5
15	5,441	943	5·5	10·5	16·0
16	2,340	650	7·2	6·7	15·4
17	6,617	1,042	16·8	21·0	37·5
18	2,887	576	6·9	12·9	19·8
19	3,356	621	9·0	12·9	21·9
20	2,843	608	11·1	16·3	26·1
21	3,907	698	14·2	16·3	27·8
22	6,567	778	19·0	17·3	29·3
23	4,607	878	10·4	15·0	24·9
24	5,124	950	9·1	17·8	26·9
25	7,610	1,128	7·4	12·0	19·4

MAP 1.
Mangalore Town divided into 25 wards.



MASS THERAPY WITH DIETHYLCARBAMAZINE.

The mass therapy campaign was commenced on September 1, 1956 and concluded at the end of February, 1957. Of the enumerated population (1,23,183), 69·1 per cent received treatment for one or more days. The drug was administered in a single daily dose for five days. The daily dosage of diethylcarbamazine was according to age* as laid down in the working notes for the National Filaria Control Programme in India.

Table II shows the percentage of the population treated for one or more days under the mass drug administration programme.

TABLE II.

Percentage of population (age-group wise) who took diethylcarbamazine.

Age-group.	Five days.	Four days.	Three days.	Two days.	One day.	Total.
I (18 years and above)	57·3	4·1	1·7	1·4	2·0	67·0
II (12—18 years)	69·9	3·9	1·9	1·6	2·1	79·4
III (6—12 years)	55·4	5·7	3·0	2·1	2·0	69·1
IV (2— 6 years)	54·3	4·2	2·1	1·6	2·6	64·3
Average	58·8	4·1	2·1	1·7	2·4	69·1

ASSESSMENT OF RESULTS OF MASS THERAPY.

Observations for assessment of control operations under the National Filaria Control Programme are confined to a specially selected portion (10 per cent of the total population of the area), called the special study area. In Mangalore Town, the special study area covered wards 11 to 14 with a total population of 13,635 (1951 census). The filariasis surveys referred to hereafter, were carried out in this special study area. Three annual surveys have so far been carried out from 1957 to 1959. The purpose of these surveys was, essentially, to assess the results of mass therapy and anti-mosquito measures undertaken in the unit area.

Night surveys were carried out during the months April—May—June in the special study area. The data collected during the 3 years of follow-up are presented in Table III.

It will be seen that the three filaria surveys carried out in the special study area, have certain important points of difference. Out of the 1,364 persons examined in 1957 only 644 persons could be re-examined in 1958 ; and again 539 could be re-examined in 1959.

It is noted that 363 persons out of the 1,364 persons examined during 1957, were repeatedly examined during 1958 as well as during 1959 surveys. The results of examination of these individuals in each of the surveys are analysed in Table IV.

*AGE-GROUP	DOSAGE OF DIETHYLCARBAMAZINE
I (18 years and above)	200 mg. daily
II (12—18 years)	150 mg. daily
III (6—12 years)	100 mg. daily
IV (2— 6 years)	50 mg. daily

TABLE III.

Microfilaria rates among the treated and untreated persons examined during the follow-up surveys.

Year of surveys.	Treated group with number of days of drug administration: 1 day: Number examined.	Number positive.	Per cent positive.	2 days: Number examined.	Number positive.	Per cent positive.	3 days: Number examined.	Number positive.	Per cent positive.	4 days: Number examined.	Number positive.	Per cent positive.	5 days: Number examined.	Number positive.	Per cent positive.	Total treated: Number examined.	Number positive.	Per cent positive.	Untreated group: Number examined.	Number positive.	Per cent positive.
1954 ...	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1008	160	16·0
1957 ...	20	4	20	9	0	..	19	2	10·5	35	3	8·6	972	73	7·5	1055	82	7·8	309	45	14·8
1958 ...	10	1	10	3	0	..	8	0	..	15	1	6·6	473	47	10·0	509	49	9·6	135	20	14·7
1959 ...	8	4	50	6	0	..	8	1	12·5	20	2	10·0	392	39	10·0	434	46	10·6	105	20	19·0

TABLE IV.

Analysis of persons examined repeatedly during all the follow-up surveys.

Year of surveys.	Treated group with number of days of drug administration: 1 day: Number examined.	Number positive.	Per cent positive.	2 days: Number examined.	Number positive.	Per cent positive.	3 days: Number examined.	Number positive.	Per cent positive.	4 days: Number examined.	Number positive.	Per cent positive.	5 days: Number examined.	Number positive.	Per cent positive.	Total treated: Number examined.	Number positive.	Per cent positive.	Untreated group: Number examined.	Number positive.	Per cent positive.
1957 ...	7	1	14·3	1	0	..	5	1	20	12	1	8·3	267	17	6·4	292	20	6·8	71	10	14·1
1958 ...	7	1	14·3	1	0	..	5	1	20	12	1	8·3	267	25	9·4	292	28	9·6	71	9	12·7
1959 ..	7	2	28·6	1	0	..	5	1	20	12	1	8·3	267	27	10·1	292	31	10·6	71	14	19·7

AVERAGE INFESTATION.

The microfilarial counts in the positive persons were also recorded during the above surveys. The numbers of microfilariae per positive smear during the follow-up surveys, as compared to the 1954 surveys, are given below :

Year.	*Average microfilaria count per positive smear.*
1954	48·9
1957	12·1
1958	8·6
1959	18·0

DISCUSSION.

In evaluating the effect of the mass drug administration campaign, the following indices have been utilised :—

(*a*) Microfilaria rates,

(*b*) Average microfilarial infestation.

The above indices have been collected from both the treated and untreated groups of residents in the special study areas. For comparison with the pre-therapy period, data collected during 1954 have been taken as the base line. The infection rate among the untreated persons during 1957 and 1958 has remained about 15 per cent, almost at the same level, compared to 16 per cent recorded during the 1954 survey (Krishnaswami, 1955 *loc. cit.*).

The general microfilaria rates in the special study area, recorded during the different surveys, are given in Table V.

TABLE V.

Infection rates (per cent) in the special study area for pre- and post-therapy period.

Survey.		Year.	Microfilaria rate.
Pre-therapy	...	1954	16·0
First follow-up	...	1957	8·8
Second follow-up	...	1958	10·8
Third follow-up	...	1959	12·2

An appreciable fall in the microfilaria rate is noticed during the first follow-up survey (1957) immediately after the completion of the mass therapy campaign. A gradual rise is noticed during the following two years, but the microfilaria rate has remained below the pre-therapy level even at the end of two years following mass therapy.

The effect of the drug administration appears to be more pronounced on the average microfilaria infestation. The average microfilaria count in the positive persons, which was recorded to be 48·9 in 1954 (Krishnaswami, 1955 *loc. cit.*), dropped to 12·1 (by more than 75 per cent) during the first follow-up survey in 1957 and rose to 18 microfilariae per positive smear in 1959.

The effect of mass drug administration in the filaria control programme is well brought out by the observations recorded above. A reduction in the reservoir of infection in the community is noted by a decline in the number of infected persons, as also a fall in microfilaria levels in the positive persons (Table VI).

TABLE VI.

Microfilaria rate (per cent), average microfilaria count per positive smear and microfilaria density in the community.

Year.	Treated persons (treated for 5 days):			Untreated persons:		
	Mf. rate.	Average Mf. count per positive smear.	Mf.* density in the community.	Mf. rate.	Average Mf. count per positive smear.	Mf. density in community.
1954	16·0	48·9	782·4	16·0	48·9	782·4
1957	7·5	8·5	63·75	11·3	21·3	240·69
1958	9·87	12·4	122·4	14·8	9·7	133·56
1959	9·9	22·2	219·8	19·04	18·2	346·53

*After Kessel (1957).

The infection rate has remained low in the treated group as compared to the pre-therapy survey (1954) whereas in the untreated group the infection rate has increased from 16 per cent to 19 per cent in the course of the five years. The average infestation has registered a fall in both the treated and untreated groups. The fall in microfilarial infestation in the untreated group is almost to the same degree as in the treated group which is inexplicable ; but the reservoir of infection, as assessed by the "microfilarial density of the community" (Kessel, 1957), is higher in the untreated group as compared to the treated group.

SUMMARY.

A review of mass therapy campaign under the National Filaria Control Programme in Mangalore Town is presented. Data collected in the follow-up surveys in the special study area over two years, following the mass therapy, have been analysed and presented.

A fall in the infection rate and average microfilarial infestation was noted in the community after mass therapy with diethylcarbamazine. A gradual rise was, however, recorded in these indices during the subsequent two years of follow-up.

Though a decline was noticed in the average microfilarial infestation even among the untreated persons examined, the "microfilarial density" in the untreated community was higher than that in the treated community.

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INTERNATIONAL ORGANIZATIONS AND FILARIASIS CONTROL.

(*WUCHERERIA BANCROFTI* AND *BRUGIA MALAYI*).

BY

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[April 4, 1960.]

For the purpose of this discussion, an international organization is defined as an agency to improve the health of inhabitants in other countries than "the home" country of the organization. Filariasis control is defined as : (1) the utilization of the available techniques to determine the distribution of clinical filariasis and of *Wuchereria bancrofti* infection among the human and mosquito populations ; (2) the application of the known measures to prevent transmission and the development of clinical filariasis ; and (3) the employment of accepted procedures to evaluate the effectiveness of control measures, as recommended by the South Pacific Conference in Tahiti, 1951 (Beye, 1953).

From the days of Sir Patrick Manson until the mid-nineteen forties, knowledge concerning these two parasites was obtained either through individuals or teams working as members of a Commission, such as the British Filariasis Commission in British Guiana, or projects sponsored by one of the Schools of Tropical Medicine, or by workers assigned to one of the semi-governmental institutes in endemic areas. Clinicians, surgeons, and laboratory investigators in the various health services also made valuable contribution.

During this period neither knowledge, organizational techniques, nor the possibility of a continuity of effort, was available for a serious consideration of filariasis control. It is of interest that, although Buxton (1927) deferred from recommending control measures in Polynesia or Melanesia, and wrote that the nature of the control problem was so difficult that time and effort could be better devoted to research, he also wrote that the disease could be prevented by clean-up of village areas. By this time Bahr (1912), O'Connor and Hulse (1932) and Buxton had implicated *Aedes polynesiensis* as a major vector and reported the short flight range for this vector.

Probably the first mosquito control program, aimed specifically against the transmission of these filarial parasites, was in Travancore and instigated upon the recommendations of Iyengar (1938). Following this, organized experimental

mosquito control programs against bancroftian filariasis were established in Fiji (1949). The Cook Islands (1949), St. Croix (1949) and American Samoa (1950).

Although those in Fiji and the Cook Islands were associated with the Pacific Health Service, and the project in American Samoa with the American Navy, none of these could be related to an international health organization.

The first effort of research and control, partially satisfying the international criteria, was probably the development in Tahiti. Here in September 1949, the Institute de Recherches Medicales de EFO, was established by décret (enactment) under the Ministre de la France d'Outre Mer. Active participation of this Institute with the Pacific Tropic Disease Foundation (at that time affiliated with the University of Southern California but later with the Department of Infectious Diseases, University of California at Los Angeles) made this association an international organization.

By this time, diethylcarbamazine had received trials by Hewitt *et al.* (1950), Maldonado *et al.* (1950), and as early as 1948 the Pacific Tropic Disease Foundation and the Service de Sante in Tahiti had started experimental programs with the ultimate objective of developing techniques for the application of control measures on that Island.

This original international effort in Tahiti between Mille, the first director of the Institute and Beye, the first field director of the Foundation, had now passed more than a decade of investigative activity. Kessel, as director of the Foundation, has provided continuity. Contributions by Galliard, Mille, Robinson, Beye, Kessel, Edgar, Rosen, Kerrest, Thooris, Bambridge, Bonnet, Hulse, Chapman, Tapu, March, and Leigert, reflect its history.

Besides the impact of diethylcarbamazine, increasing health interests in the so-called tropical areas stimulated the formation of filariasis pilot projects in other parts of the world such as India (Orissa), Ceylon, Malaya, Fiji, and West Africa.

The South Pacific Commission, and its Executive Officer for Health, Dr. Emil Massal, aware of the Tahiti developments and the importance of bancroftian filariasis in the South Pacific islands, acknowledged its official interest in 1951 by sponsoring and organizing a world-wide Conference of Specialists in filariasis and elephantiasis in Tahiti in 1951 (1953). In brief, the terms of reference of this Conference were : (1) to define the various problems of filariasis and elephantiasis ; (2) to determine the research work to be continued or undertaken ; and (3) to propose and recommend appropriate measures for the control of these diseases. Fifty-six recommendations were made. That year the South Pacific Commission authorized the appointment of Dr. J. Kerrest as specialist officer in mosquito-borne diseases. In November 1953, Dr. M.O.T. Iyengar was appointed to succeed Dr. Kerrest and has continued to hold the post up to the present. Using the 1951 recommendations

as a guide, the South Pacific Commission has been very active during these years, and a great many of the investigations and contributions of Iyengar, especially the annotated bibliographies, will be of value to filariasis workers for many years to come.

The South Pacific Commission held another Conference of Specialists in Filariasis in New Caledonia in November 1959. During this session each of the fifty-six recommendations were reviewed in terms of new information and developments. These are too detailed for discussion in the present paper, but will be published.

During this time interesting developments were taking place in India which led to the active interest in filariasis control of another international organization, the International Cooperation Administration.

In 1950, the Indian Council of Medical Research and the State of Orissa, in cooperation with Dr. Raghavan of the Malaria Institute of India, started a pilot project in Orissa to determine effective methods of filariasis control. The preliminary results were sufficiently promising and consequently in November, 1953, the Indian Council of Medical Research passed a resolution that survey and control units be set up in each State. This resolution, in turn, led to a joint agreement between the Government of India and the United States, signed, on March 25, 1955. This agreement provided that during the initial survey and experimental stage, 13 control and 22 survey units would be established in affected States, and that the Malaria Institute of India would be expanded to enable it to provide training facilities, consultation service, research facilities, and over-all direction for the program. There is little question that the jump from the 30,000 population in Tahiti to the 470 million in India is tremendous, but there is also little doubt that this large, imaginative effort is already beginning to show dividends in the way of contributions.

In addition to the financial association between the Government of India and the U.S.A., the I.C.A. provided technical assistance. Beye (1957) and Wright (1957), McGowan (1958) Burton (1959), and Contacos, have each spent various periods of time there in association with the program.

Although I.C.A is not directly engaged in filariasis research and control programs elsewhere in the world, they are interested in the filariasis problem. Surveys, as a filariasis consultant for I.C.A., have been made by Beye in Thailand (1957), Philippine Islands (1957) and Taiwan (1957).

The World Health Organization, likewise, has been following developments in filariasis within recent years, although they were not actively engaged in pilot projects or specific control programs.

The first World Health Assembly agreed that the group of parasitic diseases should be given number three priority and should include the following : ancylostomiasis, filariasis, leishmaniasis, schistosomiasis, and trypanosomiasis. The first

specific act by this organization in this field was a survey of filariasis in Thailand by Iyengar (1953) as a filariasis consultant for this organization. During this period, the World Health Organization consultants performed preliminary surveys also in Ceylon and the Maldive Islands.

In December 1955, a study group sponsored and organized by the World Health Organization met in Kuala Lumpur. (World Health Organization, 1957). Existing data and concepts were summarized. The most pertinent expression regarding control was: "Although the ultimate aim of control should be to eradicate filarial infection, the immediate aim will often be to reduce transmission of infection to a level where clinical manifestations cease to occur, and to prevent any subsequent recrudescence. Any marked and continued reduction in the reservoir of infection and in the intensity of transmission will probably be followed by a decrease in the clinical manifestations of the disease, even if the percentage of infected persons is little affected."

This was followed up in 1958 by a study tour of interested workers in South East Asia where filariasis is endemic, and technical discussions at the meeting of the World Health Organization Regional Committee for South East Asia in 1959.

In the year 1959, a discussion of *W. bancrofti* and *B. malayi* occupied a good proportion of a study group of onchocerciasis and the other filarial diseases, in Geneva. Increasing interest of the World Health Organization in filariasis in South America is reflected in a survey of the filariasis problems and control programs in British Guiana and Surinam by Beye (1959) as a World Health Organization consultant, and a recent field review in Brazil by Giaquinto, of the Central Office in Geneva.

Some of the Institutions for the investigations of tropical diseases, established around the turn of the century in Indo China, India, Tunisia, Indonesia and the Federated Malay States, have become more international in recent years.

The activities at the Kuala Lumpur Institute of Medical Research in filariasis research and control are of great significance. This Institute established in 1900 (1952) had been very active in unravelling the problems of *B. malayi* and the problems associated with control during recent years. Contributions by Wharton, Buckley, Wilson, Edeson, Turner, Jachowski, and others, emphasize the activities in progress. Table I lists major international organizations and their areas of interests.

In summary, one can state that there has been increasing interest of international organizations in the ultimate control or eradication of *W. bancrofti* and *B. malayi*. Although the future is more promising than in some other diseases, recent developments have re-emphasized the importance of basic knowledge of filariasis in nature. At the same time demonstrations in Tahiti, Surinam, and in certain areas of India, suggest that a considerable degree of *W. bancrofti* control is possible with present admittedly imperfect techniques. The importance of sound epidemiologic basic information for individual areas, before the instigation of control measures, has been demonstrated.

TABLE I.

Major International Health Agencies Associated with Filariasis Control. (W. bancrafti, B. malayi).

Agency.	Support.	Sponsor.	Area of Interest
Pacific Tropic Disease Foundation (Institute de Recherches Medicales).	FIDES*. Private subscription, U.S.P.H.S. Local government.	1. Institute de Recherches Medicales de E.F.O. 2. Department of Infectious Diseases, School of Medicine, UCLA.	1. French Oceania: Filariasis research, Filariasis control, Other public and research health programs.
South Pacific Commission.	Various governments with interest in South Pacific.	South Pacific Commission.	1. International study groups, Tahiti 1951, Noumea 1959. 2. Grant support 3. Permanent consultant in filariasis.
International Cooperation Administration.	U.S. and participating governments.	U.S. and participating governments.	1. Filariasis research and control, India. 2. Consultant interest in Thailand, Philippine Islands, India, Taiwan.
World Health Organization.	United Nations.	United Nations.	1. Consultant, Thailand, Ceylon, Maldive Islands, British Guiana, Surinam. 2. International study groups, Kaula Lumpur (1955), Geneva (1959).

*Fonds International pour le Developpement Economique Social.

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NON-PERIODIC BANCROFTIAN FILARIASIS.

BY

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INTRODUCTION.

AMONG the early European explorers and settlers who reported elephantiasis from Polynesia and other related islands of the Pacific are Lloyd (1949) from Tonga, Wilson (1799) from Tahiti, Vinson (1858) from New Caledonia, Messer (1876) from Fiji, and Koniger (1878) from Samoa. Ellis (1833) is of the opinion that elephantiasis occurred among the Polynesians from their "earliest antiquity" and Montgomery (1831) estimates that four per cent of the population of the Society Islands suffered from elephantiasis. Rosen (1954), however, makes an interesting observation concerning elephantiasis in the Marquesas Islands, which are some nine hundred miles north-east of Tahiti and which, until common communication was established between them and the Society Islands in the middle of the last century, were quite isolated and not regularly visited by other Polynesians. Rosen found 5 per cent of the population to have elephantiasis but pointed out that early European explorers did not observe elephantiasis in these islands even in the latter part of the last century. He is of the opinion that filariasis became common there only after recent communications were established. Neither is filariasis indigenous to New Zealand or the Hawaiian Islands to which islands Polynesians made early migrations. This probably resulted from the fact that suitable mosquito vectors were not present in these islands.

Although microfilariae were first described from hydrocele fluid by Demarquay (1863) in Paris and from blood in India by Lewis (1872) (*see* Manson-Bahr, 1959), it was not until after Manson (1894) requested blood films from elephantiasis cases in the Pacific Islands that Thorpe (1896) on October 3, first reported microfilariae from Tonga and Manson (1896) on November 7, in examining blood films collected in 1884 by Dr. Davis in Samoa, also reported microfilariae from the blood of persons in Polynesia. Because Manson was acquainted with the periodic type of filariasis, he assumed the microfilariae from Samoa to be nocturnal in type but Thorpe pointed out that the microfilariae from Tonga were present in the day-time as well as at night, thus first establishing the non-periodic type of filariasis. It is now well known that the nocturnal periodic type of Bancroftian filariasis occurs in Micronesia and most parts of Melanesia, while the non-periodic type is present in Fiji, New Caledonia, the Loyalty Islands and Polynesia. The two types are separated by Buxton's Line which begins at a point midway between the Gilbert Islands and the Ellice Islands and extends southward approximately along 175° E,

longitude to about 20°S. latitude, then swings westward between New Hebrides and New Caledonia-Loyalty Islands groups.

PERIODICITY.

As is well known, three types of periodicity occur among the Filarioidea, the diurnal periodic, found in human loiasis, the nocturnal periodic of Bancroftian filariasis found in most areas of the world, and the non-periodic Bancroftian filariasis found in the south-east regions of the Pacific. It is of further interest to note that Turner and Edeson (1957) have shown that Malayan filariasis, which was originally thought to exhibit nocturnal periodicity only as in Penang on the west coast of Malaya, has been found in Pahang on the east coast to demonstrate a "reduced periodicity", or continuous occurrence of microfilariae in the peripheral blood. In contrast to human loiasis, Duke (1955) reports that certain monkeys which live in the canopy of the rain forests in Africa, demonstrate a nocturnal periodic type. It has also been observed by Heish *et al.* (1959) that *B. patei* in Africa demonstrates a nocturnal periodicity at a focus along the Tana River on the mainland but exhibits no periodicity on the island of Pate. Such variations indicate that periodicity within a genus or species is by no means a rigid or permanent phenomenon.

Although the physiologic explanation of periodicity is not complete, Hawking *et al.* (1955) have shown in diurnal loiasis and in nocturnal periodic filariasis that the optimal sites for the microfilariae are the capillaries and small blood vessels of the lungs. During the active phase of the cycle, i.e., by night with *Loa loa* and by day with nocturnal *Wuchereria*, the microfilariae remain attached in the lung tissue, but are liberated during the contrasting passive phase. Edeson, Hawking and Symes (1957) report that the non-periodic *W. bancrofti* of the Pacific differs from the periodic form in Africa in that increased oxygen caused no decrease and perhaps a slight rise of the microfilarial count instead of a noticeable reduction, as found in Africa.

The origin of non-periodic Bancroftian filariasis which occurs in Polynesia and eastern Melanesia is by no means clear, but several opinions have been presented. Most anthropologists are agreed that the major migrations of Polynesians originated in south-east Asia and followed an eastward trend, touching on many islands of the central Pacific as they progressed to that eastern triangle of the Pacific, with apexes at the Hawaiian Islands on the north, Easter Island on the east and New Zealand on the south, which area is now known as Polynesia. Recent dating by Carbon 14 indicates that many islands of Polynesia were settled by the first century A.D. (Draper, 1960).

One theory which was an outgrowth of the early work of Bahr (1912) in Fiji and of the later studies of Buxton (1928) supposes that the Polynesians harboured nocturnal periodic filariasis as they migrated eastward along the northern borders of Melanesia. Upon arrival in the eastern parts of Melanesia and western borders of Polynesia, suitable night-biting mosquitoes were rare or absent. In their place, day-biting sylvatic mosquitoes, which were most active in the morning and late

afternoon and belonging to the scutellaris group, e.g., *Aedes pseudoscutellaris* and *A. polynesiensis* were prevalent. By natural selection of the microfilariae present in the peripheral blood during the day, a strain developed which is non-periodic in type.

Canet (1952) reports that non-periodic filariasis also occurs among the Mois, Polynesian-like peoples in the south-east part of Asia. If this is confirmed, it is possible that the non-periodic type could have been transported by certain of the Polynesian migrations, if suitable vectors were available enroute.

The recent studies of Symes (1960) and Burnett (1959) which emphasize the importance in the transmission of filariasis, in those parts of Polynesia where they occur, of *A. fijiensis* and other species of *Aedes* mosquitoes which may bite either by day or night, present additional factors which may have been important in the development of the non-periodic strain. Belkin (1960 : 1960*a*), in discussing the dispersal of mosquitoes of the Pacific, reviews this topic in detail and suggests "that non-periodic filariasis is not derived from the periodic type, but rather that it is a primitive generalized type which is relict in the South Pacific Area."

He supposes that one or more stocks of filaria may have been introduced by Melanesians prior to the migration of Polynesians and that the latter upon arrival in the eastern south Pacific region "merely acquired and spread the filaria already present."

DISTRIBUTION AND PREVALENCE.

Filariasis throughout the world is noted for its marked spotty distribution. The island areas of the south Pacific are no exception to this, as evidenced by reports from Fiji by Symes (1960*a*) which show a low 6·4 per cent of the population in Labasa to be positive, while 25·2 per cent are positive in Taveuni. Likewise Beye *et al.* (1953) in French Polynesia reported percentage ranges from 19 per cent in Tubuai to 44 per cent in Hitiaa. The prevalence rates in different populations would appear to be associated with the habits of the people and the density of the mosquito vectors. If highly efficient vectors in great numbers are in close proximity to the human population, greater opportunity is afforded for transmission.

Many of the earlier reports of elephantiasis in the Pacific were from small groups of a population only and therefore were not representative of the populations as a whole, e.g., a recorded elephantiasis rate of 50 per cent would probably be made from observations on a small group of older adults. Unfortunately, even in some later reports elephantiasis rates are not always given by age groups, although the overall rates are recorded from the same population group on which the microfilaremial rates were recorded.

Table I shows by age-groups recent prevalence rates for microfilaremia and elephantiasis in several representative island groups from Polynesia. It is observed that the total non-periodic microfilaremial rates ranged from 14·2 per cent in Fiji to 40 per cent in Tahiti, and elephantiasis rates from 0·8 per cent in Fiji to 7 per cent in

TABLE I.

Prevalence rates of microfilaremia and elephantiasis.

Island :	Cook Island* :		Fiji† :		New Caledonia* :		Samoa (A)‡ :		Samoa (W)* :		Tahiti* :		India*. Mangalore :	
Author and date.	Iyengar, 1957.		Nelson and Cruikshank, 1956.		Iyengar, 1954.		Jachowski and Otto, 1955.		McCarthy and Fitzgerald, 1956.		Beye *et al.*, 1952.		Krishnaswami, 1955.	
Age-group. (Years)	Per cent positive for Mic.	Per cent positive for El.	Per cent positive for Mic	Per cent positive for El	Per cent positive for Mic.	Per cent positive for El.	Per cent positive for Mic.	Per cent positive for El.	Per cent positive for Mic.	Per cent positive for El.	Per cent positive for Mic.	Per cent positive for El.	Per cent positive for Mic.	Per cent positive for El.
1— 4							0·0				4·8	0	5·5	0·0
5— 9	10·4		3·8	0	0·0		9·1		3·6		23·2	0	13·4	1·6
10—14	17·2		6·3	0	20·0		9·8		19·0		31·4	0·3	17·0	8·3
15—19			11·5	0	30·0		15·6							
20—29	39·0		17·3	0·7	47·0		26·3		30·0		45·7	1·8	15·0	10·8
30—39	37·9		22·8	1·7	57·0		33·6		44·0		54·1	8·3	14·8	12·7
40—49	40·7		26·4	3·2	47·0		36·4		50·0		55·0	19·6	15·0	14·7
50—59	46·5		29·8	4·8	53·3		40·9		40·0		57·0	32·0	17·0	16·5
60 and over			31·1	4·5			47·0							
Total	21·0	3·9§	14·2	0·8	37·0	1·0	20·3	1·3§§	23·0	2·0	40·0	7·0	15·0	8·8
Above 20 years		11·7		2·4				4·0				14·0		13·0

*Surveys by 20 c.mm. of blood. ‡Surveys by 60 c.mm. of blood. §§Murray (1948).
†Surveys by Knott Method. §Gross filarial disease including hydrocele.

Mic. = Microfilariae. El. = Elephantiasis.

Tahiti. It is of interest to compare these surveys in the south Pacific with a representative survey of nocturnal periodic Bancroftian filariasis in Mangalore, India, (last column in Table I). It is seen that the microfilaremial rate in Mangalore is 15 per cent and the elephantiasis rate 8·8 per cent. It is of interest to note that the ratio of microfilaremia to elephantiasis is only about 2 : 1 in this report of periodic Bancroftian filariasis in India while the ratios in the survey of non-periodic filariasis in the south Pacific, range from 37 : 1 in New Caledonia to 6 : 1 in Tahiti. In two areas where the elephantiasis rates were nearly the same, i.e., 8 per cent in Mangalore and 7 per cent in Tahiti, the density of microfilariae per 20 c.mm. of blood per positive individual is also much lower in India, i.e., 40 per positive in the Indian series and 110 in the Tahitian series. In the south Pacific surveys where the density of microfilariae per 20 c.mm. per positive was approximately 40, i.e., similar to the Indian series, the elephantiasis prevalence rate was shown to be only about 1 per cent (Kessel, 1957). Reasons for such spectacular differences are not clear at present and will require more study.

VECTORS.

Nocturnal periodic Bancroftian filariasis for the most part is transmitted by mosquitoes belonging to the two genera, *Anopheles* and *Culex*, which are night-biting in their habits. Non-periodic Bancroftian filariasis on the other hand is, for the most part, transmitted by members of the genus *Aedes*, some species of which may be primarily day biters while others bite either during the day or at night. Sir Philip Manson-Bahr's classical work (Bahr, 1912) demonstrated that *A. pseudoscutellaris* is a highly efficient vector of non-periodic filariasis in Fiji, and Marks (1951) subsequently differentiated therefrom the closely related *A. polynesiensis* which is more widespread throughout Polynesia than *A. pseudoscutellaris*. Infection studies by Symes (1960) and others have demonstrated that both species are excellent vectors. Other species of *Aedes* that are important vectors in Polynesia are *A. fijiensis* in Fiji (Symes, 1960 : 1960*a*), and possibly *A. samoanus* in Western Samoa. *A. vigilax* is a recognized vector in New Caledonia and the Loyalty Islands (Iyengar, 1954), and Burnett has confirmed its occurrence in Fiji although he does not consider it an important vector in that area. *A. tongae* in the Tongan group of islands, and other closely related species of the *pseudoscutellaris* complex in Niue and Rotuma and in other island groups of the area (Belkin, 1960) appear to be significant vectors in the south Pacific.

It is generally considered that Bancroftian filariasis in the north Pacific, including Micronesia, is of the nocturnal periodic type and is transmitted by *Culex quinquefasciatus* (*fatigans*). This mosquito is cosmopolitan throughout the world and is thought to have been introduced into the Pacific by early European explorers in their sailing vessels. Its occurrence in Polynesia would therefore be superimposed upon the native *Aedes* species to which non-periodic *Wuchereria* had long been adapted. Most infection experiments in Polynesia, in which both *C. quinquefasciatus* and *A. polynesiensis* have been fed on the same patient harbouring non-periodic

W. bancrofti, have demonstrated that a much higher percentage of *Aedes polynesiensis* than of *Culex quinquefasciatus* become infected. Likewise collections of naturally infected *A. polynesiensis* and *C. quinquefasciatus*, caught in and near houses where persons known to be infected with non-periodic *W. bancrofti* reside, show *A. polynesiensis* to be more heavily infected than *C. quinquefasciatus*. These observations indicate that the non-periodic strain of *W. bancrofti* in the south Pacific is better adapted to the native *A. polynesiensis* and other species of *Aedes* than to the later arrival, *Culex quinquefasciatus*. Symes (1960*b*) likewise observes infection ratios in Fiji similar to the ones mentioned above in Polynesia, but considers, because of the intimate association with the night-biting, house-resting *Culex* in houses, that its importance as a vector may be greater in that area than is indicated merely by its infection rate.

CONTROL.

In control or eradication of any arthropod-borne disease, measures must be directed toward the definitive and intermediate hosts, as well as the parasite. For any particular disease, the life history of the parasite and its epidemiologic relationships should be well determined, and measures directed against those links in the chain which can be most efficiently interrupted. In non-periodic Bancroftian filariasis in the Pacific, in so far as is known at present, only man is the definitive host and mosquitoes, which for the most part belong to the genus *Aedes*, are the vectors. Control of the disease may, therefore, be directed against the mosquitoes, against the parasite in the definitive host or against both. For a filariasis control or eradication program, measures for reduction or elimination of the vectors should be included.

In most areas where non-periodic Bancroftian filariasis is present in the south Pacific, the major vectors appear to be members of *A. pseudoscutellaris* complex. Their bionomics are similar and they may be defined as sylvatic in habit by preference but capable of peridomestic adaptations when domiciles are in close proximity to the bush. *A. polynesiensis* has the widest distribution, being found in Fiji, Samoa, Cook Islands and throughout French Polynesia, including the Marquesas. It feeds on man, domestic and wild animals, and as is shown by Bonnet and Chapman (1956 : 1958), to lay its highly resistent eggs in many varieties of natural and man-made containers, e.g., tree holes, rock holes, tin cans, pirogues, crab holes, coconuts or puddles, which hatch and develop into mature mosquitoes either in fresh or brackish water. In urban areas *A. polynesiensis* is usually replaced by the ubiquitous and more recently imported, *A. aegypti* and/or members of the *C. pipiens* complex.

As mentioned above in the section on 'vectors', *A. fijiensis* which breeds in the axils of pandanus is important in Fiji. Other members of the *kochi* group also appear to be of significance in Samoa. These complicate the problem of vector control because of their differences in breeding and biting habits and make filariasis control by mosquito eradication alone, an expensive and difficult process.

Two main long term filariasis control programs, by mosquito control alone, have been attempted in the south Pacific area during the last ten to fifteen years, one begun by Amos in Fiji in 1944 (Burnett, 1959) and the other by Davis (1949) in Rarotonga. Subsequent follow-up reports from Fiji by Burnett (1959) indicated that where mosquito control alone had been employed there was little or no measurable evidence of a decline in filariasis. In Rarotonga, eight years after Davis' report, Iyengar (1957) found a prevalence rate of microfilaremia similar to that reported by Faine and Hercus in 1951 for a survey performed before the campaign in Rarotonga was begun. While these two unsuccessful attempts do not nullify the employment of such a method in the control of filariasis, they do demonstrate the great difficulty of reducing mosquito vectors sufficiently, to make any great impression in the rapid reduction of filarial disease.

Jackowski (1950) and Kessel (1960) show that carriers of microfilariae, when exposed to little or no subsequent infection of *W. bancrofti*, become negative for microfilariae in from six to ten years. Until completely negative, such persons are a potential menace and if suitable vector mosquitoes are present in an area, transmission may occur. It is apparent in control of filariasis that a filaricide against either adult filariae or microfilariae or both would be a valuable asset.

Following the discovery by Hewitt *et al.* (1947) that diethylcarbamazine is a valuable microfilaricide, many reports have occurred in the literature concerning its use in filariasis control campaigns. Unfortunately many of them were cursory studies with inadequate dosage and often with insufficient numbers of a population treated. Also in some studies the results were not followed long enough to furnish complete and accurate long term data. Two campaigns in the south Pacific, however, are providing significant information : one by Simpson (1957) on the Island of Niue and the other in the Society Islands first reported by Beye *et al.* (1952), and later by Kessel (1957*a*). In these programs, mass treatment of the population with diethylcarbamazine was combined with mosquito control by cleaning up breeding places of the mosquitoes and are giving highly encouraging results. They would seem to demonstrate that such a combined method is the most rapid and successful procedure to use.

In most control programs to date reports of progress have been made on the basis of reductions in microfilaremial prevalence rates, or in density of microfilariae per unit of blood, e.g., 20 c.mm. Such indices are valuable, but are not the most significant in evaluation of a control program. Reductions of infection in a young age-group, e.g., 0-4 or 0-9 years, as compared with the same age group prior to inauguration of the program, give an index concerning the amount of transmission currently in progress (Kessel *et al.*, 1960), and the prevalence rates of clinical manifestations, such as elephantiasis, hydrocele and lymphangitis before and after institution of a program, provide highly significant measures concerning the clinical success of a control project (March *et al.*, 1960).

Microfilaremial rates in control program show a distinct drop immediately after administration of diethylcarbamazine. This amount of reduction in a mass-treatment

program will vary in different reports, depending upon the thoroughness of the program and is dependent upon the dosage schedule and the percentage of the population who are treated. In Tahiti, after three year's of control, Kessel *et al.* (1960) reported a drop in microfilaremial rates from 32 per cent to 3·2 per cent, and a drop in density per 20 c.mm. of blood for the whole population from 30 to 0·6.

It has been found by several workers that following treatment of known positives with diethylcarbamazine, who had become negative, a few of them after varying periods of time revert to a positive state, either as the result of new infections or by recurrence of the original infection (McGregor and Gilles, 1956; Rachou and Ferreira, 1958; and Kessel *et al.*, 1960). Assuming that most of these are recurrences, it may be hypothesized that a certain percentage of the adult worms survived the treatment with diethylcarbamazine and subsequently regained their ability to produce microfilariae.

In one series in Tahiti, 57 individuals who were positive for microfilariae with an average of 55 microfilariae per 20 c.mm. of blood, were given mass-therapy with diethylcarbamazine and 1 year after the completion of treatment were found to be negative for microfilariae. Three years later they were retested, examining 40 c.mm. of blood, and 6 of them, i.e., 10·5 per cent were found to be positive with an average microfilarial count of 1·5 per positive person.

Although these results show that 10·5 per cent of the original positives treated in a mass diethylcarbamazine campaign, without subsequent diethylcarbamazine therapy, may return to a light carrier state by the end of 3 years, such findings would not indicate that the positives so resulting would increase transmission to a degree that would jeopardize a control project. Such recurrences, however, should not be disregarded but searched for by periodic blood surveys, and then retreated. Rachou and Ferreira (1958), suggest, instead of periodic blood surveys and retreatment of positives, repeating a dose of 6 mg. of diethylcarbamazine per kg. of body weight to the whole population once every 6 months.

The current infections recorded above show that diethylcarbamazine is not a perfect drug for immediate eradication of filariasis following a single mass-treatment routine. However, until such an ideal drug is found, the advantages accruing from its use, as recommended in the program in Tahiti for non-periodic filariasis, appear to outweigh the disadvantages.

It might be pointed out for sake of contrast that in eradication campaigns against malaria, mosquito control usually precedes any drug control that may subsequently be used. In filarial control programs, however, mass treatment with diethylcarbamazine at the beginning of a campaign reduces the number of microfilariae in man to a point where fewer mosquitoes become infected. This reduces transmission to a low level and a satisfactory combination of follow-up treatments of positives, together with suitable mosquito control, has been shown to lower filariasis transmission to that point where clinical filariasis ceases to be the important public health problem that existed previously. Actual eradication of filariasis

in a given area will require continued expenditure of effort to eliminate all carriers and keep mosquitoes at that low density where transmission is reduced to an insignificant level.

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FILARIASIS RECORDS IN CEYLON.

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INTRODUCTION.

THE present Anti-Filariasis Campaign in Ceylon began only in 1947, though knowledge of the presence of the disease in its full cycle of manifestations dates from 1914 when Bahr made a survey of this country. The clinical phenomenon of elephantiasis has, of course, been known from a much earlier date and reference to it may be culled from classical Sinhalese literature.

Originally both *Wuchereria (Brugia) malayi* and *W. bancrofti* were to be found, and in the early post-war years the Campaign devoted much energy to the destruction by spraying with Phenoxylene 30 of *Pistia stratiotes* and other host plants of the insect vector (Mansonioides spp.) of *W. (Brugia) malayi*. This met with considerable success and, whilst vigilance is required to prevent the reservoirs of infection from refilling, this parasite appears now to be so rare that it can be left out of routine consideration. What follows is concerned with the Campaign's activities in the more difficult work of controlling urban filariasis due to infection by *Culex fatigans* transmitted *W. bancrofti*.

As is now standard in control programmes of this type, the Campaign is engaged in simultaneous attacks both on the vector and on the parasite. With the first of these, which is conducted partly by direct assault (mainly larvicidal) and partly indirectly through environmental sanitation, the present note is not concerned. Control of the parasite, involving the detection of microfilaria positive cases and their treatment with diethylcarbamazine, requires above all a prolonged administrative effort in that large numbers of blood films have to be taken and positive cases followed-up over an extended period of time. It is in an attempt to facilitate the organizational difficulties of this task that the record system to be described has been devised.

Bancroftian filariasis is endemic in the towns and villages of the west coast of Ceylon over a strip, a little under 150 miles long which, receiving two monsoons a year, has an annual rainfall of 80 to 120 inches. The population of the defined

administrative areas which comprise the strip is of the order of two million, though, due to local variations in terrain, not all these persons are equally exposed to risk of contracting the disease. For case finding, the Campaign has at its disposal a staff of 21 Public Health Inspectors who, besides supervising vector control, visiting known cases and conducting educational work during the day, collect blood films at night. So far as possible, they have been trained to collect standard 20 c.mm. samples. These are examined centrally and in the past, it has proved possible to collect and examine approximately 120,000 blood films per year, of which a little under 2 per cent have been positive. It is not economically possible in this situation either to take blood films from all the people, treating those found to have microfilaraemia, or to administer diethylcarbamazine to all the people (the two alternatives recommended by the W.H.O. study group on filariasis)* and the central problem of direction of this aspect of the Campaign resolves itself into the familiar one of deploying available resources to the best advantage. To achieve this and to provide information required both for the day-to-day running and the periodic evaluation of the Campaign, a new system of records has been devised, taking advantage of the possibilities offered by punch card machinery for the economical sorting and processing of mass data. The system has three main components which can be described severally.

RECORDS OF NIGHT BLOOD EXAMINATIONS.

Previously, whilst consolidation of data was limited to hand tabulation, it was possible only to analyse blood filming records by area and by positive or negative. This was unsatisfactory since it ignored the sex and age of the donor and lumped together blood films taken in the course of house-to-house surveys with those of known microfilaria cases taken after therapeutic, or their contacts after prophylactic, dosage with diethylcarbamazine. Pilot studies in two centres showed that although the incidence of microfilaria positivity continued to rise at least until the 40-49 age-group, relatively fewer samples (having regard to the estimated age-grouping of the population) were being taken from persons aged thirty and over. At the same time a disproportionate number of blood films were being taken from residential institutions, where it was relatively easy for the peripheral worker to get hold of a large number of persons simultaneously, as compared with the harder to obtain specimens from door-to-door surveys. Further analysis of the Campaign's operational activities resulted in defining eleven distinct situations in which blood films were taken. These were:

(i) Persons who do not show any manifestation of the disease and who attend voluntarily at night blood filming centres.

(ii) Persons sampled in the course of house-to-house investigations.

(iii) Persons living in residential institutions containing more than 10 persons.

* World Health Organization/Fil/17 dated 15th February, 1957.

(*iv*) Persons living in houses in or around which infected mosquitoes have been found.

(*v*)-(*xi*) Known microfilaria cases, overt cases, and their contacts subdivided as follows:

(*v*) Overt clinical cases after commencement of treatment.

(*vi*) Overt clinical cases before commencement of treatment.

(*vii*) Contacts of clinical cases after prophylactic treatment.

(*viii*) Contacts of clinical cases before prophylactic treatment.

(*ix*) Known microfilaria cases at routine intervals after treatment.

(*x*) Contacts of microfilaria cases after prophylactic treatment.

(*xi*) Contacts of microfilaria cases before prophylactic treatment.

Beginning with August, 1959 (the monthly results for which were used as a final trial of the system) a new recording routine has been adopted for the purpose of providing separate information about each of these groups.

Blood films are recorded when taken on a schedule, which contains space for some 65 names and addresses. Separate schedules are prepared for each of groups *i-iv* and a joint one for groups *v-xi*, which are however recorded opposite the individual name. The schedules are prepared in duplicate and are sent together with the relevant slides, to the central laboratory. The layout of the schedule is shown at Appendix I.

After the slides have been examined in the laboratory and the results recorded on the schedule, one copy is returned to the Public Health Inspector who originally took the samples and the other is passed on to the Medical Statistician's Office at the Department of Health. From the latter copy, a punch card is prepared for each blood film taken. The following items are recorded.

Columns	Information
1- 4	Laboratory reference number identifying the schedule.
5	Date (month).
6- 7	Medical Officer of Health area.
8- 9	Public Health Inspector responsible for taking specimens
10-11	Individual Serial Number.
12-13	Age
14	Sex
15	Group number (reason for blood filming)
16	Number of worms found.

Since, for reasons stated above, it is anticipated that *W. bancrofti* alone will be encountered, the species of microfilaria is not at present recorded on the punch

cards. However, the punch operators have instructions to refer back any positive entry on which the number of worms is not specified as bancroftian, and experience will dictate future action.

The punch card equipment available uses a card with 65 columns; accordingly, for reasons of economy the cards will be used more than once. The present intention is that tables will be prepared quarterly and two packs of punch cards will be used; cards punched during the first quarter will be analysed during the second, when a new pack will be punched; the first pack will then be used again to punch the results of the third quarter's activities and so on.

Information derived from these cards is primarily intended to provide operational statistics for regular periodic evaluation of the Campaign's work. However carefully planned, widespread activities of this kind involving the testing of many thousands of persons, cannot be easily reviewed by the responsible administrative head except in statistical terms. Peripheral workers vary in their capacity and enthusiasm, and local variations in density of population, ease of communications and similar local factors affect the extent of the work they can perform. Whilst an accountancy of this kind is no substitute for leadership, which is essentially a face-to-face activity, it can materially assist in the task of supervision. At the same time, since blood filming discloses the persons who are probably not affected, as well as those who are, analysis of the results may give clues to problems involved in the epidemiology of the disease, though in almost every case these require more detailed subsequent *ad hoc* examination.

Up to now the main experience which we have of the working of the scheme, rests on analysis of results obtained during the period September to December, 1959. During this period a total of 27,200 blood films were taken from members of the public and examined in the Campaign's laboratory at Dehiwela. This figure is considerably below that which it is hoped to reach during a similar period when the Campaign is operating at full pressure.

Just over half the blood films were taken in the course of routine door-to-door enquiries, 18 per cent came from persons who attended voluntarily at night blood filming centres and a further 12 per cent were taken from persons living in residential institutions. The remainder, a little under 20 per cent of the whole, were taken in the course of follow-up of known cases either of microfilaraemia or of overt clinical filariasis and their contacts. The number of films taken and the results obtained from them, classified according to reason for taking the blood film, are shown in Table I.

Besides showing the percentage of films found to be positive for *W. bancrofti*, Table I also shows the percentage of the positive films which were found to have more than 10 microfilariae per 20 c.mm. of blood. Allowing for the fact that specimens were taken in the field without pipettes, and that there is therefore a certain amount of variation in the amount of blood taken from each person, this proportion may be taken as a rough guide to the severity and level of infectivity of the

positives found at any given moment of time. There is of course every possibility that the case with a low level of infection would show a greater concentration of microfilariae if examined at different times; this, however, does not affect the picture statistically.

TABLE I.

Blood filming activities, September-December, 1959, by reason for taking, and results.

Reason.	Total films taken.	Per cent., positive.	Percentage of *positives* with more than 10 microfilariae per 20 c.mm.
Attendance at night blood filming centre.	5,023	3·9	33·3
Routine enquiries.	13,796	3·3	19·3
Residential institutions.	3,372	1·7	22·4
Contact of clinical case after treatment.	90	Nil	Nil
Contact of clinical case before treatment.	1,038	4·5	23·4
Contact of known microfilaraemia cases after treatment.	776	2·1	6·3
Contact of known microfilaraemia cases before treatment.	1,501	4·3	37·6
Known microfilaraemia cases after treatment.	584	18·2	21·7
Clinical cases before treatment.	546	1·1	Nil
Clinical cases after treatment.	221	0·5	(1 case only)
All reasons*	27,200*	3·5	22·8

*Includes 163 unclassified among whom 5 were positive.

It will be seen that persons who attended at night blood-filming centres, not only tended to be positive more frequently than the section of the general population examined in the course of routine inquiries (3·9 per cent as against 3·3 per cent) but a higher proportion of them were more heavily infected. A comparably high proportion of heavily infected persons is only found among contacts of known cases of microfilaraemia. It would appear, therefore, that there is a selection among persons who volunteer for examination and that a proportion of them presumably do so as a result of knowing themselves to be especially at risk.

Routine blood-filming inquiries are conducted by the Campaign's Public Health Inspectors on a house-to-house basis. Whilst the object of this activity is primarily case-finding, the results represent the nearest thing we have at present to a survey of the incidence of microfilaraemia. It must, however, be borne in mind that the level of incidence probably varies geographically within comparatively small areas and the selection of streets to be examined in this way is determined by the Public Health Inspectors themselves and does not represent a random sample of persons living within the range covered by them. Indeed any survey

concerned with a disease such as filariasis, which is localized in pockets topographically, must raise the problem of where the boundaries that define the population at risk are to be drawn.

Some light is thrown on how these routine inquiries are conducted by examination of the age distribution of the persons blood-filmed as compared with the population as a whole. Local estimates of the age and sex composition of the population of the areas, worked by the Anti-Filariasis Campaign, are not available. We can, therefore, compare them only with the estimated population for the whole of Ceylon at mid-year 1958, the latest published in the Statistical Abstract of Ceylon, 1959. Table II shows such a comparison. When we look at the full range of ages, it is evident that the section of the community which is blood-filmed is deficient in the age-group 0-9 years. This, doubtless, arises partly from the fact that the blood films are taken at an hour of the night when small children are asleep and partly because incidence in this age-group is in any case known to be very low.

TABLE II.

Age distribution of persons blood-filmed.

Age-group (Years).	Percentage in each age-group.					
	Males.			Females.		
	Ceylon mid-1958.	Blood films taken for		Ceylon mid-1958.	Blood films taken for	
		Attendance at centre.	Routine enquiries.		Attendance at centre.	Routine enquiries.
0-9	28·7	4·5	18·9	30·5	14·7	17·9
10 and over	71·3	95·5	81·1	69·1	85·3	82·1
All ages	100	100	100	100	100	100
10-19	28·7	38·4	37·6	30·5	39·0	34·0
20-29	23·1	42·7	21·6	23·9	25·0	23·1
30-39	19·2	11·7	16·0	18·9	17·4	18·1
40-49	15·0	4·8	11·4	13·6	11·8	12·1
50-59	9·1	1·9	7·2	7·9	5·1	7·6
60-69	3·8	0·4	4·7	3·5	1·3	3·6
70 and over	1·0	0·1	1·6	1·6	0·3	1·5
All ages	100	100	100	100	100	100

If we restrict our comparison to persons aged 10 years and over, it will be seen that the blood-filmed group contains an excess of persons aged 10—19 years but, except for this, approximates reasonably well to the age distribution of the population as a whole. It would appear from these comparisons that Public Health Inspectors are being reasonably successful in collecting blood films from all members of the houses visited without any systematic bias except of course with respect to the younger children.

In view of the relatively good correspondence between the age distribution of persons blood-filmed under these routine arrangements and that of the population as a whole, we can use the results to examine the incidence of microfilaraemia by age and sex. It must, however, be borne in mind that we are here lumping together blood filming activities from a number of areas among which, as we shall see, the incidence varies considerably. It is theoretically possible that disproportionately many films have been taken in a particular age/sex group in an area with particularly high (or low) incidence, and this, of course, could bias our results. It is believed to be very unlikely that any such thing has happened, but figures presented in Table III can only be accepted with this reservation in mind.

TABLE III.

Incidence of microfilaraemia among persons routinely blood-filmed, by age and sex.

Age-group (Years)	Males.		Females.	
	No. of films taken.*	Per cent., positive.	No. of films taken.†	Per cent., positive.
0–9	1,290	1·86	1,243	1·93
10–19	2,085	3·60	1,943	3·81
20–29	1,200	3·85	1,318	3·03
30–39	886	5·08	1,032	2·33
40–49	631	4·28	690	3·19
50–59	397	5·54	434	2·30
60–69	261	4·60	206	4·37
70 and over	88	2·27	84	1·19
All ages	6,838	3·70	6,950	2·93

*Excluding 4 of unspecified age.
†Excluding 4 ,, ,, ,,

From Table III we see that the incidence among females under the age of 20 years is slightly higher than among males, whereas at all succeeding age-groups it is lower. Among males the age pattern is fairly regular, rising to a peak about the age of 40 years and remaining at a fairly high level thereafter. Among females there is no such regular pattern and the incidence remains roughly constant at all ages over 10 years.

As already stated, the incidence of microfilaraemia varies considerably between areas. Table IV shows the incidence by Health Office Areas and gives separately the results of examinations from routine enquiries, of volunteers at night blood-filming centres, and of contacts both of overt cases and of cases having microfilaraemia alone. Only those results which are based on over 100 blood films, are included in Table IV. It will be observed that where evidence is available from more than one type of blood-filming the results are generally consistent. Areas where a high incidence is found as a result of routine inquiries, show also a high incidence among contacts and among persons examined at centres. In general, as

might be expected, the highest incidence is found among contacts of overt cases. Contacts of microfilaraemia cases in 4 of the 6 areas show a higher incidence than is found by routine enquiries, though not substantially so.

TABLE IV.

Incidence of microfilaraemia by area and reason for blood-filming.

Health Office Area.	Routine Inquiries.		Volunteers at night blood-filming centres.		Contacts* of overt cases.		Contacts* of microfilaraemia cases.	
	No. of films.	Per cent., positive.	No. of films.	Per cent., positive.	No. of films.	Per cent., positive.	No. of films.	Per cent., positive.
Negombo	391	1·3	..	..	..	..	..	..
Jaela	454	5·1	..	..	..	..	..	..
Kolonnawa	934	1·4	..	..	..	..	267	3·0
Dehiwela	1,108	2·1	1,531	4·0	328	4·9	..	..
Kotte	1,066	1·2	1,281	5·1	104	3·8	..	..
Moratuwa	569	3·7	1,059	2·2	..	..	..	..
Panadura	1,651	1·4	..	..	..	..	109	0·9
Kalutara	1,998	3·1	..	..	..	..	..	..
Beruwela	600	6·3	..	..	..	..	120	8·3
Ambalangoda	706	2·3	116	2·6	..	..	260	3·1
Galle	149	12·1	286	5·2	..	..	..	..
Unawatuna	1,111	6·0	..	..	..	..	233	5·2
Weligama	1,449	4·5	..	..	124	8·9	322	5·6
Matara	1,344	4·5	457	5·0	..	..	..	..

*All contacts examined before prophylactic treatment.

Microfilaria positive cases are given domiciliary treatment and their contacts, who are defined as persons living under the same roof, are treated prophylactically. The therapeutic regimen at present employed involves issuing a total of 126 tablets of diethylcarbamazine at three successive weekly visits by the Public Health Inspector, the patient being instructed to take 6 tablets per day. Contacts are given a one-week course at the same dosage.

Table V groups together the results of examination of contacts. The following points may be observed :

(*a*) No clinical contact was found to be positive after receiving prophylactic treatment. The number of cases examined after treatment is, however, disturbingly low.

(*b*) Microfilaraemia contacts, examined after treatment, show a considerably lower level of incidence than those examined before treatment. Furthermore, reference back to Table I discloses that even among those who were positive after treatment the level of infection was very much lower. Whilst not so acute as in the case of clinical contacts, the number of cases examined after treatment is too low in view of the number examined before.

TABLE V.

Results of contact examination.

	Before treatment.		After treatment.	
	Cases.	Per cent., positive.	Cases.	Per cent., positive.
CLINICAL CONTACTS.				
Males	519	5·0	36	..
Females	519	4·0	54	..
All	1,038	4·5	90	..
MICROFILARAEMIA CONTACTS.				
Males	891	3·3	351	1·1
Females	700	5·7	425	2·8
All	1,591	4·3	776	2·1

The results of therapeutic treatment of known microfilaraemia cases do not give the same reasons for encouragement as those of prophylactic treatment of contacts. Over 20 per cent of the males and nearly 18 per cent of the females continue to be positive after treatment. Table VI shows that the results, though bad in all groups, were slightly better for younger age-groups than for older and for females than for males. It is clear that more follow-up and control of this part of the Campaign's activities is needed than can be provided by blood-filming records alone. This is all the more important since the bionomics of the vector mosquito are such as to offer little hope for complete control along the lines successfully pursued by Malaria Campaigns against the relatively more vulnerable anophelines and early detection and treatment of microfilaraemia thus becomes the main hope of interrupting the cycle of transmission. To achieve this it is necessary that individual cases, once detected, should be followed-up over a period of years, and the records kept of this work should be such as to enable analyses to be made of the relative efficacy of different treatment regimes. Such a system forms the second part of our new system.

TABLE VI.

Incidence of continuing microfilaraemia among known m/f cases after treatment, by age and sex.

Age-group. (years)	Males.		Females.		All	
	Number treated.	Per cent., positive.	Number treated.	Per cent., positive.	Number treated.	Per cent., positive.
0–39	218	18·8	221	14·0	439	16·4
40 and over.	93	23·7	52	23·1	145	21·5
All ages.	311	20·3	273	15·8	584	18·2

THE CENTRAL REGISTER OF MICROFILARAEMIA CASES.

The essentials of any follow-up scheme are the same. All patients must be treated and all must be visited at regular intervals after completion of treatment. Where, as in the present case, the patient is treated at home and has no overt signs or symptoms to remind him of this disease a fairly high degree of organisation is required to ensure that he is regularly tracked down at the appropriate time. The routine we have adopted was introduced on January 1, 1960 and sufficient time has not yet elapsed to test its working in detail. Its outline is as follows :

(1) On hearing that a blood film is positive, the Public Health Inspector opens a field treatment card for the case. The layout of the card is shown at Appendix II. Two copies are prepared, one of which is filed locally in numerical order according to local reference number. Two indexes, as detailed below, are kept to this file.

(i) *A surveillance index.*—This shows the reference number of each case under the month when next due for examination. At the beginning of each month, the Public Health Inspector uses this to draw out from his main file the cards of cases due for attention during that month and, having regard to the addresses, fits them into his advance programme to ensure that follow-up action is taken at the right time without unnecessary travelling.

(ii) *An index by street or village.*—This again shows a similar reference number against the village or street name. This index enables the Public Health Inspector to answer questions about the areas within his range where filariasis is more or less prevalent. It will also enable an inspecting officer, if he so wishes, to check all cases resident in a limited area.

These indexes are maintained on sheets of foolscap paper since each Public Health Inspector will normally have a little over 100 new cases per year which, with a two year follow-up, will mean that he seldom has more than 250 cases on his register.

The other copy of the field treatment card is sent to the Superintendent, Anti-Filariasis Campaign. All copies so received are then given a central register number and filed by year under that number. The two files of cards constitute the local and central registers, and the word 'register' is used to denote these card indexes. There will be no transfer of the entries to a register in a book form.

Two indexes to the central register, as described below, are maintained. In view of the larger number involved, they are kept as card indexes.

(i) *A surveillance index similar to that maintained by Public Health Inspectors.*—Some time after the beginning of each month this is checked through and reminders sent to the Public Health Inspector concerned about any cases from whom a blood film was due and has not been received.

(*ii*) *A name index.*—This is kept in strict alphabetical order of principal name. Again, all that is shown on the cards is the full name (principal name first) and the central register number. This index is used whenever a new case is discovered to ensure that it is in fact a new case.

To keep this system going in the headquarters office of the Campaign, involves certain procedures in connection with the handling of the schedules of night blood films after the examination of specimens is completed in the laboratory.

(*i*) Whenever a positive case is recorded it must be checked with the central register and, if it is a new case, be allotted a central register number which will be entered on the Public Health Inspector's copy of the form. For this purpose a book is maintained showing in the first column the central register numbers consecutively, in the second column the schedule number on which the positive was recorded, in the third column the Public Health Inspector's name, in the fourth column the date when the Public Health Inspector's copy of the schedule was returned to him, and in the fifth column the date when the second copy of the field treatment card was received for filing in the central register.

(*ii*) All blood film schedules, relating to follow-up examination of known microfilaria positive cases, are passed on to the central register in order that the proper entry can be kept on the headquarters copy of the field treatment card.

Since it is intended that both the Public Health Inspector's local register and the central register should be filed in strict order of the relevant register number by year of detection, the following convention is used for register numbers :

> Public Health Inspectors employ a 4 digit number and the central register a 5 digit number. The first figure is always the last number of the year and the remaining figures run from 001 (Public Health Inspector's) or 0001 (for the central register) onwards. Thus the first case in the central register for 1960 is 00001 whilst the last case in 1960 will probably be about 02500 and will be followed by 10001 as the first case in 1961.

For purposes of statistical analysis the principal items recorded on the field treatment card will be put on punch cards in the Medical Statistician's Office. In order to transmit the information to him it will be transcribed from the central register on a cyclostyled transmission slip to be designed by the Statistical Office.

One of the principal intentions of the new scheme is to ensure the continued surveillance of microfilaria positive cases and their contacts with a view to reducing, by early detection and treatment, the number of potential sources of infection and thus cutting the chain of transmission. Unfortunately, persons under surveillance

do not always remain at the same address. To meet this situation, the following rules are observed :

(i) *A simple change of address within the area under the supervision of a Public Health Inspector where the whole household goes to live in a new house.*—The new address is written above the old on the field treatment card and a note made to this effect, on the next night blood-filming schedule, is sent to the Superintendent, Anti-Filariasis Campaign, regarding this case.

(ii) *A change of address within the Public Health Inspector's area where the case under surveillance moves and only some or none of the contacts move with him.*—A new field treatment card is made out for the case at the new address. Provided the case was negative at the last blood examination before moving, only those contacts who moved with him are retained under surveillance at the new address. The old card is retained for any contacts left at the old address and their surveillance will continue. If, however, the case was positive at the last examination prior to moving, all new contacts at the new address are taken under surveillance ; similarly they will, of course, be brought into surveillance if any person at the new address becomes positive subsequent to the move.

(iii) *A change of address to a place outside the Public Health Inspector's area.*—A new card is made out complete up to the time of leaving by the Public Health Inspector who has previously supervised the case and sent to the Superintendent, Anti-Filariasis Campaign, for onward transmission to the new area. In sending these new cards the initiating Public Health Inspectors are instructed to indicate with as much accuracy as possible the new address to which the patient has moved. In these circumstances surveillance of contacts is subject to the principles given in rule (ii).

(iv) When a case under surveillance moves to a residential institution, arrangements are made, if he was negative at the time of moving, for him to attend for future surveillance at a night blood-filming centre, to avoid wherever possible unnecessary disclosure to other persons in the institution that he is under surveillance.

In the foregoing, contacts have been defined simply as persons normally resident under the same roof as the original case. An exception to this will be made only when a microfilaria positive case is detected (or goes to live) in an institution having more than 10 residents. In this case, the Public Health Inspector will indicate on the back of the field treatment card, after a preliminary visit, whether all residents are to be kept under surveillance or only a defined group of them (e.g. those living in particular buildings, defined groups who do not habitually sleep under mosquito nets and so on).

CENTRAL REGISTER OF CLINICAL CASES.

Overt cases of filariasis are only recognizable as a result of clinical diagnosis by a doctor, normally following a visit to one of the Campaign's clinics. Examination and treatment is conducted in these clinics and not in the patients home and the clinical records of cases are maintained there. As a routine, new cases attending for the first time are blood-filmed and given a second appointment so that the results of the laboratory examination may be available when they attend for detailed clinical examination. From the clinics the Public Health Inspector, in whose area the patient resides, is informed so that he may place the contacts under surveillance.

Two other new cards have been devised. One, a treatment and record card for use in clinics and the second a contact record for overt cases which is maintained by the Public Health Inspectors in whose area the patient lives. Only one copy of the contact record is maintained. Two copies of the treatment and record card are prepared, one of which is maintained up to date in the clinic and the other forms part of the central register of known cases kept centrally at the office of the Superintendent, Anti-Filariasis Campaign. Copies thereof are shown at Appendices III and IV.

The intention of the central register for overt cases is, however, rather different from that of the microfilaraemia register. In the first instance, it is of course a device for detecting double counting in statistics relating to new cases detected, since each new case is checked to ensure whether or not it has already been detected, possibly through attendance at a different clinic. Beyond this, however, the procedure is different. Evaluation of treatment of the symptomless microfilaria positive case rests on a single simple laboratory criterion and so can easily be mechanised from the central register. Long-term follow-up of the overt cases is more complicated and depends on the state of the disease and the pattern of clinical manifestations at first detection. The intention of the new treatment and record card is to record the results of the first full examination in some detail so that, by use of punch card machinery, groups of cases may be selected which presented themselves initially with a common pattern of signs and symptoms and these can then be followed up *ad hoc*. At the same time, of course, examination of the proportion of cases first seen at different stages of the disease will provide a yardstick for checking the efficiency of the Campaign in early detection of cases.

For this to be successful, it is of course necessary that different observers should use a common terminology, and the same word used by different persons must describe manifestations that are similar. Doctors at clinics are asked to classify the stage of disease at first detection as lymphangitis, lymphoedema-soft, lymphoedema-solid or elephantiasis. For the present the following definition has been issued with the instructions covering the use of the cards : "for the purpose of uniformity, it is decided to define "lymphangitis" as those cases where transient

signs such as inflammation of lymph vessels and glands, associated with slight swelling and redness, are observed. When the swelling has become permanent, it could be classified as "lymphoedema..." Nevertheless it is fully appreciated that this definition, and probably any written definition, leaves much to the judgement of the examining doctor. For the present, therefore, two solutions are being pursued. The Superintendent, Anti-Filariasis Campaign, is examining personally all new cases attending the main clinic at Dehiwela in the southern suburbs of Colombo. Results from this clinic, representing the judgement of a single observer, will therefore be the first to be examined. At the same time, by visiting other clinics and discussing the problem with the doctors in charge, he hopes gradually to obtain uniformity of judgement among all who are concerned. This, however, is necessarily a slow process.

SUMMARY AND CONCLUSIONS.

The Anti-Filariasis Campaign in Ceylon, which is mainly concerned with infection due to *W. bancrofti* occurring in a thickly populated coastal area, pays particular attention to early detection and treatment of cases of microfilaraemia. This procedure necessarily requires continuous long term surveillance of affected persons. To assist in this process we have adopted recording techniques already familiar to those concerned with central registration of cancer or tuberculosis.

One point, worth special comment, is the difficulties that arise as a result of side effects from treatment with diethylcarbamazine. As originally planned, no arrangements were made in the scheme for recording these, since it was felt that to include them specifically in the record documents might draw unnecessary attention to their possible occurrence. Experience has convinced us that we were wrong. Future copies of the field treatment card for microfilaraemia will include a section for recording this information. Meanwhile, make-shift arrangements have been made to employ the last four columns provided on the present card for blood examinations. Reference to Table IV shows, by inference, how serious is the problem. Unless and until pharmacologists can provide a single dose treatment there will always be difficulty in persuading persons who feel healthy to continue with a course of treatment which makes them feel ill.

Further appraisal of the results of the present system must now wait until it has been in operation for at least a year. It is, however, hoped that the records described, whilst proving reasonably simple to maintain, will serve the dual purpose both of keeping track of affected individuals and providing statistical evidence for the future planning of the Campaign.

ACKNOWLEDGEMENTS.

Preliminary work leading to a central register was started with Dr. W.S. Perera to whom our thanks are due ; we have also drawn freely on the experience of Dr. P.K. Ratnasingham, Assistant Superintendent, Anti-Filariasis Campaign.

We are also grateful to Dr. A. Zahra, South-East Asia Regional Office, World Health Organization, for helpful suggestions. We are indebted to Dr. W.A. Karunaratne, Director of Health Services, Ceylon, for permission to publish this paper. Our thanks are due to Mr. A. Wickramasurya who has laboriously retyped successive drafts of the forms.

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APPENDIX I.

Original/Duplicate

DEPARTMENT OF HEALTH.

FILARIASIS CAMPAIGN—NIGHT BLOOD EXAMINATION SCHEDULE.

Group No. and Code Name :..... *P.H.I. Annual Serial No.* .....

Name and Designation of P.H.I. :..... *Laboratory Reference No.* .. ..

M.O.H./H.O. Area :..... ..... *Date of Blood Filming* :.....

Town or Village : ....

No.	Name	Address (including Ward, Street and Assessment No.)	Age	Sex	Reason for Blood filming	Result of Blood examination
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						
21						
22						
23						
24						
25						
26						
27						
28						
29						
30						
31						
32						
33						
34						
35						
36						

[P.T.O.

APPENDIX I. *(Contd.)*

No.	Name	Address (including Wards, Street and Assessment No.)	Age	Sex	Reason for Blood filming	Result of Blood examination
37						
38						
39						
40						
41						
42						
43						
44						
45						
46						
47						
48						
49						
50						
51						
52						
53						
54						
55						
56						
57						
58						
59						
60						
61						
62						
63						
64						
65						

SUMMARY

Group No.	Town or Village	Street or Locality	No. of Films sent	No. Positive	Group No.	Town or Village	Street or locality	No. of Films sent	No. Positive

To PHI.....

The following numbers are positive for Microfilaria *bancrofti/malayi*. The central register number is entered against each case.....

.....

.....

Date : .....

Signed : .....
Superintendent, Anti-filariasis Campaign.

APPENDIX II.

MONTH AND YEAR WHEN NEXT DUE FOR EXAMINATION

P. H. I. Ref. No. ..

DEPARTMENT OF HEALTH

Field treatment card for a microfilaria case

Full name of patient : .....

.....

Address : .....

.....

Residence during five years prior to onset—

First .....

Second .....

Third .....

Fourth .....

Fifth .....

New case | | Transfer from P.H.I. .....

FIRST BLOOD EXAMINATION

Date taken	Date Received	Result

METHOD OF DETECTION	tick
Attendance at centre ..	
Routine survey ..	
Institutional survey ..	
Special survey ..	
Clinical contact—treated ..	
Clinical contact—untreated ..	
Microfilaria contact—treated ..	
Microfilaria contact—untreated ..	
Referred from outside source ..	

Central Register Number

Age at detection ..

Year of detection ..

Sex { Male .. .. / Female .. ..

P. H. I. (F. C.) Area : .....

.....

Town/Village : .....

.....

M.O.H./H.O. Area : .....

.....

COURSE OF TREATMENT	DATE	BLOOD EXAMINATIONS		2	3	4	5	6	7	8	9	10
First		Date ..										
		Result ..										
Second ..		Date ..										
		Result ..										
Third ..		Date ..										
		Result ..										
..		Date ..										
		Result ..										

RECORD OF CONTACTS

NAME	Sex	Age	Relationship to head of household		First blood film	Prophylactic treatment	POST TREATMENT BLOOD EXAMINATIONS 1	2	3	4	5	6	7	8	9
				Date											
				Result		X									
				Date											
				Result		X									
				Date											
				Result		X									
				Date											
				Result		X									
				Date											
				Result		X									
				Date											
				Result		X									
				Date											
				Result		X									
				Date											
				Result		X									
				Date											
				Result		X									
				Date											
				Result		X									
				Date											
				Result		X									
				Date											
				Result		X									
				Date											
				Result		X									

Appendix III.

DEPARTMENT OF HEALTH

FILARIASIS CLINIC—TREATMENT AND RECORD CARD

CLINIC REGISTER No. ..

CENTRAL REGISTER No. ..

Full name of patient .....

Address : .....

Residence during five years prior to onset—

First : .....

Second : .....

Third : .....

Fourth : .....

Fifth : .....

Date of birth ..

Year of onset of clinical symptoms ..

Dates of—	Date	Month	Year
First clinic visit ..			
First full examination			

Stage of disease at first detection:
- lymphangitis ..
- lymphoedema-soft ..
- lymphoedema-solid ..
- elephantiasis ..

Age at onset ..

Sex: Male .. / female ..

PHI(FC) Area: .....

Town/Village : .....

MOH/HO Area : .....

FINDINGS AT FIRST FULL CLINICAL EXAMINATION

PARTS AFFECTED

	Left	Right	Both	Neither
Leg(s) ..				
Arm(s) ..				
Testis(es)/Mammary Glands				
			yes	no
Scrotum/Labia				

ENLARGED GLANDS

	Left	Right	Both	Neither
Epitrochlear ..				
Axillary ..				
Inguinal ..				
Popliteal ..				

OTHER SIGNS AND SYMPTOMS

	History only	Seen at Clinic	Absent
Red patches ..			
Painful spots ..			
Filarial fever ..			
Funiculitis right ..			
Funiculitis left ..			
Epididymitis-right ..			
Epididymitis-left ..			
Orchitis-right ..			
Orchitis-left ..			
Chyluria ..			
Hydrocele-right ..			
Hydrocele-left ..			
Fungoid growths			
Ulceration			

LABORATORY FINDINGS

Date	Test	Result
	W.B.C. total count	
	D.C.	%
	polymorphs	
	lymphocytes ..	
	neutrophils	
	eosinophils	
	Microfilaria	

SPECIAL NOTES OR COMMENTS : .....

RECORD OF TREATMENT

TREATMENT	DATE GIVEN
Metrazan tablets issued	
Antimony preparation,* injections given	
*specify	

DATE	PROGRESS AND OTHER TREATMENT	DATE	PROGRESS AND OTHER TREATMENT

APPENDIX IV.

MONTH AND YEAR WHEN NEXT DUE FOR EXAMINATION *P. H. I. Ref. No.*

DEPARTMENT OF HEALTH

Contact record for a case of filariasis

Name of primary patient : .....

Address : .....

Clinic attended : .....

Stage of disease of primary patient :

- lymphangitis
- lymphoedema—soft
- lymphoedema—hard
- elephantiasis

CONTACTS

NAME	Sex	Age	Relationship to head of household		First blood film	Prophylactic treatment	POST TREATMENT BLOOD EXAMINATIONS 1	2	3	4	5	6	7	8	9
				Date											
				Result		X									
				Date											
				Result		X									
				Date											
				Result		X									
				Date											
				Result		X									
				Date											
				Result		X									
				Date											
				Result		X									
				Date											
				Result		X									

RECORD OF CONTACTS

NAME	Sex	Age	Relationship to head of household		First blood film	Prophylactic treatment	POST TREATMENT BLOOD EXAMINATIONS 1	2	3	4	5	6	7	8	9
				Date											
				Result		X									
				Date											
				Result		X									
				Date											
				Result		X									
				Date											
				Result		X									
				Date											
				Result		X									
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				Date											
				Result		X									
				Date											
				Result		X									
				Date											
				Result		X									
				Date											
				Result		X									

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LABORATORY AND FIELD STUDIES ON THE EFFECTIVENESS OF ORGANOPHOSPHORUS INSECTICIDES IN THE CONTROL OF *C. FATIGANS**.

BY

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[August 6, 1960.]

INTRODUCTION.

CONTROL of *C. fatigans*, the vector of bancroftian filariasis, has not been successfully achieved by residual spraying of houses with DDT (Wharton, 1951 ; Brown and Williams, 1949 ; Davidson, 1952 and Sharma *et al.*, 1957). Dieldrin was found to be effective against this species (Davidson, 1952 *loc. cit.*; Sharma and Krishnamurthy, 1954 and Jaswant Singh *et al.*, 1956) but in recent years there have been many reports of selection of resistant strains of *C. fatigans* to this insecticide as well as to other chlorinated hydrocarbon insecticides in many parts of the world, including India (Sharma, 1958 and Brown, 1958). More effective insecticides other than chlorinated hydrocarbons are, therefore, needed in case its successful control is desired through residual spraying of houses. Reid and Lim (1959) have reported that malathion at a dosage of 100 mg. per sq. ft. was quite effective against *C. fatigans*. On the other hand, Krishnan *et al.* (1958) found malathion and diazinon each at a dosage of 25 mg. per sq. ft. to be effective only for 3-4 weeks against it.

Organophosphorus compounds, in general, have been stated to have low residual effect against insects and high mammalian toxicity (Metcalf, 1955). A newer organophosphorus compound, namely, Bayer-S-1752 or Baytex† has recently been synthesised in the laboratories of Farbenfabriken Bayer AG. Germany. This compound was found to have long residual toxicity against insects of public health importance and low mammalian toxicity (Jung, 1960). The water dispersible powder of baytex (Baytex-40w), when applied at a dosage of 100 mg. per sq. ft. in certain areas of Belgian Congo, was observed to control *C. fatigans* for a period of about six months (Cerf and Lebrun, 1959). Two other newer organophosphorus compounds, namely, trithion and methyltrithion have been reported to be quite

* The investigations were in part financed by a grant from the Council of Scientific & Industrial Research.

† 0, 0-dimethyl-0-4-(methyl mercapto)-3-methyl phenyl thiophosphate.

stable to alkali and, therefore, their deposits on mud surfaces have the possibility of residual toxicity against insects for long periods.

Laboratory and field studies were, therefore, carried out to evaluate the comparative effectiveness of these compounds and diazinon and malathion as adulticides against *C. fatigans*.

The control of *C. fatigans* by anti-larval measures may be the method of choice in certain urban areas where the ratio of house-wall surface to breeding areas is usually very high. Tests were, therefore, carried out to determine their relative effectiveness as larvicides also. In addition to these compounds, phosdrin (Shell) was also evaluated as a larvicide. The results are presented in this publication.

EVALUATION AS LARVICIDES.

RELATIVE TOXICITY OF ORGANOPHOSPHORUS INSECTICIDES TO *C. FATIGANS* LARVAE.

The method recommended by the World Health Organization (1959) for assessing the susceptibility of mosquito larvae to insecticides was used for determining the relative toxicity of the compounds. The compounds tested were baytex, trithion, diazinon, malathion and phosdrin. p-p′ DDT was also included for purposes of comparison.

Various concentrations of the insecticides were prepared by mixing insecticidal solutions in alcohol (1 ml.) and 250 ml. of tap water in glass beakers (capacity 500 ml.). Batches of thirty third-instar (late) *C. fatigans* larvae* were introduced into each beaker and exposed to the insecticide for 24 hours, after which mortality in them was recorded. The water temperature during the exposure period varied between 24 and 30°C. About 60 to 150 larvae were tested at each concentration of the different insecticides. The percentage mortality in larvae, obtained at various concentrations of the insecticides (concentration expressed as parts per million), are shown in Table I.

The results obtained were analysed by the probit analysis method (Finney, 1952). The equations of the fitted lc-pr lines, along with median lethal concentration (MLC) and regression coefficient values, are given in Table II. The data obtained showed that, of the insecticides tested, baytex and trithion were most toxic to *C. fatigans* larvae. Both these compounds had an identical MLC value of 0·003 ppm. and were, therefore, equally toxic to mosquito larvae. The MLC of phosdrin was 0·021 ppm. which was found to be next in order of toxicity. The MLCs of diazinon and malathion were 0·035 and 0·031 ppm. and these compounds, therefore, were about one-tenth as toxic as baytex and trithion. DDT with the MLC value of 0·3 ppm. was about one-hundredth as toxic as baytex and trithion and, therefore, out of the compounds tested DDT was least toxic as a larvicide.

From Table II it may also be seen that malathion had the highest regression coefficient value (4·9±0·6). The regression coefficient values of baytex, diazinon and phosdrin varied from 3·04 to 3 9. Trithion had the smallest regression coefficient value of 2·0±0·2, indicating thereby that the widest range of dosages of this

* Obtained from the colony maintained at the Malaria Institute of India, Delhi.

compound was required to increase the kill as compared to the other organophosphorus compounds tested. It may also be inferred that for 100 per cent level of larval mortality, which should be the aim of effective control programme, baytex will prove more toxic than trithion.

TABLE I.

Percentage mortality in C. fatigans *larvae at different concentrations of different insecticides.*

INSECTICIDES.											
BAYTEX :		TRITHION :		DIAZINON :		MALATHION :		PHOSDRIN :		DDT.	
Concentration (ppm.).	Per cent mortality.	Concentration (ppm.).	Per cent mortality.	Concentration (ppm.).	Per cent mortality.	Concentration (ppm.).	Per cent mortality.	Concentration (ppm.).	Per cent mortality.	Concentration (ppm.).	Per cent mortality.
0·0019	30·0 (90)	0·00095	15·6 (90)	0·015	10·0 (90)	0·015	12·5 (120)	0·0078	10·0 (90)	0·125	30·7 (150)
0·0039	63·3 (150)	0·0019	38·3 (120)	0·03	43·3 (90)	0·03	46·0 (150)	0·015	33·6 (150)	0·25	48·7 (150)
0·0078	88·3 (90)	0·0039	53·3 (120)	0·06	76·6 (90)	0·06	97·1 (90)	0·03	76·0 (90)	0·5	62·7 (150)
0·0156	100·0 (60)	0·0078	90·3 (120)	0·12	100·0 (60)	..	..	0·06	100·0 (90)	..	..
..	..	0·0156	93·3 (120)	..	..	..	..	..	..	..	..

Note :—1. Figures in parenthesis denote the number of larvae used in the test.
2. Larval mortality in control tests was nil in all the cases.

TABLE II.

Equation of the fitted lc-pr line, median lethal concentration with fiducial limits and regression coefficient values regarding C. fatigans *larvae exposed to different insecticides.*

Insecticide.	Equation of the fitted lc-pr line.	MLC (ppm.)	Fiducial limits.	Regression coefficient.	Factor by which the dosage was multiplied.
Baytex	$Y=3{\cdot}5366+3{\cdot}04x$	0·003	0·0027 – 0·0034	3·04 ± 0·3	1,000
Trithion	$Y=4{\cdot}9091+2{\cdot}0\ x$	0·003	0·0028 – 0·0034	2·0 ± 0·2	1,000
Diazinon	$Y=3{\cdot}0405+3{\cdot}5\ x$	0·035	0·032 – 0·04	3·5 ± 0·4	100
Malathion	$Y=2{\cdot}6019+4{\cdot}9\ x$	0·031	0·028 – 0·033	4·9 ± 0·6	100
Phosdrin	$Y=-0{\cdot}1192+3{\cdot}9x$	0·021	0·019 – 0·023	3·9 ± 0·3	1,000
DDT	$Y=3{\cdot}0878+1{\cdot}4\ x$	0·23	0·19 – 0·28	1·4 ± 0·1	100

RELATIVE TOXICITY OF ORGANOPHOSPHORUS INSECTICIDES TO *C. FATIGANS* PUPAE.

The relative toxicity of baytex, trithion, diazinon, malathion and phosdrin was also assessed against *C. fatigans* pupae. The same technique as described for the larval tests was followed, with the modification that instead of 24 hours the exposure period in this case was reduced to 4 hours. Thirty *C. fatigans* pupae were exposed for 4 hours to each of the concentrations of insecticides tested. After exposure, they

were washed with water and kept in clean water (without any insecticide) under observation for 24 hours when mortality in them, as well as in the adult mosquitoes hatching from them, was recorded (Table III).

The mortality data of both pupae and adults were combined and taken as the total kill produced by the insecticides*. The results were analysed by probit analysis method. The median lethal concentration and regression coefficient values of various insecticides are depicted in Table IV. It may be seen from the results obtained that, of the insecticides tested, baytex and trithion were most toxic to pupae (results similar to their larval toxicity). The MLC of 0·58 ppm. for baytex was about 190 times of the MLC (0·003 ppm.) obtained against the third-instar larvae. Other insecticides, i.e., diazinon, malathion and phosdrin were found to be about equal in their toxicity to pupae. The MLCs varied from 2·1 to 2·5 ppm. The dosages of these compounds, required to produce the toxic effect on pupae, were very high.

FIELD EVALUATION OF ORGANOPHOSPHORUS INSECTICIDES AS LARVICIDES.

Field studies to assess the efficacy of organophosphorus compounds as larvicides against culicine larvae were conducted during March-April, 1960, in a number of stagnant water collections near Ghaziabad (Meerut District, Uttar Pradesh). The compounds tested were baytex, trithion, malathion and phosdrin. Their 20 per cent emulsifiable concentrates were prepared in xylol, using triton-x 100 as the emulsifying agent. The requisite concentration of ready-to-use emulsion was prepared by dilution with water for each dosage of the insecticide.

The rate of application of the ready-to-use formulation was kept constant in each case at one gallon per 100 sq. yds. of water surface and the applications were made with a stirrup pump fitted with a fish tail nozzle.

For the estimation of larval density, dips with a laddle (3·5 inch diameter) were made along the edges of each breeding place. At least five dips were taken in each of the breeding places. Prior to initial insecticidal application, profuse mosquito breeding was observed in the experimental water collections and, therefore, the average larval density per dip was estimated approximately. Dips were taken daily after treatment till such time that pupae were observed in the water collections.

As in actual practice mosquito breeding places require retreatment one day before the appearance of pupae, the number of days for the initial appearance of pupae minus one was considered to be the period for which a particular insecticidal dosage was effective.

During the course of these studies, freshly-hatched larvae were observed in most of the breeding places one day after treatment. The compounds, therefore, did not exhibit any significant ovicidal action against *C. fatigans* at the dosages applied. In certain breeding places, the first-instar larvae developed in a normal

* Even when adult mortality was excluded and pupal mortality data alone were analysed, the values obtained were similar to the ones derived from combined data.

TABLE III.

Percentage mortality in C. fatigans *pupae and adults at different concentrations of insecticides.*

BAYTEX :			TRITHION :			DIAZINON :			MALATHION :			PHOSDRIN :		
Concentration (ppm.)	Percentage mortality as pupae.	Percentage mortality as adults.	Concentration (ppm.).	Percentage mortality as pupae.	Percentage mortality as adults.	Concentration (ppm.).	Percentage mortality as pupae.	Percentage mortality as adults.	Concentration (ppm.).	Percentage mortality as pupae.	Percentage mortality as adults.	Concentration (ppm.).	Percentage mortality as pupae.	Percentage mortality as adults.
0·25	6·6 (60)	6·6 (60)	0·25	16·7 (120)	8·3 (120)	1·0	10·0 (90)	5·6 (90)	1·0	13·3 (90)	..	1·0	26·7 (90)	4·4 (90)
0·5	43·3 (90)	21·1 (90)	0·5	33·3 (60)	25·0 (60)	2·5	35·5 (90)	6·7 (90)	2·5	35·3 (90)	2·2 (90)	2·5	34·0 (90)	11·1 (90)
1·0	77·8 (90)	11·1 (90)	1·0	80·0 (120)	7·5 (120)	5·0	65·4 (90)	5·6 (90)	10·0	91·1 (90)	3·3 (90)	10·0	62·2 (90)	10·0 (90)
2·0	98·1 (90)	1·1 (90)	2·5	83·3 (60)	15·0 (60)	20·0	100·0 (90)	..	40·0	98·9 (90)	..	40·0	100·0 (90)	..

Note :—1. Figures in parenthesis denote the number of pupae used in the test.
2. Pupae and adult mortalities in control tests were nil in all the cases.

TABLE IV.

Equation of fitted lc-pr line, median lethal concentration with fiducial limits and regression coefficient values regarding C. fatigans *pupae exposed to different insecticides.*

Insecticide.	Equation of the fitted lc-pr line.	MLC (ppm.).	Fiducial limits.	Regression coefficient.	Factor by which the dosage was multiplied.
Baytex	$Y=2{\cdot}1821+4{\cdot}1\,x$	0·58	0·52—0·63	4·1±0·4	10
Trithion	$Y=3{\cdot}1215+2{\cdot}99x$	0·5	0·36—0·56	3·0±0·3	10
Diazinon	$Y=1{\cdot}632+2{\cdot}4\,x$	2·7	2·4 —3·2	2·4±0·2	10
Malathion	$Y=1{\cdot}4078+2{\cdot}4\,x$	3·1	2·5 —3·7	2·4±0·2	10
Phosdrin	$Y=3{\cdot}0041+1{\cdot}4\,x$	2·8	2·1 —3·6	1·4±0·1	10

way into a second-instar in a day or two (3-4 days after treatment) whereas in some of the places second-instar larvae were noticed after 6 to 15 days after treatment. The laying of eggs was found to be a regular phenomenon and freshly-hatched larvae were noticed almost daily in almost all the breeding places. The late appearance of second-instar larvae was, therefore, due to the persistent toxic effect of insecticides in the breeding places. Record was, therefore, kept of the number of days after which the second-instar larvae appeared in each of the experimental breeding places to assess the persistence of toxicity, if any. Due to the absence of ovicidal action, presence of first-instar larvae was not considered as an index of the effectiveness or otherwise of the treatment. First-instar larvae were, therefore, not included in the pre- and post-treatment data. The pre-treatment larval density, its reduction one day after treatment, and the period in days when second-instar larvae and pupae appeared, at various dosages of the insecticides in all the breeding places, are depicted in Table V.

The results obtained with the different insecticides are briefly discussed below.

BAYTEX.

Baytex was tried at dosages of 0·05, 0·1, 0·3 and 0·6 lb. per acre of water surface. In all, twelve water collections were treated with this insecticide. From the data it may be seen that its application at dosages of 0·1, 0·3 and 0·6 lb. per acre of water surface gave hundred per cent reduction in the larval density. A large number of dead pupae were observed in all the breeding places one day after treatment but their reduction was not markedly apparent from their average number per dip. The likely reason being that the proportion of larvae as compared to pupae was very high before treatment as compared to after treatment. The sampling method used, therefore, could not possibly provide any definite estimate about the relative reduction in the population of pupae. Moreover, after the elimination of larvae from the breeding places, the pupae concentrated at the various spots in the breeding areas which were usually sampled when the dips were taken and, therefore, pupae were recorded in large number. So this method could not give any definite idea about the toxic effect of insecticides on the pupae.

In the water collections treated at a dosage of 0·05 lb. of insecticide per acre of water surface, second-instar larvae were noticed on the third or fourth day after treatment which is the normal period for the first-instar larvae to develop into the second-instar larvae; thereby showing that at this dosage the compound did not show persistence of toxicity. The pupae were noticed after seven to nine days of treatment. At this dosage, therefore, retreatment of the breeding places was required after about seven days of initial treatment.

The second instar larvae were noticed on the sixth day after treatment in the breeding places which were treated at a dosage of 0·1 lb. of insecticide per acre of surface though the first-instar larvae were observed even on the first and second day after treatment. This clearly showed that baytex, when applied at this dosage,

TABLE V.

Effectiveness of various organophosphorus compounds as larvicides in the field.

Insecticide.	Dosage lb./acre.	Pretreatment average density per dip:		Percentage reduction after one day of treatment:		Days after treatment on which:			Remarks.
		Larvae.	Pupae.	Larvae.	Pupae.	1st instar larvae appeared.	2nd instar larvae appeared.	Pupae appeared.	
Baytex	0·05	1,200	90	99·92	22·0	1	3	7	
	,,	1,600	400	99·25	25·0	2	4	8	
	,,	1,000	200	100·0	60·0	1	3	7	
	0·1	1,785	8	100·0	100·0	4	6	11	
	,,	1,600	10	100·0	100·0	2	5	9	
	,,	2,050	150	99·99	34·6	1	5	9	
	0·3	940	330	100·0	36·3	1	9	14	
	,,	1,000	114	100·0	73·0	4	10	15	
	,,	750	60	100·0	100·0	..	..	..	Pit dried up.
	0·6	1,100	150	100·0	94·7	1	15	20	
	,,	800	100	100·0	98·0	2	15	20	
	,,	1,300	56	100·0	94·0	9	16	20	
Trithion	0·1	1,400	270	99·3	63·0	1	3	8	
	,,	2,180	180	95·0	59·0	..	..	..	Pit dried up.
	,,	1,700	300	100·0	66·6	1	2	7	
	,,	728	80	100·0	0·0	1	3	6	
	0·3	1,650	120	100·0	88·9	2	6	9	
	,,	1,345	150	100·0	97·7	1	3	7	
	,,	1,600	200	100·0	87·5	3	6	9	
	,,	997	48	100·0	60·0	1	6	9	
	0·6	1,670	50	100·0	90·0	1	5	9	
	,,	1,130	50	100·0	80·0	1	4	8	
	,,	840	37	100·0	100·0	..	..	..	Pit dried up.
Malathion	0·1	650	50	93·9	30·0	..	..	..	Pit dried up.
	,,	1,620	85	100·0	82·0	2	4	8	
	,,	1,200	10	100·0	100·0	..	..	..	No fresh breeding occurred.
	,,	1,130	40	87·5	25·0	1	3	6	
	0·3	1,500	80	99·7	100·0	1	3	8	
	,,	1,700	75	100·0	90·0	1	3	7	
	,,	1,300	10	97·4	50·0	..	..	..	Pit dried up.
	,,	1,440	40	100·0	81·3	..	..	..	,,
	0·6	2,500	80	100·0	96·3	1	4	8	
	,,	2,100	217	100·0	88·9	2	5	9	
	,,	1,140	81	100·0	55·0	1	3	8	
Phosdrin	0·1	1,200	76	95·0	66·0	1	3	7	
	,,	1,800	200	91·7	..	..	..	..	Pit dried up.
	,,	950	80	100·0	50·0	2	4	8	
	,,	1,750	50	94·5	80·0	1	4	8	
	0·3	1,250	370	100·0	35·0	1	3	6	
	,,	930	94	100·0	100·0	..	..	..	Pit dried up.
	,,	1,250	66	100·0	66·8	2	4	8	
	,,	1,120	400	100·0	87·5	2	4	7	
	0·6	1,340	82	100·0	86·8	..	..	..	No breeding occurred.
	,,	880	96	100·0	94·95	2	5	9	
	,,	1,480	104	100·0	100·0	1	4	8	

showed residual toxicity as a larvicide for about 3 days. Pupae were found in these breeding places on 9th to 11th day of treatment. At this dosage, therefore, the retreatment of the breeding places was required after about 9 days of the initial treatment.

In the case of breeding places treated at dosages of 0·3 and 0·6 lb. of the insecticide per acre of water surface, second-instar larvae appeared on tenth and fifteenth day of treatment respectively. The pupae at these dosages were recorded on the fifteenth and twentieth day of treatment, respectively. The first-instar larvae were noticed in a number of water collections almost daily during the period of observation. At these dosages, therefore, retreatment of the breeding places was required after about 15 to 20 days of the initial treatment.

From this it may be concluded that the initial toxic effect of different dosages, i.e., 0·1, 0·3 and 0·6 lb. of baytex per acre of water surface was observed to be the same but its residual effect as a larvicide increased with the increase in the dosage applied.

TRITHION, MALATHION AND PHOSDRIN.

Trithion, malathion, and phosdrin, each were tested at dosages of 0·1, 0·3 and 0·6 lb. of the insecticide per acre of water surface. About twelve places for each insecticide were treated. From the results it may be seen that the effect of all these insecticides was almost similar (Table V). The breeding places were found to be free from larvae* one day after treatment at dosages of 0·3 and 0·6 lb. of the insecticides per acre of water surface. When these insecticides were applied at a dosage of 0·1 lb. per acre of water surface, reduction in larval density varied from 87·0 to 100·0 per cent. These insecticides showed no persistent toxic effect and the water collections required retreatment every week.

EVALUATION AS ADULTICIDES.

RELATIVE TOXICITY OF ORGANOPHOSPHORUS INSECTICIDES TO *C. FATIGANS* ADULTS.

The relative toxicity of baytex, trithion, methyltrithion, diazinon and malathion was assessed against *C. fatigans* adults by using a technique similar to the one described by Busvine and Nash (1953).

Filter papers (Whatman No. 1, diameter 11 cm.) were impregnated with 1 c.c. of varying concentrations of solutions of technical insecticides in ethylene dichloride. The treated filter papers were cut to the requisite size to line 3 inch × 1 inch specimen tubes. Two to three days old glucose-fed *C. fatigans* (♀♀) were exposed to the impregnated papers for one hour, after which they were removed and kept under observation for 24 hours when their mortality was recorded. The percentage mortality figures at various concentrations of different insecticides tested are given in Table VI. The results were analysed by probit analysis method. The equations of fitted lc-pr lines, along with median lethal concentration (MLC) and regression coefficient values, are given in Table VII.

* This only included second, third and fourth-instar larvae.

TABLE VI.

Percentage mortality in C. fatigans (♀♀) at different concentrations of different insecticides.

INSECTICIDE.									
BAYTEX.		TRITHION.		METHYLTRITHION.		DIAZINON.		MALATHION.	
Concentration, per cent.	Corrected percentage mortality.	Concentration, per cent.	Corrected percentage mortality.	Concentration, per cent.	Corrected percentage mortality.	Concentration, per cent.	Corrected percentage mortality.	Concentration, per cent.	Corrected percentage mortality.
0·0003125	6·1 (108)	0·0625	9·7 (180)	0·0625	12·0 (108)	0·005	2·8 (72)	0·0039	8·3 (36)
0·000625	34·7 (216)	0·125	55·1 (180)	0·125	40·0 (108)	0·01	11·4 (108)	0·0078	38·9 (72)
0·00125	74·9 (216)	0·25	93·6 (180)	0·25	80·0 (108)	0·02	66·6 (108)	0·0156	84·7 (72)
0·0025	90·6 (256)	0·5	99·3 (180)	0·5	94·4 (108)	0·04	100·0 (36)	0·031	100·0 (72)
0·005	99·3 (144)		c=1·1*		c=2·5*		c=0*		c=0*
	c=0·46*								

* Insect mortality in control tests.

Note :—Figures in parenthesis denote the number of insects used in the test.

TABLE VII.

Equation of the fitted lc-pr line, median lethal concentration with fiducial limits and regression coefficient values regarding C. fatigans (♀♀) exposed to different insecticides.

Insecticide.	Equation of the fitted lc-pr line.	MLC (per cent).	Fiducial limits.	Regression coefficient.	Factor by which dosage was multiplied.
Baytex	Y= 1·8192+3·3x	0·0009	0·00085—0·001	3·3±0·2	10,000
Trithion	Y= 0·1500+4·5x	0·12	0·11 —0·13	4·5±0·3	100
Methyltrithion	Y= 1·2882+3·2x	0·15	0·13 —0·16	3·2±0·2	100
Diazinon	Y=—0·8346+4·4x	0·016	0·015—0·018	4·4±0·4	100
Malathion	Y= 0·8656+4·3x	0·009	0·008—0·01	4·3±0·5	1,000

From the data it may be seen that out of the insecticides tested, baytex with MLC of 0·0009 per cent was found to be the most toxic against *C. fatigans* adults; and trithion, which was found as toxic as baytex against larvae and pupae, was found to be the least toxic against the adult mosquitoes. Its MLC (0·12 per cent) was about hundred times that of baytex. The order of toxicity of methyltrithion (MLC 0·15 per cent) was also the same as that of trithion. The MLC of malathion was 0·009 per cent showing that this compound was one-tenth as toxic as baytex. Malathion was found to be slightly more toxic than diazinon (MLC 0·015 per cent). The regression coefficient values of baytex, trithion, methyltrithion, malathion and diazinon varied from 3·3 to 4·5 and did not differ much from one another. The regression coefficient values of organophosphorus compounds were quite high, indicating that a small range of dosages would produce partial and complete mortalities in test insects.

SUSCEPTIBILITY OF MULTI-RESISTANT *C. FATIGANS* TO BAYTEX AND DIAZINON.

C. fatigans from village Khuraji (Delhi) was reported to be highly resistant to DDT, dieldrin and gamma BHC (Sharma, 1958 *loc. cit.*; and Sharma and Joshi, 1960). Pupae obtained from this village were hatched into adults. Two to three days old glucose-fed *C. fatigans* (♀♀) were exposed for one hour to filter papers impregnated with baytex and diazinon by following the technique described above. The percentage mortality in insects was recorded after 24 hours of observation period. The results were analysed by probit analysis method and MLCs with regression coefficient values were determined (Table VIII).

From the data it may be seen that the MLC of baytex against resistant *C. fatigans* was 0·007 per cent. The MLC in the case of susceptible *C. fatigans* was found to be 0·009 per cent. These values did not differ significantly from each other. The MLC of diazinon (0·015 per cent) for resistant *C. fatigans* also did not differ significantly from the value obtained for the susceptible population (0·016 per cent).

These results indicated that *C. fatigans*, resistant to chlorinated hydrocarbon insecticides, did not show any increased tolerance towards baytex and diazinon.

RESIDUAL PERSISTENCE OF ORGANOPHOSPHORUS INSECTICIDES ON MUD SURFACES.

Mud panels (6 inch × 6 inch) were prepared from the clay used in the construction of rural houses. The panels, when completely dry, were sprayed with suspensions in water prepared from water dispersible powders* of baytex, trithion, methyltrithion, diazinon and malathion. Dosage of application in each case was 100 mg. of the insecticide per sq. ft. of surface. Two to three days old laboratory-reared *C. fatigans* (♀♀) were exposed to the treated panels for one hour in accordance with the technique described by Pal (1951). Twenty insects were used in each test.

*The water dispersible powders used were baytex-40W (40 per cent), trithion 50 per cent, methyltrithion 50 per cent, diazinon 50 per cent and malathion 25 per cent.

TABLE VIII.

Results of susceptibility tests on the DDT- and dieldrin-resistant C. fatigans (♀♀) *against baytex and diazinon.*

Insecticide.	Concentration, per cent.	Percentage mortality.	Equation of the fitted le-pr line.	MLC (per cent).	Fiducial limits.	Regression coefficient.	Factor by which the dosage was multiplied.
Baytex	0·0003125	9·7 (72)	Y=1·3176+4·33x	0·0007	0·0005—0·001	4·3±0·4	1,0000
	0·000625	30·6 (72)					
	0·00125	91·7 (72)					
	0·0025	98·7 (72)					
Diazinon	·005	2·8 (36)	Y=1·0545+3·3x	0·014	0·012—0·021	3·3±0·5	100
	·01	27·8 (36)					
	·02	61·1 (36)					
	·04	100·0 (36)					

Note :—Figures in parenthesis denote the number of insectes used in the test.
Mortality in insects in control tests was nil in all the cases.

TABLE IX.

Corrected percentage mortality in C. fatigans (♀♀) *exposed on mud panels sprayed at the rate of 100 mg. per sq. ft. with different insecticides.*

Days after treatment.	INSECTICIDES :					Percentage mortality on control.
	Baytex.	Trithion.	Methyl trithion.	Diazinon.	Malathion.	
1	100·0	67·3	71·8	100·0	100·0	5·0
10	100·0	35·1	41·8	100·0	100·0	10·0
20	100·0	10·8	22·2	94·0	100·0	0·0
30	100·0			83·2	68·3	5·0
40	78·0			53·4	79·5	10·0
50	89·2			24·4	63·3	5·0
60	71·5			8·5	26·5	5·0
70	73·0			10·0	53·0	0·0

For each insecticidal application, five replicates were done. An average percentage mortality in mosquitoes was recorded after 24 hours holding. Same panels were used for subsequent tests at 10 days interval. The results are summarised in Table IX. It may be seen from the data obtained that trithion and methyltrithion exhibited significant toxicity towards test insects only for one day after spraying. After 10 days of spraying, mortality figures in mosquitoes were 35 and 43 per cent for trithion and methyltrithion respectively. In the case of baytex, 100 per cent mortality in test insects was obtained up to 30 days after spraying and even after 70 days the mortality was as high as 73 per cent. Mortality in test insects was above 50 per cent in the case of diazinon and malathion for 40 and 50 days after spraying, respectively.

FIELD EVALUATION OF BAYTEX AND TRITHION AS ADULTICIDES AGAINST *C. FATIGANS*.

Field investigations on the comparative efficacy of baytex (baytex-40W) and trithion (50 per cent water dispersible powder) against *C. factigans* were carried out in three villages of Meerut District, Uttar Pradesh, during March-June, 1960. These villages were completely isolated from each other and consisted mainly of houses with mud walls and roofs made of thatch or wooden planks. About 20 per cent of the structures in each of the villages were pucca. The number of houses, total population and previous history of insecticidal spraying of experimental villages are depicted in Table X. Two villages were sprayed with the two insecticides and the third was left unsprayed for purposes of comparison.

TABLE X.

Particulars of experimental villages.

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

SUSCEPTIBILITY STATUS OF *C. FATIGANS* OF THE AREA.

Prior to insecticidal spraying, the susceptibility levels of *C. fatigans* adults collected from village Garhi to baytex and from village Milkh to trithion were determined. Villages Garhi and Milkh were later sprayed with baytex and trithion, respectively.

The technique used for determining the insecticide susceptibility of adults was the same as used in the laboratory studies. Blood-fed *C. fatigans* (♀♀), collected from the two experimental villages, were used in these tests.

TABLE XI.

Susceptibility status to baytex and trithion of C. fatigans (♀♀) *collected from experimental villages.*

Insecticide.	Concentration, per cent.	Corrected percentage mortality.	Equation of the fitted lc-pr line.	MLC with fiducial limits per cent.	Regression coefficient.	Factors by which the dosage was multiplied.
Baytex	0·0003125	16·8 (36)				
	0·000625	25·1 (36)	Y=2·1866+2·3x	0·001 (0·0008–0·0012)	2·3±0·4	10,000
	0·00125	50·3 (36)				
	0·0025	86·6 (36)				
	0·005	100·0 (36)				
		C=5·6*				
Trithion	0·06125	70·0 (36)				
	0·125	100·0 (36)	..	<0·06125	..	..
	0·25	100·0 (36)				
	0·5	100·0 (36)				
		C=0*				

*Insect mortality in control tests.

Note :—Figures in parenthesis denote the number of insects used in the test.

The MLC of baytex (0·001 per cent) did not differ significantly from the value obtained in the laboratory studies with the normal strain of *C. fatigans* (0·0009 per cent). The MLC of trithion of the field strain (<0·06125 per cent) was even less than that obtained with the normal laboratory strain (0·12 per cent). These investigations showed that previous insecticidal spraying of villages did not result in increased tolerance towards the respective insecticide which was later sprayed in the village.

INSECTICIDAL FORMULATIONS AND DOSAGES APPLIED.

Both baytex and trithion were sprayed at the rate of 100 mg. of insecticide per sq. ft. of surface. Ready-to-use suspensions in water were prepared from water dispersible powders of baytex (baytex 40W) and trithion (50 per cent). Spraying was carried out with stirrup pumps fitted with fish tail nozzles by experienced workers. In the case of village Milkh, all the structures, except 3, were sprayed with trithion ; whereas only about 80 per cent of the houses in the other village, Garhi, could be sprayed with baytex*. Much difficulty was experienced while spraying ready-to-use formulation (5 per cent suspension) of baytex because it was highly viscous.

ENTOMOLOGICAL DATA.

During the period of these investigations, mosquito catches from the experimental villages were found to consist almost exlusively of *C. fatigans*.

* The whole village could not be sprayed because sufficient quantity of the insecticide was not available.

In each of the experimental villages, six suitable catching stations were established. In each treated village, three catching stations were left unsprayed. Mosquito catches were made from all catching stations by means of a sucking tube. The mosquitoes so caught were kept in cages in suitable unsprayed houses in the villages for recording the 24 hours' survival rates in them. The gonotrophic condi tion of female mosquitoes, i.e., unfed, fully fed, half gravid and gravid were recorded in the case of mosquitoes caught from sprayed and unsprayed catching stations (Tables XII, XIII, XIV).

TABLE XII.

Number of mosquitoes caught (average of 3 catching stations) and ratio of mosquitoes caught from sprayed and unsprayed catching stations.

Days after treatment.	BAYTEX 100 mg. per sq. ft.			TRITHION 100 mg. per sq. ft.			UNSPRAYED: COMPARISON.
	Sprayed catching stations.	Unsprayed catching stations.	S/Us.	Sprayed catching stations.	Unsprayed catching stations.	S/Us.	Catching stations.
−7	130·5	118·2	1·1	62·7	69·0	0·91	73·7
0	128·0	95·0	1·3	69·0	74·4	0·93	85·0
Sprayed							
1	0·0	21·3	..	5·0	32·0	0·16	..
5	2·0	24·7	0·08	13·3	122·0	0·11	77·9
8	6·7	45·0	0·15	73·7	121·0	0·61	234·0
15	10·3	56·0	0·18	105·3	162·0	0·65	127·0
21	11·7	85·0	0·14	143·0	152·3	0·94	188·0
28	21·7	66·0	0·33	79·0	85·3	0·93	144·3
35	16·3	53·0	0·38	69·7	67·7	1·03	110·0
42	9·0	65·7	0·14	72·6	75·3	0·96	81·6
50	12·0	51·7	0·23	..	..	..	67·3
57	11·7	38·3	0·30	..	..	..	24·0

S = Sprayed. Us. = Unsprayed.

The results obtained with the two insecticides are briefly discussed below :

BAYTEX.

In the village treated with baytex, density of mosquitoes came down to nil in sprayed catching stations. It remained at a very low level during 57 days' (8 weeks) period of observation, after which there was natural decline in the mosquito population of the area. There was considerable reduction in mosquito density in the unsprayed catching stations also. In the unsprayed catching stations, the mosquito density rose to 50 per cent level of the pre-spray density in about 8 days, but as compared to the density in the unsprayed village it was quite low (Graph 1).

Twenty-four hours' survival rate data of the mosquitoes collected from the sprayed catching stations showed that no mosquito survived up to 21 days after spray (Table XIII). After 28 days, the survival rate amongst *C. fatigans* (♀♀) was 1·7 per cent which increased in subsequent observations and by 57 days the survival rate was 34·4 per cent. The survival rate amongst the male mosquitoes collected remained nil up to 42 days of treatment and by 57 days it was 15·8 per cent.

TABLE XIII.

24 hours' survival rate in mosquitoes collected from sprayed and unsprayed catching stations.

Days after treatment.	BAYTEX, 100 mg. per sq. ft.				TRITHION 100 mg. per sq. ft.				UNSPRAYED COMPARISON:	
	Sprayed catching stations.		Unsprayed catching stations.		Sprayed catching stations.		Unsprayed catching stations.		Catching stations.	
	Male.	Female.	Male.	Female.	Male.	Female.	Male.	Female.	Male.	Female.
1	..	..	0·0 (17)	0·0 (47)	0·0 (5)	0·0 (10)	0·0 (40)	0·0 (56)	..	..
5	0·0 (2)	0·0 (4)	0·0 (25)	0·0 (49)	0·0 (12)	17·85 (28)	17·2 (128)	35·2 (138)	96·4 (55)	98·6 (179)
8	0·0 (6)	0·0 (14)	12·8 (47)	43·2 (88)	11·4 (88)	30·1 (133)	96·9 (159)	98·9 (184)	91·7 (256)	96·3 (446)
15	0·0 (4)	0·0 (27)	18·8 (64)	57·7 (104)	100·0 (129)	100·0 (187)	100·0 (198)	100·0 (287)	93·1 (160)	96·8 (221)
21	0·0 (5)	0·0 (30)	83·3 (66)	94·2 (189)	91·3 (185)	93·4 (244)	88·3 (187)	94·8 (270)	93·1 (204)	97·8 (360)
28	0·0 (9)	1·7 (56)	87·6 (57)	73·1 (141)	89·9 (79)	96·8 (158)	85·8 (84)	93·3 (172)	76·2 (165)	95·7 (268)
35	0·0 (18)	6·4 (31)	91·7 (48)	93·7 (111)	87·0 (69)	92·9 (140)	82·0 (161)	95·1 (142)	82·4 (102)	95·2 (228)
42	0·0 (10)	23·3 (17)	67·7 (62)	62·2 (135)	91·3 (69)	86·6 (149)	83·6 (67)	91·9 (159)	61·3 (93)	90·9 (152)
50	10·0 (10)	15·6 (26)	68·8 (48)	86·0 (107)	..	..	..	..	85·7 (68)	92·2 (134)
57	15·8 (12)	34·4 (23)	87·5 (35)	90·0 (80)	..	..	..	..	83·0 (28)	92·5 (44)

Note :—Figures in parenthesis denote the number of mosquitoes kept for recording the survival rate.

TABLE XIV.

Percentage of gravid and half-gravid mosquitoes in relation to total mosquitoes collected.

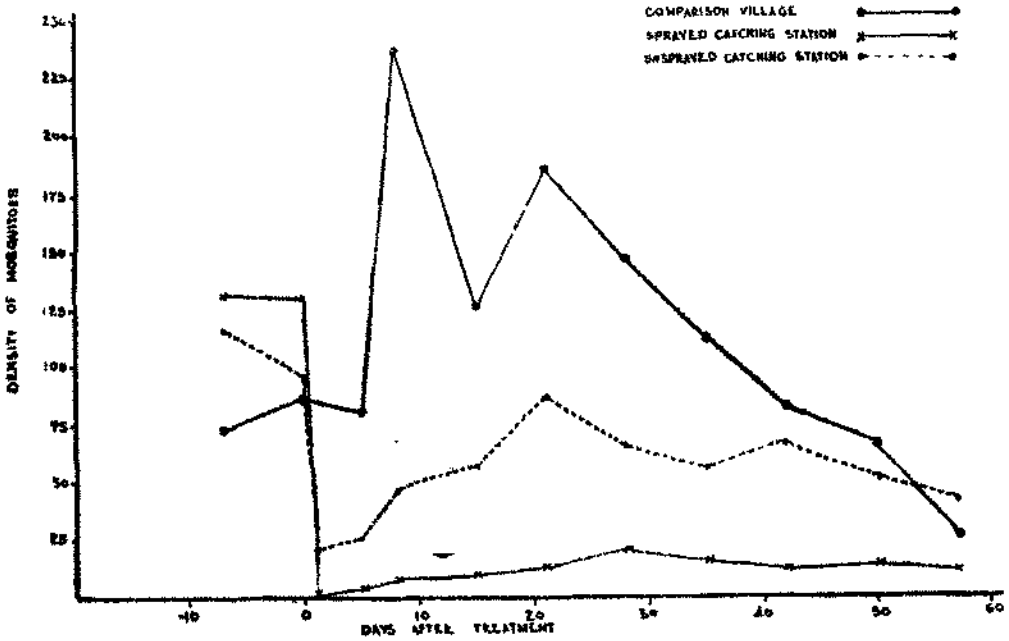
Days after treatment.	Baytex 100 mg. per sq. ft.										Trithion 100 mg. per sq. ft.										Unsprayed comparison.				
	Sprayed.					Unsprayed.					Sprayed.					Unsprayed.									
	UF.	FF.	HG.	G.	Per cent of HG & G.	UF.	FF.	HG.	G.	Per cent of HG & G.	UF.	FF.	HG.	G.	Per cent of HG & G.	UF.	FF.	HG.	G.	Per cent of HG & G.	UF.	FF.	HG.	G.	Per cent of HG & G.
0	77	67	83	44	46·9	50	52	70	20	46·9	41	53	54	20	44·04	51	72	35	8	25·9	6	125	50	31	38·2
Sprayed																									
1	..	..	..	..	..	9	17	18	3	44·7	3	2	4	1	50·0	28	10	16	2	32·1	104	43	113	17	46·9
5	3	1	..	..	0·0	29	14	5	1	12·2	8	6	8	6	50·0	82	23	26	7	23·9	317	80	31	18	11·0
8	10	3	1	..	7·1	38	26	13	11	27·3	108	3	10	12	16·5	154	10	10	10	10·9	43	45	104	29	60·1
15	18	4	4	1	18·5	23	28	43	10	51·0	54	70	46	17	33·7	83	58	104	45	50·9	85	185	60	30	25·0
21	18	10	1	1	6·7	31	100	41	17	30·7	70	113	60	21	30·7	56	88	89	37	46·7	38	96	77	57	50·0
28	29	20	5	2	12·5	35	52	34	20	38·3	38	69	36	15	32·3	30	65	54	23	44·8	54	89	55	30	37·2
35	10	17	2	2	12·9	36	28	30	17	42·3	14	57	48	31	52·7	11	54	53	24	54·2	25	61	42	24	43·4
42	6	6	4	1	29·4	21	66	32	16	35·6	20	73	34	22	37·6	22	80	35	22	35·8	16	72	26	20	34·3
50	8	10	6	2	30·8	16	53	23	15	35·5	..	..	..	..	..	..	..	..	..	..	11	22	8	3	25·0
57	5	10	5	3	34·8	12	28	28	12	50·0	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..

UF=Unfed FF=Fully fed HG=Half-gravid G=Gravid

Therefore, after 8 weeks of spray some loss in the biological efficacy of the insecticidal deposits was apparent. The proportion of half-gravid and gravid mosquitoes* of the total mosquitoes caught from sprayed catching stations remained low for a period of 35 days, after which there was a considerable rise (Table XIV, Graph 2). Considering all the criteria and keeping in view the fact that about 20 per cent of the houses in the village could not be sprayed, it may be concluded that baytex at 100 mg. per sq. ft. was effective for at least 8 weeks against *C. fatigans*.

GRAPH 1.

Density of C. fatigans (average of 3 catching stations) in catching stations of comparison village and sprayed and unsprayed catching stations of the village treated with baytex.



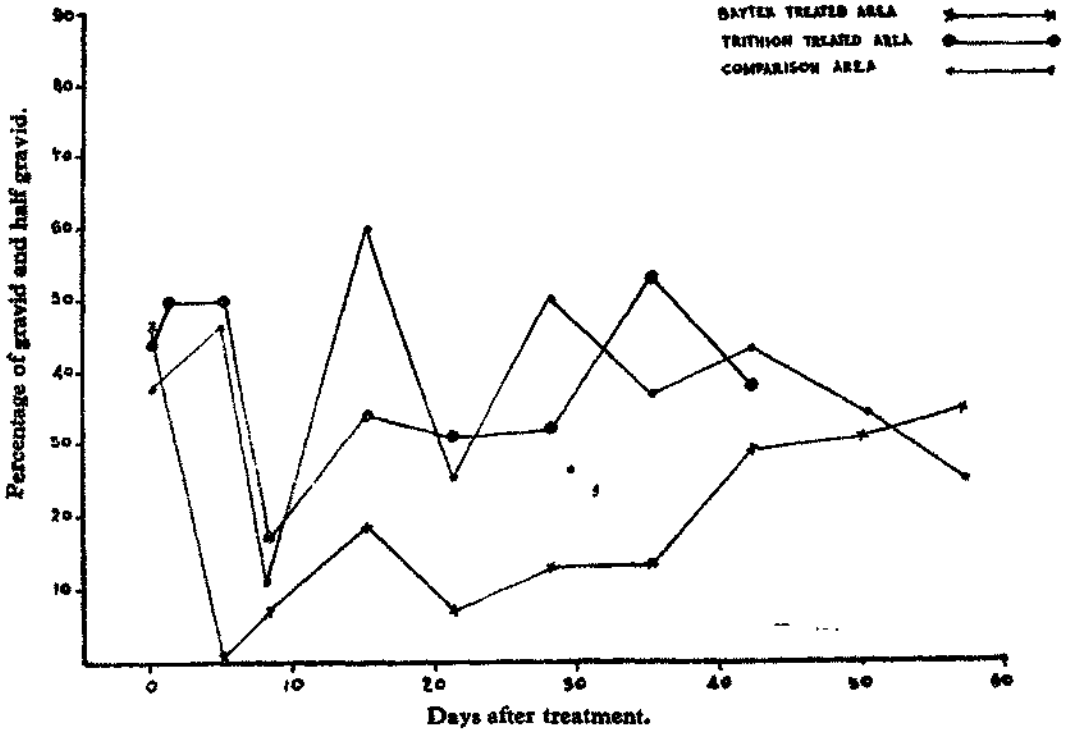
TRITHION.

In the village treated with trithion the density of mosquitoes both in the sprayed and unsprayed catching stations remained low only for a week after spray (Table XII). Twenty-four hours' survival rate of mosquitoes, collected from sprayed catching stations, showed significant increase on the 8th day and by the second week it was 100 per cent (Table XIII). There was not much difference in the density of mosquitoes in the trithion treated village and in the comparison village one week after spraying (Graph 3). The percentage of gravid and half gravid mosquitoes in relation to the total mosquitoes collected, also did not show any difference in the two villages (Table XIV, Graph 2).

* Increase in the proportion of gravid and half gravid mosquitoes indicated that some mosquitoes could survive for two days or more.

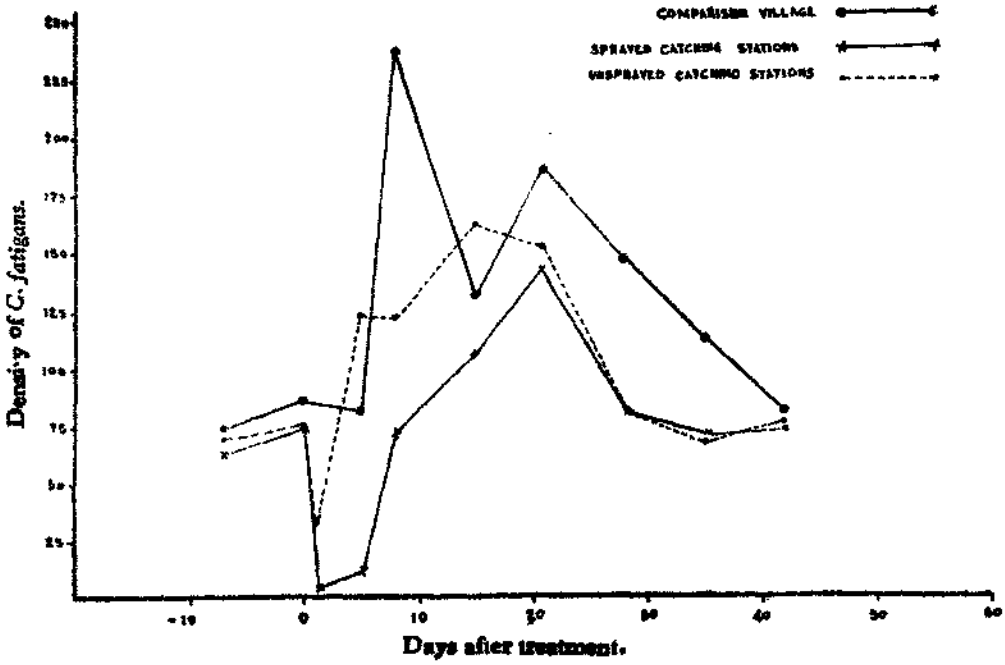
GRAPH 2.

Percentage of the gravid and half gravid mosquitoes in relation to the total mosquitoes collected from sprayed catching stations and catching stations of the comparison village.



GRAPH 3.

Density of C. fatigans (average of 3 catching stations) in catching stations of comparison village and sprayed and unsprayed catching stations of the village treated with trithion.



Trithion, therefore, was not effective as a residual spray against *C. fatigans.*

TOXICITY TO WILD LIFE.

In the village treated with baytex about 50 house sparrows, two pigeons and one crow were reported dead one day after spray. Their death most probably was due to their having fed on dead insects. There was quite a large population of rats in the village but no death amongst them was reported. In the trithion treated village, four house sparrows were similarly reported dead on the day following insecticidal spray. There was no poultry in the two villages and effect, if any, on these birds, therefore, could not be observed.

DISCUSSION.

Organophosphorus compounds have been reported to be quite effective for the control of insects resistant to chlorinated hydrocarbon insecticides. Diazinon, malathion, and dicaptnon, each at 100 or 200 mg. per sq. ft., were found to have sufficient residual toxicity against dieldrin-resistant *A. quadrimaculatus* (Mathis and Schoof, 1959; Labrecque *et al.*, 1958; and Gahan and Hofman, 1959). Investigations on this group of insecticides had not been carried out very extensively for the control of *C. fatigans.*

During the present studies on the relative toxicity of organophosphorus compounds against *C. fatigans* it was observed that baytex and trithion were equal in toxicity to *C. fatigans* larvae and were more toxic than diazinon, malathion and phosdrin, when these were compared at the Lc_{50} level. In view of the high value of regression coefficient in the case of baytex, for higher level mortality of 99 or 100 per cent., baytex is likely to prove more toxic than trithion. The pupae were much more difficult to kill than the larvae and required much heavier dosages. According to Brown (1951) also, amongst the various stages of holometabolous insects, leaving apart the eggs, the larvae are most susceptible and pupae the most resistant to insecticides. The order of toxicity of various compounds tested against pupae was similar to that observed against larvae. But the difference in the relative toxicity of various organophosphorus compounds against larvae and pupae was no of such at high order as was observed in the case of adult mosquitoes. Moreover the order of toxicity of various organophosphorus compounds was found to be different against adult mosquitoes from that against larvae and pupae. Baytex was found to be 100 times more toxic than trithion against *C. fatigans* adults though both were equally effective against larvae and pupae. The results showed that the relative toxicity of insecticides was considerably influenced by the developmental stage of the insect against which these were applied. Preliminary work on the ovicidal action against *C. fatigans* egg rafts also showed that baytex was considerably more toxic than trithion.

In the antilarval operations oils are generally preferred over synthetic insecticides. Experience in the field has shown that there is a great possibility of quick

selection of resistant strains of mosquitoes when the synthetic insecticides are used as larvicides. The application of oil, though effective, would be difficult from the point of view of procurement, storage, transportation and application. Before oils can be applied the water surfaces are required to be prepared by the removal of scum, aquatic and marginal vegetation and other floating materials. Moreover oils cannot be used over waste waters which are utilized for irrigation purposes. In such situations use of chemicals may be more economical and practical than oil application. There are many reports about the selection of resistant strains of mosquitoes in different parts of the world, wherever chlorinated hydrocarbon insecticides were used as larvicides (Sharma, 1958 *loc. cit.*; Brown, 1958 *loc. cit.*). Their use for this purpose, therefore, is usually not recommended. Present studies showed that baytex, trithion, phosdrin and malathion were quite effective for larval control at dosages of 0·1, 0·3 and 0·6 lb. per acre of water surface. Baytex at a dosage of 0·05 lb. per acre was also found to be quite effective. No residual effect was observed in the case of trithion, phosdrin and malathion. Therefore when these insecticides are used, water collections are to be retreated every week for effective larval control. Toxicity of baytex was found to persist and at a dosage of 0·6 lb. per acre of water surface the kill in the larvae was observed up to 15th day after treatment. Reapplication of this insecticide at 0·6 lb. per acre of water surface may, therefore, be carried out about every 3 weeks. Synthetic insecticides as larvicides are principally used for their immediate toxic effect and for weekly application of larvicides, therefore, baytex may be used at a dosage of 0·05 lb. per acre of water surface or the other insecticides may be employed.

Instances have been reported of *Culex tarsalis* and *Aedes nigromaculis* becoming resistant to malathion and parathion respectively when these compounds were used as larvicides (Gjullin and Isaak, 1957 ; and Lewallen and Brawley, 1958). Therefore when organophosphorus insecticides are put into large scale use as larvicides, this possibility and their toxic hazards have to be kept in view.

In certain rural areas, where implementation of antilarval measures is difficult use of residual insecticides against *C. fatigans* adults may be preferred. None of the chlorinated hydrocarbon insecticides have proved useful because of selection of resistant strains of *C. fatigans* after they have been in use for sometime. There is urgent need of an insecticide by which its effective control can be achieved. Amongst the organophosphorus compounds tested, baytex was found to be the most effective as a residual spray against *C. fatigans*. The residual toxicity of this compound at a dosage of 100 mg. per sq. ft. was found to last for at least 8 weeks. More extensive trials are indicated to explore the potentialities of baytex as residual spray for the control of mosquitoes. Reports of resistance in insects to organophosphorus compounds also are being received in increasing numbers as their use is extended. However, these compounds have an advantage over chlorinated hydrocarbons, i.e., resistance in their case is comparatively unstable and its quick reversion is possible if the use of the particular insecticide is discontinued. Moreover

insects, resistant to one organophosphorus compound, do not show any significant increase in tolerance towards any other compound of this group (March, 1958 : 1959). If these assumptions are tenable a few insecticides of this group can be utilized for the effective control of *C. fatigans*. In case of appearance of tolerance in mosquitoes to any one compound, the residual spraying of houses can be substituted by some other insecticide of the group. However, the data available are meagre at present and detailed investigations are needed to understand these aspects of the problem properly.

SUMMARY.

Laboratory and field studies in the evaluation of a number of organophosphorus compounds, i.e., baytex, trithion, methyltrithion, diazinon, malathion and phosdrin as larvicides and adulticides, for the control of *C. fatigans*, are described.

Tests were carried out in the laboratory to assess the relative toxicity of these compounds against larvae and pupae of *C. fatigans*. Trithion and baytex were found to be more toxic than the other compounds tested both against larvae and pupae.

Baytex, trithion, malathion and phosdrin, when tested in the field as larvicides, were found to be effective for the control of *C. fatigans* at dosages of 0·1, 0·3 and 0·6 lb. per acre of water surface. Baytex at a dosage of 0·05 lb. per acre was also found to give satisfactory control. In the case of trithion, malathion and phosdrin no persistent toxic effect was observed even at the highest dosage applied and the breeding places required retreatment every week for the effective control of *C. fatigans*. Residual effect was observed in case of baytex and the persistence of toxicity to larvae increased with the increase in the dosage applied.

Baytex, as adulticide, was found to be the most toxic of all the compounds tested. Trithion, which was observed to be as toxic as baytex against larvae and pupae of *C. fatigans*, was one-hundredth as toxic as baytex when tested against adults. *C. fatigans* (♀♀), resistant to DDT and dieldrin, did not show any increased tolerance towards baytex and diazinon. Laboratory studies on the residual effectiveness of baytex, trithion, methyltrithion, diazinon and malathion, each at a dosage of 100 mg. of insecticide per sq. ft., showed baytex to be the most persistent of all the insecticides tested.

In the field, two insecticides, i.e., baytex and trithion, each at a dosage of 100 mg. per sq. ft., were evaluated as a residual spray against *C. fatigans*. Trithion was not found to be effective at all as a residual insecticide for the control of *C. fatigans*. The toxic effect was found to last for at least eight weeks against *C. fatigans* in the village sprayed with baytex.

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PERIODICITY OF MICROFILARIAE.

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In 1879, Manson found that the microfilariae of *Wuchereria bancrofti* were numerous in the blood of infected patients during the night but they become rare or absent during the day. This phenomenon of alternate increase and decrease in number according to a 24-hour cycle is known as the periodicity of microfilariae. It has been found to occur to a greater or lesser extent in most (but not all) species of filarial worms and it is clearly of fundamental importance in their biology. It is linked with the transmission by arthropod vector, the period of swarming in the blood being timed to coincide with the habitual time of biting by the vector. The earlier literature on the subject was reviewed by Hawking and Thurston (1951*b*) and by McFadzean and Hawking (1956) and reference to these reviews will be employed in the present article to limit repetition.

A typical curve for the microfilariae of *W. bancrofti* is shown in Graph 1. It should be noted that the microfilaria count has begun to increase at 8·00 p.m., some hours before the man retired to sleep, and it does not return to its minimal level until 10·00 a.m., some hours after the man has begun to walk about. There is a tendency for such curves to show two peaks with a small depression between them at about midnight or 2·00 a.m. It is not clear whether such a depression is an artefact (due to variation in sampling) or a genuine phenomenon (as is more probable). At any rate, it seems unimportant.

The first problem was :—What happens to the microfilariae when they are scanty in the peripheral blood ? There were two main theories (*a*) that the microfilariae were destroyed every morning and a new brood was produced every evening (Theory of Myers and of Lane) ; and (*b*) that the microfilariae migrated backwards and forwards between different parts of the body, their total number remaining approximately constant (Manson's theory).

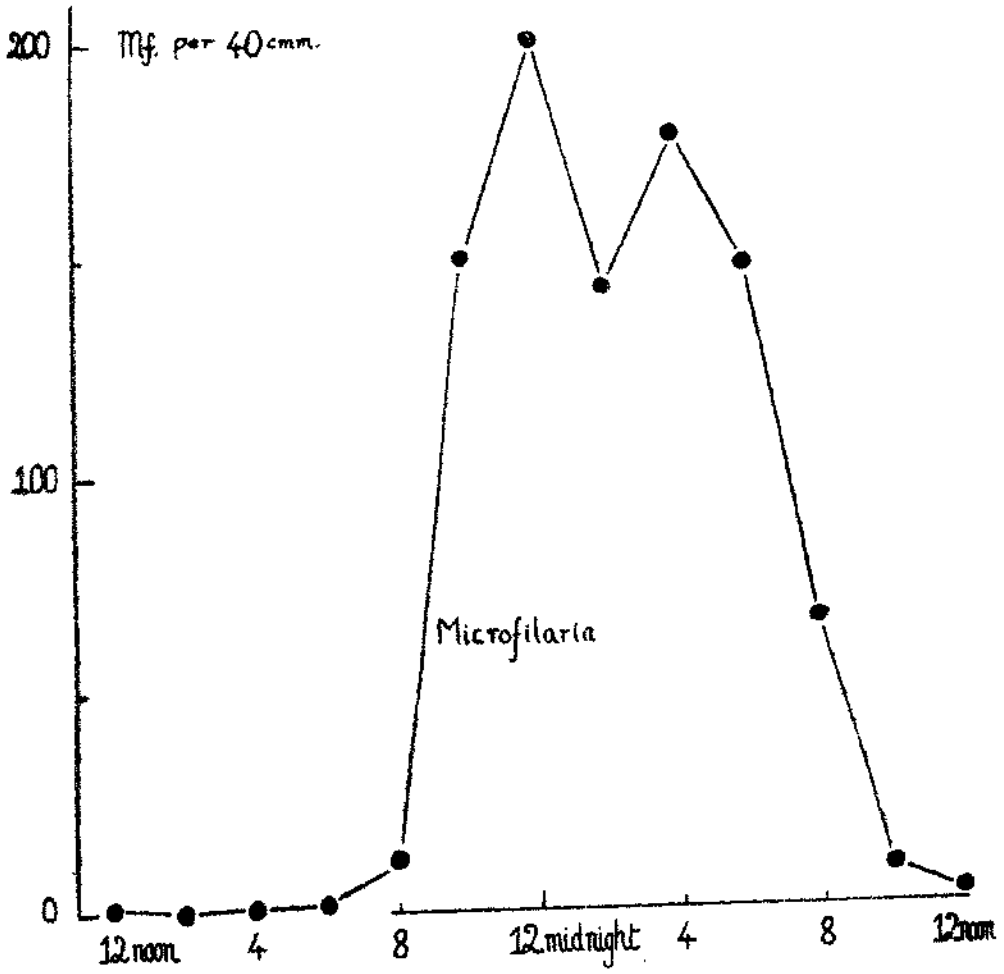
The latter theory has been proved to be correct, because :

(i) If microfilariae are transferred into a clean host they persist for many days in the absence of adult worms and they often show periodicity (Knott, 1935 ; Hawking, 1940 ; Hawking, 1953 ; and others).

(ii) By biopsies and autopsies on monkeys and dogs with filarial infections, it has been shown (*a*) that microfilariae accumulate in the lungs during the day time when they are scanty in the peripheral blood, and (*b*) that the increase of number in the lungs during the day is sufficient to account for the number which have disappeared from the blood (Hawking and Thurston, 1951*a*).

GRAPH 1.

Periodicity of *Microfilaria bancrofti* in human blood.



In histological preparations taken from lung biopsies or from freshly killed animals, the microfilariae are found in the capillaries and small blood vessels (presumably venules). It is difficult to tell their exact position during life, since the interruption of the circulation, when the biopsy is taken or the animal is killed, causes a great shift of the microfilariae down stream. This explains why at post-mortem examination microfilariae are often found in great numbers in the left auricle and ventricle (and even aorta) although they have been scanty or absent from the blood examined during life. Apparently the microfilariae normally lie in the capillaries or other small vessels of the lungs. How they hold themselves there against the circulation, is not known. They have no obvious hooks for holding on (especially if they are inside a sheath); and attempts to prove the presence of an

electrostatic charge, which might be attracted by an opposite charge in the capillary wall, have been unsuccessful (unpublished experiments).

The lung is the only organ in which there are more microfilariae by day than by night. In all other organs, the number of microfilariae is very much smaller than in the lungs and it passively follows the number in the blood. Consequently it is clear that the periodic fluctuation in the number of microfilariae in the peripheral blood is due to their accumulation in the lungs during the day-time and to their liberation therefrom at night. Two phases of the cycle may be distinguished. There is a passive phase in which the microfilariae are fairly evenly distributed throughout all the blood and hence they appear numerous in the peripheral circulation ; and there is an active phase, in which the microfilariae are concentrated in the lungs. The purpose of the passive phase is to give an opportunity for the microfilariae to be sucked up by an arthropod vector and it is timed accordingly ; (with microfilariae transmitted by nocturnal mosquitoes it occurs at night ; with *Loa loa* which is transmitted by the diurnal fly, *Chrysops*, it occurs by day). The purpose of the active phase (of concentration in the lungs) is less clear ; we must assume that lungs are the most favourable site in the body for the microfilariae—perhaps because of the high oxygen tension, etc. But if the microfilariae spent all their time in this favourable site, they would never meet a mosquito or get transmitted. Accordingly periodicity may be conceived as a compromise by which the microfilariae spend the daytime in the lungs benefiting from the good conditions there ; they come out at night and circulate in the peripheral blood so that they can be sucked up by a mosquito ; and in the morning if this has not happened, they return to the lungs to rest until the next night. But even in the night-time the lungs are never emptied of all their microfilariae ; even when the number in the blood is at its highest the lungs still contain 10-40 per cent of the whole body content. Duke (1960) has shown that when monkeys are first infected with *Loa* the microfilariae pass from the adult worms in subcutaneous tissues to the lungs and accumulate there for some time before they begin to appear in the peripheral blood ; he suggests that they may require a period of further development or maturation in the lungs before they are ready to participate in these periodic migrations. Probably the distribution of microfilariae between lungs and periphery depends upon a dynamic equilibrium rather than upon a static one, the number in the lungs at any given moment being the difference between those arriving and those departing ; the total number may remain constant for appreciable periods, but the individuals may keep changing.

[The accumulation of microfilariae in the lungs raises the question whether their presence produces any pathological lesions therein. In histological examinations of the lungs of patients, monkeys, dogs and other animals infected with filariae such lesions have been rare, although occasionally in dogs a few small granulomatous masses, containing disintegrating microfilariae, have been found (*see* Hawking and Thurston 1951*a*, Fig. 8). In two out of four human volunteers inoculated with infective larvae of *B. malayi* or *B. pahangi*, there was a dry cough which

developed at about the time (10th week) when microfilariae might be expected to reach the lungs ; this might be ascribed to inflammatory reactions directed against microfilariae in the lungs (Edeson, Wilson, Wharton and Laing, 1960). Furthermore in tropical eosinophilia, which now appears to be somehow due to nematode (filarial) infection, there seems to be an abnormal relation between the patient and the filarial worms, either due to the filariae being of non-human type, or due to an allergic state in the patient. In this condition the lungs (more than other organs) contain great numbers of eosinophilic granulomatous masses which may be formed around microfilariae (Webb, Job and Gault, 1960). Accordingly, it may be concluded that although the accumulation of microfilariae in the lungs usually does not cause damage, it may do so on certain occasions or in certain individuals.]

The periodic cycle of the microfilariae is orientated not to the alternation of day and night, but to the 24-hour habits of the host. This was originally shown by Mackenzie and it has since been confirmed by many others including Era (1959). When a man or animal is made to reverse his routine so that he sleeps by day and moves about by night, the periodicity of the microfilariae alters accordingly. This alteration takes about a week, showing that the microfilariae are not affected by sleeping and waking as such but only by the whole 24-hour rhythm of the body of the host. This 24-hour rhythm affects most of the functions of the body in man and higher mammals. Thus during the night the temperature falls, the carbon dioxide pressure rises, the oxygen pressure falls, the body becomes more acidic, the kidney secretes less water and chlorides, the adrenal is less active, etc. One or more of these 24-hour alterations presumably affects the microfilariae so that the periodic cycle is produced.

It has recently been suggested by Wang, Lin and Ch'en (1958) that the periodicity of microfilariae depends upon the size of the microfilariae relative to that of the capillaries of the lung. This seems unlikely, since (1) in histological sections the microfilariae always appear much narrower than the capillaries in which they lie, and (2) when the circulation of the living dog's mesentery is studied under the microscope, the microfilariae are seen to flash past without any hindrance. In any case the concentration of microfilariae in the lungs cannot depend upon any opening or shutting of capillaries by the host, since the microfilariae of *Loa* and of *Wuchereria*, which are of the same size and shape, behave quite differently in the same host, i.e., man. One swarms in the peripheral blood during the day and the other during the night. It is difficult to believe that the small blood vessels of a man in West Africa behave differently according to whether he is infected by *bancrofti* or by *Loa*. Similarly, there are some strains of *Loa* with diurnal periodicity in monkeys, and others with nocturnal periodicity in identical animals (Duke, 1960). Consequently, we believe that the accumulation of the microfilariae in the lungs (and conversely their liberation therefrom) depends upon the microfilariae themselves and not upon the host. The microfilariae must give an active response to some stimulus or stimuli provided by the 24-hour rhythm of their

environment. As said above, the nature of this active response, whereby the microfilariae hold themselves in the capillaries of the lung, is unknown.

We have spent much effort in trying to identify the effective stimulus in question (Hawking, 1956; McFadzean and Hawking, 1956). But the response to certain standard stimuli varies according to the species of microfilariae involved (*see* Table I); and it seems probable that the stimuli controlling periodicity may be different for different worms. In considering the data of Table I, it must be remembered that during the active phase of the cycle, the microfilariae are held in the lungs by their own active response. Therefore any agent, which is a general depressant to cellular activity, would be expected to depress this response, liberating microfilariae from the lungs and causing a rise in the microfilariae count. This explanation would cover the increases in the count which occur during anoxia and during anaesthesia with many species. Such effects on the count could be regarded as non-specific and unlikely to have special significance for the production of periodicity. Similarly conditions which cause dilation of cutaneous capillaries, e.g., local warmth or sweating, might lead to the flushing out of these capillaries and might cause small apparent falls in the microfilarial count as judged by puncture of the finger or ear; such effects would clearly lack real significance. On the other hand, stimuli which cause marked *falls* in the microfilaria count, i.e., accumulation of microfilariae in the lungs, are likely to be highly significant especially if the stimulus is physiologically bland and non-irritant. Judged by this criterion the effect of oxygen (and of exercise) in

TABLE I.

Comparison of the responses of various species of microfilaria to selected stimuli.

[The figures show the increase (+) or decrease (−) of the microfilaria count, calculated as a percentage of the maximum observed count].

Periodicity		Oxygen	Anoxia	Carbon dioxide	Anaesthesia	Exercise
Nocturnal	*W. bancrofti* (African)	−60	0	−18	0	−56
Absent	*W. bancrofti* var. pacifica	+38	+45	−14		−62
Nocturnal	*B. malayi*	−91		0		−22
Diurnal	*L. loa*	0	0	0	−60	0
Absent	*A. perstans*	0	0	0		0
Nocturnal	*Dirofilaria aethiops*	+35	+57	0	++	−22
Nocturnal	*D. immitis*	+67	+48	0	++	+40
Nocturnal	*D. repens*	+42	+88	0	++	+40
Absent	*D. magnilarvatum*	0	0	0	0	
Absent	*Monnigofilaria setariosa*	+26	+48	0	0 (Nembutal) +42	
Absent	*Dipetalonema vite*	+47	+17	0	+78	
Absent	*L. carinii*	0	0	0	−33	

Most of these figures are taken from the papers by the author already quoted. *D. magnilarvatum* is a filaria of monkeys described by Hawking (1959). *Monnigofilaria setariosa* is a filaria of mongooses (unpublished experiments); its microfilaria count did not respond to ether anaesthesia but increased by 42 per cent when nembutal was given.

lowering the microfilaria count of *W. bancrofti* and *B. malayi* is likely to be significant, and it deserves more detailed description. In the case of African *W. bancrofti*, an increase in the oxygen concentration of inspired air, produces a marked fall in the microfilarial count at night (i.e., a marked accumulation of microfilariae in the lungs). This effect is exerted by any concentration of oxygen above 230 mm. Hg. (normal air 150 mm. Hg.) and it begins within a few minutes of the oxygen being breathed ; the microfilaria count falls as low as 20-30 per cent of its maximum value, but it never falls as low as the normal mid-day level (approximately zero). Moderate falls in the microfilarial count are also produced by hyperventilation (which lowers alveolar CO_2 pressure). In the Pacific type of *W. bancrofti* (in which there is a slight increase of the microfilarial count during the daytime), breathing oxygen causes a rise of the microfilarial count by day (and possibly a fall by night) (Edeson, Hawking and Symes, 1957). With *Loa* (which has a diurnal periodicity) breathing oxygen has no significant effect. With *Dirofilaria aethiops* of monkeys or *D. immitis* of dogs, oxygen causes a rise of the microfilaria count. The sensitivity of *W. bancrofti* to oxygen is certainly curious ; but it is difficult in our present state of knowledge to frame an explanation of periodicity which depends upon it, for the fluctuations of oxygen pressure which occur naturally in human lungs during the 24-hour rhythm do not usually amount to more than 5 mm. Hg.

Numerous other stimuli have been investigated (for details *see* Hawking, 1956 ; McFadzean and Hawking, 1956), but apart from the stimuli mentioned above, none seemed to have a significant effect on the microfilarial count. Heparin was said by Yoeli (1957) to cause a partial release of microfilariae of *W. bancrofti* into the peripheral blood during the day, but this was not confirmed by Gilles and Williams (1958).

SUMMARY.

The daily periodic disappearance and reappearance of microfilariae in the peripheral blood is due to their alternate accumulation in the small vessels of the lung (disappearance)and their liberation therefrom (reappearance). This accumulation of microfilariae in the lungs is believed to be due to the microfilariae themselves. which give an active response to some stimulus provided by the physiological 24-hour rhythm of the host. The exact nature of this stimulus has not yet been identified, but it may have some connection with the oxygen pressure in the lungs.

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IMMUNITY TO INEECTION WITH *WUCHERERIA*.

BY

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THERE has been a considerable amount of speculation in the literature concerning the possibility of immunity to infections of *Wuchereria*, but there are few facts providing evidence either for, or against, its existence. Neither is there much evidence that the various immunological reactions which have been described, really reflect any true protective immunity. This paper is concerned solely with aspects of such protective immunity, and does not consider the immunological reactions, even though these are recognized to be of great importance from other points of view.

The lack of evidence for immunity to *Wuchereria* infections does not argue against its existence. The epidemiology of hookworm disease was studied for many years before sound knowledge of immunity and its profound relation to nutritional status was revealed. Once demonstrated, this immunity seemed so obvious that it immediately became important in the field of public health.

The word immunity has been used in many senses. Protection against infection should be clearly distinguished from protection against the effects of infection. Natural and acquired immunity, although conceptually distinct, are often indistinguishable as far as their effects are concerned. Either may theoretically be complete or partial, and either one or both combined may be involved in effects described as age immunity, sexual immunity, etc. Local immunity is often considered as distinct from parenteral immunity. Various types of immunity may affect, or be induced by, any one or several stages in the life history of the parasite. A number of mechanisms of immunity have been suggested with respect to helminths. Some of these involve antigen-antibody reactions. Whether all will eventually be shown to have this basis, is still uncertain.

Any attempt to present a classification of immunity must be unsatisfactory both because of our incomplete knowledge and because the effects are not all mutually exclusive. The classification which follows is presented merely as a framework for discussion.

(*A*) Protection from the effects of infection.

(*B*) Protection against acquisition, establishment or persistence of infection.

1. Protection against mosquito bites.

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2. Protection against penetration of the skin by infective larvae.
3. Enhancement in infected individuals of cellular reactions against larvae during migration.
4. Reactions associated with the presence of previous infection which retard the rate of growth and development of the parasite, either temporarily or permanently.
5. Reactions which reduce the fecundity of the parasites.
6. Reactions which reduce the length of life of the parasite.
7. Other or unspecified reactions producing various alleged effects attributed to "immunity".

(A) PROTECTION AGAINST THE EFFECTS OF INFECTION.

Kessel (1957) states that "the disabling effects of clinical filariasis in most tropical communities are difficult to evaluate exactly." Until more detailed knowledge of pathogenesis becomes available, it will be even more difficult to determine how much individuals vary naturally in their response to infection, or whether one infection may modify this response to subsequent infections and produce a reduction of the effects. There is some evidence that the converse is true, namely that some people can become so sensitized that the allergic or other manifestations of subsequent infections are more severe than those of primary infections. It can be argued that these increased effects are in themselves protective against further infection, but this is a subject appropriately discussed under other headings.

(B) PROTECTION AGAINST ACQUISITION, ESTABLISHMENT OR PERSISTENCE OF INFECTION.

1. PROTECTION AGAINST MOSQUITO BITES.

Individuals are often considered to differ in their attractiveness to mosquitoes. It is clear that they differ with respect to the reaction to bites, but the supposition that there is a variation in repellency or attraction does not seem to be supported by much objective data. Observations made under natural conditions are often subject to diverse interpretations, but experiments may be equally difficult to perform. Well controlled experiments would need to be very carefully designed to distinguish the effects of the specified factors from those resulting from epidemiological or environmental factors. Muirhead-Thomson (1954) has presented observations which seem to show that in parts of tropical Africa where *W. bancrofti* is anopheline-borne, older children and adults are bitten to a much greater extent than infants and very young children, whereas in regions where *Culex fatigans* is the vector, no such difference has been observed. He believes that these observations will explain the lower rate of microfilaremia in young children in many parts of the world. Whether the observed differences represent differences in attractiveness, or are based on environmental factors, is not clear.

2. PROTECTION AGAINST PENETRATION OF THE SKIN BY INFECTIVE LARVAE.

It is possible that there could be natural differences in the skins of various individuals or racial stocks which would affect the ease of penetration. On the basis of experiments in mice, Yokagawa (1939) states that "when the exudate of lymph resulting from a bite is poor and the skin is dry, the larva set free from the proboscis of the mosquito during the act of blood sucking remains practically inactive ; on the contrary, when much lymph is exuded, the larva immediately exhibits its inherent property of lymphotaxis, follows the lymph, and finally makes its way to the lymph spaces of the deeper tissues." If these conclusions could be shown to hold true for human skin, the facts might have a bearing on this aspect of natural or acquired immunity. This is a field in which further experimentation would seem to be indicated, both in connection with immunity, and because of the possible relationship with epidemiological factors associated with the degree of atmospheric humidity. Here individual variation in propensity to react to the bite might play an important role.

3. ENHANCEMENT IN INFECTED INDIVIDUALS OF CELLULAR REACTIONS AGAINST LARVAE DURING MIGRATION.

It is quite possible that an immune reaction develops against *Wuchereria* as it migrates through the skin, although to date no information on the point seems to be available. The demonstration by Sarles and Taliaferro (1936) that precipitates in and around larvae of *Nippostrongylus muris*, migrating through tissues of infected animals, were accompanied by increased cellular reaction leading to immobilization has been repeated for a number of species of nematodes. Similar oral precipitates have been shown to form *in vitro* on infective larvae of one filariid, *Litomosoides carinii*, when placed in serum of infected animals (Scott, 1952 ; Briggs, 1958). No precipitates were observed within the intestinal tract in this species. Evidence regarding the number completing migration (Macdonald and Scott, 1953) indicates that immobilization in the tissues does not play a major role in immunity to this species. Now that animal hosts are available for experimental infections with certain species of *Wuchereria*, we may expect to hear of results more nearly analogous to the situation in human tissues.

4. REACTIONS ASSOCIATED WITH THE PRESENCE OF PREVIOUS INFECTION WHICH RETARD THE RATE OF GROWTH AND DEVELOPMENT OF THE PARASITE EITHER TEMPORARILY OR PERMANENTLY.

As in the case of many other nematodes, the growth rate of one filariid, *L. carinii*, is retarded in superimposed infections (Macdonald and Scott, 1953) and in some cases even after the worms of the primary infection are dead (Macdonald and Scott, 1958). Bertram (1953) has shown that in super-imposed infections the ultimate length reached by the female worms is less than in most single infections. It may be questioned whether this latter effect is entirely due to the presence of a previous infection, or partly because of crowding, since in some heavy single infections the worms were also shorter than in highly infected animals. Some would

question our right to call this retardation and stunting as immunity, since they cannot see how the effect can be protective. On the other hand, the reaction apparently occurs only as a result of previous infection, and in this sense can be called an expression of immunity. It is doubtful whether it will ever be possible to determine whether a similar effect occurs in the human infections.

5. REACTIONS WHICH REDUCE THE FECUNDITY OF THE PARASITES.

Bertram (1953) has also shown a reduction in fecundity in superimposed infections. The comments made immediately above with regard to interpretation of stunting as immunity, could also be applied to reduction of fecundity. In addition, however, it is clear that if such a phenomenon exists in the human infections, it might easily be the explanation of some observations on reduced microfilarial counts in advanced cases, which are often interpreted as expressions of immunity. Without question it would also be of importance in the epidemiological aspects of infection.

6. REACTIONS WHICH REDUCE THE LENGTH OF LIFE OF THE PARASITE.

In many nematode infections one of the expressions of immunity is a reduced length of life of the parasites. In the case of *L. carinii* this is markedly true with respect to the natural immunity of the white rat (Olson, 1959), but it has been impossible to show whether or not it is true in acquired immunity of the normal host. Bertram (1953) has shown a shorter period of microfilarial production in the pleural cavity, but not any earlier death of worms. In view of these facts it will obviously be difficult, if not impossible, to show whether there is in human infections a reduced length of life of worms as a result of immunity. In other words, absence of microfilariae cannot be taken as evidence of death of worms. It is well established that many worms are eventually involved in tissue reactions leading to their destruction, but whether this tendency is enhanced as the result of previous infection is not clear.

7. OTHER OR UNSPECIFIED REACTIONS PRODUCING VARIOUS ALLEGED EFFECTS ATTRIBUTED TO "IMMUNITY".

A number of persons have made observations in epidemiological studies of human filariasis which apparently could not be explained in terms of environmental factors, and have sought to explain these observations in terms of immunity. Some of these cases would obviously fall into the categories discussed above if the underlying facts were sufficiently understood. Others would perhaps come under aspects of immunity not categorized in this limited classification.

A problem of longstanding interest has been the relative scarcity of infection with *W. bancrofti* among very young children in the Pacific. Buxton (1928) considered immunity as one possible explanation. "One may suppose the children in the Pacific have a considerable degree of natural immunity ; this cannot be disproved, but it does not appear probable." Another similar problem, the difference in infection rate between the sexes, has also been explained by postulating

immunity. Murray (1948), for example, states, "There is little doubt but that the lower rate of infection in women as compared with men is due to a greater immunity on the part of the female." Jachowski and Otto (1953) have reviewed additional literature on these problems. As a result of their extensive epidemiological investigations they conclude that in Samoa the observed age and sex differences in microfilaria rates can be satisfactorily explained on the basis of environmental factors. They imply that there is no need to postulate age or sexual immunity to explain the observed differences. Since then, Muirhead-Thomson (1954) has, as mentioned above, concluded that the age difference can be explained on the basis of differential biting habits of the mosquitoes. The important point is that when immunity is invoked as an explanation for such problems, it is merely for lack of a better explanation, and is without one shread of positive evidence.

Another problem of longstanding interest is the absence of microfilariae in the blood of many cases with severe elephantiasis. Manson (1898) mentions the problem and may have been the first to recognize it. Low (1908) was probably the first to present statistical evidence of this negative correlation. At the same time he emphasized the general positive correlation between the prevalence of microfilariae and clinical symptoms in the general population. Among the many others who have presented data on the paradox, Iyengar (1938) has supported his conclusions by a very large series of observations. He states, "While in the individual there is a negative correlation between the occurrence of filarial infection and that of disease, there appears to be a definite positive correlation between the incidence of filarial infection and of filarial disease in the community." Kessel (1957) has also come to similar conclusions on the basis of extensive studies based on the use of microfilarial density rates, and reviews some of the literature on the various explanations which have been offered.

Manson (1898) concluded that elephantiasis is a response to dead worms and causes a blockage of microfilariae unless infection is extensive or reinfection common. Bahr (1912) expressed a similar opinion as have many others, some of whom have emphasized different aspects of the many pathological variations described. Others have offered the counter-argument that there should be worms in other locations not so severely affected. Another argument is that since severe elephantiasis develops after many years of infection, the most severely affected individuals may have changed their habits to the extent that they are no longer exposed to reinfection and all of the original worms have died. Kessel (personal communication) believes this argument has no basis in the areas he has studied so intensively.

The explanations for the scarcity of microfilariae in severe cases of elephantiasis which are based on concepts of immunity can be roughly divided into three types. Napier (1944) states that "It seems that one must visualize a general reaction of an allergic or antibody nature on the part of the host." In a footnote explaining this statement he says that it seems possible that microfilariae retained

behind the obstruction in the lymphatic vessels or in the subcutaneous tissues are actively destroyed and thus provide "the necessary sensitizing (*sensu lato*) stimulus." In conditions such as chyluria and lymph varix the microfilariae reach the blood stream where they circulate until they are absolete and are subject to gradual absorption with other circulating debris. The implication seems to be that in elephantiasis, as contrasted to the other clinical manifestations, the destruction of trapped microfilariae sets up a reaction resulting in destruction of the microfilariae which are not trapped. Iyengar (1938) apparently has a similar opinion, but he believes that the adhesion phenomenon of Pandit *et al.* (1929) may be the mechanism on which the reaction is based.

The second type of explanation is similar in many respects, but the end result is visualized as prevention of reinfection rather than merely destruction of microfilariae. Jordan (1955) quotes Napier and points to the similarity of their views. He then states, "It is suggested that the low incidence of microfilaremia in elephantiasis associated with *W. bancrofti* may be due to the death or sterilization of most of the adult worms in the body brought about by some factor at present unknown. It may be that the changes which lead to elephantiasis promote an immunity reaction in the body preventing reinfection." He continues that this theory is not supported by the work of McFadzean (1953) who found little or no immunity to *L. carinii* in cotton rats. Our work with this species, and that of Bertram (1953 *loc. cit.*) mentioned above, indicate that there is a very definite immunity in cotton rats, even though it is of low level compared to that against some other nematodes. Comparisons of this type between animal and human parasites can hardly be very fruitful since the location in the body of the two types of parasites and the reactions to them are so different.

The third type of explanation is based on postulation of inherent differences in individuals. Markell (1957) states, "In the hypersensitive individual, a relatively few microfilariae or adult worms may produce tissue reactions leading to elephantiasis.....while the non-reactor may harbour large numbers of worms without any symptoms." Napier (1944), in another section of the paper mentioned above, discusses suggested explanations for differences in lesions in different individuals in terms which resemble the statement of Markell (1957 *loc. cit.*) just quoted. If the complication of sepsis is excluded, there are two factors: (*a*) tolerance of the individual to filarial metabolites, and (*b*) intensity of infection to which the individual is subject. Based on combinations of these factors the human hosts fall into the following four categories.

1. Tolerant individuals subject to few bites: little tissue reactions, few symptoms, microfilariae in the blood when worms mature.
2. Tolerant individuals subject to heavy infection: in time mechanical blockage of lymph nodes may cause static oedema or lymph varix but not necessarily lymphangitis or fever.

3. Intolerant individuals subject to few bites at long intervals : little damage to distal lymph nodes since they have time to recover, and therefore larvae pass through and cause reaction in deeper nodes which leads to blockage with such conditions as chyluria and lymph varix, but the blockage is incomplete and microfilariae appear in the blood.
4. Intolerant individuals subject to many bites throughout the year : early damage to distal lymph nodes obstructs passage of larvae which mature in nodes of limbs causing lymphangitis and fever. Some nodes become completely blocked, causing elephantiasis and allowing few microfilariae to reach the blood.

Napier does not consider these categories to be sharply defined and sees no reason to believe that tolerance is a fixed quality. Those at first tolerant may become intolerant. Although he does not use the term immunity in this connection, it seems obvious that if there are such differences in tolerance as he suggests, they should certainly be included in immunity in the broad sense. Both natural and acquired immunity would be involved in the situations he describes.

There is one further type of observation which might be considered as evidence of immunity. In some areas where a large proportion of the population is exposed to the vectors, only relatively few people show evidence of infection. For example, Causey *et al.* (1945) report that of 5,000 people examined in Belem, Brazil, only 12 per cent showed either microfilaremia or clinical manifestations. In the houses in which these people lived, night captures during the first seven months of the year revealed an average of 334 mosquitoes per house, of which 99 per cent were *C. fatigans*. Calculations based on the infection rate of this species showed that in the highest month, April, each family would be exposed, on the average, to bites of 67 infected mosquitoes per night. One would think that under these conditions a much larger proportion of the population would become infected. Of course there are many hazards for the infective larvae to overcome before they can pass from the mosquito and become established in a new definitive host, hazards which have nothing to do with immunity *per se*. Judging from the results of experiments with animals, however, one might predict a higher infection rate in the people.

In summary, the result of this exploration of the evidence for protective immunity to *Wuchereria* infection is quite unsatisfactory. Much of the suggested evidence is based entirely on the lack of any other explanation for certain observations. Other evidence is of an extremely hypothetical nature. Some of the postulations may be validated as new facts are acquired, but the facts needed to support the others appear to be essentially inaccessible. An impression is gained that a certain amount of immunity does exist, but both the degree and nature of this immunity are at present unknown.

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THE IDENTIFICATION OF FILARIAL LARVAE IN THEIR VECTORS.

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IN the investigation of the transmission of vector-borne diseases, whether they be viral, bacterial, protozoal, or helminthic, there is one question which must be answered before any deductions can be made from the observations :

CAN THE ORGANISMS UNDER INVESTIGATION BE CORRECTLY IDENTIFIED IN THEIR INTERMEDIATE HOSTS ?

It is essential to answer this question at the outset of all epidemiological studies of vector-borne diseases. In practically all groups of organisms, those infecting man represent only a small proportion. The intermediate hosts of the human parasites are frequently infected with related organisms from mammals, birds or even reptiles. The investigator, who is too anthropocentric in his approach, will encounter many pitfalls.

At least 150 species of filariae are known ; the life cycle of only 38 have been studied and of these, 23 complete their development in mosquitoes. New species are being discovered every year. Undoubtedly many more species will be shown to undergo development in mosquitoes.

In this paper it is proposed to summarise the methods we have used for establishing the identification of filarial larvae in their vectors.

Until quite recently most epidemiologists, investigating the transmission of filariasis, were satisfied with circumstantial evidence. If man biting species of insects were found infected with filariae it was assumed that the filariae were of human origin, especially if precipitin tests suggested that the particular species of insect was primarily anthropophilic.

On the Kenya Coast, where there is a high infection rate of *W. bancrofti* in man, one of the commonest mosquitoes found in the houses is *Aedes pembaensis*. On the island of Faza, precipitin tests suggested that man was the primary host of this mosquito. We found that more than 2 per cent of *Aedes pembaensis* contained infective filarial larvae and at first we thought that this was a new vector of *W. bancrofti*. Subsequent investigations, however, demonstrated that 32 per cent of the larvae were *Brugia patei*, from cats, dogs or genets ; 28 per cent either *Dirofilaria immitis* or *D. repens* from dogs and cats ; 10 per cent were *D. corynodes* from monkeys ; 18 per cent were *S. equina* from donkeys and the rest were animal filariae which have not yet been identified. More than 10,000 *Aedes pembaensis* have been

dissected but none contained infective larvae of *W. bancrofti*, and we have failed to get complete development in the laboratory. The vector on this island is *C. fatigans*. During preliminary investigations in another village on the coast where *W. bancrofti* was present, *Mansonia uniformis* and *M. africanus* were the only mosquitoes found infected in the houses, but again these larvae were shown to be *Brugia patei*. Subsequent investigations in this area suggest that the main vectors of *W. bancrofti* are *A. gambiae* and *A. funestus*. *Brugia patei* has never been found in man. A detailed account of this work can be seen in the papers by Heisch, Nelson and Furlong (1958) and Nelson (1959).

THE IDENTIFICATION OF FIRST AND SECOND STAGE LARVAE.

Here we are concerned with the practical problem of identifying the larvae. The identification of first and second stage larvae may be useful but the results may be misleading ; for example *Aedes pembaensis* often contains first and second stage *W. bancrofti* but these never develop to the infective stage. It is, however, of value if we can determine the particular species of filaria as this will indicate the preferred hosts of the mosquitoes dissected. Where filarial infection rates are high, species identification may be a better guide to host preference than precipitin tests.

If there are microfilariae in the stomach these can be identified by making smears ; these should be kept over-night to ensure adherence to the slide, then dehaemoglobinised in water, fixed with methyl alcohol and stained with Giemsa. It is an advantage to add a little fresh human blood to the smears. We have had the best results with 1 in 10 Revector Giemsa buffered at pH 6·5. The sheath of *Brugia* spp. and *Setaria* spp. stain intensely with this method, whilst the sheath of *W. bancrofti* is usually only a pale pink colour.

The site of development of the larvae in the vector may help in species identification ; for example *Wuchereria bancrofti*, *Setaria* spp. and *Mf. fijiensis* all develop in the thoracic muscles. *Dirofilaria immitis*, *D. repens* and *D. magnilarvatum* develop in the malpighian tubules and *D. corynodes*, *Dipetalenoma arbuta* and *Foleyella* spp. develop in the fat body cells. The position of development is only of value with first and second stage larvae ; the third stage larvae are found throughout the body of mosquitoes.

It is the species which develop in the thoracic muscles which concern workers interested in human filariasis. The early stages of *Brugia* in the thorax can easily be distinguished from *Wuchereria* ; they retain the two characteristic terminal nucleii throughout the first stage. The early stages of *Setaria* spp., are, however, very similar to those of *Wuchereria* and there are probably other unrecognised species which also develop in the thoracic muscles.

The recognition of early stages of filarial larvae may help the entomologist to calculate survival times of mosquitoes in the same way as oocyst rates help in malariology.

THE IDENTIFICATION OF THIRD STAGE INFECTIVE LARVAE.

The main problem in the assessment of vector transmission is the identification of infective larvae. All third stage larvae are regarded as infective They are easily distinguished from second stage larvae which are fat and sluggish with a fragile cuticle and an anal plug in a gaping anal canal. The infective larvae have shed the cuticle and anal plug with the second ecdysis and are active and snake-like with a tough cuticle ; the caudal papillae, if present, are well developed and the anal canal is narrow.

Identification might be achieved by inoculating the infective larvae into susceptible animals as has been done by Edeson and Wharton (1957) with *Brugia* spp. Unfortunately man is the only known susceptible host of *Wuchereria bancrofti* and in any case the long prepatent period makes this approach generally impracticable. In routine investigations it is necessary to rely on morphological characters.

Infective larvae cannot be identified when they are wriggling all over the place in a freshly dissected mosquito, they must be preserved for more careful study. Laboratory staff must be trained to make permanent preparations of all infective larvae. We have used two methods described below which can be used in field investigations.

(1) THE GLYCERINE ALCOHOL TECHNIQUE FOR FRESH DISSECTIONS.

The mosquitoes are dissected in saline. The infective larvae are transferred to a fresh drop of saline by picking them up with a fine entomological pin. They are killed by adding a drop of 70 per cent alcohol containing 5 per cent glycerine. They can then be transferred to a very small drop of the glycerine alcohol on a coverslip ; the alcohol evaporates rapidly and the larvae are left in a *minute* drop of glycerine ; the coverslip is then inverted over a cavity slide which has been ringed with "Euparol" ; or, if only temporary mounts are required, "Vaseline". The larvae, after fixation and evaporation of the alcohol, can also be mounted in glycerine under a coverslip on an ordinary slide, ringing the preparation with "Euparol" or "Glyceel". With this method there is a tendency for gradual skrinkage and after several years valuable material may be lost, but it is a perfectly satisfactory technique for routine surveys.

(2) THE HAEMALUM STAINING TECHNIQUE FOR MOSQUITOES PRESERVED IN ALCOHOL.

It is not always convenient to collect mosquitoes and dissect them "on the spot", or transport them alive to the laboratory. We, therefore, developed a routine method for the examination of mosquitoes preserved in alcohol. A preliminary note on this technique, which is a modification of the method used by Lebied (1950) for studying Onchocerca larvae in the thorax of *Simuliidae*, has already been published (Nelson, 1958), but since the original paper may not be readily available and since we have had more experience with this method, it may be of value to briefly repeat the details. During the past two years we have dissected more than 20,000 preserved mosquitoes, using the haemalum-glycerine method.

The entomological staff in the field collect the mosquitoes alive, identify them and transfer them immediately to McCartney bottles containing 80 per cent pure ethyl alcohol with approximately 100 mosquitoes in each bottle. The relevant details of species and locality, etc., are written in pencil on a piece of paper which is put inside the bottle with the specimens. The specimens are then sent to the central laboratory for further examination. The identification is checked by an entomologist. (We have been very fortunate in having the assistance of Mrs. E. van Someren for this work). The mosquitoes can remain in the alcohol indefinitely.

The staining method is very simple. The alcohol is removed by taking the specimens through descending dilutions down to water ; they are then stained for three days in Mayer's acid haemalum, differentiated for three days in distilled water and transferred to glycerine to await dissection. It is important to make fresh acid haemalum every two or three months as the stain gradually deteriorates.

The stained mosquitoes are dissected in glycerine. All stages of development in the thorax are readily seen and the infective larvae can be picked out and transferred to a fresh drop of glycerine for examination under a coverslip with higher magnifications. If the mosquito has recently fed and the abdomen is full of blood this is fixed in a solid mass and it is usually impossible to identify first and second stage larvae in the abdomen ; third stage larvae in the abdomen are often distorted. This is of little practical importance since it is very rare to find infective larvae only in the abdomen. In routine surveys for detecting species of vector and assessing transmission rates we are usually only concerned with the infective larvae. Wharton (1959*a*) has described a similar method for preserving mosquitoes in formalin-glycerol ; he has had no difficulty in demonstrating infective larvae in subsequent dissections. These techniques for the delayed dissection of vectors of filariae have greatly facilitated routine survey work ; they are also very useful for teaching purposes.

The next step in identification, once the larvae have been mounted by either the glycerine alcohol method or the Haemalum method, is to examine their morphology and compare them with known infective larvae. Sixteen species of filarial worms from mammals, four from frogs, two from lizards and one species from a bird are known to develop in mosquitoes. The morphology of infective larvae of several of the species have been adequately described but only scanty information is available for others. It is important in describing the development of new species in their vectors to give a clear description of the characteristic features of the infective larvae.

In Kenya we are making a reference collection of infective larvae by feeding arthropods on animals infected with known species of filariae in an attempt to find specific or generic characteristics. We have been fortunate in getting material from other workers, but there are still many species requiring further study and there are several species with characteristic morphology in our local mosquitoes which have not yet been identified.

The larvae are measured with a micrometer eyepiece.

The characteristic features of the caudal extremity are best seen with an oil immersion lens.

The characteristics which have proved most valuable in differentiation are :—

(1) LENGTH

The infective larvae of *Wuchereria bancrofti* and *Brugia* spp. which average more than 1,100 microns in length can be immediately distinguished from *Dirofilaria* spp. which average less than 1,100 microns in length. The larvae of *Setaria equina* are, however, about the same size as *Wuchereria* and *Brugia*

(2) THE POSITION OF THE ANUS.

The anus is much nearer the caudal extremity in *Dirofilaria* spp. than in *W. bancrofti*, *Brugia* spp., or *Dipetalonema* spp. The "Anal Ratio" introduced by Wharton (1957) is a useful characteristic and is defined as the distance from the anus to caudal extremity divided by the breadth of the larvae half way from the anus to the caudal extremity. In *Dirofilaria* spp. the ratio averages approximately 2 ; in *Wuchereria* and *Brugia* it is nearer 4.

(3) THE SHAPE OF THE CAUDAL EXTREMITY.

The most important feature for diagnosis is the shape of the caudal extremity. The difference in some species transmitted by mosquitoes can be seen in Plate I.

In well preserved material there is no difficulty in recognising the characteristic rounded knobs at the extremity of the infective larvae of *W. bancrofti*. In a single specimen all the knobs are not always visible but usually several specimens are available and this species is easy to identify. Unfortunately no differences have been noted between *Brugia malayi* and *Brugia pahangi* although the larvae of *B. patei* are slightly different. The larvae of *Dirofilaria immitis* are readily distinguished from *D. corynodes* from monkeys, but they are practically identical with the larvae of *D. repens* from dogs and cats.

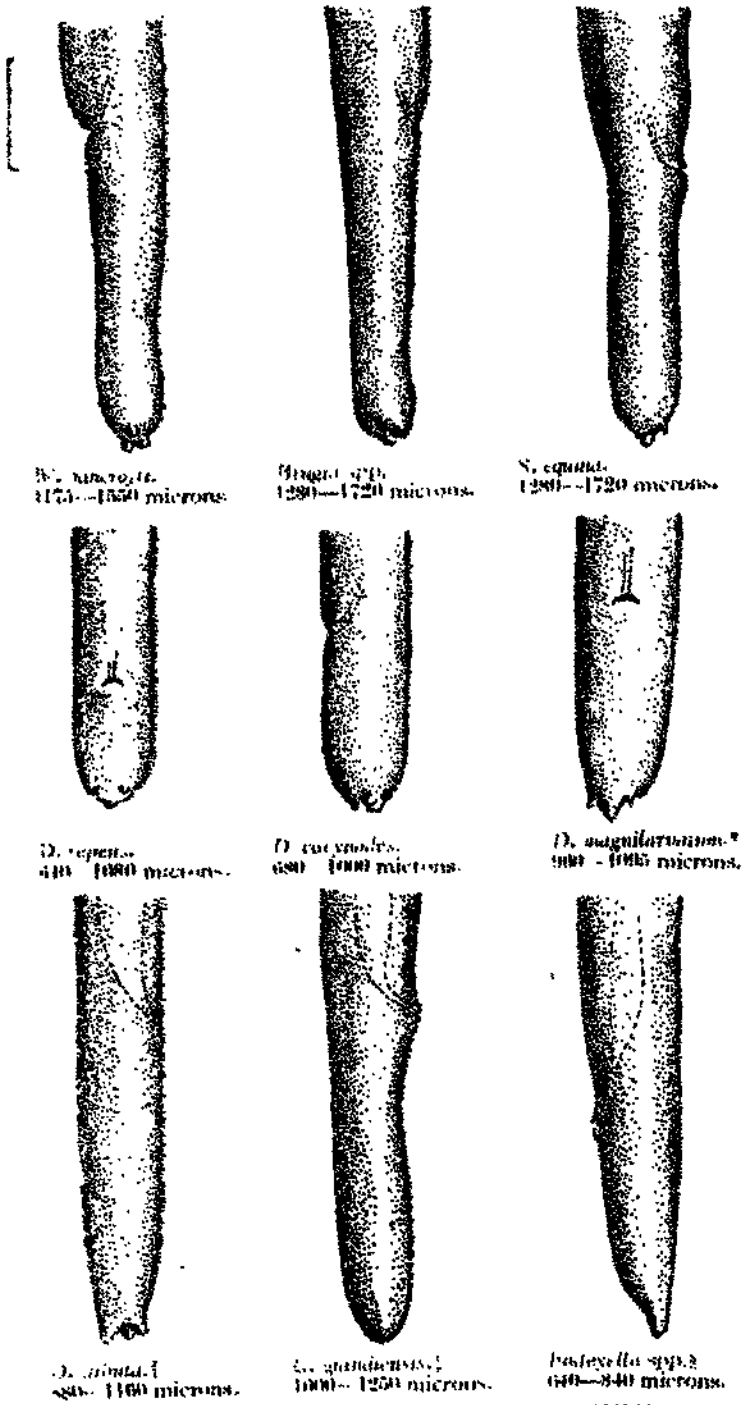
A more detailed description of the species found in mosquitoes, together with a provisional key, can be seen in a recent paper by the author (Nelson, 1959).

The problem involved in the identification of filarial larvae is equally relevant to studies of other genera such as *Dipetalonema* and *Onchocerca*.

In a study of the development of filariae in fleas it was the discovery of a quite typical infective larva which first suggested that dogs in the Nairobi area of Kenya were infected with a *Dipetalonema* and not *Dirofilaria immitis* or *Dirofilaria repens*. The larvae were identical with those obtained by feeding fleas on a spring hare infected with another species of *Dipetalonema*.

On Mount Elgon where onchocerciasis is prevalent in man the vector *Simulium neavei* is fairly heavily infected with two types of infective larvae, one is much shorter than the other. These probably represent different species but as suggested by Crosskey (personal communication) they might possibly represent male and female larvae of *Onchocerca volvulus* which shows very marked difference in size in

PLATE I.


N. equina.
1280—1720 microns.
D. repens.
640—1080 microns.
D. magnilarvatum.*
900—1085 microns.
1000—1250 microns.
Footnotes
*D. magnilarvatum after WHARTON (1950).
†D. ghanbiensis after PANDIT et al. (1929)
‡Folleyella spp. after WITENBERG and GERICHTER (1944).

the male and female adult worms. Chabaud (1954) has noted sex differences in the infective larvae of *Dipetalonema vite*. If the sexing of living infective larvae proves practicable a number of interesting studies will be possible in the field of experimental parasitology and therapeutics, especially with *Brugia* spp. which are readily transmitted to animals in the laboratory.

In conclusion it is suggested that before initiating control measures, infective larvae from many of the so-called vectors of human filariae should be re-examined to eliminate the possible misidentification of species.

SUMMARY.

The incrimination of particular species as vectors of human filariae and the determination of intensity of transmission is only possible when the infective larvae have been correctly identified.

The identification of first and second stage larvae by their morphology or site of development in the vector is sometimes possible and may be of value in indicating host preferences and vector survival times but in routine surveys emphasis must be laid on the correct identification of infective larvae.

Infective larvae should be preserved for examination under high magnification. Details are given of the glycerine alcohol technique for fresh dissections and the haemalum technique for insects preserved in alcohol; the latter method has the advantage that all dissections can be performed under ideal laboratory conditions.

The characteristics most useful in identifying the species of infective larvae are the length, the position of the anus and, most important, the shape of the caudal extremity. Some characteristic larvae are illustrated.

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A PRELIMINARY NOTE CONCERNING DEVELOPMENT OF LARVAL STAGES OF *BRUGIA MALAYI* AND *WUCHERERIA BANCROFTI* IN THE BEDBUG IN COASTAL FILARIAL AREAS OF KERALA STATE, SOUTH INDIA.

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[August 31, 1960.]

In August, 1960, the author collected 60 bedbugs from cots on which slept carriers of *Brugia malayi* and *Wuchereria bancrofti* microfilariae. These were mostly *Cimex lectularius*, the common bedbug, but three specimens were identified as *Cimex hemipterus*, the oriental bedbug.

Due to the flattened nature of the insect, the exoskeleton appeared completely transparent, so that the larval stages could be made out within the undissected insect. Dissection and maceration were also carried out in regular fashion.

The following table summarizes the extent to which larval development was found to have taken place :

Number of bedbugs dissected (all stages) ...	60	
Number showing microfilariae still alive in blood mass (dissected about 18 hours after feeding) ...	6	(10 per cent.)
Number positive with *B. malayi* sausage stages (in tibia and tarsus of legs, no more than 2 per bedbug)	5	(8·3 per cent.)
Number positive with *B. malayi* infective larvae (in antennae, abdomen, and prothorax, only 1 per bedbug seen)	4	(6·6 per cent.)
Number positive with *W. bancrofti* sausage stages (in macerated tissues of thorax and legs, 3 forms found)	1	(1·7 per cent.)
Number positive with *W. bancrofti* infective larvae (only 1 larva seen)	1	(1·7 per cent.)
Number positive with sausage and/or infective larvae of *B. malayi*	9	(15 per cent.)
Number positive with sausage and/or infective larvae of *W. bancrofti*	2	(3·3 per cent.)
Total number positive with sausage or older stages ...	11	(18·3 per cent.)

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

Although only one infective larva was seen per bedbug having this stage, others may have already escaped. No emergence of infective larvae was seen to occur naturally in undissected insects. This phenomenon remains to be actually observed and described.

Approximately 80 per cent of all the bedbugs dissected showed one or more dead microfilariae in the abdominal blood mass, indicating that they had at least fed on a person having microfilaraemia.

Differentiation of sausage and infective larvae was made by examination of the posterior portion, and by measurements of specimens, as already described by Feng (1936) and Kobayasi (1940), and brought up-to-date by Iyengar (1957). Details concerning the morphology and behaviour of the various stages of *B. malayi* and *W. bancrofti* mentioned herein will be presented with photomicrographic illustrations in a forthcoming paper.

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ON THE BIONOMICS OF VECTORS OF HUMAN FILARIASIS IN ERNAKULAM (KERALA), INDIA.*

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[June 30, 1960.]

INTRODUCTION.

STUDIES on the bionomics of *C. fatigans* (vector of *W. bancrofti*) and *M. annulifera* and *M. uniformis* (vectors of *B. malayi*) were undertaken in Ernakulam, Kerala State, during the period July, 1958 to December, 1959.

Ernakulam is endemic for filariasis (Jaswant Singh *et al.*, 1956). Both *W. bancrofti* and *B. malayi* are prevalent but the incidence of the former is comparatively very much higher. The latter infection is mostly confined to the peripheral part of the area, where semi-urban and rural conditions prevail.

METEOROLOGICAL DATA.

The annual average rainfall ranges between 120 and 150 inches. The heaviest rainfall is between June and September. The monthly mean maximum and minimum temperatures, relative humidity and rainfall are shown in Table I. The maximum temperature was 33·1°C. during March and April and it ranged from 27·0°C. to 33·1°C. The minimum temperature was 21·9°C. and it ranged between 21·9°C. and 25·9°C. The relative humidity is high throughout the year and during the rainy months of June to October, it is well over 80 per cent.

The present studies on the bionomics of *C. fatigans*, *M. annulifera* and *M. uniformis* pertained to (1) Seasonal prevalence, (2) Resting places, (3) Time of entry and feeding, (4) Blood preferences, and (5) Infection and infectivity rates.

METHODS.

Six adult collecting stations were selected in different parts of Ernakulam Town. Collections were made once a week for half-an-hour in each station with

* An Enquiry under the Director, Malaria Institute of India, but sponsored by the Indian Council of Medical Research.

suction tubes. The day collection started at 07·00 hours. In order to determine the time of entry and feeding, the night collections were made between 18·00 and 06·00 hours. These collections were made at two-hourly intervals and the abdominal conditions of mosquitoes collected were recorded. Mosquito dissections were carried out on the same day of collection and the different developmental stages of the filaria larvae were recorded for determining the infection rates.

TABLE I.

Meteorological data—Ernakulam (Kerala State).

Year and month.	Temperature 0°C (mean)		Relative humidity, per cent (Average).	Rainfall (average) in inches.
	Maximum.	Minimum.		
1958				
July	29·6	24·5	84	14·88
August	28·5	23·9	90	18·00
September	29·9	24·3	79	4·36
October	29·7	24·9	82	5·67
November	30·7	24·0	77	6·38
December	31·9	23·0	67	6·3
1959				
January	32·9	22·8	79	"t"
February	32·2	23·8	77	0·0
March	33·1	25·5	71	0·50
April	33·1	25·3	73	4·42
May	32·3	26·3	81	14·92
June	27·0	25·9	85	36·67
July	28·7	23·5	88	33·41
August	30·9	23·6	85·8	17·6
September	27·9	24·0	86·9	13·17
October	29·9	23·4	87·0	5·94
November	31·0	23·8	83·7	1·75
December	31·4	22·3	85·0	2·36

RESULTS.

CULEX FATIGANS.

(1) *Seasonal prevalence.*—Only the data obtained from January to December, 1959, have been analysed as the period of observation in the year 1958 covers only a part. From the data (Table II), it will be observed that *C. fatigans* is prevalent throughout the year. The highest density of 58·8 per-man-hour is reached in February. The density has been high in the months of January to March. During the rainy months of April to October, the density is comparatively low, except in the months of June and July.

(2) *Resting places.*—The indoor resting habits of *C. fatigans* were studied while making weekly adult collections. The collections were classified under, (*a*) mosquitoes resting on walls below or above 5 feet height, (*b*) on the roof, (*c*) on hanging objects like ropes, clothing and others, (*d*) on furniture, and (*e*) on other places. The possible outdoor places, round about habitations, like stone crevices, fencing made of leaf and mat, shrubs etc., were searched, for locating any outdoor resting places.

TABLE II.

Seasonal prevalence of C. fatigans, M. annulifera *and* M. uniformis.

Year and month.	PER-MAN-HOUR DENSITY :		
	C. fatigans.	*M. annulifera.*	*M. uniformis.*
1959			
January	32·9	1·17	0·69
February	58·8	0·4	0·41
March	45·8	0·08	0·08
April	26·2	0·1	0·1
May	19·8	0·0	0·0
June	46·8	0·18	0·09
July	34·6	0·08	0·0
August	28·2	1·9	0·3
September	23·8	4·85	0·85
October	16·9	13·6	1·1
November	25·8	8·6	1·0
December	26·3	1·5	0·36

In the indoor shelters, nearly 52 per cent of adult *C. fatigans* rested on hanging objects while 33·7 per cent on the walls. Only 13·6 per cent were found resting underneath the roof (Table III).

In order to determine whether indoor residual spraying in any way altered the resting places of *C. fatigans*, the catching stations were sprayed with BHC at a dosage of 40 mg. gamma isomer per square foot. Before the treatment, 47·3 per cent rested on walls and roof. But after spraying, 35·1 per cent rested on these treated surfaces. No significant change was noted in the resting habit. The hanging objects, even after spray, showed 57·1 per cent of *C. fatigans* resting on them (Table III).

In the outdoor collections, 146 man-hours were spent but only 12 specimens of *C. fatigans* were collected the per-man-hour density being 0·082. When compared with the per-man-hour density of *C. fatigans* resting indoors, the outdoor collection formed only 0·26 per cent (Table V). Thus it would seem that this species is predominantly an indoor rester.

(3) *Time of entry and feeding.*—*C. fatigans* enters the shelters throughout the night. A higher proportion (23·6 per cent) of them entered before 18·00 hours. The feeding took place throughout the night but the peak feeding was recorded in the collection made from mid-night to early morning (00·00 hours to 06·00 hours). More than 50 per cent of *C. fatigans* were found with engorged blood in the collection made after mid-night (Table VI).

(4) *Blood preferences*—842 blood meals of *C. fatigans* were tested to determine the source of their meal. Out of this, 342 were from Ponnani areas and the rest 500 were from Ernakulam. The anthropophilic index for Ponnani was 85·2, while for Ernakulam it was 50·6 (Table VII). The difference may perhaps be due to a higher animal population in Ernakulam.

TABLE III.

Number and percentage of C. fatigans, M. annulifera *and* M. uniformis *caught from various indoor resting places.*

Species.		Number Caught	Walls: Below 5 feet.	Walls: Above 5 feet.	Roof.	Hanging objects: Rope.	Hanging objects: Clothing.	Hanging objects: Other places.	Furniture.	Miscellaneous.
C. fatigans	Pre-spray	4,201	829 (19·7)	590 (14·0)	572 (13·6)	1,022 (24·3)	820 (19·5)	345 (8·2)	4 (0·1)	10 (0·5)
	Post-spray	3,020	499 (16·5)	323 (10·7)	262 (8·7)	800 (21·5)	646 (21·4)	430 (14·2)	15 (0·5)	45 (1·4)
M. annulifera	Pre-spray	1,459	1,039 (71·2)	79 (5·4)	24 (1·6)	65 (4·5)	212 (14·5)	24 (1·6)	13 (0·9)	3 (0·2)
	Post-spray	381	191 (50·13)	42 (11·0)	2 (0·5)	22 (5·8)	83 (21·8)	40 (10·5)	1 (0·3)	.. ..
M. uniformis	Pre-spray	178	135 (75·9)	12 (6·7)	2 (1·1)	6 (3·4)	22 (12·3)	.. ..	1 (0·6)	.. ..
	Post-spray	47	30 (63·8)	4 (8·5)	.. ..	2 (4·2)	8 (17·0)	3 (6·5)	.. ..	.. ..

Figures in brackets indicate percentage in relation to the total number of mosquitoes caught.

TABLE IV.

Outdoor collection of C fatigans, M. annulifera *and* M. uniformis *in Ernakulam during the period July, 1958 to December, 1959.*

Species.	Total number collected.	Places from where collected: Crevices in stones.	Fence (made up of leaf and mat).	Shrubs.	Grass.
C. fatigans	12	3 (25·0)	2 (16·7)	4 (33·3)	3 (25·0)
M. annulifera	618	7 (1·1)	66 (10·7)	345 (55·8)	200 (32·4)
M. uniformis	966	11 (1·14)	118 (12·2)	296 (30·8)	539 (55·8)

Figures in brackets indicate percentage in relation to the total number of mosquitoes of each species collected.

TABLE V.

Comparative densities of C. fatigans, M. annulifera *and* M. uniformis *in outdoor and indoor shelters in Ernakulam (Kerala State).*

Species.	INDOOR SHELTERS :			OUTDOOR SHELTERS :			PROPORTIONAL RATE OF PER-MAN-HOUR DENSITY :	
	Total time spent.	Number collected.	Per-man-hour density.	Total time spent.	Number collected.	Per-man-hour density.	Indoor.	Outdoor.
C. fatigans	230	7,221	31·4	146	12	0·082	99·74	0·26
M. annulifera	230	1,840	8·0	146	618	4·23	65·4	34·6
M. uniformis	230	225	0·98	146	966	6·62	12·9	87·1

(5) *Infection rates in* C. fatigans.—*C. fatigans* is infected in nature throughout the year. The highest infection rates are in the months of November and December (Table VIII). The total infection rate for the year is 5·1. High humidity seems to favour the transmission of filarial infection. It was also observed that there is no corresponding increase in the infection rates along with a rise in the density of *C. fatigans*.

M. ANNULIFERA AND *M. UNIFORMIS*.

(1) *Seasonal prevalence.*—*M. annulifera* appeared in low numbers during the year 1959. *M. uniformis*, however, was present in much smaller numbers than *M. annulifera*. In the months of February and August the density of *M. annulifera* was extremely low. Their numbers started building up in August and the per-man-hour density reached the highest figure of 13·6 in October (Table II). It was thus observed that the incidence of *M. annulifera* was low during the monsoon months and the numbers increased in the post-monsoon months. The indoor density of *M. uniformis* did not go beyond 1·1 per-man-hour though this species was encountered during all the months of the year. This species is known to be an outdoor rester which fact will be discussed under resting habits. Thus the number caught, inside houses, do not reflect fully the numerical prevalence. It does not appear that temperature and relative humidity *per se* influence the seasonal prevalence of these two species. Rainfall seemed to have direct bearing on the seasonal fluctuations. *M. annulifera* and *M. uniformis* breed in small ponds in association with *Pistia stratiotes*. It is a well-known fact that organic pollution in the ponds has a positive correlation with the intensity of breeding of the two species. During heavy rains, the ponds are flooded and the organic contamination gets diluted to a great extent. These two factors are inimical to the breeding of these two species. Soon after monsoon months, conditions become favourable for the breeding, and the density builds up gradually. During very hot months, with no rainfall, their numbers are low. This is probably due to the drying up of a number of breeding areas.

TABLE VI.

Time of entry and feeding of C. fatigans, M. annulifera *and* M. uniformis *at Ernakulam (Kerala State) : July, 1958 to December, 1959.*

Species.	Total number that entered.	Time of entry during night : 18·00 hours.		20·00 hours.		22·00 hours.		0·00 hours.		2·00 hours.		4·00 hours.		6·00 hours.	
		Entered.	Fed.	Entered.	Fed.	Entered.	Fed.	Entered.	Fed.	Entered.	Fed.	Entered.	Fed.	Entered.	Fed.
C. fatigans	1,543	365 (23·6)	66 18·1*	211 (13·7)	46 21·8*	173 (11·2)	44 15·4*	177 (11·5)	92 51·9*	244 (15·8)	128 12·4*	176 (11·4)	92 52·2*	197 (12·8)	107 54·3*
M. annulifera	257	21 (8·2)	9 42·8*	36 (14·0)	9 25·0*	25 (9·7)	11 44·0*	32 (12·4)	16 50·0*	44 (17·1)	39 88·5*	51 (19·8)	42 82·3*	48 (18·7)	38 79·1*
M. uniformis	1,257	27 (2·1)	12 44·4*	154 (12·2)	30 19·5*	310 (24·7)	87 28·1*	314 (24·9)	152 48·1*	191 (15·2)	160 83·6*	170 (13·5)	141 82·9*	91 (7·2)	78 85·7*

( ) Figures in brackets indicate percentage in relation to the total number of mosquitoes of each species that entered during the whole night.

* Figures with asterisks denote percentage in relation to the number of mosquitoes collected at the corresponding time.

TABLE VII.

Result of precipitin tests.

Species.	Area.	Place of capture.	Number of blood meals tested.	Number positive for : Human.	Bovine.	Dog.	Goat.	Anthropophilic index.
C. fatigans	Ponnani	Human dwelling	236	199	..	..	..	84·3
	Ponnani	Mixed dwelling	106	93	..	..	..	87·7
	Ernakulam	Human dwelling	500	253	8	1	..	50·6
		Total :	842	545	8	1	..	64·7
M. annulifera	Ernakulam	Human dwelling	431	187	14	..	5	43·4
	Ernakulam	Cattle-shed	128	23	21	1	11	18·0
		Total :	559	210	35	1	16	37·5
M. uniformis	Ernakulam	Cattle-shed	584	11	433	..	..	1·88
	Thuravur	Human dwelling	21	2	4	..	4	14·30
	Thuravur	Cattle-shed	960	32	289	9	49	3·3
		Total :	1,565	45	726	9	53	2·8

TABLE VIII.

Percentage infection rates in C. fatigans, M. annulifera *and* M. uniformis.

Year and month.	PERCENTAGE INFECTION RATES :		
	C. fatigans.	*M. annulifera.*	*M. uniformis.*
1959			
January	5·2	15·4	0·0
February	2·5	12·5	3·6
March	1·3	0·0	0·0
April	0·0	0·0	0·0
May	1·7	0·0	0·0
June	2·3	0·0	0·0
July	2·6	0·0	0·0
August	5·8	25·0	30·0
September	6·4	33·3	16·1
October	7·9	25·7	5·3
November	15·9	23·3	8·1
December	15·9	19·2	4·6
Total number dissected.	3,309	1,158	856
Number positive.	110	322	51
Infection rate.	5·1	27·8	5·9

(2) *Resting places.*—From indoor shelters 1,459 *M. annulifera* were collected; out of this 78·2 per cent were found resting on the walls and roofs and 14·5 per cent on hanging-clothes, the rest on other miscellaneous objects (Table III). After treating the shelters with BHC at 40 mg. gamma isomer per square foot, 61·6 per cent of the adults still rested on the walls and roofs. There was thus a reduction of 16·5 per cent in the mosquitoes resting in such situations as compared to the pre-spraying figures. In general, *M. annulifera* was noted to prefer walls and roofs for resting than the other objects like clothes, rope, furniture etc.

165 *M. uniformis* were collected from indoor shelters. 83·7 per cent of them were found resting on walls and roofs and this habit is more or less similar to *M. annulifera*. After treating the shelters with BHC the percentage of adults, resting on walls and roofs, was 72·3 which showed a very small decrease from the pre-spraying density.

Outdoor resting places.—618 specimens of *M. annulifera* were collected from outdoor shelters, such as crevices, fence, shrubs and grass (Table IV). Out of this 55·8 per cent were found resting on shrubs, 32·4 per cent on grass, 10·7 per cent on fence and 1·1 per cent in crevices. The per-man-hour density of indoor and outdoor resting *M. annulifera* was 4·23 and 8·00 respectively. The proportional rate of outdoor to indoor resting works out to 34·6 (Table V), showing that a good proportion of this species rests outdoors. It was found, however, that comparatively a much higher degree of outdoor resting was observed in *M. uniformis*.

966 specimens were obtained in a search of 146 man-hours in outdoor shelters (Table V). The per-man-hour catch for outdoor shelters works out to 6·62. This density, when compared with the indoor density of 0·98, gave a proportional rate of 87·1 for outdoor resting (Table V). This clearly shows that *M. uniformis* is mainly an outdoor rester in this area. The most favoured resting place was grass which yielded 55·8 per cent of adults, shrubs 30·8 per cent, while fences 12·2 per cent; stone crevices yielded only 1·14 per cent (Table IV).

(3) *Time of entry and feeding.*—*M. annulifera* enters the indoor shelter all through the night but the highest number was noted to come in between midnight and 06·00 hours (Table VI). Feeding starts early. The bulk of feeding, however, was from midnight to 06·00 hours as nearly 70 to 80 per cent of mosquitoes, collected during this period, were fully-fed. *M. uniformis* like *M. annulifera* enters houses throughout the night. But the largest percentage, i.e., 24·7 to 24·9 per cent were found to enter between 22·00 hours and midnight. Thereafter there is a gradual decline. The highest number of fully-fed specimens were collected between 02·00 and 06·00 hours, the percentage of fed mosquitoes being as high as 83 to 85·7 per cent.

(4) *Blood preferences.*—559 blood meals of *M. annulifera* were tested for their source. The anthropophilic index worked out to 37·5 per cent (Table VII). This anthropophilic index is as to be expected of *M. annulifera* which is the primary vector of *B. malayi* in Kerala. *M. uniformis*, on the other hand, feeds poorly on humans; the anthropophilic index was only 2·8 per cent out of 1956 blood meals tested.

(5) *Infection rates.*—*M. annulifera* was found infected in nature in all the months except March to July. The highest infection rate of 33·3 per cent was in September (Table VIII). The gross infection rate for the year was 27·8. It was observed that the infection rates were closely related to the adult density ; with a higher density the infection rate also registered an increase. In *M. uniformis* natural infection was observed in all the months except February to June. The highest infection rate of 30·0 per cent was in October but the gross infection rate for the year was only 5·9 per cent.

DISCUSSION.

In general the highest prevalence of *C. fatigans* is in the non-monsoon months. The main breeding grounds of *C. fatigans* are cess pools, drains, dung pits and other collections of polluted water. During rains, all these breeding places are flushed and consequently the density is comparatively low. The density shows an upward trend after the cessation of the rains Subramaniam and Tampi (1958) have reported that in Mangalore, there is a negative correlation between the density of *C. fatigans* and filarial infection. A more or less similar feature has been observed in Ernakulam as mentioned earlier. The filarial infection in mosquitoes was high when the relative humidity was over 80 per cent.

Chow and Thevasagayam (1957) in Kurunegala (Ceylon) have shown that about 70 per cent of female *C. fatigans* rested on hangings, clothes and furniture. In the present studies, it was observed that more than 50 per cent rested on such situations. The resting places did not vary very much and after residual indoor spraying there was only a slight difference if any, in the percentage of mosquitoes resting on treated walls. Chow and Thevasagayam (1957 *loc. cit.*) have also stated that the resting habits of *C. fatigans* were similar in both the insecticide-sprayed and unsprayed areas.

M. annulifera and *M. uniformis* are the two known vectors of *B. malayi* in India. Of these two, *M. annulifera* is the primary vector and *M. uniformis* plays a secondary role in the transmission. In the present studies, dissections of these two species, over a period of 12 months have shown that the overall infection rate in *M. annulifera* is very much higher than in *M. uniformis*. The total infection in the former was 27·8 while in the latter it was 5·9. The studies have also revealed that there is a wide difference in the adult habits of these two closely allied species. *M. annulifera* is more domestic with a higher anthropophilic index, while *M. uniformis* rests mostly outdoors and has a low anthropophilic index.

SUMMARY.

The seasonal prevalence, the resting habits, the time of entry and of feeding, the blood preferences and the natural infections of *C. fatigans*, *M. annulifera* and *M. uniformis*, the vectors of human filariasis in India, were studied in Ernakulam, Kerala State, during the year 1959.

Culex fatigans was prevalent throughout the year but its density during the monsoon months was low.

Nearly 51 per cent of *C. fatigans* rested on hanging objects while about 33·7 per cent rested on walls. Very insignificant numbers rested outdoors. No appreciable change in the indoor resting habit of this species occurred after treating the shelters with residual insecticides.

C. fatigans entered indoor shelters throughout the night but a higher percentage entered in the early part of the night.

Though feeding also took place throughout the night, the peak feeding was from 00·00 to 06·00 hours.

The overall anthropophilic index of *C. fatigans* was 64·7.

Natural infection in *C. fatigans* was noted throughout the year but the highest infection rate was in the months of November and December.

The density of *M. annulifera* was highest in October. From February to August, their numbers were very low.

78·2 per cent of *M. annulifera* rested on walls and roofs in the indoor shelters. 35 per cent of this species rested outdoors.

Though this species entered the dwellings throughout the night, a higher percentage entered between 02·00 and 06·00 hours. Feeding started early but the peak was reached between 02·00 and 06·00 hours.

M. annulifera had an anthropophilic index of 37·5 per cent. Natural infection occurred in this species in all the months except March to July. Infection rates were correlated with the density.

M. uniformis did not have very high density at any time of the year. The highest density recorded was 1·1 per-man-hour in the post-monsoon months.

This species was predominantly an outdoor rester. Nearly 87·1 per cent of the adults rested in outdoor shelters.

M. uniformis entered dwellings throughout the night but the majority of them entered between 22·00 and 00·00 hours. The bulk of feeding took place after midnight which went on till 06·00 hours.

The anthropophilic index of *M. uniformis* was 2·8 per cent.

Natural infection in *M. uniformis* occurred in all the months except February to June.

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STUDIES ON CULICINE MOSQUITOES.

1. Preferential indoor resting habits of *Culex fatigans* Wiedmann, 1828, near Ghaziabad, Uttar Pradesh.

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INTRODUCTION.

About 85 per cent of the mosquitoes so far recorded from India belong to the tribe *Culicini*. However, due to the importance and prevalence of malaria throughout the country, most of the published work on Indian mosquitoes refers to the tribe *Anophelini*. The fact that filariasis is spread over larger areas in India than hitherto known and also that culicines are known vectors of some of the helminthic and viral diseases of man, it has become imperative to know more about the systematics, distribution and bionomics of culicines in India.

The most comprehensive work on Indian culicines is that of Barraud (1934) which deals mainly with some aspects of systematics of the group with short notes on habits and distribution of the species. Recently Rao and Rajagopalan (1957) published observations on some aspects of the bionomics of culicines of Poona District. During the past few years a series of laboratory and field observations were undertaken at the Malaria Institute of India to gain knowledge about various aspects of the biology and bionomics of culicine mosquitoes.

Among culicines, *Culex fatigans* Wied. is the most prevalent and widely distributed species throughout India. Increasing industrialisation and urbanisation makes this mosquito still more prevalent. This species is the main natural vector of Bancroftian filaria in areas where favourable conditions for the transmission of the disease occur. Even where filariasis may be absent the nuisance due to this species is well known. Therefore, a great deal of work that has been undertaken revolves around this species.

The present paper deals with the data collected on the resting habits of adult *C. fatigans* in a semi-urban† area, about 15 miles from Delhi, during January, 1958 to October, 1959. From January to July 1958, the observation villages were unsprayed after which they were sprayed in August, 1958 and in July and October, 1959 with DDT at the rate of 100 mg./sq. ft. Therefore, the data presented in this paper include the resting habits of *C. fatigans*, before and after the insecticidal application.

* The assistance of Shri K.M.S. Bedi for statistical analysis of data is greatly appreciated.

† The observation villages are being encroached upon by the fast developing Ghaziabad Township. The normal village huts are rapidly giving way to the pucca brick houses. It seems that within a few years the villages may become part of Ghaziabad Township itself.

METHODS.

Prangarhi and Ramagarhi, two villages unsprayed in January, 1958, were selected for these observations. The villages are situated about 15 miles off Delhi, near Ghaziabad Town of Uttar Pradesh (Map 1). The village Prangarhi starts a few furlongs from Ghaziabad Town and extends up to the road to Dasna. On the other side of the road is Ramgarhi. There are about 350 dwellings in the two villages, 80 per cent of which are mud-plastered huts with mud, thatch or wooden ceilings. The rest are brick houses with mud or cement plastered walls and ceilings. The villages are surrounded by agricultural fields, mainly growing vegetables.

The source of mosquito breeding is provided by open drains and pits containing sullage water and rain water collections. Preliminary surveys had shown *Culex fatigans* to be the main mosquito species breeding in the area.

Mosquitoes were collected once a week from human dwellings and cattlesheds for one hour each during 7 a.m. to 10 a.m. The time of observation was, as far as possible, equally divided for collecting mosquitoes from various structures inside a shelter. *C. fatigans* were collected while resting on the following structures :—

Ceiling,
Cobwebs hanging from ceiling,
Hanging objects (clothes, umbrellas, etc.),
Walls,
Corners,
Shelves,
Objects on floor (fire-wood, cowdung, empty baskets* and earthen pots*).

In addition, one-hour search for mosquitoes was made in the likely outdoor shelters like the hedges, trees, grasses, and other plants in the vicinity of the dwellings.

The equipment used for catching adult mosquitoes for the present study was aspirator tubes, flash lights and test tubes. Sometimes butterfly nets were also used to collect mosquitoes resting in the vegetation. Throughout these observations the same personnel were employed for making catches and recording the data.

METEOROLOGICAL DATA (TABLE I).

During the period of observations, the total annual rainfall averaged 79·73 cm (31·4 inches). The average mean temperature varied from 15·2°C. (59·3°F.) in January to 34·5°C. (94·1°F.) in June and the average relative humidity varied from 24·5 per cent in May to 78·0 per cent in September. The saturation deficiencies for the period of observation were calculated with the help of a nomogram, based on the vapour pressure tables of the Smithsonian Institution (1918) and reproduced by Hicks and Majid (1937).

* Empty baskets and earthen pots were provided by the authors for these observations and it was ensured that the same would not be shifted during the course of these observations.

MAP 1.

Location of observation villages.

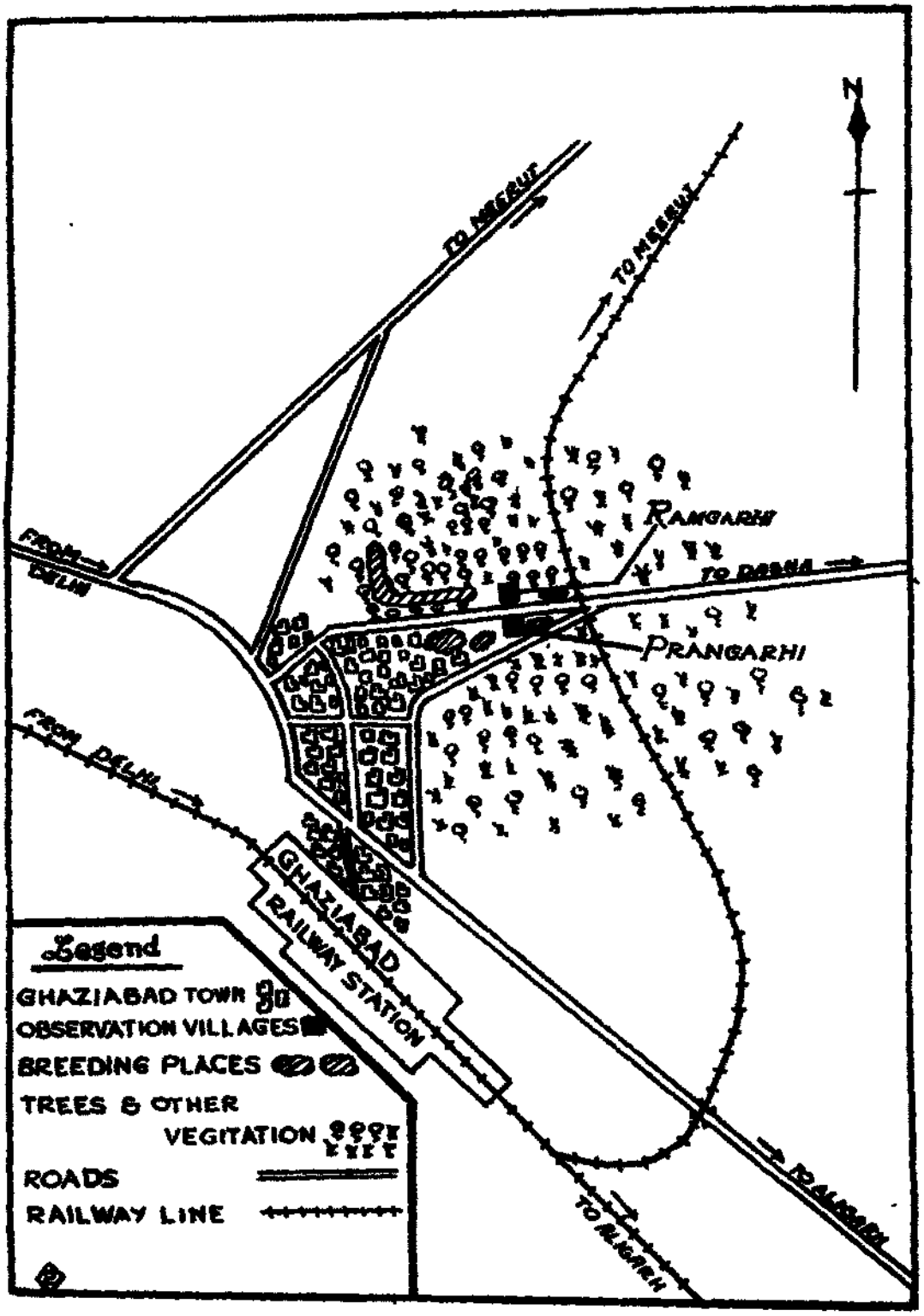

TABLE I.

Meteorological data for Delhi (January, 1958 to October, 1959).

Months.	Mean temperature (°C.)			Mean relative humidity (per cent.)			Total rainfall (cm.)			Saturation deficiency (mm.)	
	1958.	1959.	Average.	1958.	1959.	Average.	1958.	1959.	Average.	1958.	1959.
January	16·2	14·3	15·2	65·5	70·5	68·0	2·64	4·06	3·35	4·8	3·6
February	17·6	16·5	17·0	46·0	53·6	49·8	0·1	0·08	0·09	8·5	7·2
March	23·9	22·8	23·3	40·4	38·0	39·2	1·84	0·31	1·08	12·2	13·2
April	30·9	26·9	28·9	28·0	27·0	27·5	0·05	0·0	0·02	24·1	20·0
May	33·0	31·4	32·2	21·5	27·5	24·5	0·8	2·2	1·5	29·5	25·2
June	35·3	33·8	34·5	33·0	37·0	35·0	0·79	2·59	1·69	28·7	24·4
July	30·4	29·2	29·8	74·5	64·0	69·3	43·92	12·82	28·37	8·5	10·8
August	28·8	30·3	29·5	74·0	71·5	72·8	23·92	21·81	22·86	7·8	9·4
September	29·1	29·7	29·4	76·5	79·5	78·0	28·87	11·34	20·1	7·2	6·5
October	25·8	27·1	26·4	57·5	70·5	64·0	0·19	0·0	0·19	10·8	8·2
November	20·3	..	20·3	53·0	..	53·0	0·24	..	0·24	8·5	..
December	16·4	..	16·4	70·5	..	70·5	0·24	..	0·24	4·3	..

TABLE II.

Per-man-hour density of C. fatigans *in two villages near Ghaziabad, Uttar Pradesh. (January, 1958 to October, 1959).*

Unsprayed/ sprayed.	Year.	Months :											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
Unsprayed	1958	12·9	16·1	17·3	27·4	25·8	18·2	9·8	..	..	..	..	..
Sprayed	1958	..	..	..	..	..	..	..	7·9	8·9	15·6	16·3	13·9
Sprayed	1959	6·4	8·0	13·5	22·5	17·4	12·6	9·6	*	5·1	11·6	†	..

Total mosquitoes collected : 2,521
Per-man-hour density : 14·3

* Observations not made. † Observations stopped.

OBSERVATIONS.

(i) DENSITY.

Between January, 1958 and October, 1959, 2,521 adult *C. fatigans* were collected in 88 searches lasting 176 hours from human dwellings and cattle-sheds in the two observation villages. This species as mentioned earlier, was the most dominant mosquito in the area and was found in fairly large numbers throughout the period of study. The per-man-hour density ranged from 9·8 in July to 27·4 in April during unsprayed period and 5·1 in September to 22·5 in April during the sprayed period (Table II). The indoor per-man-hour density for the period of observation was 14·3.

(ii) PREFERENCE FOR SHELTER.

As mentioned earlier, the indoor resting habits of *C. fatigans* were studied both in human dwellings and cattlesheds. More* specimens (57·2 per cent) were found resting in cattle-sheds than in human dwellings (42·8 per cent). The preference for resting sites and the difference during the unsprayed and sprayed periods in the two types of shelters (mentioned below) were observed to be similar. Therefore, the data obtained from both types of shelters were combined for analysis.

(iii) INDOOR RESTING PREFERENCES.

Table III indicates the preferential resting habits of adult *C. fatigans* in the indoor shelters during the unsprayed (January to July, 1958) and the sprayed periods (August, 1958 to October 1959). The data indicate some differences in the resting habits of this species during the unsprayed and sprayed periods. During the former period, the percentage of mosquitoes found resting on ceiling and cobwebs hanging from the ceiling (18·1) and walls and corners (50·3) were more than during the sprayed period (4·2 and 42·1 respectively). However, during the sprayed period the percentage of mosquitoes found on objects on floor (30·1) and hanging objects (12·5) were more than found resting on these objects during the unsprayed period (13·4 and 10·8 respectively). These differences in the preference for resting sites during sprayed and unsprayed periods were also evident when the data for the same seasons during the unsprayed (January to July, 1958) and sprayed (January to July, 1959) periods were compared (Table III).

By regrouping of these data on the basis of surfaces likely to be sprayed or left unsprayed† during insecticidal treatment (Table IV), it was observed that the differences between the preferences for resting sites during the two periods were statistically significant.

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* Statistically significant at 5 per cent level.

† The baskets and earthen pots kept by the authors remained unsprayed during the period of observation. The other objects on the floor such as cowdung and firewood are either not sprayed during the spray operation or do not remain sprayed because of their constant replacement with fresh stock.

TABLE III.

Adult C. fatigans collected on different surfaces inside village dwellings. (Figures are in per cent).

Total caught.	Time spent, (hours).	RESTING PLACES : Ceiling and cobwebs.	Hanging objects.	Walls and corners.	Shelves.	Objects on floor*.
(A) UNSPRAYED PERIOD (January to July, 1958).						
1063	58	18·1	10·8	50·3	7·4	13·4
(B) SPRAYED PERIOD (January to July, 1959).						
765	58	5·2	15·7	45·0	11·6	22·5
(C) SPRAYED PERIOD (August, 1958 to October, 1959).						
1458	118	4·2	12·5	42·1	11·1	30·1

* Includes mosquitoes found resting on fire-wood, cowdung, earthen pots and empty baskets. The last two were provided by authors for observation.

TABLE IV.

Adult C. fatigans collected on surfaces normally sprayed or left unsprayed inside village dwellings during insecticidal treatment. (Figures are in per cent).

Total caught.	Time spent (hours).	Sprayed surfaces*.	Unsprayed surfaces†.
(A) UNSPRAYED PERIOD (January to July, 1958).			
1063	58	75·8	24·2
(B) SPRAYED PERIOD (January to July, 1959).			
765	58	61·8	38·2
(C) SPRAYED PERIOD (August, 1958 to October, 1959).			
1458	118	57·4	42·6

* Includes ceiling and its cobwebs, walls, corners and shelves.

† Includes hanging objects, and objects on floor, as given in Table III.

(A) and (C) Differences significant at 1 and 5 per cent levels.

(A) and (B) Differences significant at 5 per cent level only.

During the observations out of 2,521 *C. fatigans*, 1,148 were collected from the four walls and the corners in the indoor shelters searched. Data were maintained on the basis of mosquitoes resting at 0 to 2, 2 to 4, 4 to 6 and over 6 feet height from the floor. From these data, it was observed that the mosquitoes preferred to rest at different heights from the floor depending on the prevalent temperature and the saturation deficiency.

Tables V and VI indicate the correlation of the atmospheric temperature and the calculated saturation deficiency with the resting preference for height of mosquitoes on the wall surface. In the case of effects of temperature [Table V (*A*)] it was found that up to a range of 27·0°C. (average monthly mean temperature), significantly more mosquitoes preferred to rest above 4 feet level whereas above this range mosquitoes preferred to rest below four feet level. To bring out these differences, the data were rearranged [Table V(*B*)] on the basis of the two temperature levels, viz , below 27°C. and above 27°C. The significance test of means (*t*-test), applied to the data, showed that the preference for lower height at temperatures above 27·0°C. and vice versa was highly significant.

TABLE V.

The relationship between atmospheric temperature and* C. fatigans *resting at different heights on wall surface.*

(A)

Height (feet).	Temperature (°C.)					Total
	12·1—17·0	17·1—22·0	22·1—27·0	27·1—32·0	32·1—37·0	
0·1—2·0	47 (23·3)	16 (13·1)	44 (18·6)	147 (37·1)	101 (52·6)	355
2·1—4·0	61 (30·2)	34 (27·9)	57 (24·2)	126 (31·8)	59 (30·7)	337
4·1 and above	94 (46·5)	72 (59·0)	135 (57·2)	123 (31·1)	32 (16·7)	456
Total	202	122	236	396	192	1,148

*Average monthly means.

Figures in brackets indicate percentage of the total number of *C. fatigans* resting at the height-group mentioned in Column 1.

(B)

Height (feet).	Temperature (°C.)		Total
	12·1—27·0	27·1—37·0	
0·1—2·0	107 (19·1)[1]	248 (42·2)[4]	355
2·1—4·0	152 (27·1)[2]	185 (31·5)[5]	337
4·1 and above	301 (53·8)[3]	155 (26·3)[6]	456
Total	560	588	1,148

Figures in brackets indicate percentage of the total number of *C. fatigans* resting at the height-group mentioned in Column 1.

t values :

1 and 2= 3·2	4 and 5=3·8
1 and 3=12·9	4 and 6=5·8
2 and 3= 9·6	5 and 6=2·0

Similarly, it was observed [Table VI (*A*), (*B*)] that at saturation deficiencies higher than 20·0 mm., significant numbers of *C. fatigans* preferred to rest below 4 feet level, particularly between 0 - 2 feet level ; and at saturation deficiencies lower than 20·0 mm., there was a tendency to rest at the higher portions of the wall, particularly above 4 feet level.

TABLE VI.

The relationship between saturation deficiency and* C. fatigans *resting at different heights on wall surface.*

(A)

Height (feet).	Saturation deficiency (mm.).						Total.
	0·1—5·0	5·1—10·0	10·1—15·0	15·1—20·0	20·1—25·0	25·1—38·0	
0·1—2·0	38 (23·6)	99 (29·4)	49 (25·5)	9 (11·4)	88 (46·3)	72 (38·1)	355
2·1—4·0	51 (31·7)	103 (33·6)	49 (25·5)	24 (30·4)	41 (21·6)	69 (36·5)	337
4·1 and above.	72 (44·7)	135 (40·0)	94 (49·0)	46 (58·2)	61 (32·1)	48 (25·4)	456
Total	161	337	192	79	190	189	1,148

*Determined from the average monthly means of humidity and temperature.

Figures in brackets indicate percentage of the total number of *C. fatigans* resting at the height-group mentioned in Column 1.

(B)

Height (feet).	Saturation deficiency (mm.).		Total.
	0·1—20·0	20·1—38·0	
0·1—2·0	195 (25·4)[1]	160 (42·2)[4]	355
2·1—4·0	227 (29·5)[2]	110 (29·0)[5]	337
4·1 and above	347 (45·1)[3]	109 (28·8)[6]	456
Total	769	379	1,148

Figures in brakets indicate percentage of the total number of *C. fatigans* resting at the height-group mentioned in Column 1.

t values :

1 and 2 = 1·8	4 and 5 = 3·8
1 and 3 = 8·3	4 and 6 = 3·9
2 and 3 = 6·4	5 and 6 = not significant.

(iv) OUTDOOR RESTING.

During the period of observation, a search for adult *C. fatigans* was made in the likely outdoor shelters within the villages and in their vicinity. The collections, made for 88 hours, revealed the presence of only 43 specimens of *C. fatigans* (Table VII). 55·8 per cent of these were collected on grass and the remaining on trees and other plants (vegetables, etc.).

DISCUSSION.

Culex fatigans has been referred to as one of the most abundant of Indian *Culicini*, found in all parts of the Indian region and largely a domestic mosquito (Barraud, 1934). The experience of most observers and the published data (Horsfall, 1955) confirm the impression that this mosquito is a domestic species. In the observations reported here the overall density in the indoor shelters was

14·3 per-man-hour(Table II) as against 0·5 (per-man-hour.) recorded from the likely outdoor shelters (Table VII). Ramachandra Rao and Rajagopalan (1957 *loc. cit.*) reported similar findings but slightly higher average per-man-hour densities, i.e., 3·5 for outdoor shelters and 14·6 (per-man-hour) for indoor shelters in Poona City, Manjri, Baramati and Bhorghat in Bombay State. The study area of these authors was different in topography and provided extensive breeding places surrounded by large number of trees and other vegetation.

TABLE VII.

Adult C. fatigans *collected from outdoor shelters near Ghaziabad, Uttar Pradesh. (January, 1958 to October, 1959).*

Number of collections.	Time spent (hours).	Resting places: Grass.	Trees.	Other vegetation.	Total.
88	88	24 55·8	15 34·9	4 9·3	43 (Number). 100·0 (Per cent).

Per-man-hour density = 0·5.

As to the prevalence of this species in human dwellings and cattle-sheds, Basu (1957), working in Bokakhat area in Assam, reported that 74·8 per cent of *C. fatigans* were found in human dwellings and only 25·2 per cent in cattle-sheds. In the present studies, more mosquitoes (57·2 per cent) were found resting in cattle-sheds than in human dwellings (42·8 per cent). The preference shown for cattle-sheds was found to be statistically significant That more mosquitoes were found in cattle-sheds than human dwellings may be due to the presence of smoke from kitchen in such shelters and the constant disturbance caused by normal activities of the house-holders.

There are different reports about the resting habits of *C. fatigans* in indoor shelters. Chow and Thevasagayam (1957), while working in Ceylon, observed that 60 per cent of the mosquitoes preferred to rest on clothes and other hanging objects, 9 per cent on furniture, 8 per cent on the ceiling and 23 per cent on the walls. Of the mosquitoes resting on walls, more were found below 6 feet level from the floor than above it. However, Deane (1951), working in Brazil, reported that only about 30 per cent of female *C. fatigans* rest behind and under furniture and on clothes, 20 per cent above 6 feet level from the floor and 50 per cent on the lower portions of walls. Rachou and Lima (1953), also from Brazil found about 60 per cent of female *C. fatigans* resting on the ceiling and upper parts of walls and the rest on lower walls, and curtains and furniture. In India, Sharma *et al.* (1957), while working in Meerut District near the area where the present observations were made, found 6·6 per cent *C. fatigans* resting on ceiling, 83·9 per cent on walls and 9·5 per cent on the articles on the floor.*

* The observations made by Sharma *et al.* (1957) were made during 2 months only and did not include the resting surface provided in the present studies. Therefore the present studies are not comparable with the observations reported by these authors.

In rural or semi-urban villages in India there is very little furniture in the rooms, and there are only a few hanging objects such as clothes, umbrellas. etc. The present observations indicated that 10·8 – 12·5 per cent *C. fatigans* preferred to rest on hanging objects, 4·2 – 18·1 per cent on the ceilings and cobwebs hanging from it, 42·1—50·3 per cent on walls and corners, 7·4 – 11·1 per cent on shelves and 13·4—30·1 per cent on the floor and objects lying on it*.

Chow and Thevasagayam (*1957 loc. cit.*) mentioned that the resting habits of *C. fatigans* in Ceylon were similar in both insecticide (D.D.T.) sprayed and unsprayed areas. However, Rachou (1958) in Brazil observed a change in the indoor resting behaviour of this species in the dieldrin and D.D.T. treated shelters. Whereas in unsprayed houses 80·2 per cent of *C. fatigans* preferred to rest on walls and ceilings and only 19·8 per cent on furniture and clothes, in the dieldrin sprayed houses a reversal of this habit was observed. In the treated houses only 20·4 per cent were found to rest on the walls and ceiling while the remaining 79·6 per cent of the mosquitoes preferred to rest on furniture and clothing, which evidently were unsprayed. After dieldrin spraying was withdrawn, the area was sprayed with D.D.T. for three years after which further observations were made. These observations revealed a slight change from the dieldrin-spray period in that 57·1 per cent were found to rest on walls and ceiling and only 42·9 per cent on furniture, clothes, etc. In either case (D.D.T. and dieldrin sprayed) it seemed that the normal resting habit of *C. fatigans* had undergone a change which Rachou (1958 *loc. cit.*), considered as a manifestation of behaviouristic resistance.

During the present investigations the observation area remained unsprayed from January to July, 1958 and thereafter was sprayed three times, between August 1958 till the end of the observations in October, 1959, with D.D.T. at the rate of 100 mg./sq. foot. It was observed (Table III) that the preference for resting of *C. fatigans* differed during unsprayed and sprayed periods. Whereas during unsprayed period more mosquitoes were found resting on ceiling and cobwebs hanging from it (18·1 per cent), and walls and corners (50·3 per cent), during the sprayed period only 4·2 and 42·1 per cent of the mosquitoes were observed to rest on these structures respectively. However, during the unsprayed period few mosquitoes were found resting on hanging objects (10·8 per cent) and the objects on the floor (13·4 per cent) as compared to the sprayed period when 12·5 and 30·1 per cent were found resting on these structures respectively. The mosquitoes resting in the shelves were more during sprayed period (11·1 per cent) than during unsprayed period (7·4 per cent). This was probably due to the top portions of the shelves not being completely covered by the insecticidal treatment.

From these observations it was evident that the structures likely to be sprayed under the regular indoor insecticidal sprays had less *C. fatigans* resting on them than those likely to remain unsprayed. The data on the mosquitoes resting on

* Range indicates the differences in preference for resting sites observed during sprayed and unsprayed periods.

ceiling and its cobwebs, walls, corners, and shelves (structures likely to be sprayed) and those resting on hanging objects and objects on floor (likely to remain unsprayed) (Table IV) indicated that there was a significant tendency of *C. fatigans* resting in greater numbers on likely unsprayed surfaces during the sprayed period. The differences would appear more marked if the mosquitoes, found resting on shelves, were grouped with the other structures likely to remain unsprayed. The tendency towards more mosquitoes resting on the likely unsprayed surfaces during the sprayed period, may be due to the excito-repellant effect of D.D.T.

Thomson (1938), in a series of observations of caged *C. fatigans* females, showed that humidity and temperature play an important role in the selection of resting sites. A relative humidity of 60-70 per cent and temperature of about 25°C. was preferred to higher or lower temperatures or higher humidities. In the present observations it was noticed that the percentage of females, found resting at different heights from the floor on the wall surface, differed with the prevalent temperature and saturation deficiency. The temperature and humidity data obtained from the meteorological observatory and the calculated saturation deficiencies for the period of observation (Table I), when correlated with the density of mosquitoes resting at 0·1—2·0, 2·1—4·0 and over 4 feet levels from the floor, showed that over a temperature of 27°C. (Table V) and over 20·0 mm. saturation deficiency (Table VI), significantly more mosquitoes preferred to rest below 4 feet level from the floor. The differences in mosquitoes found resting at these intervals were significant. The temperature was above 27·0°C. during April-September, 1958, and May-October, 1959, whereas the saturation deficiency was above 20·0 mm. during April-June, 1958 and May-June, 1959.

SUMMARY.

Indoor and out-door searches, to study the resting habits of *C. fatigans*, were made during January, 1958 to October, 1959 in two villages, namely, Prangarhi and Ramagarhi near Ghaziabad in Uttar Pradesh, India. The observation villages, a semi-urban area, were unsprayed up to July, 1958, after which they were sprayed with D.D.T. at the rate of 100 mg. per sq. ft. in August, 1958 and in July and October, 1959. The observations revealed that *C. fatigans* is a domestic species with an indoor density of 14·3 per-man-hour as against 0·5 (per-man-hour) recorded from the likely outdoor shelters. Significantly more mosquitoes (57·2 per cent) were observed to rest in cattle-sheds than in human dwellings (42·8 per cent). However, no marked difference was observed in the selection of resting sites (structures, heights, etc.) within cattle-sheds and human dwellings.

The studies revealed that, whereas during unsprayed period the per cent *C. fatigans* found resting on ceiling (including cobwebs hanging from it), hanging objects, four walls and corners, shelves and objects on floor were 18·1, 10·8, 50·3, 7·4 and 13·4 respectively, *C. fatigans* resting on these structures during D.D.T.-sprayed period were 4·2, 12·5, 42·1, 11·1, and 30·1 respectively. It was found that during the sprayed period more *C. fatigans* (42·6 per cent) preferred to rest on

likely unsprayed surfaces such as hanging objects and objects on floor than during the unsprayed period, when only 24·2 per cent *C. fatigans* were found resting on these structures. The differences in the resting habits of *C. fatigans* during unsprayed and sprayed periods were found statistically significant.

The present investigation also showed that the prevalent temperature and saturation deficiency influence the resting of *C. fatigans* at different heights on the walls and corners. It was found that above average monthly mean temperature of 27°C. and saturation deficiency above 20 mm., there was a significant tendency towards resting on lower portions of the walls. At temperatures and saturation deficiencies lower than 27·0°C. and 20 mm., the trend for preference of 'resting height' was reversed.

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SOME OBSERVATIONS ON FILARIAL INFECTION IN CEYLON WITH SPECIAL REFERENCE TO ITS HISTOPATHOLOGY.

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FILARIASIS is a major public health problem in Ceylon and other Asian countries. In Ceylon, in the year 1954, there were approximately 7,133 clinical cases under observation and it was found that, out of 84,728 blood films examined, 2,355 were positive, giving a microfilaria rate of 2·8 per cent (de Silva, 1956). Unless effective preventive measures are taken, there is the likelihood of an increase in the incidence of urban filariasis in this country, as it has been noticed that the breeding places of *Culex fatigans*, the vector of *W. bancrofti* responsible for urban filariasis, have increased by some 50 per cent from 1950-54, in spite of the efforts taken by the Anti-Filariasis Campaign and the local Municipalities.

The rural type of filariasis in Ceylon is caused by *Wuchereria* (*Brugia*) *malayi*, whose vector is the *Mansonia* group of mosquitoes, *Taeniorhynchus* (*Mansonioides*) *uniformis* and *Taeniorhynchus* (*Mansonioides*) *annuliferi*, which breed in the plant *Pistia stratiotes* growing exuberantly in tanks, streams and pools of water in the southern, north-western and eastern parts of Ceylon. The destruction of the *Pistia* plant by means of the weedicide "Phenoxylene 30" has resulted in the reduction of the rural type of filariasis while the urban type caused by *W. bancrofti* remains an unsolved problem.

Little attention has been paid to the tissue reactions in filarial infections for the simple reason that adult filarial worms or microfilariae are found in human tissues only by chance. The rarity of such cases has hindered the study of tissue responses to the presence of this parasite. An attempt is made in this paper to describe the development of pathological changes in tissues where filarial worms or remnants of such worms have been found. During the period 1947-1960, forty-eight cases in which filarial worms, alive, dead or calcified or microfilariae were present in the tissues, were studied.

REVIEW OF THE LITERATURE.

O'Connor and Hulse (1932) described in detail one case where adult filarial worms were found in the axillary, epithrochlear, and inguinal glands. Pasternack (1943) invited the attention of pathologists to filarial epididymo-funiculitis and described two cases where dead filarial worms, associated with fibrosing lymphangitis, were discovered in the spermatic cord of two seamen in the American Expeditionary Force. Hartz (1944) gave an account of the histopathology of filariasis

basing his observations on three patients in whose tissues macro or microfilariae were found. Wartman (1944) reported 17 cases of acute lymphangitis of varying severity. Filarial worms were seen in lymph glands in 5 cases. The tissue changes in these cases have been described in detail. De Saram and Peiris (1946) reported the presence of an adult filarial worm in the epitrochlear gland of a Ceylonese patient while Clayton Lane (1948) has discussed the biological mechanisms that underlie the periodicity and the clinical manifestations of Bancroftian filariasis. His observations were based on several tissue sections showing macro- and microfilariae. Paul (1950), in describing the blood and lymph pathways in the spermatic cord, drew attention to the presence of adult worms in lymphatics of this structure. Dhayagude and Amin (1942) described multiple gross nodular lesions in the spleen of eleven patients from Bombay, which lesions were believed to be caused by microfilaria and hence termed microfilarial granulomata.

THE DIAGNOSIS OF FILARIAL INFECTIONS.

Table I gives a short summary of the clinical and pathological findings in the 48 cases studied. In all excepting two cases, live, dead or calcified adult filaria worms were found in the tissues, the exceptions being the finding of microfilariae in two cases. From a perusal of Table I, it becomes evident that the clinical diagnosis of a filarial infection cannot be arrived at with any degree of precision. In this series only ten cases were thought to be of filarial origin clinically, although filarial worms were later found in the tissues. The presence of such worms brings about protean manifestations such as lymphadenopathy, testicular and inguinal swellings and hydrocoeles and although the clinician in the tropics is mindful of filariasis as being the cause, a certain diagnosis is not always possible.

Table II gives a summary of the presenting symptoms. The most frequent were lymphadenopathy, enlargement of the testicle and epididymis, swelling in the groin, and hydrocoele. It is of interest to note that 5 of the 14 females in the series presented with tumour-like enlargements of the breast 4 of which simulated mammary carcinoma and one fat necrosis. The spermatic cord showed nodular thickening in 2 cases. There was one example each of inguinal abscess, salivary gland tumour and ectopic gestation while in one case microfilariae were found in the routine examination of a spleen removed during the operation of total gastrectomy for carcinoma. As, in all excepting 10 cases, the diagnosis of a filarial infection was made only after a histological examination of the tissue after operation, it is of importance to recognise the histological changes that occur in filarial infected tissues. Although such changes are thought to be non-specific and not typical, it is possible, by studying a series like the one presented in this paper, to establish certain criteria whereby the pathologist could arrive at a diagnosis of this condition. These changes, (*vide infra*), if found in any of the tissues mentioned, should be considered as indications for a thorough search of the adult worm or microfilariae in the tissues. Such a search becomes imperative if the patient presents with the symptoms listed in Table II, particularly lymphadenopathy, epididimo-orchitis, inguinal swellings, hydrocoeles and breast tumours.

TABLE I.

Short summary of clinical and pathological findings in 48 cases in which filarial worms, alive, dead or calcified or microfilariae were present in the tissues.

Number.	Age (years).	Sex.	Presenting symptoms.	Clinical diagnosis.	Biopsy.	Histological findings.
1	34	M	R. hydrocoele	Hydrocoele	Inguinal lymph node	Live macrofilaria in lymph gland.
2	20	M	Enlarged left testicle, thickening of cord. Craggy epididymis	Tb. epididymo orchitis	Thickened cord	Calcified macrofilaria.
3	14	F	L. inguinal hernia	Inguinal hernia with filarial lymph adenitis.	Inguinal lymph gland	Live macrofilaria in lymph gland.
4	2½	F	Enlarged axillary lymph glands	Axillary lymph adenitis.	Axillary lymph gland	Dead worm macrofilaria.
5	36	F	Lower abdominal pain	Ectopic pregnancy	Twisted ovarian cyst	Live macrofilaria in ovarian tissue.
6	15	F	Enlarged axillary glands	? Tuberculosis ? Hodgkins disease	Axillary lymph gland	Live macrofilaria in lymph gland.
7	19	M	L. hydrocoele and hernia	Inguinal hernia and hydrocoele	Spermatic cord	Dead macrofilaria.
8	20	F	Enlarged gland in neck and inguinal region	Tuberculous lymphadenitis	Inguinal lymph gland	Live macrofilaria in lymph gland.
9	27	M	R. hydrocoele	Hydrocoele (filarial)	Spermatic cord	Calcified macrofilaria.
10	40	M	Swelling inguinal region	Funiculitis inguinal hernia (filarial)	Spermatic cord	Dead macrofilaria.
11	25	M	Enlarged inguinal gland	Lymphadenitis	Inguinal lymph gland	Live macrofilaria.
12	18	M	Enlarged R. inguinal gland, oedema of R. leg.	Filarial lymphadenitis	Inguinal lymph gland	Dead macrofilaria.
13	20	M	Bilateral hydrocoele	Filarial hydrocoele	Spermatic cord.	Dead macrofilaria.
14	30	M	L. inguinal hernia	Inguinal hernia (Filarial lymphadenitis).	Inguinal lymph gland	Dead macrofilaria.
15	19	M	Enlarged cervical and inguinal L. glands	Hodgkin's disease	Inguinal lymph gland	Dead macrofilaria.
16	47	M	Bilateral enlargement of testis	Syphilitic orchitis	Spermatic cord	Calcified macrofilaria.
17	13	F	Bilateral enlarged inguinal gland	Inguinal lymphadenitis	Inguinal lymph gland	Dead macrofilaria.
18	42	M	Nodule in inguinal region	Bubonocoele	Spermatic cord	Dead macrofilaria.
19	20	M	Abscess L. inguinal region	Suppurative adenitis	Inguinal lymph gland	Dead macrofilaria.
20	46	F	Nodule R. breast	Carcinoma of breast	Breast	Dead macrofilaria with adenosis of breast.
21	55	F	Tumour of breast.	Fat necrosis of breast	Breast	Dead macrofilaria.
22	32	M	Swelling of testicle	Tuberculous epididymitis	Spermatic cord	Dead macrofilaria.
23	27	M	Swelling submandibular region	Salivary gland tumour	Submandibular lymph gland	Dead macrofilaria.
24	58	M	Hydrocoele	Hydrocoele (filarial)	Scrotal lymphatics	Dead macrofilaria.
25	28	F	Swelling of left arm with enlarged epitrochlear gland	Filarial elephantiasis	Epitrochlear gland	Dead macrofilaria.

(Contd.)

TABLE I (*Concld*).

Number.	Age years.	Sex.	Presenting symptoms.	Clinical diagnosis.	Biopsy.	Histological findings.
26	14	M	Swelling inguinal region	Funiculitis	Lymphatics of scrotum	Partly calcified macrofilariae.
27	28	M	Swelling inguinal region	Funiculitis	Spermatic cord	Dead macrofilaria.
28		M	Nodules on spermatic cord	Funiculitis	Spermatic cord	Dead macrofilaria.
29	30	M	Swelling inguinal region	Funiculitis	Spermatic cord	Dead macrofilaria.
30	18	F	Enlarged cervical glands	Tb. adenitis	Cervical lymph gland	Dead macrofilaria.
31	32	M	Enlarged testicle	Epididymitis (filarial)	Vas deferens	Dead macrofilaria.
32	30	M	Nodule in vas	Funiculitis.	Vas deferens	Dead macrofilaria.
33	43	M	Swelling of scrotum	Filarial elephantiasis of scrotum	Spermatic cord	Dead macrofilaria.
34	30	M	Swollen testicle	Filarial lymphangiectasis	Spermatic cord	Dead macrofilaria.
35		M	Swelling in inguinal region	Funiculitis	Spermatic cord	Dead macrofilaria.
36	15	M	Swelling in testicle	Epididymitis	Spermatic cord	Partly calcified macrofilaria.
37	42	M	Swelling in inguinal region	Funiculitis	Spermatic cord	Dead macrofilaria.
38	42	F	Swelling of breast	Carcinoma of breast	Breast	Dead macrofilaria.
39	20	M	Swelling of testicle	Epididymitis	Spermatic cord	Dead macrofilaria.
40	36	M	Hydrocoele	Hydrocoele	Spermatic cord	Dead macrofilaria.
41	60	M	Enlarged testicle	Filarial orichitis	Spermatic cord	Dead macrofilaria.
42		M	Swelling inguinal region	Funiculitis	Spermatic cord	Dead macrofilaria.
43	10	F	Enlarged cervical lymph gland	Hodgkin's disease	Cervical lymph gland	Dead macrofilaria.
44	40	F	Tumour of breast	Breast carcinoma	Breast	Dead macrofilaria.
45	36	F	Hard nodule in breast	Carcinoma of breast	Breast	Dead macrofilaria.
46	33	M	Hydrocoele	Hydrocoele	Sac wall	Microfilaria amidst necrotic tissue.
47	60	M	..	Carcinoma of stomach. Splenectomy done during operation of gastric resection.	Spleen	Microfilarial granuloma.
48	12	M	Swelling inguinal region	Inguinal adenitis	Inguinal gland	Live macrofilaria.

The sites where adult worms, live, dead or calcified, were found, are given in Table III. It will be noticed that the spermatic cord in the male was the most frequent site and the lymphatic glands in both males and females the next in order of frequency. Adult worms were found in the female breast in 5 cases. Craig and Faust (1937) state that, in women, the mammary gland is not infrequently involved. An unusual finding was that of an adult filarial worm in an ovarian cyst which case presented as an ectopic gestation. There were 2 cases where adults were found in the vas deferens and two more in the lymphatics of the scrotal skin. The preponderance of

inguinal gland involvement may possibly be due to the biting habits of the mosquito and to the anatomical arrangement of lymphatics.

TABLE II.

Presenting symptoms in 48 cases.

Presenting symptoms.	Number of cases.
Enlarged lymphatic glands ...	11
Enlarged testicle and epididymis ...	8
Swelling in inguinal region ...	8
Hydrocoele ...	7
Breast tumours ...	5
Nodular thickening of cord ...	2
Inguinal hernias ...	2
Abscess in inguinal region ...	1
Scrotal swelling ...	1
Salivary gland tumour ...	1
Lower abdominal pain ...	1
Carcinoma of stomach ...	1
Total ...	48

TABLE III.

Sites where adult worms, live, dead or calcified, were found in 48 cases.

Site of filarial worms.		Number of cases.
Spermatic cord ...		20
Lymph glands		
Inguinal	10	
Axillary	2	
Cervical	2	16
Epitrochlear	1	
Submandibular	1	
Female breast ...		5
Vas deferens ...		2
Scrotal lymphatics ...		2
Ovarian cyst ...		1
Hydrocoele sac ...		1*
Spleen ...		1*
Total		48

*Microfilariae.

HISTOLOGICAL CHANGES.

The description which will be given is a composite one of all the material examined. It has been possible by a study of this series of cases to describe the morphology of the adult worms within lymphatics, the development of changes in filarial infected tissues and the genesis of lymphatic obstruction.

The adult worm is usually seen within a markedly dilated lymphatic, frequently a subcapsular or periglandular lymph vessel. It is elongated and cylindrical in shape (Figs. 1 and 2) with a smooth non-nucleated cuticle. The

worm which was alive at the time of fixation can be made out by the presence in its uterine tubes, the wall of which can be distinctly identified, of a number of ensheathed microfilariae which may be cut longitudinally in section as in Fig. 1 or transversely as in Fig 2. The characteristic features of the microfilariae inside the live worm are :— (1) they are even in outline; (2) possess deeply staining nuclei; and (3) can be distinguished individually. In this early stage, apart from the dilatation of the lymphatic vessel, which is probably a compensatory phenomenon, to allow for the free flow of lymph, there is no evidence of lymphatic obstruction. The lymph gland shows a marked degree of follicular hyperplasia (Fig. 3) as well as the presence of a fairly large number of giant cells lining the lymph sinuses (Fig. 4). These changes are probably brought about by the secretory and excretory products of the microfilariae expelled by the live filarial worm during their migration in the lymphatic glands. The presence of follicular hyperplasia and a giant cell reaction indicate that the tissues should be examined more thoroughly, and if necessary, by serial sectioning. At this stage, when the adult worm is alive, there is little or no eosinophilic cell infiltration and no granulomatous reactions are seen.

In the case of recent death of the adult worm the uterine wall can still be identified but only a few embryos can be made out as separate individuals while a larger number is in the form of ill-defined basophilic masses. The lymphatic vessel remains dilated and therefore affords no obstruction but there is some fibroblastic proliferation around it and commencing lymph thrombosis within (Fig. 5). When some time elapses between the death of the parasite and fixation of the tissues, it is only possible to define parts of the wall of the uterine tubes. No individual microfilariae can be made out, the uterine contents appearing as dense basophilic masses (Fig. 6).

Tissue reactions appear to result from the presence of dead worms which appear in section as ill-defined structureless masses. In the early stages the tissue reaction is characterised by the appearance of giant cells which are seen lining the lymphatic vessel (Fig. 7). The sub-intimal tissues are infiltrated with inflammatory cells amongst which a large number of eosinophils are seen. Partial occlusion of lymphatic vessels is now apparent.

The lymphatic vessels show a great variety of lesions, the recognition of which will enable the pathologist to make a diagnosis of filarial infection even in the absence of live or dead filarial worms. A marked cellular reaction develops in the lymphatic vessel in the sub-endothelial connective tissue which takes the form of a granulomatous reaction characterised by the presence of epitheloid and giant cells. Such a reaction which is very helpful in the diagnosis of a filarial infection is shown in Fig. 8. Sometimes the granulomatous reaction which develops around a dead parasite causes complete obliteration of the lymphatic vessel and in order to overcome this obstruction the muscle coat undergoes hypertrophy and appears more prominent (Fig. 9).

Fig. 1.

Fig. 1. Lymph gland. Live microfilaria within a dilated lymphatic microfilaria in longitudinal section can be distinguished individually.

Fig. 2. Lymph gland—Live microfilaria in dilated lymph vessel. Microfilariae in transverse section can be distinguished individually.

Fig. 2.

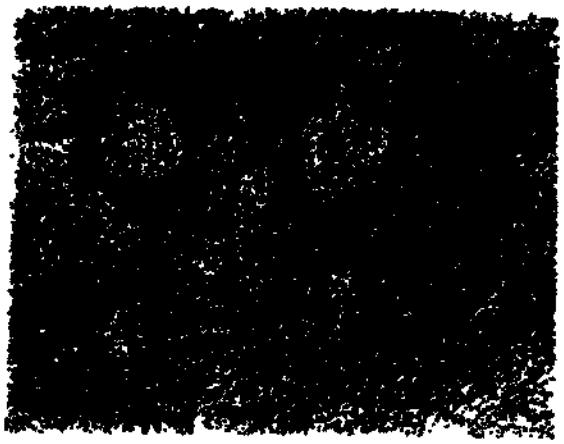

Fig. 3.

Fig. 3. Lymph gland showing well marked follicular hyperplasia with prominent germinal centres.

FIG. 4.

FIG. 4. Lymph gland showing a giant cell projecting into a lymph sinus.

FIG. 5.

FIG. 5. A living macrofilaria within a dilated lymphatic in which lymph thrombosis is commencing.

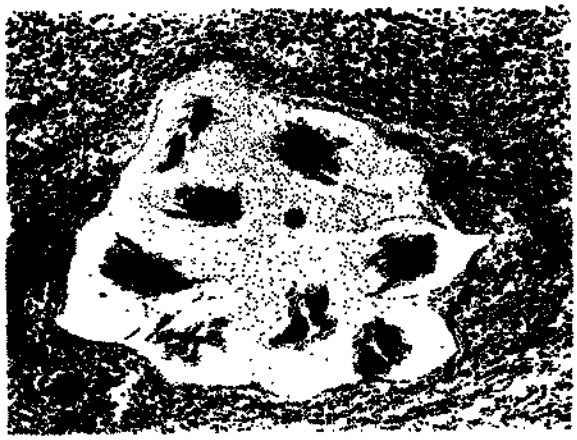

FIG. 6.

FIG. 6. A dead macrofilaria within a dilated lymphatic. Uterine contents converted to a homogeneous basophilic mass. Microfilariae cannot be distinguished individually.

Fig. 7.

Fig. 7. A giant cell granulomatous reaction developing around a dead macrofilaria represented by a structureless mass within a lymphatic which is partially occluded.

Fig. 8.

Fig. 8. Epithelioid and giant cell reaction in the wall of a lymphatic.

Fig. 9.

Fig. 9. A dilated and occluded lymphatic with accentuation of the muscle coat showing a marked inflammatory reaction around a dead macrofilaria.

FIG. 10.

FIG. 10. The granulomatous reaction around the dead macrofilaria has destroyed the lymphatic. A few strands of muscle can still be made out (bottom right)

FIG. 11.

FIG. 11. Dead tissue showing a granulomatous reaction around a dead parasite. Lymphatics are unrecognisable.

FIG. 12.

FIG. 12. Granulomatous reaction around a dead parasite. The cells with dense staining are eosinophils.

In other cases the granulomatous reaction destroys the surrounding muscle, saving only a few fibres which still remain recognisable, and which merges with surrounding inflammatory cells (Fig. 10). At a later stage the components of lymphatic vessels become unrecognisable, the picture presented being an intense cellular reaction around dead worms (Fig. 11 and 12). The presence of eosinophil leucocytes amongst these cells, as well as in areas some distance from the dead parasites, is a characteristic feature. Some cases show this endolymphangitic reaction in several lymph vessels which appear in sections as well-defined granulomatous masses with central necrosis (Fig. 13), some showing slit like spaces which represent lymphatic lumina (Fig. 14).

In long standing infections, the dead parasite lying within the lymphatic vessel undergoes calcification. The cellular granulomatous reaction seen in the early stages is replaced by the formation of concentric layers of a cellular hyaline fibres (Fig. 15). As no musculo elastic tissue is identified in such lymphatics it must be presumed that, on account of the lack of a propulsive force, lymph stasis occurs in them although their lumina still show some patency. Finally the calcified contents completely obliterate the lymphatic (Fig. 16).

Lymphatic obstruction.—It is quite clear from the foregoing description that living adult worms do not effectively block a lymphatic as shown earlier by O'Connor and Hulse (1932 *loc. cit.*) and by Clayton Lane (1948 *loc. cit.*). Lymphatic obstruction results from the granulomatous reaction (endolymphangitis) which develops after the death of the worm (Fig. 9 to 14) and calcification with sclerosing lymphangitis (Fig. 15 and 16). Obstruction to lymphatics results in lymph thrombosis and the lymph thrombi appear in sections as structureless homogeneous material within dilated lymphatics (Fig. 17). In the smaller sinuses of lymphatic glands the lymph thrombi appear in sections as deeply staining lymphocytes which have conglutinated to form large masses (Fig. 18). Obstruction to lymphatic flow promotes the formation of collateral lymphatic channels which become conspicuous (Fig. 17). These appearances in histological material which indicate lymphatic obstruction are important diagnostic criteria of a filarial infection and should prompt the pathologist to search the tissue more carefully for the presence of adult worms.

Microfilariae in tissues is a rare finding. In this series only two such cases have been encountered—one in the spleen (Fig. 19) removed during a total gastrectomy for carcinoma of the stomach and the other amidst necrotic tissue removed from the wall of a hydrocoele sac (Fig. 20). The infrequency of the presence of microfilariae is due to (1) in a majority of the cases in this series the adult worm was dead; (2) the development of occlusive lymphangitis which blocks the passage of microfilariae; (3) destruction of microfilariae by the reticulo endothelial system. Although Craig and Faust (1937*b*) state that living circulating microfilariae do not cause tissue alterations, Dhayagude (1942 *loc. cit.*) has described microfilarial granulomata in the spleen. In a suspected case of filariasis a careful search for microfilariae in the tissues should be made by the pathologist.

SUMMARY AND CONCLUSION.

1. Filariasis is a major problem in Ceylon and other Asian countries.

2. The rarity of the finding of adult filarial worms and microfilariae in tissues has acted as an impediment to the study of the histopathological reactions in this disease. Only a few cases of such findings are reported in the literature.

3. This paper deals with the tissue responses in filarial infections, the study being based on 48 cases. *Adult filariae* were found in the tissues in 46 cases and *microfilariae* in 2 cases.

4. These cases presented with protean manifestations such as lymphadenopathy, epididymo-orchitis, groin swellings, hydrocoeles and breast tumours, salivary gland tumour and ectopic gestation. As, in only ten cases was the diagnosis established clinically, it becomes important to recognise the histopathological changes in affected tissues in order that the correct diagnosis may be arrived at by the pathologist after an examination of tissues removed at a surgical operation or biopsy.

5. The spermatic cord, lymphatic glands, and the female breast were the most frequent habitat of adult worms. In the case of the lymphatic glands, the inguinal glands were most frequently involved.

6. The morphology of the live parasite and the metamorphosis that take place during death have been described. In this series 7 were alive, 34 were dead and 5 were calcified at the time of fixation of tissues while the 2 remaining cases showed *microfilariae* in tissues. In such cases the histological diagnosis of a filarial infection is readily made.

7. Death of the parasite takes place as the result of tissue activity which initially takes the form of endolymphangitis. This process causes a considerable degree of lymphatic occlusion which is compensated to some extent by the dilatation of collateral lymphatics and the formation of new lymphatic channels.

8. The migration of microfilariae within a lymphatic gland brings about follicular hyperplasia as well as a giant cell reaction in the lymph sinuses.

9. The lymphatics later become unrecognisable. However, in some cases remnants of the hypertrophied muscle coat of the lymphatic vessel can still be made out.

10. A granulomatous reaction, characterised by the presence of epithelioid cells and giant cells resembling a tubercle follicle, develops around the dead parasite. The presence of eosinophil leucocytes amidst this granulomatous tissue as well as in the immediate vicinity is a characteristic feature. These granulomata completely obliterate the lumen of lymphatic vessels or reduce the lumen to a mere narrow slit.

11. Finally the lymphatic vessel becomes permanently occluded by deposition of calcium in the granulomatous mass.

12. The above appearances are characteristic and should help the pathologist in the diagnosis of a filarial infection.

Fig. 12.

Fig. 13. Lymphangitis including spermatic cord lymphatics.

Fig. 14.

Fig. 11. Lymphangitis causing partial occlusion of spermatic cord lymphatics.

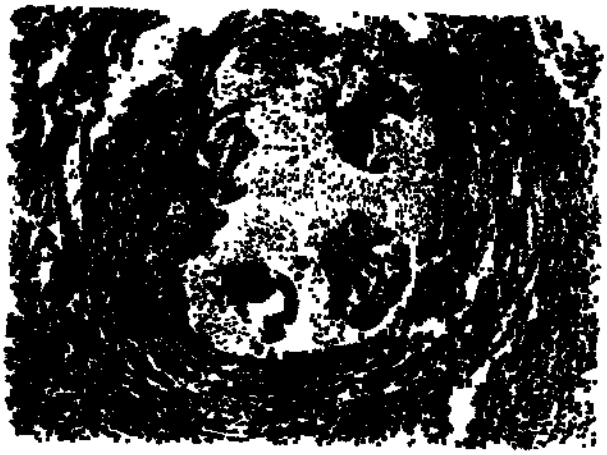

Fig. 13.

Fig. 15. Spermatic cord—Calcification of the parasite with formation of concentric layers of acellular hyaline fibres.

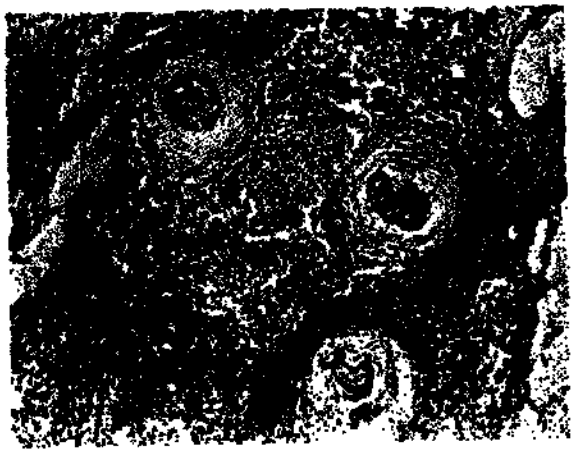

FIG. 16.

FIG. 16. Complete obliteration of spermatic cord lymphatic vessels by calcium deposits.

FIG. 17.

FIG. 17. Lymph gland showing dilated lymphatics with lymph thrombi. Formation of several patent collateral channels.

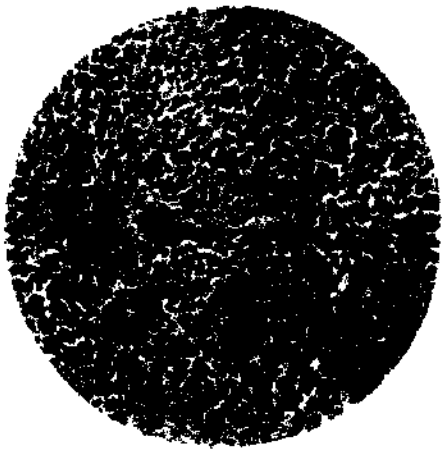

FIG. 18.

FIG. 18. Two lymph thrombi in the sinuses of a lymph gland.

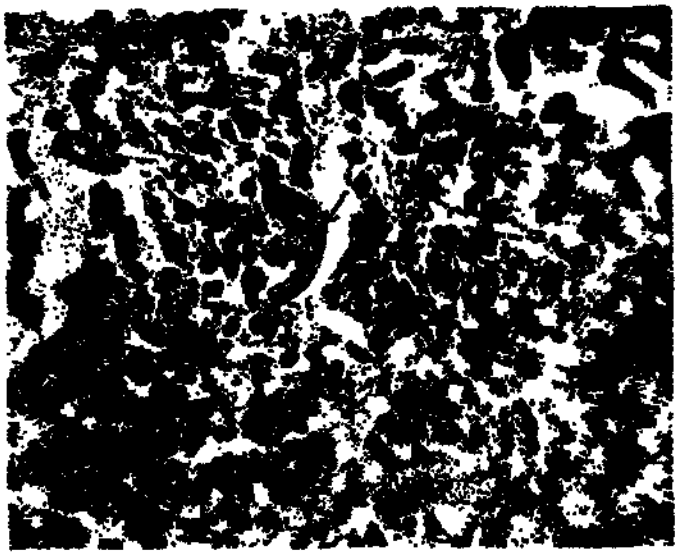

FIG. 19.

FIG. 20.

FIG. 19. Microfilaria in spleen indicated by arrow.
FIG. 20. Microfilariae in necrotic tissue from a hydrocele sac

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AETIOLOGY OF TROPICAL EOSINOPHILIA.

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[October 8, 1960.]

INTRODUCTION.

FRIMODT-MOLLER and Barton (1940) described a series of cases which they called a pseudo-tuberculous condition with widespread *x*-ray shadows, pronounced eosinophilia but no tubercle bacilli in the sputum. The syndrome (tropical eosinophilia) which has since been diagnosed in many parts of India, Ceylon, Malaya, the West Indies and the islands of the Pacific, is marked by cough often associated with severe asthmatic attacks and responds fairly readily to arsenic therapy. In this respect it differs from the disease described by Loeffler of Zurich. Great interest has been shown in the aetiology of the disease. Allergy was suggested but no specific allergic agent has been identified. The presence of mites in the sputum from patients was a primary field of study but the ubiquitous occurrence of these insects threw doubt on the significance of these observations. The possibility of a helminth infection as the cause has attracted many workers since both *Ascaris lumbricoides* and *Ankylostoma duodenale* spend part of their life cycle in the lungs. Ball (1950), however, in his very thorough review of the condition, was unable to find a greater incidence of these infections among patients with tropical eosinophilia than in the general community.

Microfilariae were demonstrated by Meyers and Kouwenaar (1939) in eosinophil aggregates in the lymph nodes of Javanese patients, who presented with enlarged lymph nodes and massive eosinophilia in the blood. They demonstrated degenerating larvae in these lymph nodes surrounded by deeply acidophilic necrotic material which have been called Meyers-Kouwenaar bodies. In 1952 Beaver and his colleagues demonstrated the formation of eosinophil granulomata in the liver and lung and a marked eosinophilia in the blood of children in the Southern United States infected with the larvae of *Toxocara canis*. They suggested that the larvae were rapidly destroyed instead of circulating in the peripheral blood and that a similar host reaction might account for the syndrome of tropical eosinophilia. In recent years a microfilarial basis has been suggested by two pieces of

evidence. Firstly tropical eosinophilia responds remarkably well to a potent antifilarial drug, diethylcarbamazine. Secondly Danaraj and his colleagues (1958 : 1959) have shown that many cases of tropical eosinophilia have present in their sera complement fixing antibodies against a filarial antigen made from *Dirofilaria immitis*.

CLINICOPATHOLOGICAL INVESTIGATION.

These findings suggested to us a study of material from cases of tropical eosinophilia in order to discover the type of lesion present and any indication of an organismal basis in these lesions. All the patients studied were typical cases clinically of tropical eosinophilia in whose blood microfilariae could not be demonstrated.

LIVER BIOPSIES.

Our first interest lay in the examination of liver biopsies from children with tropical eosinophilia, as liver enlargement was not an uncommon finding in such cases. The first specimens were obtained by needle biopsy but these revealed little beyond an eosinophilic infiltration of the portal tracts. We then decided to perform open liver biopsies through an upper abdominal incision and seven livers were examined in this way. In each case, the liver showed a number of small white spots 2 – 5 mm. in diameter, scattered over the serous surface. We decided to make serial sections of the biopsies so that if any trace of a larva was found, it could be followed through from one section to another. A variety of lesions were seen in the livers. The general pattern of the liver lobules was preserved. There was irregular infiltration of the portal tracts by eosinophils but in addition there were lesions varying from lakes of eosinophils to granulomas with central collections of foreign body giant cells surrounded by epithelioid cells and eosinophils at the periphery. A careful search in these eosinophil aggregates under oil immersion magnification revealed the presence of one microfilaria which could be followed through nine serial sections. The organism could be clearly recognised by its cylindrical outline and the black dots in its body. The total length was at least 160 microns and the greatest width 6 – 7 microns. The constant picture of eosinophil granulomas in the liver of cases of tropical eosinophilia made us anxious to secure biopsies of the lung in such cases.

LUNG BIOPSIES

We recalled the removal of a lung in 1950 which showed eosinophil aggregates. We were also fortunate in obtaining a biopsy from a lung in a patient, who had tropical eosinophilia but who was also operated on for a patent ductus arteriosus and from another patient, who also had a lung cyst. Finally in a fourth patient an open lung biopsy was performed. In all these cases, serial sections were taken of the biopsy specimen or from representative pieces of the resected lung.

The lung tissue showed small nodules 3—5 mm. in diameter separated by air containing lung. The nodules were 1 – 3 cm. apart and in one case there were

larger areas of consolidation. On histological examination, some of these nodules consisted of collections of eosinophils. In some places the walls of the alveoli were destroyed so that the nodule formed an eosinophil abscess. These resembled closely similar nodules seen in the liver. However, there were some nodules in the centre of which epithelioid cells were surrounded by masses of eosinophil staining hyaline material and others where giant cells and epithelioid cells formed giant cell systems surrounded by a few eosinophils. Careful scrutiny of the sections revealed fragments of larvae in the nodules of three out of the four lungs examined. In one case an almost complete larva was followed through three sections having a length between 200 and 230 microns and a width of approximately 7 microns. Larvae were not found in granulomas where a giant cell reaction had taken place.

LYMPH NODES.

Enlarged lymph nodes in cases of tropical eosinophilia had been noted by Ball (1950 *loc. cit.*). In fourteen cases, lymph node biopsy was performed. In seven cases (two without and five with lung involvement) the nodes contained eosinophil aggregates and granulomas resembling those seen in the lung and the liver. In all of these lymph nodes, portions of microfilariae were found in the eosinophil aggregates. Teased preparations in saline were examined for the presence of live microfilariae and these were found in five cases.

DISCUSSION.

These results are only presented in summary as fuller clinical and pathological reports have already been published. It has been shown that granulomas, varying from collections of eosinophils to giant cell systems, occur as nodules in the liver, lung and lymph nodes of typical cases of tropical eosinophilia. Such lesions have already been described but the finding of microfilariae in association with these lesions is a new and important observation which has only been possible by careful and thorough examination of literally thousands of serial sections. The presence of microfilariae appears to excite an eosinophil reaction but with the death of the larvae a granulomatous reaction occurs. We presume that these larvae are being constantly released, trapped and destroyed and the result of this is to produce reactions in the tissues and a generalised blood eosinophilia. In most cases the major lesion is found in the lung but occasionally lesions are found more markedly in the liver or the lymph nodes.

We have not been able to identify the type of infection. The larvae closely resemble the microfilariae of *W. bancrofti* but in Malaya, Edeson, Wharton and Buckley (1955) have reported the recovery of sheathed microfilariae indistinguishable from human *W. malayi* from five different species of animal. The microfilariae seen in lung, liver and lymph node may therefore represent an unusual form of infection in which the body reacts to their presence with the formation of eosinophil aggregates and their death by granulomatous formation. We have not

been able to identify an adult source of these microfilariae as yet and a thorough search for this and for all possible sources of infection needs to be undertaken.

SUMMARY.

Nodules consisting of eosinophils and granulomas containing giant and epithelioid cells have been found in biopsy material from the liver, lung and lymph nodes of typical cases of tropical eosinophilia. In the centre of some of these nodules (but not in those with a giant cell reaction) fragments of microfilariae have been found. The microfilariae resemble the sheathed microfilariae of *W. bancrofti* but they may well belong to a closely allied species.

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ENDEMIC LYMPHANGITIS IN FRENCH GUIANA AND ITS RELATION TO *W. BANCROFTI* FILARIASIS*.

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[April 4, 1960.]

A HIGH incidence of "large legs" is found to occur in the population of Antillian group of islands and of the north-east of South America (region sometimes also improperly called "Carribean"). The condition is often (fortunately) limited and the deformity does not reach the stage of true elephantiasis. This malformation is generally considered to be due to the repeated attacks of lymphangitis and the term "tropical endemic lymphangitis" seems particularly justified for these recurrent crises which are observed in this geographical region.

The relationship between the tropical endemic lymphangitis (and its eventual termination in elephantiasis) and filariasis has been dealt with in numerous publications and has provoked a great deal of discussion.

The protagonists of filarial aetiology have advanced serious arguments. However, during early years of the century, many authors have equally vehemently defended the infectious microbial origin of the tropical endemic lymphangitis.

No conclusion has been reached between the protagonists of the different pathogenic theories : univocal theory (exclusively filarial or exclusively bacterial) and dualist theory with a combination of the two aetiological agents.

The French physicians were, in general, amongst the first protagonists of the bacterial aetiology.

The endemicity of tropical endemic lymphangitis in French Lesser Antilles, Martinique and Guadeloupe, is described below.

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* The original manuscript of this paper was received from the authors written in French.

Thanks are due to Mr. Bal Raj, M.A., Indian Council of Medical Research, Malaria Institute of India, Delhi, for kindly translating the manuscript from French to English.—EDITOR.

In Martinique, the incidence of "large legs" is high. The microfilaria rate (*W. bancrofti*) continues to be between 7 and 10 per cent. This is in spite of the use of contact insecticides. The possibility of ineffective application of the insecticides has to be considered. It would appear that women are more affected than men and this observation has been made also in other zones of the region. The conclusion that it is so, may be hasty due to the fact that the observation of the condition is easier in women than in men due to differences in the way of dressing. It can also be due perhaps to the fact that women are more concerned and, therefore, consult physicians to a greater extent than men. It is, however, difficult to explain the comparatively higher incidence of the disease in the left leg of both the sexes.

About 2 to 5 per cent of the hospital cases have elephantiasis. These cases seem to be hospitalised for various other conditions. They do not seem to be keen on the treatment of elephantiasis due to previous experience of failures. Besides, the disease is ignored from the point of view of Social Security.

The physicians see less number of acute lymphangitis than in the past. This may be due to the fact that many patients used domiciliary treatment with sulphonamides, perhaps resulting in a reduction in the duration of attack. This does not, however, seem to prevent recurrence. In retrospect, the situation with regard to the disease seems to be stationary for the past many years.

The microfilaria rate in Guadeloupe also seems to have been stationary. It is about 15 per cent on the whole. The infection seems to be also confined to certain families in certain localities. About 8 per cent of the total attendance in dispensaries is for lymphangitis or elephantiasis of various degree. Two-thirds (2/3rd) of the patients are women. It would seem that the frequency of acute attacks of lymphangitis is gradually decreasing. At present a patient seems to have one or two attacks per year ; in some cases there seems to be several years of remission in between the attacks. This state of affairs have slowed down the progress of the disease to its terminal stage, which is now becoming rare. Most often only the intermediary stages like inguinal or axillary lymphangitis, intermittent swellings often leaving residual oedema, are observed. These conditions seem to be related to menstruation in women. Prolonged standing also seems to contribute to these conditions. Chronic oedema without pachydermia is common.

In French Guiana, the microfilaria rate seems to be on the decrease. From 27·7 per cent in 1917, it came down to 18·6 per cent in 1934 and 14·3 per cent in 1947. This improvement of the situation could be attributed to the decrease in the prevalence of *C. fatigans* from 1949 to 1954. In the earlier period the decrease was due to the action of DDT., and later, when the insect became resistant to DDT, the reduction was maintained by antilarval measures using HCH. In 1944-1945, the infection rate of *W. bancrofti* in *C. fatigans* was found to be 11 per cent (Floch and Abonnenc, unpublished result). In 1951, no infection was detected in 50 specimens of *C. fatigans* which were dissected (unpublished result). New cases of elephantiasis have been very rare for the last 12 years.

I. ARGUMENTS IN FAVOUR OF FILARIAL THEORY.

1. GEOGRAPHICAL ARGUMENTS.

The incidence of acute lymphangitis is known to be more frequent in populations living in areas endemic for *W. bancrofti* filaria. It is to be remembered that the absence of micro-filariae in the blood of cases of lymphangitis is not a proof of absence of filarial infection.

2. PARASITOLOGICAL ARGUMENTS.

A number of authors have observed microfilariae of *W. bancrofti* in the blood of persons with lymphangitis. Adult worms have been observed in fluids obtained by puncture of the affected tissues. In many of the instances, no bacterial organisms were found.

The Island of Guadeloupe, in 1914, was definitely considered to have a greater incidence of elephantiasis than Martinique. In that year, the microfilaria rate was 15·33 per cent in the former and did not exceed 5·47 per cent in the latter.

In 1917, the microfilaria rate was 28 per cent in French Guiana. Bremont and Leger (1917) found *W. bancrofti* infection in 18 cases of elephantiasis examined by them. These authors did not, however, state whether they attempted to isolate microbial organisms by culture of blood or lymph.

Mackinley (1931), working in Puerto Rico, was not able to culture any microbial organism from the blood of 38 cases of acute lymphangitis. Culture of serous fluid aspirated from the sites of pain and inflammation also produced negative results. Skin clippings from 11 cases were also cultured but no streptococci were isolated. On the contrary, 7 out of 9 clear cases of septic lymphangitis yielded streptococci in culture. Similar have been the findings of Giglioli (1933) who obtained positive cultures only from fluids drawn from inflammatory tissues and abscesses.

Thooris (1956), working in French Oceania, examined 45 cases of lymphangitis and observed that 91 per cent of the blood cultures and 77 per cent of the lymph cultures were negative. Six out of 30 patients examined (20 per cent) were positive for microfilaria.

3. CLINICAL ARGUMENTS.

According to a number of authors, including Thomson, Rifkin and Zarrow (1945), clinically, filarial lymphangitis differs markedly from the common bacterial lymphangitis. In the former, fever is not so high and other general symptoms are also less noticeable. Removal of the patient from the area of transmission contributes to a definite improvement, free from relapses. In cases of abscesses the fluid is not real pus. It is a clear liquid, without smell and it contains red cells and lymphocytes, and sometimes microfilaria.

4. BIOLOGICAL AND PATHOLOGICAL ARGUMENTS.

Rose, Culberston and Lipman (1945), during their investigations on American Military people who had acquired established *W. bancrofti* infection in the Pacific Theatre, could not find any evidence of antibodies against streptococcus in 7 out of the 8 subjects examined. Wartman (1947) confirmed these observations.

At present, the tendency is to attribute filarial lymphangitis to allergy. The evolution seems to be an allergic stage followed by a symptomatic stage (funiculitis, epididymitis, periorchitis, hydrocele, etc.), and later by the stage of obstruction with irreversible lesions during which the microfilariae in the blood diminish or disappear altogether.

O'Connor and Hulse in 1935 considered that the acute inflammatory syndrome (fever and lymphangitis) was associated with the degeneration of dead worms and the absorption of the decomposed products (allergic or anaphylactic phenomenon).

Rodhain (1953) considered the anaphylactic phenomenon initiating in the foci in which the filarial worms are in a way to disintegration. According to him, the reactions would be due to toxins or proteins from the dead adult worms and microfilariae. He did not consider any bacteria to be responsible for the reactions, in view of the fact that cultures from such cases were negative. On radiographical and histological evidences he concluded that elephantiasis could be caused by repeated attacks due to hyperfilarial conditions.

Other authors like Thomas, Rifkin and Zarrow (1945) were of the opinion that anaphylactic reaction was due to sensitisation to metabolic products and toxins excreted by the living adult worm, resulting in lymphatic and perilymphantic changes.

Huntington, Eichold and Scott (1945) and Manson Bahr (1950) also considered that the attacks of lymphangitis were due to severe allergic reactions.

Superinfections by infective larvae, production of new generations of microfilariae by adult worms, and destruction of microfilariae have been considered to explain the filarial origin of lymphangitis.

According to these authors the condition due to living or dead worm is a filarian syndrom *per se*. According to O'Connor and Hulse (1935) lymphangitis is characterised by constant pain at a precise point (focal spot), repeating itself at each recurrence. It should also be added that blood eosinophilia is considered as an evidence of filarial origin of tropical lymphangitis.

Thooris (1956) observed eosinophilia in patients, but the eosinophilic count was considerably higher in the patients with acute microfilarial manifestations. In such cases he also noted that the neutrophil count remained approximately normal or even slightly lower than in controls. Thooris (1956 *loc. cit.*) viewed such a blood picture in favour of the condition being allergic rather than a bacterial origin.

5. THERAPEUTIC ARGUMENTS.

The use of antibiotics in the treatment of lymphangitis was tried by Thooris (1956 *loc. cit.*). No appreciable results were produced by either penicillin or terramycin which were used in 72 and 10 cases respectively and so the use of antibiotics appeared to be limited to cases with secondary infection. On the contrary, excellent results were achieved with anti-histaminic preparations combined with aspirin in serious cases. According to Thooris (1956 *loc. cit.*) the inefficacy of antibiotics in such cases was a proof of the non-bacterial origin of the filarial lymphangitis. In conclusion, Thooris considered that the aetiology of lymphangitis was filarial due to an allergic type of lymphangitis crisis. His antifilarial treatment by Notezine and Thiacetarsamide as well as the desensitization by *Dirofilaria immitis* antigen, resulting effectively on the onset of lymphangitis, appears to support his view. This is further supported by the successful reduction by 80 per cent of the incidence of lymphangitis by chemotherapy as a result of the reduction of microfilaria rate as well as the average infestation of mosquitoes.

II. ARGUMENTS IN FAVOUR OF THE BACTERIAL THEORY.

1. GEOGRAPHICAL ARGUMENTS.

It is well-known that the incidence of tropical lymphangitis is correlated to endemic filariasis. However, there is no significant difference in the *W. bancrofti* microfilaria rate in carriers and non-carriers suffering from lymphangitis. According to Floch (1947), in French Guiana the rate was 19 per cent in the former and 13 per cent in the latter. Even if it is conceded that in established elephantiasis the microfilariae diminish in number or even disappear, it is difficult to explain such an occurrence during an attack of lymphangitis which is supposed to be an early manifestation of filariasis. In other words, in areas of filarial endemicity the presence of microfilariae in the blood of persons with lymphangitis may only be a matter of coincidence but actually not a causative factor.

2. BACTERIOLOGICAL ARGUMENTS.

As indicated by Sabouraud (1892) and Vincent (quoted by Noc and Stevenel, 1913) the emphasis on bacterial aetiology of lymphangitis is on streptococcal infection.

Le Dantec (1907) and later on Dufougere (1907) isolated from the tissue fluid as well as from blood of lymphangitis cases, a coccus. The former author named it "dermocoque" and the latter "lymphocoque". As we shall see later, both of them are streptococcus in their "mucoid" phase.

Dubruel (1909) in Tahiti observed that the tissue fluid of elephantoid limbs contained streptococcus more often during the course of acute lymphangitis than at other times.

A case of streptococcal lymphangitis, which finally led to elephantiasis, was described by Abadie (1912) in Algeria.

Noc and Stevenel in Martinique, in the year 1913, observed 12 cases of filariasis with chyluria as well as hematochyluria. Oedema was not noticed in any of the cases, neither microfilariae during attacks of lymphangitis acute elephantiasis. During the period 1909 to 1913, however, they observed a series of 88 cases of endemic lymphangitis. During the course of the disease, they noticed 73 acute attacks with "tendency to elephantiasis". Five patients had established elephantiasis with a history of recurring attacks of acute lymphangitis. Ten other patients had confirmed elephantiasis without recurrence of acute lymphangitis. None of the 88 cases showed microfilariae in the blood even during the acute attacks although it was possible to isolate "dermocoque" from blood and lymphnodes.

Marchoux (1913) also did not come across with microfilariae either in the blood at night or in fluid aspirated from lymphnode in 8 cases of lymphangitis from Antilla and Guiana. In Martinique, Montestruc and Bertrand in 1935 isolated a hemolytic streptococcus (type "A" of Lancefield) from a patient with repeated attacks of lymphangitis. They could not, however, find microfilariae in the night blood of this patient. In 10 other patients they failed to find microfilariae during the intervals between attacks of lymphangitis. On the contrary, in 108 subjects with no history of lymphangitis, microfilariae (*W. bancrofti*) were found in the night blood. They again confirmed in 1937 in Martinique the role of streptococcus in provocation of lymphangitis. They were able to isolate the organism from the pus of a crural abscess after the attack of lymphangitis. At the same time, microfilariae (*W. bancrofti*) were also seen in the pus. They, therefore, concluded that the traumatising action of the parasite in the lymphatic system, favoured the development of bacteria.

It has already been stated that the Island of Guadeloupe, with a microfilarial rate of 15 to 33 per cent in 1914, was considered more endemic to elephantiasis than Martinique where the rate did not exceed 5·47 per cent and where a few cases of lymphangitis were observed. Advier (1937) examined a number of patients with lymphangitis in the bacteriological laboratory at Pointe a Pitre in Guadeloupe. These patients were kept under close surveillance subsequently. A systematic search for microfilariae in the blood of these patients, both during the febrile period and during the afebrile interval, showed that proportion of individuals with microfilariae (*W. bancrofti*) was the same as among other inhabitants of Guadeloupe. On the contrary, cultures of blood and lymph taken at the time of febrile attacks frequently showed a pure culture of streptococci. Such cultures were frequently positive even after the attacks in fluid drawn from the axillary or inguinal glands. In some patients these glands showed hypertrophy during long time.

In French Guiana, the microfilaria rate was found to be 28 per cent in 1916. In 1934 the rate was 18·6 per cent, but 20 per cent only of the patients with lymphangitis were carriers. Besides, in 1947, the microfilaria rate in persons with and without lymphangitis was the same, *viz.*, 14·3 per cent. In the same territory, Floch (1938) isolated streptococcus from the blood or lymph of lymphangitis

cases. He found that the morphological, cultural, biochemical and virulence characters of the organism, studied in mice and rabbits, were those of streptococcus (type "A" of Lancefield). He thus confirmed the findings in Martinique and Guadeloupe. He further found that the streptococci isolated by him were voluminous, round in shape and frequently showed division similar to that of diplococci. This appearance combined with the fact that the strains of isolated streptococci frequently appeared in "mucoid phase", probably explains the description "dermocoque" of LeDantec and "Lymphocoque" by Dufougere referred to earlier. Floch was not able to find microfilariae in the blood of cases of lymphangitis any more frequently than in the blood of persons without lymphangitis. Thus, according to him, the aetiology of endemic lymphangitis in hot countries was considered to be due to bacteriae. Continuing his work at the Pasteur Institute of Cayenne in 1917, in collaboration with Lajudie, he wrote :

> "It is not possible for us to admit the filarial aetiology of tropical lymphangitis. The predominance of the bacterial factor in them is evident. We favour the streptococcal origin of tropical lymphangitis. This tropical lymphangitis should not be considered as a manifestation of "elephantiasis pus" but deserves to be considered as a condition *per se*."

In 8 cases of lymphangitis, Tisseuil (1936) did not come across in Guiana either microfilaria or streptococcus. It is true that he looked for streptococcus only in blood culture. Negative blood cultures are well known when the fluids obtained from lymph nodes of the same patients are positive. He tested 7 cases of lymphangitis for Frei's intradermal reactions and found one positive. He concluded that in French Guiana, endemic lymphangitis was not related to filariasis and that the disease as well as elephantiasis was due to a virus resembling the one described by Nicolas and Favre. This conclusion, as far as we know, has not been confirmed by anyone else.

The role of the streptococcus in the causation of lymphangitis had been stressed by workers in many other countries [Laber and Prowazek (1911) in Samoa, Bahr (1912) in Fiji, Wise and Minett (1912) and Anderson (1924) in British Guiana, Acton and Rao (1929) in India]. Suarez (1930), working in Puerto Rico, isolated streptococcus from patients with acute lymphangitis in 54 out of 60 cases. Sice (1927) in Madagascar was able to isolate streptococcus in 5 patients with endemic lymphangitis.

Grace *et al.* (1932) in British Guiana recorded the presence of streptococcus (haemolytic streptococcus "beta"). The microfilaria rate in this area was 23·1 per cent. They recorded similar findings at St. Kitts (also in British Guiana) where the microfilaria rate was 19·3 per cent. They emphasized the difficulty of differential diagnosis due to the fact that infestation due to *W. bancrofti* is often sub-patent without clinical symptoms and microfilaria. The same authors observed that lymphangitis was rare in Jamaica, where filariasis was unknown. They, however, noted the presence of 3 case of elephantiasis and considered to be *nostras elephantiasis* of non-tropical regions for which the streptococcus is considered as one of the aetiological agents.

At Puerto-Rico, Pomales Lebron (1940) isolated streptococcus from 28 cases of lymphangitis of leg. He also noted that there were pustules or small ulcers on the limbs. Twenty-nine per cent of the lymphangitis cases had streptococcus in the throat, versus only 9 per cent of the controls. Lichtenberg and Medina (1957) confirmed the role of streptococcus in the causation of lymphangitis and elephantiasis in Puerto-Rico.

Languillon (1957) noted in Cameroons that *W. bancrofti* was relatively more tolerable in the northern region with warm and dry climate which was not favourable for streptococcal infections. This, he considered a limiting factor for elephantiasis, which was rare. Here as well as in British Guiana and Puerto-Rico, economic backwardness, lack of footwear etc., perhaps play role in the microbial agression and streptococcal aetiology of lymphangitis.

In this connection it should be noted that in countries like Martinique or Guadeloupe, with now a highest standard of living, cases of lymphangitis are still found in native functionaries, men and women, who, since a long time, have not been bare-footed. Certain authors are of the view that streptococcus can go deep into the skin and remain latent in foci and manifest clinically when there is a balance disorder. This is in accordance with the view expressed by Acton and Rao (1933) with reference to septic focal infection.

In this connection, it is necessary to bear in mind the role of the individual and his reaction to antigenic stimulus.

It should also be pointed out that Sabouraud (1892, *loc. cit.*), Bockart (quoted by Suarez, 1936), Le Dantec (1907) and Floch (1938) were able to experimentally produce lesions lymphangitis and elephantiasis-like in animals by inoculation of hemolytic streptococcus. The role of the streptococcus in the aetiology of endemic lymphangitis was not accepted without discussion. Thooris (1956 *loc. cit.*) for example, writes with reference to filariasis in the Pacific :

> "Besides it is not surprising that, especially a few years ago, several workers frequently isolated the organism in cultures. In those days, some of the present day research facilities were not available and contamination at various stages, namely skin, syringe, culture medium, etc., was possible. It is rather surprising that such sources of contamination were not responsible for more positive cultures".

So, according to Thooris (1956 *loc. cit.*), the haemolytic streptococcus (group "A" of Lancefield), virulent for the laboratory animals and isolated by many authors in cases of endemic lymphangitis, would be only a germ grown in improperly-sterilised cultures with poor technique. In Martinique, during the last 25 years and more, less than 2 per cent of the several thousands of blood cultures were contaminated. Not one, however, showed the haemolytic streptococcus. Authors like Le Dantec, Dufougere, Marchoux, Sice, Advier, Floch and many others were well warned of the Pasteurian technique, and the observations of Thooris cannot be taken into consideration.

3. CLINICAL ARGUMENTS.

According to Floch and Lajudie (1947), acute lymphangitis looks like a microbial infection and not a parasitic infestation. There are acute onset, high fever, local signs of inflammation, general signs of infection, frequent suppurated complications.

In 1935, Dufougere declared that lymphangitis of hot countries is not only of microbial nature, but is also transmissible from man to man directly. Certainly there does not exist any established proof of such transmission. However one of us knows a number of families with lymphangitis, in Martinique, supporting such possibilities of contagiousness.

A study published by one of us in 1939 appears to confirm the views of Dufougere. This refers to a case of streptococcal meningitis in an infant of one year of age. Some days before the beginning of the meningitis, the child had a lymphangitic patch on one leg. This child used to be carried by his aunt who, on December 25, 1938, had suffered from an attack of typical acute lymphangitis on her fore-arm. The lymphangitic patch on the child's leg was seen at the point of contact with the fore-arm of the aunt with lymphangitis. The direct transmission of the streptococcus from the skin of the fore-arm of the aunt to the nephew's skin on the leg, is evident. The streptococci isolated from the aunt's lymph fluid, on the one hand, and the cerebro-spinal fluid of the child on the other hand, and streptococci isolated a few weeks later from a third patient of lymphangitis, were studied in detail. The cultural, morphological, biochemical and immunological characters as well as the virulence to rabbit of the streptococci isolated from the three sources, were identical.

4. BIOLOGICAL ARGUMENTS.

Floch (1947), working in Guiana, pointed out a number of cases of lymphangitis with neutrophil leucocytosis. It is only logical to think that filariasis, at least in the early manifestation, would show eosinophil leucocytosis. The results of his studies of Dick test in 1945 on cases of acute lymphangitis, as well as in healthy subjects, are in favour of a streptococcal infection in the former. From a similar point of view, one of us found in 1944 in Martinique that injections of streptococcic anatoxin produced local reactions only in cases of lymphangitis, and sometimes in such cases the reactions were very virulent.

Same observations on the Dick test were made by Ruiz Nazario and Morales Otero (1936) in Puerto-Rico. The latter author in collaboration with Pomales Lebron (1936) showed the increasing of anti-streptolysin levels in the blood of patients with spreading lymphangitis.

5. THERAPEUTIC ARGUMENTS.

Of all the arguments put forward by the protagonists of the bacterial aetiology of tropical lymphangitis, those based on therapy are perhaps the most weighty.

Physicians in the practice of tropical medicine since the beginning of the modern period of anti-bacterial therapy by sulphonamides and their derivatives at first, and anti-biotics later, cannot but record the spectacular results of this therapy for lymphangitis. The tropical lymphangitis formerly not yielding to treatment and recurring with severity, spectacularly became amenable to treatment by sulphonamides and sulphones since 1936 in French Antilles (Floch, 1936; Advier, 1937 and Montestruc, 1938). The pain disappeared 24 hours after therapy and temperature became normal. This almost constituted a veritable revolution in the treatment of lymphangitis in the Antilles. These results have been confirmed by Berny and Gippet (1937) in Guiana, by Chabeuf (1938) in Cameroon as well as by a number of other authors. While the sulphonamides have been good for the individual attack of lymphangitis, it is without any marked effect on the rate of recurrence. On the other hand, the anti-microbial vaccine prepared from streptococci isolated from the individual, seems to increase the interval between attacks although its result on the attack recurrence is not as spectacular as that of chemotherapy.

Floch (1945) and Montestruc (1944) supplemented the vaccine with streptococcal anatoxin and by a combination of the two types of immuno-therapy, obtained best results. Pons (1936) in Puerto-Rico obtained good therapeutic results by combination of vaccine as well as filtrates prepared from strains of haemolytic streptococcus isolated from the patients themselves. Both the chemotherapeutic as well as the biological therapy and their good results support the bacterial aetiology in at least a proportion of cases of tropical lymphangitis.

III. ARGUMENTS IN FAVOUR OF DUALISTIC OR PLURALIST THEORY.

In contrast to those who hold a unitary aetiology basis for lymphangitis, *viz.*, (*a*) streptococcal, or (*b*) filarial, there are others who hold that lymphangitis may be caused by combination of both the above factors as well as perhaps others.

According to these authors, the filarial origin of lymphangitis and subsequently elephantiasis, which cannot be explained by lymphatic obstruction alone, is not exclusive. The other provocatory causes are not clear and the bacterial infection seems to be only one of them, but the more frequent. It is so, that the authors who consider lymphangitis attacks as exclusively of filarial origin, with possible onset resembling septic abscess and emphasise the existence of sterile filarial abscesses, admit eventual bacterial complications of filarial parasitism.

Menon (1929), Castellani (1934), O'Connor and Hulse (1935) thus invoked microbial complications of filariasis, sometimes leading to a severe thrombophlebites and emboli.

According to Acton and Rao (1933), the role of streptococcus in the causation of lymphangitis and elephantiasis is undeniable. They collected the worms from foci with streptococcus. We have already stated that the physical absence of any wound

or ulcer at the time of lymphangitis attack, is not sufficient by itself to deny the attack being caused by the organism. In fact, streptococcus is capable of existing in latent state in the deep of the derm and manifesting itself pathogenically under different causes, the exact nature of which is unknown. However, Acton and Rao (1933) could explain only 5 per cent of the cases of elephantiasis studied by them on the basis of microbial infection. Ninety-five per cent of the cases appeared to be due to obstruction. Grace and his collaborators (1932) refused to consider lymphangitis and elephantiasis to be simply as a result of infestations of streptococcal infections of *W. bancrofti* and they observed in Guiana, with a microfilaria rate of 23·1 per cent, that streptococci were present in 33·2 per cent individuals, while in Jamaica where the microfilaria rate was nil, 6 per cent only of the inhabitants showed streptococci. They concluded that streptococcus could be "excitant" although the relationship between them and *W. bancrofti* remained obscure. It was logical, therefore, to seek an explanation to the clinical facts, more particularly with reference to a geographical region, and suspect a greater susceptibility of certain population to streptococcus or to consider a special strain of the filarial parasite with greater pathogenicity.

Thus, the contradictory arguments advanced by certain authors to explain the cause of lymyhangitis can be summarized as follows :

(1) The presence of filaria and streptococcus either alone or together in such cases ;

(2) The clinical picture of severe attacks of lymphangitis and their infectious nature ;

(3) The more or less elephantiasis state according to the filarial aetiology or the bacterial aetiology ;

(4) The blood picture indicating either an infectious state (neutrophil leucocytosis) or of parasitism (eosinophilia) ;

(5) Spectacular results obtained by chemotherapy and antibiotics ; on the contrary the failure of this medication and the success of specific anti-filarial and anti-allergic therapy and desensitisation ;

(6) The possible existence of special susceptibility in populations or the existence of special strains of the parasites.

It would, therefore, appear that the tropical lymphangitis is aetiologically not a single condition. Two pathogenic agents, namely *W. bancrofti* and streptococci, either exclusively or in association, can give rise to identical clinical conditions according to the geographical region concerned.

Galliard (1948), a protagonist of filarial aetiology of lymphangitis and elephantiasis, recognises different clinical syndromes in different regions :

> "It is necessary to take into account the variety of clinical manifestations of filariasis. They vary in the different regions of the world : a greater incidence of tropical lymphangitis in Antilles and Guiana ; frequency of elephantiasis and chyluria in certain Pacific islands and the frequency of lymph-scrotum in China. It is possible that the difference may lie in the racial difference of susceptibility to infestations, and, therefore, it would be proper to specify if the manifestation occurs in Whites, autochthonous or mixed blood patients. Buxton has even hypothesized that there may exist racial differences in the anatomy of lymphangitis system. Besides, the existence of different biotypes of *W. bancrofti* is probable. The specific role of *B. malayi* gives indication of this."

It seems difficult to do otherwise than to consider the Caribbean Region filarian endemic region, as a specific zone in which streptococcus, presumably due to special susceptibility of the population, causes recurring attacks of lymphangitis, terminating in moderate elephantiasis.

The role of the streptococcus in the same geographical area is also unliable in the aetiology of funiculitis which adopts there a special clinical aspect.

The term "tropical endemic lymphangitis" can, therefore, be retained to describe in the Caribbean Region recurrent lymphangitis as a clinical entity by itself and different from the bancroftian parasitism alone or secondary infected. The streptococcus plays an exciting and a dominant part in this condition.

Appendix I indicates the broad outlines of differential diagnosis based on aetiology.

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(*See* Appendix I on p. 650).

Appendix I.

Broad outlines of differential diagnosis based on aetiology.

Filarial origin. I.	Streptococcal origin. II.	Mixed origin. III.
	Can occur, in endemic area, in carriers of filaria.	Most often septic complication of the condition mentioned in Column I. (Secondary infection in injured lymphatics).
Possibility of elephantiasis without attacks of lymphangitis (primary elephantiasis).	..	..
Fever (not high) and minimum general symptoms during attacks.	Very high fever, severe general symptoms.	High fever, severe general symptoms.
Frequent presence of *W. bancrofti*, especially at the time of early attacks.	Presence of *W. bancrofti* is possible in highly endemic areas.	As in Column I.
Absence of streptococci. Possibility of "abscess" with adult worm or fragments of worm, and lymphocytes without pus.	Isolation of streptococcus in lymphatic tissue and secretions.	Generally, more or less large abscess with pus of bacterial origin. Localization of lower limbs (external infection).
Eosinophilia in the blood.	Polymorphonuclear leucocytosis.	Eosinophilia in the blood.
Failure of antibacterial therapy. Success of mass anti-filarial chemotherapy.	Spectacular results by anti-bacterial therapy.	Temporary results of anti-bacterial therapy.
Feeble transitory oedema between the early attacks. Arrest of progressive elephantiasis and allergic processes if the infected person quits the endemic region before 2 years of exposure. Progressive development of elephantiasis and attack of lymphangitis possible if the infected person continues to stay in the same area (secondary elephantiasis stage). Ofted verrucose aspect. Disappearance of microfilariae.	More or less persistent oedema after the attacks. Progressive development of the "large leg". Generally not true elephantiasis, but oedema more or less continuous, caused by fatigue, standing position, etc. The "elephantiasis nostras" of the nonfilarial regions can be provoked by streptococcus (recurrent erysipelas) but it is caused by lymphatic lesions of diverse aetiology, followed by sclerosis or cicatrices occurring in the lymphatic system.	Progressive establishment of elephantiasis as indicated in Column I, independent of bacterial lymphangitic attacks.

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FILARIASIS IN BRITISH GUIANA.

SOME INDUSTRIAL MEDICAL PROBLEMS.

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BANCROFTIAN filariasis has long been known as one of the more important public health problems of the Coastlands of British Guiana; it is more prevalent in the urban and suburban population and in the larger villages; less so in the smaller rural communities and isolated settlements. In the Amerindian population of the sparsely inhabited interior it does not occur, but asymptomatic infections with *D. perstans* and *M. ozzardi* are very common.

In 1948 attention was drawn (Giglioli, 1948) to the very considerable reduction in the microfilaraemia rate in the city of Georgetown brought about by the installation of a modern sewer plant between 1926 and 1932. Anderson, in 1921 surveyed a centrally situated ward of the city by house to house visits and examined a total of 515 persons; he reported a general M.F. rate of 30·5 per cent and a rate of 19·5 per cent in children under 10 years of age. The highest rate, 37·4 per cent, occurred in the 11-20 years age-group.

In 1947, in the same ward, 1,801 persons were examined with a technique comparable to the one used by Anderson; the general M.F. rate was found to be 12·1 per cent In children under 10 years the rate was only 4·6 ; the highest rate, 18·0 per cent, fell in the 51 to 60 age-group.

In 1947 surveys were also carried out in the suburbs of Georgetown, in New Amsterdam and at Buxton, largest of the rural villages; 3,910 persons were examined at random by house visits. In all of these localities sewage disposal is by pit latrine and cess pools; M.F. rates were consistently high, averaging 22·6 per cent; unfortunately there were no previous figures available for comparison.

A recent house-to-house survey has been carried out by W. Adams in 1955-57. The technique was generally comparable with the one used in 1947, but in some areas three slides were taken from each subject, thus the probabilities of recording positive results were increased rather than reduced. Adams' findings are consistently much lower than any of those recorded in 1947 as shown in Table I.

The incidence of microfilaraemia in children under 10 years of age also appears to have declined. Out of 1,006 children in this age-group examined in

1947, 124 or 12·3 per cent were found positive; out of 2,735 recorded by Adams in 1955-56 there were only 143, or 5·2 per cent positive. Dr. C. Romiti (verbal communication) reports a drastic reduction over the last 15 years in the incidence of all forms of clinical filariasis reporting for treatment.

TABLE I.

Comparative incidence of Bancroftian filariasis on the British Guiana Coastlands as indicated by Microfilaraemia rates taken in 1947 and in 1955-1956.

Localities surveyed.	GIGLIOLI (1947):			ADAMS (1955-56):		
	Examined.	M.F. positive.	M.F. rate per cent.	Examined.	M.F. positive.	M.F. rate per cent.
Georgetown City	6329	906	14·3	..	..	..
Georgetown City Suburbs:						
Kitty village	1136	281	24·7	8958	..	8·3
Campbellville	..	..	..	4981	..	8·3
Newtown	..	..	..	2651	..	8·4
Lodge village	1022	219	21·4	3089	503	16·3
La Penitence	1513	196	12·9	..	..	..
New Amsterdam	549	126	22·9	..	..	..
Buxton village	1206	257	21·2	2812	266	9·5
Cane Grove	..	..	..	2887	264	9·1
Queenstown Essequibo	..	..	..	1593	327	20·0

This decline in the incidence of both parasitological and clinical filariasis may find its explanation in three factors.

1. *Improved sanitation.*—Great improvement in rural housing has developed during the last ten years. General sanitation has progressed in parallel; on the sugar estates (aggregate population 105,000), 10,000 worker families, previously residing in dilapidated ten-room barrack buildings, have been moved to new housing areas. House lots have a surface of 1/10 of an acre and only one building per lot is allowed. The houses have been erected with the assistance of long term, interest free loans, and they are the property of the workers. This has brought about the substitution of the old time communal latrines, built over the main drainage canals and subject to periodical flushing, by some 10,000 individual family pit latrines. While this is a change for the better in many ways, it is certainly not an improvement as far as *Culex* breeding is concerned; on the low-lying impermeable coastal lands, in fact, the water table is high, and even shallow pit latrines are usually flooded for long periods during the May—July and November—January rainy seasons. These highly polluted waters are the favourite breeding sites of *Culex quinquefasciatus*, at present the only known local vector of filariasis.

2. *Treatment by diethylcarbamazine.*—This drug was used experimentally on 296 cases in British Guiana by Hewit and Kenny in 1949; it has since been prescribed extensively by medical practitioners. However the M.F. surveys made by Adams in 1955-56, and shown in our Table, were all collected before any mass chemotherapeutic campaign was undertaken. While treatment has undoubtedly had some influence in reducing the M.F. rates, it is doubtful that this has been a major factor in the rural areas; a large proportion of persons who seek treatment moreover, are suffering from the more advanced lesions of filarial disease in which M.F. rates tend to be low.

3. *The D.D.T. spraying campaign for the control of malaria.*—This campaign began in 1945 and reached a successful conclusion with the eradication of malaria and its vector *A. darlingi* from the coastlands. Spraying operations were closed down in 1951. *A. darlingi* has been proved to be very efficient vector of *Wuchereria* (Giglioli, 1948) and was certainly an important carrier in suburban and rural areas. In these situations its eradication must be regarded as a factor of some importance in the reduction of transmission.

The effect of D.D.T. campaign on the transmission of *Wuchereria* by *Culex quinquefasciatus* is more difficult to assess. This mosquito proved *ab initio* much less susceptible to the insecticide than either *A. darlingi* or *Aedes aegypti*. After a single application of 100 mg. per sq. foot to the interior of houses, house infestation was back to normal within six months. However, D.D.T. spraying, as long as it lasted (5 to 6 years), certainly brought about a drastic reduction in the bed-room population of this species. In a Georgetown suburb, for instance, the average *Culex* capture before D.D.T.-spraying averaged 162 mosquitoes per bedroom throughout the January to October period in 1945; for the same period in 1947, after four spraying cycles, the average capture was only 2 mosquitoes per room. In 1953 Charles reported average capture in this same area of 49·7 mosquitoes per room after 10 spraying cycles with D.D.T.; nearly two years however had elapsed since spraying had been discontinued.

In 1950 and 1951, i.e., during the last phases of spraying operations, on a total of 200,000 *Culex* collected, Charles (1953) reported an average room population of only 4·38 and 4·14 mosquitoes per room by hand capture and 17·88 and 8·89 per room by flit capture. With the closing down of routine spraying on the coastlands in 1951 the *Culex* room population obviously has tended to return to its pre-D.D.T. level.

Charles also carried out a promising experiment towards *Culex* eradication with Dieldrin on a small island on the Essequibo estuary; however, a more recent experiment reported by Adams in Buxton village proved disappointing.

To conclude, the reduction in the incidence of microfilaraemia, which appears to have occurred fairly uniformly throughout the coastlands, is probably the result of several factors; the elimination of *A. darlingi* and the curtailment of the

Culex house population during the 5 to 6 years of D.D.T.-spraying operations, would appear however to be of preponderant importance.

On the sugar estates, which carry approximately one-third of the rural coastal population, the incidence of filaria is tends to be low in the more isolated communities and quite high where an estate community adjoins a large and old established village. This, as has been mentioned, is probably related to the absence, up to recently, of pit latrines in the old-time estate layouts.

At Blairmont Estate, on the Berbice Estuary, in 1936 a rate of only 2·18 per cent was found in 642 residents examined at random (Giglioli). On the same estate, Subryan recorded a rate of 1·6 per cent in 561 persons examined in 1959.

In 1947 at Lusignan Estate on the East Coast of Demerara, the rate was 4·49 per cent in 846 persons examined. Port Mourant, on the Corentyne Coast, on the contrary, is a large estate community, which is in direct contact with Rose Hall, an old established village where pit latrines abound. This area was studied by Edgehill in 1958-59. Out of 3,572 persons examined at random, he found 399, i.e. 11·9 per cent with microfilariaemia ; in the section directly adjoining Rose Hall the rate was 16·2 per cent. Negroes (24·4 per cent) were affected nearly three times as frequently as East Indians (8·7 per cent). No positive cases were found in 67 children under 2 years ; one only in 72 children aged 3 and one only in 81 children aged 4 years. In the fifth year the incidence rose steeply to 8·4 per cent ; it remained fairly stable, around 12 per cent., between 11 and 60 years. Above 60 the rate rose to 20 per cent in 66 persons examined.

Clinical manifestations of filarial disease, active or latent, were found in 768 persons with a total of 937 lesions :

Lymphadenitis (including fibrous and varicose glands)	Inguinal	24 per cent
	Axillary	1·1 ,, ,,
	Epitrochlear	10·9 ,, ,,
	Popliteal	0·3 ,, ,,
Lymphangitis	... ...	4·0 ,, ,,
Funiculitis	... ...	20·0 ,, ,,
Hydrocoele	... ...	16·1 ,, ,,
Elephantiasis and oedema ...	... ...	19·5 ,, ,,
Abscess	... ...	4·5 ,, ,,

The fluid of five, out of 85 hydrocoeles which were examined, was found positive for microfilariae (5·6 per cent).

Table II summarises findings concerning the relations of microfilaraemia and the clinical signs of filarial disease in a series of 2,815 residents examined at random on Port Mourant Estate :

From Table II it appears that 32·3 per cent of the persons examined by Edgehill at Port Mourant presented parasitological or clinical manifestations of filariasis.

TABLE II.

Port Mourant Estate—Microfilaraemia and clinical signs of filarial disease in a random sample of 2,815 residents (Edgehill, 1960).

	RACE :			
	East Indians.	Negroes.	Mixed and others.	All races.
Number examined :	2,390	356	69	2,815
Number with microfilariae only :	158 (6·6 per cent)	67 (18·8 per cent)	9 (13·0 per cent)	234 (8·3 per cent)
Number with microfilariae and clinical signs of filarial disease :	54 (2·3 per cent)	21 (5·9 per cent)	5 (7·2 per cent)	80 (2·8 per cent)
Number negative for M.F. with clinical signs of filarial disease :	475 (19·8 per cent)	98 (27·5 per cent)	20 (28·9 per cent)	593 (21·0 per cent)
Number negative for both M.F. and clinical signs of filarial disease :	1,703 (71·2 per cent)	170 (47·1 per cent)	35 (50·7 per cent)	1,908 (67·7 per cent)

This high incidence of filarial infection and disease is acquiring progressively more importance in industrial medical practice ; a new aspect has developed with the introduction of Workmen's Compensation in 1934, its extension to agricultural workers in 1947 and to all manual workers, whatever their remuneration, and other workers earning up to $2,700 per annum in 1960.

According to the prevailing and deeply ingrained popular belief, "cold" is the main precipitating factor of a filarial attack ; a wetting, working in water or even standing barefooted on a concrete floor ; successive exposure to heat and cold is particularly dreaded, thus a maid who has been cooking or ironing cannot be made to open a refrigerator.

Genital affections in the male, including urethritis and epididimo-orchitis, on the contrary, are traditionally attributed to vague back injuries usually described as a "jerk", a "slip" or "strain". Thus filarial disease affecting the lymphatics of the spermatic cord also tend to be attributed to some similar mechanism ; where workmen's compensation is involved, the introduction of a traumatic etiological factor in respect to filarial funiculitis becomes even more attractive and profitable !

Lymphangitis and lymph varix of the funiculus is a very common manifestation of filarial disease in British Guiana ; this is well indicated by Edgehill's data (Table II). Romiti (1935), on an exceptionally wide surgical experience, has long insisted that the lymphatics of the funiculus in the male and of the broad ligament in the female, constitute the selective "habitat" of the adult worms in the human body.

Claims for compensation for this particular form of filariasis are frequent and are tending to increase as the law-courts, on what appears to be rather vague medical evidence, issue judgements in favour of workers.

On a group of estates in East Demerara, aggregating a weekly pay roll of 5,167, and with a general microfilaria rate which is probably considerably below average, during the period 1953 to 1959, the following claims under the Workmen's Compensation Ordinance were made for filarial disease :

Condition.	Not compensated	Compensated.	Total claims.
Filarial Funiculitis	107	72	179
Lymphangitis & Oedema	6	0	6
Hydrocoele	2	9	11
Total	115	81	196

The injury which was alleged to have precipitated the condition was described as —

Direct	in 85 cases	43·3 per cent.
Indirect (jerk—strain)	in 92 cases	46·9 per cent.
Doubtful (falls)	in 19 cases	9·8 per cent.
Total	in 196 cases	100·0 per cent.

Disability averaged 20 days and ranged from 4 to 60 days.

During 3 years (1957-1959) 137 other cases of filarial disease were seen by the same Medical Officer in none of which any form of traumatism was reported :

Funiculitis	60 cases
Hydrocoele	33 cases
Lymphangitis and oedema	43 cases
Chyluria	1 case
	137 cases

In the literature of filarial disease trauma has been frequently discussed, not however as a precipitating factor in the pathogenesis of an attack, but as providing a *portal of entry* for an associated or complicating streptococcal infection. Grace and Berman (1931) made a special study of the bacterial complications of filariasis in British Guiana. None of the traumas alleged by our cases are of this nature ; their action, if any, would be more or less direct on the parasite itself. We find it very difficult to visualise the mechanism by which a "jerk" or "back strain" could have repercussions on the worms inhabiting the funicular lymphatics. The case may be different in rare cases in which the injury consists of a direct contusion of the inguino-scrotal area.

The sex and age incidence of filariasis and of its more advanced manifestations in particular, does not in itself point to traumatism as an important factor in the pathogenesis of lymphangitic attacks.

In principle our policy has been to reject claims unless there is evidence of direct traumatisms of the inguino-scrotal region. Many cases, however, have had to be referred for a second opinion, and in these, the certificate issued by the Consultants generally gave the worker the benefit of the doubt on the grounds that the *alleged* traumatism, *might* have been the cause of the condition.

The significance of microfilaraemia and of clinical signs of filariasis in assessing the fitness of workers, in the course of medical examinations, poses yet another problem. The examination of 155 candidates for recruitment as farm labourers in the U.S.A. in 1958 offers a good example. These men had already been selected by visual examination in respect to physical development and healthy appearance ; in spite of this, 55, i.e., 37·4 per cent were rejected on the grounds of microfilaraemia.

The high incidence of filariasis, in conclusion, poses frequent and difficult problems for the industrial medical officer :

(*a*) What is the significance of filariasis—parasitological or clinical—in the routine physical examination of workers for employment ?

(*b*) What is the fair evaluation of compensation claims in respect to cases of filarial disease of alleged traumatic origin ?

The two problems are obviously connected. If filariasis continues to constitute a considerable compensation hazard for employers, the employment of filarial subjects will have to be avoided ; this would evidently cause much hardship, wherever the disease is widespread. A better understanding of the role of trauma in the pathogenesis of filarial disease is required.

Therapeutics could obviously be suggested as an answer to our difficulties. Antifilarial drugs have a dramatic effect on circulating microfilariae ; the majority of cases with clinical signs of the disease, however, have no circulating microfilariae: the effect of the drug on the adult worms is less clear as some treated cases continue to suffer clinical attacks even after microfilariae have disappeared. Finally the administration of a course of treatment lasting 10 to 21 days and which may have to be repeated, to people who are not aware of any active illness, is by no means an easy matter; swallowing of every tablet needs to be supervised ; handing over the tablets to the patient for self treatment at home is, with very rare exceptions, a waste of drug, money and time.

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A RAPID METHOD OF PREPARING DRY MOUNTS OF FILARIAL LARVAE (A PRELIMINARY NOTE).

BY

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[August 8, 1960.]

For purposes of study and demonstration, it is often necessary to prepare permanent or semi-permanent mounts of filarial larvae obtained by dissection of infected specimens of mosquitoes. Being very delicate objects, they need to be handled with extreme care during the various steps of preparation, to obviate distortion and damage. Obviously, the less they are handled during preparation, the better would be the results.

Recently, Nelson (1958) and Wharton (1959) described two methods of making semi-permanent mounts of filarial larvae. Nelson's technique consists of *in situ* fixation and staining of the larvae. The killed mosquitoes are passed through descending dilutions of alcohol, starting from 80 per cent ethyl alcohol, to distilled water. They are then stained in Mayer's acid haemalum for 3 days, differentiated in distilled water for another 3 days, and stored in pure glycerol until dissection. On dissection, the stained filarial larvae are easily spotted and if necessary mounted in glycerol.

Wharton (1959 *loc. cit.*) proceeds as follows : The freshly killed mosquitoes are dissected in normal saline and the larvae fixed by the addition of a drop of Bles fluid (70 per cent alcohol, 90 parts; formaldehyde, 7 parts; glacial acetic acid, 3 parts) After 2 or 3 minutes, the larvae are transferred to a drop of formalin glycerol, the mounting medium, which consists of 20 parts of formaldehyde, 5 parts of glycerol and 85 parts of distilled water with 0·0003 part methylene blue added for stain. The cell is then closed with a cover slip and sealed with a quick drying cement.

The afore-mentioned methods are 'wet' processes and the final preservation of the larvae is done in fluid mountants which need to be hermetically sealed.

The present author has obtained satisfactory results in the preparation of dry mounts of third (infective) stage filarial larvae of *Wuchereria bancrofti*, by the following simple method :

The freshly killed mosquito is dissected in a large drop of saline placed near the end of a clean slide. The dissection is done under a stereoscopic microscope (magnification 40×), with a pair of fine, clean needles made of No. 12 entomological pins mounted on wooden holders.

As soon as the filarial larvae are found, three or four drops of blood drawn by pricking a finger are placed at the centre of the same slide. The filarial

larvae, dead or alive, are picked up one by one on the dissecting needles and quickly transferred to the blood, before it dries up. To ensure that the larvae are safely deposited in the blood the slide may be moved sideways to bring the blood into the field of the microscope so that the needle point, carrying the larva, could be watched while it is lowered into the blood.

After the transference of the filarial larvae, the remains of the dissection are wiped off with a rag, and the blood spread in the manner of a thick smear, by means of a dissecting needle. While spreading the smear, it should be watched through the microscope to ensure that the larvae are not cut up in the process. The smear is now left aside to dry, at room temperature.

When perfectly dry, the smear is treated as follows :

1. Dehaemoglobinize in normal saline.
2. Wash gently in distilled water by immersion for about 2 minutes.
3. Fix in Bles solution for 10 minutes.
4. Wash in two changes of distilled water for 5 minutes each.
5. Stain for 30 minutes in 1 per cent Giemsa's stain prepared by adding one drop of stock solution to every 5 drops of phosphate buffer (pH 7·2).
6. Differentiate in 1 : 1,000 magnesium sulphate.
7. Wash in tap water and dry.

The dry smear is examined without cover slip, like ordinary blood smears. But it is essential to coat the smear with a film of immersion oil even while using the dry objectives, in order to clear the larvae and bring out their structural details. The oil may be removed after examination, as usual, with xylene, and the slide stored away.

Obviously, after fixation of the smear, it could be stained in any suitable stain including the haematoxylins; but the author has not yet tried them. The preparations that the author has so far made are of third-instar larvae, and these have come out satisfactorily as may be seen from the accompanying photomicrographs (Plate I) which were made from 3 months old preparations. Figure 1 shows an early third-instar larva, and Figure 2 its posterior end highly magnified under oil immersion. In Figure 2, the three terminal papillae (*P*) so characteristic of the infective larvae of *W. bancrofti* (Iyengar, 1956) can be made out in their formative stage. Figure 3 shows a late third-instar larva lying in a loop. In Figure 4, the head of this larva is shown magnified under oil immersion. The numerous spots seen around the larvae in Figures 1 and 3 are the nuclei of leucocytes, some of which can be seen enlarged in Figures 2 and 4.

Further study is required to find out if the method described above is suitable for the early instars of filarial larvae as well.

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The author is thankful to Dr. C. Joseph, Deputy Director of Health Services (Malaria and Filariasis), Kerala, for permission to publish this note.

PLATE 1

The measuring of filarial larvae (W. bancrofti).

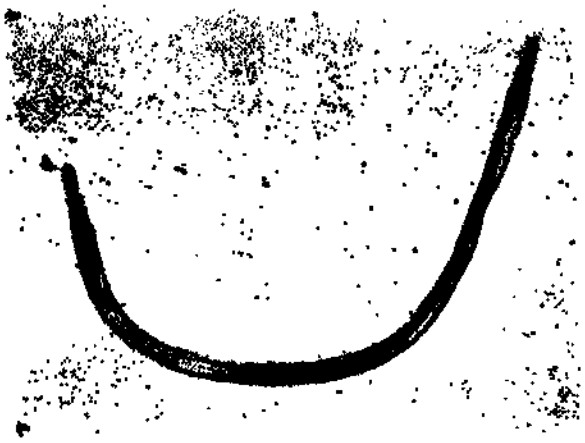

FIG. 1.

FIG. 2.

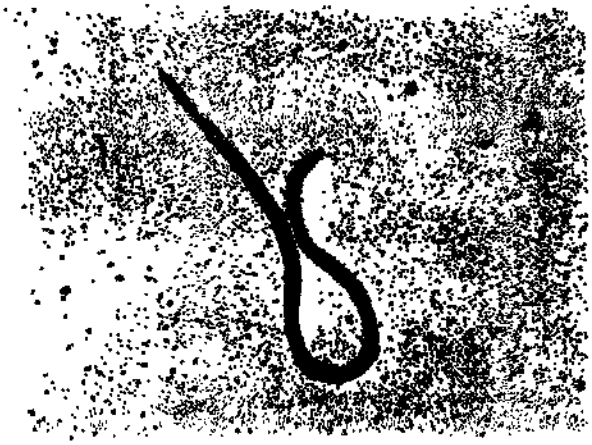

FIG. 3.

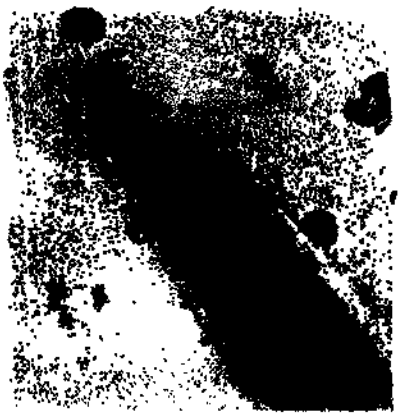

FIG. 4.

FIG. 1. An early third-instar filarial larva (100×).
FIG. 2. Tail end of the same larva showing developing terminal papillae (*P*) (1000×).
FIG. 3. A late third-instar larva (100×).
FIG. 4. The head of the same larva (1000×).
N.B. The spots seen around the larvae are the nuclei of leucocytes present in the blood smear.

He is also grateful to Dr. M. Thankavelu, Principal, Medical College, Trivandrum, for facilities to take the photomicrographs.

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PILOT STUDIES ON THE CONTROL OF FILARIASIS DUE TO *BRUGIA MALAYI* (BRUG, 1927) IN KERALA.

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[August 5, 1960.]

INTRODUCTION

THE State of Kerala is a narrow strip of land situated in the south-western part of peninsular India. It is the smallest of the States which constitute the Indian Union, having an area of 14,992 square miles. Its population is 15 million and is one of the densest in the world, being over a thousand per square mile. Physiographically, Kerala is cut off from the rest of India by a chain of hills, the Western Ghats, in the east and north-east. In the west it is flanked by the Arabian Sea. The coastal belt which is particularly thickly populated with a density of about two thousand persons per square mile, represents the main endemic tract of filariasis in Kerala. Cocoanut is the main crop of this region and a large section of the indigenous population is engaged on the manufacture of coir and coir-products.

The species of filaria responsible for human filariasis in Kerala are *Wuchereria bancrofti* (Cobbold) and *Brugia malayi* (Brug). According to Iyengar (1938) *W. bancrofti* is essentially an urban infection while *B. malayi* is mainly rural. Indeed, the coastal tract of Kerala represents the largest single tract of *B. malayi* infection so far reported in India (Raghavan, 1957). Even though the demarcation of areas subject to *B. malayi* infection in the State is yet incomplete the total affected area may be safely estimated at 700 square miles and the population at risk, at 1·5 million. The average endemicity rate for the entire endemic tract would roughly be 20 per cent. There are localities where it is as high as 60 per cent (Iyengar, 1938 *loc. cit.*; Jaswant Singh *et al.*, 1956*a* : 1956*b*).

The epidemiology of filariasis due to *B. malayi* has been worked out in detail by Iyengar (1934 : 1938). The primary vector of *B. malayi* is *Taeniorhynchus (Mansonioides) annuliferus*, a species which is very common in the *B. malayi* infected areas of Kerala. *T. (M.) uniformis* which occurs in much smaller numbers is also a good vector but actually plays only a secondary role in transmission because of its comparative paucity and preference for cattle blood. There are three essential factors which determine the endemicity of *B. malayi* infection in Kerala. They

are : (i) the presence of fresh water ponds suitable for the breeding of the vector species of *Mansonioides* ; (ii) the presence in the ponds of the water weed, *Pistia stratiotes*, which harbours the aquatic stages of *Mansonioides* ; (iii) the presence of organic matter in high concentration in the water. Iyengar (1934 : 1938) showed how the local practice of retting cocoanut husks in the ponds for the manufacture of coir helps keep a high degree of organic contamination in the water in the ponds and thus facilitates *Mansonioides* breeding all the year round. He also demonstrated (Iyengar, 1934) how *Mansonioides* breeding could be successfully prevented by the cheap and simple biological method of keeping the ponds and other water collections free from *Pistia* growth through frequent clearing. Sweet and Pillai (1937) later proved that the transmission of *B. malayi* infection could be effectively checked by controlling *Mansonioides* through *Pistia* clearance.

However, in the context of the National Filariasis Control Programme now being carried out in India since 1956 for the control of *W. bancrofti*, it was considered desirable to study the feasibility of extending the measures under this programme, namely, indoor residual spraying with dieldrin and mass therapy with diethylcarbamazine, to areas with *B. malayi* as well. The suitability of these measures for the control of *B. malayi* infection had not been sufficiently studied in India.

EXPERIMENTAL MEASURES OF CONTROL.

The pilot studies reported in this paper were designed to assess the relative merits of indoor spraying with dieldrin* as compared to *Pistia* clearance in the control of *Mansonioides* and thus in the prevention of *B. malayi* transmission, and also to study how far mass therapy with diethylcarbamazine adopted as a supplementary measure to each of the aforesaid antivector measures could further help prevent transmission. The studies, inclusive of preliminary observations for baseline data, covered a period of 20 months beginning January, 1958. The following are the measures tried out :

(i) *Mansonioides* control by indoor residual spraying of dieldrin with 2·5 per cent watery suspension prepared from 50 per cent water dispersible powder :—

The spraying was done in two cycles at a dosage of 50 mg. per square foot during each cycle. The first round was done in May-June, 1958, just before the onset of the south-west monsoon which exercises a natural seasonal check on the mosquito population, especially of the vector *Mansonioides*. The second round was done in January, 1959.

(ii) *Mansonioides* control as above, accompanied by mass therapy :—

For mass therapy, the drug diethylcarbamazine was administered according to the following dose schedule for 5 days :

*Jaswant Singh *et al.* (1956*b*) found that dieldrin, applied as an indoor residual spray at 50 mg. per square foot, remained effective against *Mansonioides* for 20 weeks.

For adults—200 mg. ;

For adolescents— 150 mg. ;

For children between 6 and 12 years—100 mg. ;

For children between 2 and 6 years—50 mg. ;

Children under two years of age and pregnant women were not given treatment. Blood examination of each treated individual was not done before or after treatment. But a general blood survey of about 10 per cent of the population concerned was done just before mass therapy and 12 months after.

(*iii*) *Mansonioides* control through *Pistia* clearance :—

The method consisted of the manual removal of *Pistia* from water surfaces, the implements used being a long rope for hauling the weed towards the shore, a basket to collect and carry the plants, and a spade to dig a pit to bury the weed. The weed collected was immediately buried deep, sufficiently away from the water, and the people around, especially the owners, were specifically requested not to dig them up and put them back into the water. This was necessary because *Pistia* is a good fertilizer for kitchen vegetables and cocoanut trees. The annual number of rounds of *Pistia* clearance would depend on the re-appearance of the weed. *Pistia* growth is generally faster and far more luxurient during the rainy season than during the dry months of the year. Every effort was made to maintain the water surfaces free from *Pistia* throughout the period of the studies.

(*iv*) *Mansonioides* control through *Pistia* clearance as described above, supplemented by mass therapy with diethylcarbamazine.

LAY OUT OF THE EXPERIMENTS.

The experimental measures were tried out in Sherthallai and Ambalapuzha, two areas about 22 miles apart, situated in the coastal filarial belt. Both these areas are typical *malayi* areas and are alike as regards physiography and climate. The meteorological data for the experimental period are furnished in Table I.

In Sherthallai, two contiguous villages (I and II) were selected for the trial of the first two measures mentioned above, *viz.*, indoor dieldrin spraying, and indoor dieldrin spraying supplemented by mass therapy. Similarly, two adjacent villages (III and IV) were selected in Ambalapuzha, for *Pistia* clearance and *Pistia* clearance plus mass therapy, respectively.

In villages I and II *Pistia* clearance for filariasis control had been done during the period 1934-45. This measure was withdrawn by the beginning of 1946 and nothing had been done since then. until the commencement of the present studies.

But in experimental villages III and IV in Ambalapuzha, *Pistia* clearance for filariasis control had been going on since 1946. And thus, the work of *Pistia* clearance done as part of the pilot studies reported here was but a continuation of the previous work. However, the field staff engaged on the work was reorganized and the work itself has been carried out more systematically and efficiently than before. It would probably have been better to select a virgin area for the purposes of the studies, but for administrative reasons Ambalapuzha had to be taken.

MAP I.

Pilot Study Areas—Control of *B. malayi*.

Scale : 1 inch=8 miles.

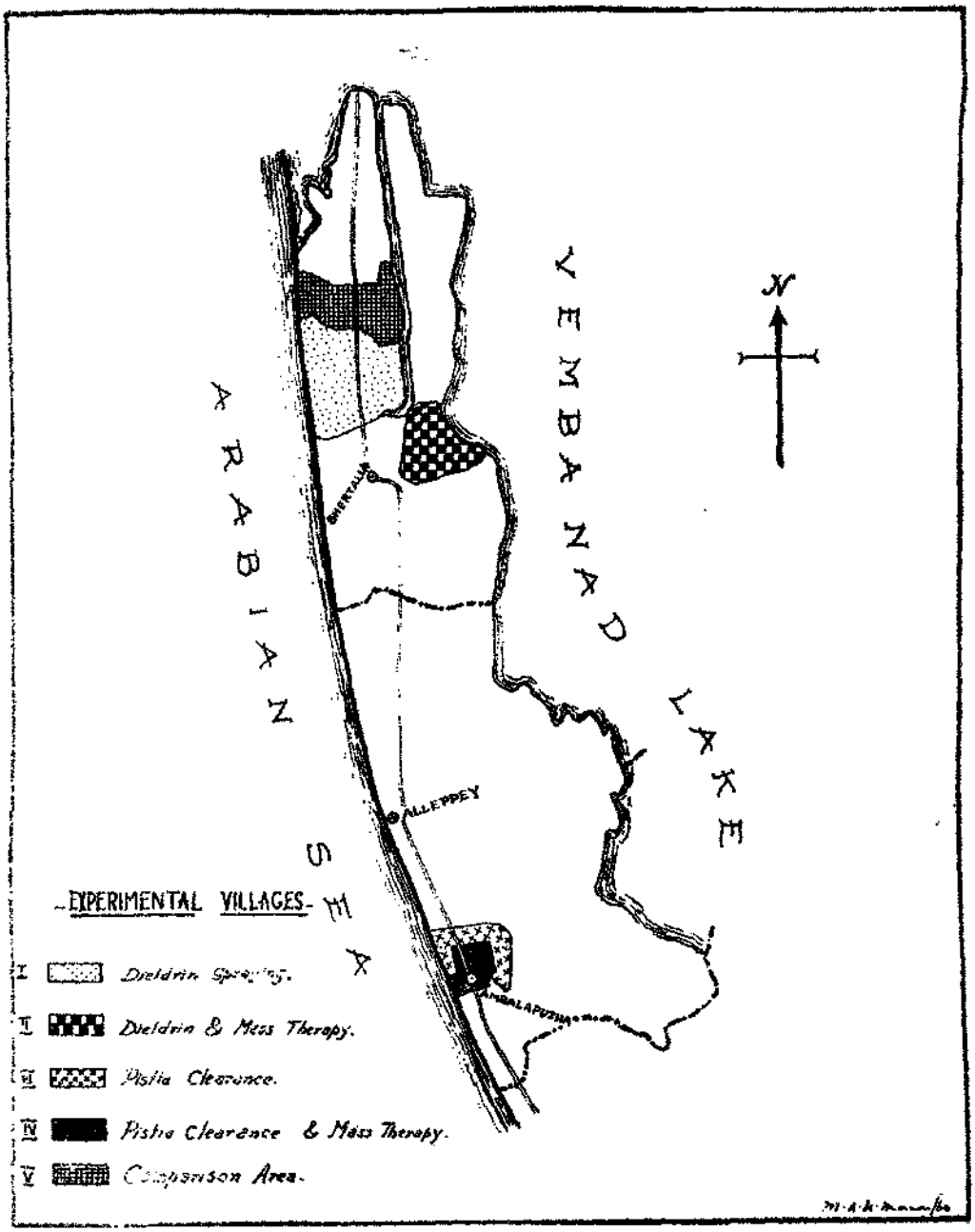

TABLE I.

Meteorological data—January, 1958 to August, 1959.

Year and month.	Temperature (in shade): Max. (F°)	Min. (F°)	Mean monthly temperature.	Relative humidity: At 9 a.m.	At 5 p.m.	Average monthly humidity.	Rain-fall: Number of rainy days.	In inches.
1958—								
January	..	No data	..	..	..	No data	..	1·24
February	89·0	82·0	85·5	79·0	..	79·0	..	0·33
March	90·0	84·5	87·25	78·1	..	78·1	..	1·64
April	88·7	83·8	86·25	85·0	81·0	83·0	14	8·12
May	85·8	81·2	83·50	84·0	75·0	79·5	22	37·55
June	82·6	78·6	80·60	87·0	80·0	83·5	26	14·25
July	81·9	78·1	80·00	84·0	80·0	82·0	25	1 ·49
August	80·5	76·6	78·60	87·0	83·0	85·0	27	14·73
September	83·3	79·9	81·60	80·0	73·0	76·0	12	4·67
October	82·5	78·9	80·70	80·0	77·0	78·5	17	10·47
November	83·1	79·7	81·40	80·0	73·0	76·0	16	5·12
December	85·1	80·9	83·00	70·0	65·0	67·5	3	1·03
1959—								
January	85·5	79·1	82·30	73·0	62·0	67·5	2	0·62
February	86·1	81·7	83·90	73·0	65·0	69·0	3	1·97
March	87·8	84·0	85·90	71·0	65·0	68·0	3	1·03
April	88·6	83·8	86·20	75·0	62·0	68·5	10	4·11
May	86·2	81·7	83·95	84·0	78·0	81·0	21	21·35
June	81·1	77·4	79·25	87·0	84·0	85·5	27	37·60
July	78·6	75·7	77·30	92·0	84·0	88·0	28	40·37
August	81·9	78·2	80·50	83·0	80·0	81·0	18	12·57

In addition to the aforesaid four experimental villages, a "Comparison Village" (V) was also fixed in Sherthallai, some six miles distant from Village I.

After fixing the five villages as mentioned above, mosquito catching stations were established in each for periodical mosquito collections, to determine fluctuations in mosquito densities and natural transmission indices. The catching stations were of two categories, houses and cattlesheds. The majority of houses in the villages have thatched roofs and a considerable proportion are mere huts made almost entirely of plaited coconut fronds. The cattlesheds seldom adjoin the houses proper, but are situated some distance from them. The total number of catching stations fixed was 60 and their distribution among the five villages, together with the area and population of each village, is shown in Table II.

TABLE II.

Area name.	Experimental village (Number).	Control measure.	Area (miles²)	Population.	Number of catching stations.
Sherthallai	I	Indoor dieldrin spraying	9·1	21,471	20
Sherthallai	II	Dieldrin and mass therapy.	3·0	7,580	15
Ambalapuzha	III	*Pistia* clearance.	7·5	15,000	5
Ambalapuzha	IV	*Pistia* clearance and mass therapy.	2·0	5,223	5
Sherthallai	V	Comparison village (no control)	7·3	14,537	15

Before the introduction of the experimental measures in the respective villages, a pre-control survey was done in each of the five villages, in order to obtain base line data regarding vector mosquito prevalence, degree of natural transmission, and filarial infection, morbidity and endemicity among the population. To assess the results of the control measures the entomological observations were continued throughout the observation period and a parasitological and clinical survey was done a year after the introduction of control measures.

PRE-CONTROL SURVEY.

The pre-control survey of the five experimental villages was carried out during January through April, 1958. The survey of Villages I and II extended from January to the end of April, that of III and IV from March through April and of Village V from February to the end of April, 1958.

(i) ENTOMOLOGICAL.

(*a*) *Mosquito densities.*—For routine collections every catching station of each village was searched for mosquitoes once a week by the same Insect Collector. The collection was made between 7 and 10 a.m., and the time spent for actual collection in a catching station was 20 minutes. The day's collection was taken to the field laboratory at Sherthallai and the specimens identified and dissected for filarial infection on the same day.

Altogether 14 species of mosquitoes, 9 culicines and 5 anophelines, as listed below, were recorded from the routine catches from the five villages.

CULICINES.

1. *Culex* (*C*) *vishnui*
2. *Culex* (*C*) *gelidus*
3. *Culex* (*C*) *sitiens*
4. *Culex* (*C*) *fatigans*
5. *Culex* (*L*) *fuscanus*
6. *Taeniorhynchus* (*M*) *annuliferus*
7. *Taeniorhynchus* (*M*) *uniformis*
8. *Taeniorhynchus* (*M*) *indiana*
9. *Armigeres obturbans.*

ANOPHELINES.

1. *Anopheles barbirostris*
2. *Anopheles hyrcanus* var. *nigerrimus*
3. *Anopheles jamesi*
4. *Anopheles subpictus*
5. *Anopheles vagus.*

The bulk (60 to 70 per cent) of the catches consisted of pest species of *Culex* like *C. vishnui* and *C. gelidus*, which are not concerned with filarial transmission. *C. fatigans* occurred in negligible numbers. So did *Armigeres obturbans* in which, according to Iyengar (1938), *W. malayi* could develop to the infective stage under

laboratory conditions. Anophelines occurred in considerable numbers, the predominant species beirg *A. subpictus* and *A. vagus*. *A. hyrcanus* var. *nigerrimus* and *A. barbirostris* species which can harbour the parasite up to the infective stage (Iyengar 1938 *loc. cit.*), were comparatively rare *A. jamesi*, too, was scarce. *Mansonioides* species, the vectors, were common in the collections from Villages I, II and V in Shertballai, but comparatively rare from III and IV in Ambalapuzha. The low incidence of *Mansonioides* in Ambalapuzha was only to be expected in view of the fact mentioned earlier that *Pistia* clearance was going on in that area for several years past.

Table III shows the ten-man-hour d·nsities of the adults of the three species of *Mansonioides* prevalent in the five experimental villages. The most common species was *M. annuliferus*, the primary vector of *B. malayi*. It exhibited a consistent preference for human dwellings to cattlesheds and is evidently a domesticated species. On the other hand the secondary vector, *M. uniformis*, which occurred in much smaller numbers than *M. annuliferus*, was observed to rest in cattlesheds as well. *M. indiana* was only very rarely obtained in the collections.

TABLE III.

Pre-control 10-man-hour densities of adult Mansonioides *and* C. fatigans.

Experimental village.	January, 1958:								February, 1958:							
	Houses.				Cattlesheds.				Houses.				Cattlesheds.			
	MA.	MU.	MI.	CF.	MA.	MU.	MI.	CF.	MA.	MU.	MI.	CF.	MA.	MU.	MI.	CF.
I	7·3	1·1	0·0	10·8	8·6	8·6	..	8·5	24·8	2·7	0·0	6·4	10·7	11·4	0·0	4·2
II	57·0	7·5	0·0	6·0	..	..	..	0·0	62·0	0·7	0·0	1·3	8·2	0·0	0·0	0·0
III	..	..	..	..	..	..	..	..	ND	ND	..	..	..	..	..	..
IV	..	..	..	..	..	..	..	..	ND	ND	..	..	..	..	..	..
V	..	..	..	ND	ND	..	..	..	58·7	0·0	0·0	4·1	24·5	5·5	0·0	0·0

TABLE III. *Concld.*

Experimental village.	March, 1958:								April, 1958:							
	Houses.				Cattlesheds.				Houses.				Cattlesheds.			
	MA.	MU.	MI.	CF.	MA.	MU.	MI.	CF.	MA.	MU.	MI.	CF.	MA.	MU.	MI.	CF.
I	23·1	1·9	1·9	3·1	10·0	7·5	0·0	2·5	30·0	1·9	0·0	3·1	0·0	0·0	0·0	0·0
II	49·7	0·9	0·0	1·7	0·0	0·0	0·0	0·0	43·3	0·0	0·0	0·0	0·0	0·0	0·0	0·0
III	1·9	1·9	0·0	1·9	0·0	0·0	0·0	0·0	7·5	0·0	0·0	0·0	0·0	0·0	0·0	0·0
IV	2·5	0·0	0·0	2·5	0·0	0·0	0·0	0·0	30·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
V	84·9	2·6	0·0	0·0	37·5	5·0	0·0	0·0	192·0	1·5	0·0	0·0	18·4	1·9	0·0	0·0

MA = *Mansonioides annuliferus*. MU = *Mansonioides uniformis*.
MI = *Mansonioides indiana*. CF = *Culex fatigans*. ND = Not done.

(*b*) *Natural filarial infection in mosquitoes.*—In dissecting mosquitoes for filarial infection, the developmental stages of *B. malayi* encountered in the abdomen, thorax and the head, were recorded separately. The Malphighian tubules of each

specimen were dissected and examined for possible infection with *Dirofilaria* or similar animal filariae. Particular care was taken to identify the developmental forms found in the thorax and head on the basis of the differential characteristics described for *B. malayi* by Iyengar (1956).

None of the mosquito specimens dissected revealed any filarial infection other than *B. malayi*. Developmental stages of *B. malayi* were found in all the three species of *Mansonioides* prevalent in the experimental villages. Infective stage larvae were, however, seen only in *M. annuliferus* and *M. uniformis*. The natural infection and infectivity rates in respect of these two species are presented in Table IV from which it is clear that, as observed by previous workers (Iyengar *loc. cit.* and Jaswant Singh *et al., loc. cit.*), *M. annuliferus* and *M. uniformis* are the primary and secondary vectors respectively, *M. indiana* being of little consequence in transmission because of its scanty occurrence.

TABLE IV.

Natural filarial infection and infectivity rates in Mansonioides annuliferus *and* M. uniformis.

(Pre-control data).

Experimental village.	JANUARY, 1958 :						FEBRUARY, 1958 :					
	M. annuliferus.			*M. uniformis.*			*M. annuliferus.*			*M. uniformis.*		
	D.	IR.	IFR.	D.	IR.	IFR.	D.	IR.	IFR.	D.	IR.	IFR.
I	9	11·1	..	4	25·0	..	53	24·5	3·9	11	9·1	..
II	39	7·7	..	5	40·0	..	80	18·8	..	1	..	..
III	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
IV	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
V	ND	ND	ND	ND	ND	ND	93	12·9	5·3	2	50·0	..

TABLE IV (*Concld.*).

Experimental village.	MARCH, 1958 :						APRIL, 1958 :					
	M. annuliferus.			*M. uniformis.*			*M. annuliferus.*			*M. uniformis.*		
	D.	IR.	IFR.	D.	IR.	FR.	D.	IR.	IFR.	D.	IR.	IFR.
I	44	18·2	9·7	7	..	..	19	21·1	..	2	..	..
II	58	18·9	5·1	1	..	..	26	23·1	7·6	..	..	..
III	1	..	..	1	..	..	8	..	..	..	..	..
IV	1	..	..	..	..	..	1	..	..	..	..	..
V	147	10·9	3·3	6	..	..	131	13·8	3·6	1	..	..

D = Number dissected. IR = Infection rate (Per cent).
IFR = Infectivity rate. ND = Not done.

It is note-worthy that during the pre-control survey no infected mosquitoes were obtained from the two experimental Villages (III and IV) in Ambalapuzha where, as already seen, *Mansonioides* species were very rare as a result of *Pistia* clearance. In contrast to this, the other 3 villages in Sherthallai, especially the

comparison village (V), showed considerable infection among *Mansoniotdes* during all the four months of the survey.

(ii) PARASITOLOGICAL AND CLINICAL.

About 10 per cent of the population of each village was examined. The collection of blood samples was done between 8 p.m. and mid-night. Approximately 20 c.mm. of blood was taken to prepare a smear. The clinical survey was done together with the blood survey. Only the gross clinical symptoms of filariasis, such as swelling and elephantiasis of the extremities, were recorded.

The blood films were stained routinely in 0·1 per cent methylene blue in water. Every positive slide was examined carefully for the morphological characteristics of the microfilariae to determine the species of infection. All positive specimens, except one of *W. bancrofti* from Village III (Ambalapuzha), were of *B. malayi*. The results of the clinical and parasitological surveys are presented in Table V. It will be seen from the table that the villages in Sherthallai, especially Villages II and V, were more filarious than III and IV of Ambalapuzha. In Villages I and II the infection rates were 22·4 per cent and 15·4 per cent respectively. In Village V (comparison village) it was 23·3 per cent. On the other hand, Villages III and IV of Ambalapuzha had infection rates respectively of 11·3 per cent and 10·6 per cent. The disease and endemicity rates were like-wise proportionately higher in Villages I, II and V than in III and IV. These observations are in accordance with those in regard to the relative prevalence of the vector mosquitoes and the natural infection rates found in them (*vide supra.*)

TABLE V.

Pre-control parasitological and clinical data.

Experimental village.	Population.	Number of persons examined.	Number with microfilaria.	Microfilaria infection rate.	Number with elephantiasis.	Disease rate.	Microfilarial density.
I (Dieldrin)	21,471	3,513	806	25·7	397	11·3	21·1
II (Dieldrin+Mass therapy)	7,580	1,496	231	15·4	146	9·7	28·5
III (*Pistia* clearance)	15,000	1,512	161	10·6	107	7·1	10·6
IV (*Pistia* clearance+Mass therapy)	5,223	770	87	11·3	42	5·4	12·5
V (Comparison village)	14,537	1,509	352	23·3	196	12·9	17·6

INTRODUCTION OF THE EXPERIMENTAL CONTROL MEASURES.

Indoor dieldrin spraying.—The first round of spraying in experimental Villages I and II was carried out between May 20, 1958 and July 26, 1958. The total number of houses sprayed was 10,224. To start with, the spraying operation was welcomed by the people. The percentage of refusals was only 4·5 per cent. The second round was done between January 24, 1959 and April 22, 1959, the total number of houses sprayed being 9,232. This time the percentage of refusals increased to

12·3 per cent. The main reason for this fall in the popularity of the measure was the appearance of *C. fatigans* in very large numbers in the sprayed areas following the first round and the unbearable nuisance caused by this mosquito to the local residents. This development will be discussed further, later on (*vide infra*).

The routine mosquito catching stations in Village I were sprayed during the first round, between May 31, 1958 and July 26, 1958. Those in Village II were sprayed between May 20, 1958 and May 30, 1958. During the second round of spraying, however, the catching stations in both these areas were sprayed on the same day, i.e., on January 29, 1959.

Pistia clearance.—Since May, 1958, when the respective control measures were introduced in the other experimental villages, six rounds of *Pistia* clearance have been done in the two villages. On an average, it took about two months to finish one round, the strength of the manual labour employed for the work being 16 field-workers. The total area of water surfaces cleared of *Pistia* during the six rounds was 1,53,865 sq. yards or 31·8 acres. Ponds formed 90 per cent of the waters. There was no opposition from the local people against the clearance operations.

Mass therapy was conducted in experimental Village II between April 15, 1958 and April 27, 1958 and in Village III between May 4, 1958 and May 25, 1958. In Village II the number of persons who underwent complete course of treatment was 4,109 i.e., 54·2 per cent. Partial treatment was taken by 1,019 persons or 13·4 per cent ; and 2,452 (32·4 per cent) did not receive any treatment. In Village III the number of persons who had complete course of treatment was 3,095, i.e., 59·1 per cent of the population. 506 persons (10·6 per cent) had partial treatment and 1,622 (31 per cent) had no treatment at all. Table VI furnishes details regarding the number of individuals of different age-groups who took complete, partial or no treatment. It can be seen that in both villages, the largest number of persons who had full or partial treatment belonged to the adult group, above 15 years of age. This is obviously because of the fact that the adult population is by far greater than that of any of the earlier age-groups and is naturally more represented in the treated section of the population. The number of persons who refused to take treatment also consisted more of adults than of juveniles. In either village, for every 10 adults who took partial or full treatment there were about 7 who refused it, whereas for every 10 juveniles who took treatment only about 3 refused it.

In village II, 1,913 (37·4 per cent) persons out of those treated showed reactions of various types, to the drug, and in Village III, 1,310 (36·4 per cent). The first reactions were usually reported within 12 to 24 hours of taking the drug, the maximum cases of reaction occurring on the second, and third days of therapy. The reactions usually took the form of fever, headache, diarrhoea, vomiting, pruritus and body pain and swelling of limbs. All cases were amenable to symptomatic treatment and subsided quickly. There were no fatal complications following therapy. The percentage of reactions in each age-group is given in Table VII.

TABLE VI.

Mass therapy in Villages II and IV.

(A) VILLAGE II.

Population.	Age-group.	Number of persons who took the drug.	Number received drug for: 5 days.	4 days.	3 days.	2 days.	1 day.	Number of refusals to take drug.	Number of persons with reaction.
7,580	I	2,663	2,111	257	45	130	120	1,747	1,115
	II	561	437	60	11	25	28	213	214
	III	1,084	890	94	27	36	37	248	362
	IV	820	671	67	24	24	24	244	222
Total:		5,128	4,109	478	107	215	219	2,452	1,913

(B) VILLAGE IV.

Population.	Age-group.	Number of persons who took the drug.	Number received drug for: 5 days.	4 days.	3 days.	2 days.	1 day.	Number of refusals to take drug.	Number of persons with reaction.
5,223	I	1,796	1,495	176	26	36	63	1,221	67[illegible]
	II	457	399	35	3	9	11	116	210
	III	728	644	51	6	9	18	147	251
	IV	620	557	39	5	12	7	138	171
Total		3,601	3,095	301	40	66	99	1,622	1,310

Age-group I=18 years and above. II=12–17 years. III=6–11 years. IV=2–5 years.

TABLE VII.

Percentage of persons who showed reactions to treatment.

Village.	Age-group*: Adults.	Adolescents 12–18 years.	6–12 years.	2–6 years.
II	58·2	11·2	18·9	11·5
VI	51·7	16·0	19·1	13·8

The incidence of reactions was highest in the age-group "Adult", i.e, among persons over 18 years of age. In both villages more than 50 per cent of the reactions occurred in this age-group. The reactions, following diethylcarbamazine, therapy, are due to disintegration and destruction largely of microfilariae and are thus a sensitizing phenomenon (Raghavan, 1956). The remarkably higher incidence of reactions among adults is therefore attributable to the higher incidence of filarial infection among them.

POST-CONTROL OBSERVATIONS.

ENTOMOLOGICAL*.

The effect of indoor spraying of dieldrin and *Pistia* clearance respectively on the incidence of adult *Mansonioides annuliferus*, the primary vector, and on *M. uniformis*, the secondary vector, is demonstrated in Tables VIII (*a*) to VIII (*e*) which furnish the monthly 10-man-hour densities of the vector species for the different experimental villages in comparison with those of "other culicines" and anophelines. It should be noted that "other culicines" exclude *C. fatigans* for which densities are given in a separate column.

(*a*) *Effects of dieldrin spraying.*—Following the first round of dieldrin spraying, the density of *M. annuliferus* in human dwellings dropped to 0·0 in both the villages where this measure was applied. This species was practically absent from the routine catches from these villages, throughout the 14 months since the first round of spraying, except for a slight sporadic appearance in Village I during July, 1959.

The density of *M. uniformis* also dropped to 0·0 and remained so for about two months after the first round of spraying. But later, from September, 1959 onwards this species began to appear in progressively appreciable numbers especially in cattlesheds where, as shown earlier, it likes to rest. Its density within cattlesheds in Village I was 41·5 and 56·3 respectively in December, 1958 and January, 1959. It is noteworthy that these figures are higher than the densities recorded in this village, for any month during the pre-control survey. But the second round of spraying during January, 1959, brought down the density of *M. uniformis* to 0·0 which remained so for 5 months, i.e., until July, 1959 when it started re-appearing in small numbers. In Village I also, *M. uniformis* began to reappear in small numbers two months after the first round of spraying. In November and December, 1958, the densities within cattlesheds in village I were, respectively, 8·2 and 15·0. Following the second round of spraying, however, the density of *M. uniformis* fell to 0·0. It tended to reappear only in June, 1959, i.e., four months after the second round of spraying. The above findings in regard to the post-control prevalence of vectors were further checked by general collections of mosquitoes from sprayed and unsprayed structures in the dieldrin-treated Villages I and II. Altogether 213 structures were searched on 15 occasions during the post-control observation period. Only 3 specimens of *M. annuliferus* and 6 of *M. uniformis* could be collected.

It may be seen from Tables VIII (*a*) and VIII (*b*) that the effect of dieldrin on "other culicines" is far less marked than on *Mansonioides*. There is definitely a fall soon after spraying but the build-up thereafter is so fast that the pre-control density levels are attained by the second month after spraying. One point worthy of note is that the fall in density after the second round is much less striking than that following the first round.

* Mosquito collections had to be suspended in all the five villages during May and June, 1958, because the Insect Collectors had to be temporarily assigned for supervision of spraying and assistance in the mass therapy programme.

TABLE VIII (*a*)

Pre-control and post-control mosquito densities (10-man-hours).

VILLAGE I—(INDOOR DIELDRIN SPRAYING).

Year and month.	*Mansonioides annuliferus*		*Mansonioides uniformis.*		ALL OTHER CULICINES :		ALL ANOPHELINES.		*Culex fatigans.*	
	HD.	CS.	HD.	CS.	HD.	CS.	HD.	CS.	HD.	CS.
1958—										
January	7·5	8·6	1·1	8·6	91·1	72·9	5·4	12·8	10·8	8·5
February	24·6	10·7	2·7	11·4	141·4	199·3	19·8	70·7	6·4	4·2
March	23·1	10·0	1·9	7·5	126·9	162·5	71·9	127·5	3·1	2·5
April	30·0	0·0	1·9	0·0	69·9	108·0	60·0	78·0	3·1	0·0
May* } June* }	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
July*	0·0	12·0	0·0	0·0	3·4	18·0	0·0	0·0	6·0	0·0
August	0·0	0·0	0·0	8·0	111·8	0·0	0·0	0·0	0·0	0·0
September	0·0	0·0	0·0	21·0	14·0	54·0	0·0	6·0	16·9	6·0
October	0·0	0·0	1·0	13·1	15·0	123·1	2·0	0·0	124·0	56·2
November	0·0	2·0	2·0	20·0	1·5	168·0	1·0	18·0	328·0	64·0
December	0·0	0·0	2·3	41·5	30·6	260·1	18·4	60·0	229·6	55·3
1959—										
January†	0·0	0·0	2·8	56·3	36·6	309·5	2·8	7·5	300·4	50·6
February	0·0	0·0	0·0	0·0	13·3	120·0	1·9	3·8	96·5	52·5
March	0·0	0·0	0·0	0·0	5·6	75·0	7·5	7·5	125·6	90·0
April	0·0	0·0	0·0	0·0	9·4	245·0	32·7	84·5	184·6	180·0
May	0·0	0·0	0·0	0·0	32·5	126·0	38·7	54·0	88·7	0·0
June	0·0	0·0	0·0	0·0	30·0	162·9	6·4	12·9	45·0	30·0
July	0·8	0·0	0·8	3·3	20·7	33·3	13·5	46·6	24·1	26·7
August	0·0	0·0	1·3	0·0	7·0	80·0	53·5	80·0	15·6	0·0

HD = Human dwellings. CS = Cattle-sheds

NC = No collections.

* First round of spraying.

† Second round of spraying.

Anophelines, on the other hand, have exhibited a higher susceptibility to dieldrin than *culicines* as is evident from their total disappearance from collections immediately after the first application of dieldrin and their comparatively low densities until November, 1958, i.e., for about 5 months after spraying. That the effect of the insecticide was obviously less after the second round, is indicated by the persistence of *Anophelines* immediately after the second application and their steady build-up thereafter.

A very significant observation made during the course of these studies was the unexpected appearance in enormous numbers of *Culex fatigans* in the dieldrin-treated Villages I and II. This species was extremely rare in these villages before the indoor application of dieldrin. In the other two experimental villages too, as well as in the comparison village (V), *Culex fatigans* had been rare during the pre-control survey, but has remained so all along in striking contrast to the sprayed areas.

In the wake of the first round of spraying, *C. fatigans* was totally absent or very rare in the collections for two months, i.e., until the end of September, 1958. But by October, 1958, the density of this species exhibited a sharp rise in Village I where its density reached the unprecedented levels of

TABLE VIII (*b*).

Pre-control and Post-control Mosquito Densities (10-man-hours).

VILLAGE II—(DIELDRIN and MASS THERAPY).

Year and month.	*M. annuliferus.*		*M. uniformis.*		ALL OTHER CULICINES.		ALL ANOPHELINES.		*Culex fatigans.*	
	HD.	CS.	HD.	CS.	HD.	CS.	HD.	CS.	HD.	CS.
1958—										
January	57·0	0·0	7·5	0·0	118·5	306·0	3·0	60·0	6·0	0·0
February	62·8	8·2	0·0	0·0	263·7	310·7	14·6	154·3	1·4	0·9
March	49·7	0·0	0·9	0·0	176·9	223·3	15·4	173·3	1·7	0·0
April	43·3	0·0	0·0	0·0	145·0	180·0	58·2	252·0	0·0	0·0
May* } June* }	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
July*	0·0	0·0	0·0	0·0	15·0	60·0	0·0	0·0	0·0	0·0
August	0·0	0·0	0·0	0·0	18·2	75·0	0·0	0·0	0·0	0·0
September	0·0	0·0	1·2	0·0	51·2	172·3	1·2	0·0	18·8	0·0
October	0·0	0·0	0·0	0·0	29·2	170·0	0·8	8·3	19·3	0·0
November	0·0	0·0	2·7	8·2	33·4	136·4	0·0	16·4	37·5	14·6
December	0·0	0·0	1·1	15·0	45·0	200·7	1·1	17·1	90·5	4·3
1959—										
January†	0·0	0·0	0·0	0·0	95·6	191·3	0·9	15·0	112·5	30·0
February	0·0	0·0	0·0	0·0	22·5	46·7	0·0	6·7	69·2	33·3
March	0·0	0·0	0·0	0·0	4·6	78·0	4·6	30·0	130·0	78·0
April	0·0	0·9	0·0	0·0	27·9	136·4	25·8	84·6	107·4	35·5
May	0·0	0·0	0·0	0·0	41·3	237·0	27·9	78·0	45·6	6·0
June	0·0	0·0	0·6	3·0	41·5	141·0	10·9	105·0	13·3	0·0
July	0·0	0·0	0·6	0·0	19·4	75·0	12·4	87·5	15·2	3·8
August	0·0	0·0	0·7	2·5	18·7	107·0	6·5	32·5	13·7	2·5

HD = Human dwellings. CS = Cattle-sheds.
NC = No collections.
* First round of spraying.
† Second round of spraying.

124·0 and 56·4 respectively, for human dwellings and cattlesheds. This upward trend continued in the succeeding months. The remarkable rise in the prevalence of *C. fatigans* was first taken to be a localized phenomenon. A careful survey of the breeding places revealed heavy breeding in some ponds and channels situated around a match factory which were used for soaking match wood. But the soaking of wood in these ponds and channels had been going on for at least one year before the flare-up of *C. fatigans* and could not have been the immediate cause of it. Further, breeding was not confined to the situations mentioned above, but was present also in ponds and channels with other types of organic contamination, such as retting cocoanut husks and leaves. Again, in Village II also a definite trend of increase in the prevalence of *C. fatigans* had been noticed since September, 1958 even though the densities were considerably less than those recorded for Village I. In Village II, the breeding sites were mostly ponds with soaking cocoanut husks and leaves. It was thus clear that the increase of *C. fatigans* was a generalized phenomenon affecting the sprayed areas. The second round of spraying done in January, brought down the incidence of *C. fatigans* to some extent, but not to any satisfactory degree. By March the upward trend was again evident. The presumption

was therefore inevitable that a strain of *C. fatigans*, resistant to dieldrin, was getting itself established in the areas treated with this insecticide, a development similar to that reported by Wharton (1958) from Malaya. Twenty-four hour mortality tests, done with specimens of *C. fatigans* collected from the sprayed areas soon after the second round of spraying, confirmed the inference.

(*b*) *Effect of* Pistia *clearance.*—In experimental villages III and IV, as has been pointed out earlier, *Pistia* clearance was already in progress when the observations under the Pilot Studies were started. So we do not have for comparison any pre-control or base line data as regards the normal prevalence of *Mansonioides*. We have therefore to take for purposes of comparison the monthly mosquito densities recorded for the comparison village (V) [*See* Table VIII (*e*)].

The monthly ten-man-hour densities of *M. annuliferus* and *M. uniformis* in the *Pistia* clearance villages have been invariably far below those of the comparison area. In some months *Mansonioides* mosquitoes were totally absent from collections made from the *Pistia* clearance villages in contrast to the collections from the comparison area which always contained considerable numbers of them.

Removal of *Pistia* does not inhibit or increase the breeding of other species of mosquitoes, anopheline or culicine [compare Tables VIII (*c*) and VIII (*d*) with VIII (*e*)].

TABLE VIII (*c*)

Mosquito densities (10-man-hours).

VILLAGE III—PISTIA CLEARANCE.

Year and month.	*M. annuliferus.*		*M. uniformis.*		ALL OTHER CULICINES.		ALL ANOPHELINES.		*Culex fatigans.*	
	HD.	CS.	HD.	CS.	HD.	CS.	HD.	CS.	HD.	CS.
1958—										
January	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
February	1·9	..	1·9	..	135·0	337·5	15·0	15·0	1·9	..
March	7·5	..	..	..	60·0	82·5	45·0	..	..	..
April										
May*	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
June*	7·5	..	..	..	..	..	..	..	7·5	..
July*	15·0	10·0	10·0	..	7·5	40·0	12·5	10·0	5·0	..
August	32·5	..	22·5	..	..	60·0	10·0	..	..	20·0
September	11·3	..	30·0	..	3·8	75·0	22·5	..	3·8	..
October	20·0	..	42·5	..	5·0	100·0	7·5	..	10·0	..
November	..	..	10·0	..	15·0	160·0	15·0	..	..	10·0
December										
1959—										
January†	..	..	15·0	..	30·0	120·0	22·5	..	..	..
February	..	..	..	..	112·5	330·0	..	..		..
March	3·0	..	..	..	141·0	210·0	10·5	..	1·5	..
April	11·3		..	..	97·5	1[illegible]5·0	22·5	..	..	..
May	5·6	..	1·9	..	50·6	187·5	41·3	7·5	..	..
June	1·5	..	..	..	27·0	90·0	12·0	..	1·5	..
July	7·5	..	7·5	..	2·7	22·5	2·7	..	17·5	..
August	17·5	..	3·5	..	12·4	30·0	..	..	17·6	..

HD = Human dwellings CS = Cattle-sheds.
ND = Not done. NC = No collections.
* First round of spraying in Villages I and II.
† Second round of spraying in Villages I and II.

TABLE VIII (*d*).

Mosquito densities (10-man-hours).

VILLAGE IV—PISTIA CLEARANCE AND MASS THERAPY.

Years and Month.	*M. annuliferus.*		*M. uniformis.*		ALL OTHER CULICINES.		ALL ANOPHELINES.		*Culex fatigans.*	
	HD.	CS.	HD.	CS.	HD.	CS.	HD.	CS.	HD.	CS.
1958—										
January } February }	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
March	1·9	..	1·9	..	135·0	337·5	15·0	15·0	1·9	..
April	30·0	..	..	..	82·5	165·0	108·8	45·0	..	..
May* } June* }	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
July*	20·0	10·0	..	..	..	10·0	20·0	..	5·0	..
August	27·5	..	2·5	..	..	..	30·0	..	..	..
September	20·0	..	5·0	..	2·5	7·5	35·0	7·5	5·0	7·5
October	5·0	..	5·0	..	7·5	10·0	55·0	20·0	12·5	..
November	7·5	7·5	5·6	..	30·0	67·5	..	..	3·8	7·5
December	5·0	..	5·0	30·0	22·5	230·0	57·5	10·0	15·0	..
1959—										
January†	..	..	..	..	82·5	120·0	75·0	60·0	..	..
February	..	..	..	..	112·5	210·0	52·5	..	..	..
March	31·6	..	..	..	94·7	132·0	23·7	..	..	42·0
April	..	..	..	..	78·7	90·0	33·8	45·0	..	..
May	..	..	..	..	38·0	90·0	40·0	15·0	..	..
June	1·6	0·0	3·2	0·0	4·8	0·0	22·4	0·0	1·6	0·0
July	1·9	..	1·9	..	9·4	7·5	3·8	7·5	1·9	..
August	..	..	6·4	..	6·4	52·5	6·4	15·0	4·3	..

HD = Human dwellings. CS = Cattle-sheds.
ND = Not done. NC = No collections.
* First round of spraying in Villages I and II.
† Second round of spraying in Villages I and II.

NATURAL FILARIAL INFECTION IN *MANSONIOIDES*.

In Table IX, are shown the monthly dissection results in respect of the two vectors, *M. annuliferus* and *M. uniformis*, for the post-control observation period July, 1958 through August, 1959. As observed earlier, the density of the vectors was very low in the dieldrin-sprayed areas and so, only a small number of female specimens were altogether available for dissection. This was particularly true of *M. annuliferus*. From Village I (dieldrin-sprayed) out of 4 female specimens of *M. annuliferus* dissected, one was positive for filaria larvae. This infected specimen was obtained in July, 1958. None of the 48 specimens of *M. uniformis* dissected, showed any infection. In Village II (dieldrin-sprayed and mass therapy) no female specimens of *M. annuliferus* were available for dissection and all the 23 specimens of *M. uniformis* dissected were negative for filaria larvae.

In the two Villages (III and IV) where the anti-vector measure employed was *Pistia* clearance, more specimens of the two vectors were obtained for dissection than from Villages I and II. In Village III (*Pistia* clearance alone) 50 *M. annuliferus* and 44 *M. uniformis* were dissected, all being negative for filarial infection,

TABLE VIII (*e*).

Mosquito densities (10-man-hours).

VILLAGE V—(COMPARISON AREA).

Year and month.	*M. annuliferus.*		*M. uniformis.*		ALL OTHER CULICINES.		ALL ANOPHELINES.		*Culex fatigans.*	
	HD.	CS.	HD.	CS.	HD.	CS.	HD.	CS.	HD.	CS.
1958—										
January	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
February	58·6	24·5	..	5·5	80·5	212·7	64·8	100·9	4·1	..
March	84·9	37·5	2·6	5·0	95·5	160·0	115·5	235·0	2·6	..
April	192·0	18·0	1·5	6·0	88·5	90·0	45·0	78·0	..	..
May* } June* }	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
July*	42·4	5·5	2·9	2·7	24·9	79·1	16·8	49·0	..	..
August	34·4	6·7	24·7	3·3	14·1	136·6	17·6	56·6	2·6	..
September	38·2	3·0	26·3	12·0	10·5	66·0	12·5	33·0	..	3·0
October	59·4	9·2	35·2	4[illegible]·2	24·8	11[illegible]·4	17·3	46·2	2·9	2·3
November	54·3	5·0	24·3	50·0	19·8	95·0	1·3	52·5	7·0	10·0
December	81·7	13·3	30·8	76·7	15·0	123·3	25·0	40·0	0·8	..
1959—										
January†	70·0	90·0	6·6	97·5	52·5	153·8	45·0	30·0	13·1	3·8
February	30·0	15·0	3·8	3·8	43·1	12[illegible]·0	124·7	86·3	..	..
March	23·2	5·5	2·0	..	52·5	133·6	90·0	90·0	2·0	..
April	64·5	18·0	2·3	..	90·0	156·0	28·5	60·0	..	..
May	77·9	13·6	1·4	..	46·4	120·0	31·4	30·0	..	..
June	60·6	19·1	3·4	..	11·9	68·2	9·6	16·4	..	5·5
July	15·5	2·1	1·1	2·1	8·0	32·1	5·4	17·1	..	2·1
August	30·7	2·7	5·0	2·7	8·6	65·5	40·0	24·5	..	..

HD = Human dwellings. CS = Cattle-sheds.
ND = Not done. NC = No collections.
* First round of spraying in Villages I and II.
† Second round of spraying in Villages I and II.

On the other hand from Village IV (*Pistia* clearance and mass therapy) 7 out of 41 *M. annuliferus* and 2 out of 20 *M. uniformis* were found to be positive. Of the 7 *annuliferus* positives, two were recorded in July, 1958, 4 in August, 1958, and 1 in December, 1958. Of the two positive specimens of *M. uniformis*, 1 was obtained in August, 1958 and the other in August, 1959.

As against the absence or mere sporadic occurrence of infected specimens of the vectors in experimental Villages I to IV, their incidence in the comparison village (V) was continuous and high. Practically every catch contained infected specimens of the vectors, whose prevalence was, as already shown, always considerable in the area. Out of a total of 962 *M. annuliferus* dissected, 227 (23·6 per cent) were found to be infected. The natural infection rate in this species ranged from 36·7 per cent in July, 1958, to 13·1 in January, 1959. Of 327 *M. uniformis* specimens dissected, 37 (11·3 per cent) showed filarial infection.

TABLE IX.

Natural filarial infection rate in vectors (Post-control period).

Month and year.	Village I:						Village II:						Village III:					
	M. annuliferus.			*M. uniformis.*			*M. annuliferus.*			*M. uniformis.*			*M. annuliferus.*			*M. uniformis.*		
	D.	P.	IR%.	D.	P.	IR%.	D.	P.	IR%.	D.	P.	IR%.	D.	P.	IR%.	D.	P.	IR%.
1958—																		
July	2	1	50·0	..	..	0·0	..	..	0·0	..	..	0·0	1	..	0·0	..	..	0·0
August	..	..	0·0	..	..	0·0	..	..	0·0	..	..	0·0	7	..	0·0	4	..	0·0
September	..	..	0·0	7	..	0·0	..	..	0·0	2	..	0·0	12	..	0·0	5	..	0·0
October	..	..	0·0	5	..	0·0	..	..	0·0	..	..	0·0	3	..	0·0	8	..	0·0
November	1	..	0·0	11	..	0·0	..	..	0·0	7	..	0·0	8	..	0·0	17	..	0·0
December	..	..	0·0	22	..	0·0	..	..	0·0	9	..	0·0	..	..	0·0	4	..	0·0
1959—																		
January	..	..	0·0	..	..	0·0	..	..	0·0	..	..	0·0	..	..	0·0	2	..	0·0
February	..	..	0·0	..	..	0·0	..	..	0·0	..	..	0·0	..	..	0·0	..	..	0·0
March	..	..	0·0	..	..	0·0	..	..	0·0	..	..	0·0	2	..	0·0	..	..	0·0
April	..	..	0·0	..	..	0·0	..	..	0·0	..	..	0·0	3	..	0·0	..	..	0·0
May	..	..	0·0	..	..	0·0	..	..	0·0	..	..	0·0	..	..	0·0	..	..	0·0
June	..	..	0·0	..	..	0·0	..	..	0·0	2	..	0·0	1	..	0·0	1	..	0·0
July	1	..	0·0	..	..	0·0	..	..	0·0	1	..	0·0	3	..	0·0	1	..	0·0
August	..	..	0·0	..	..	0·0	..	..	0·0	2	..	0·0	10	..	0·0	2	..	0·0
Total :	4	1	25·0	45	..	0·0	..	..	0·0	23	..	0·0	50	..	0·0	44	..	0·0
Pre-control total :	120	23	15·0	23	2	8·7	203	35	17·2	7	2	28·6	9	..	0·0	1	..	0·0

TABLE IX. (*Concld.*)

Month and year.	Village IV :						Village V :					
	M. annuliferus.			*M. uniformis.*			*M. annuliferus.*			*M. uniformis.*		
	D.	P.	IR%.	D.	P.	IR%.	D.	P.	IR%.	D.	P.	IR%.
1958—												
July	9	2	22·2	..	..	10·0	60	22	36·7	5	2	40·0
August	11	4	36·4	1	1	100·0	41	9	21·9	29	6	20·7
September	8	..	0·0	4	..	0·0	52	16	30·8	39	4	10·3
October	2	..	0·0	2	..	0·0	104	20	19·2	77	10	13·0
November	5	..	0·0	3	..	0·0	87	23	26·4	58	4	8·3
December	2	1	50·0	5	..	0·0	102	26	25·6	60	2	3·3
1959—												
January	2	..	0·0	..	..	0·0	99	13	13·1	32	1	3·1
February	..	..	0·0	..	..	0·0	36	9	25·0	5	1	20·0
March	2	..	0·0	..	..	0·0	27	7	25·9	3	2	66·7
April	..	..	0·0	..	..	0·0	91	20	21·9	3	2	66·7
May	..	..	0·0	..	..	0·0	107	20	18·7	2	1	50·0
June	1	..	0·0	1	..	0·0	96	26	27·1	5	1	20·0
July	1	..	0·0	1	..	0·0	30	8	26·7	3	..	0·0
August	..	..	0·0	3	1	33·3	30	8	26·7	8	1	12·5
Total :	43	7	16·3	20	2	10·0	962	227	23·6	329	37	11·3
Pre-control total	2	..	0·0	..	..	0·0	371	46	12·4	9	1	11·1

D = Number dissected ; P = Number positive ; IR% = Infection rate, per cent.

CHART I.

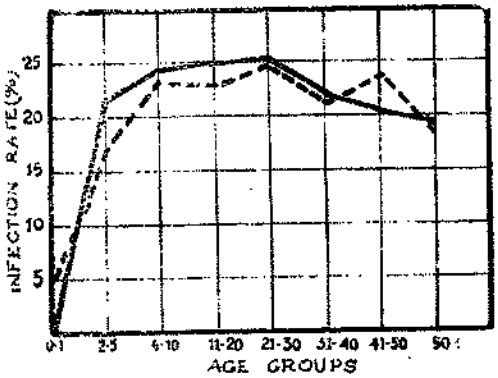

FIG. 1. Village I—(Dieldrin).

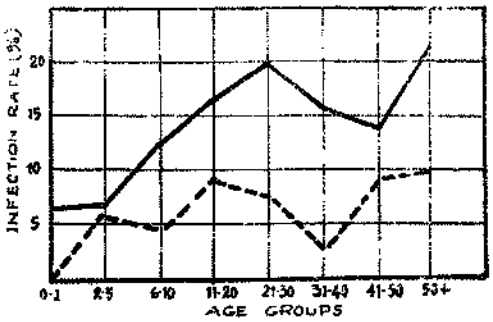

FIG. 2. Village II—(Dieldrin plus mass therapy).

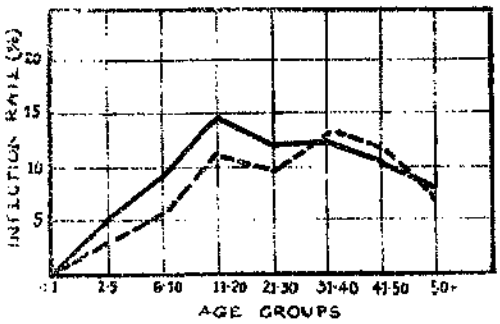

FIG. 3. Village III—(*Pistia* clearance).

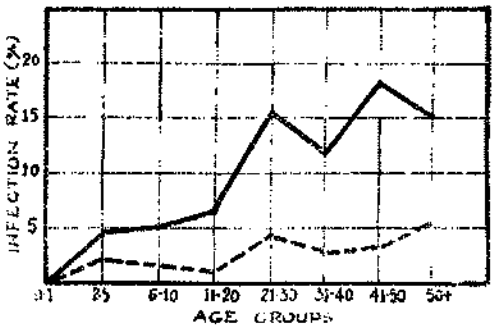

FIG. 4. Village IV—(*Pistia* clearance plus mass therapy.

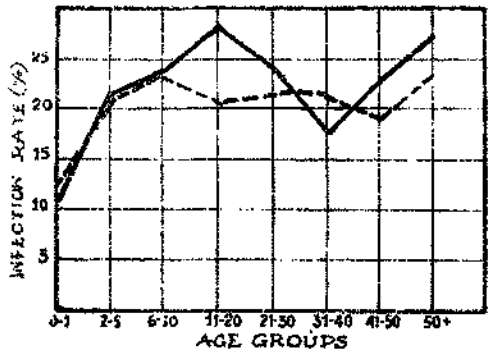

FIG. 5. Village V—(Comparison).

Explanation.

Pre- and post-control infection rates in experimental villages.

Pre-control (1958)—Unbroken line.

Post-control (1959)—Broken line.

(iii) PARASITOLOGICAL.

A second parasitological and clinical survey of the five study villages was done during May—June, 1959, i.e., a year after the introduction of the respective experimental measures. In each village the number of persons surveyed was approximately the same as that during the first survey. Even though the samples of the population examined in the two surveys are not identical, a considerable number of persons examined during the first survey were included in the second survey also. The gross results of the two surveys are compared in Table X.

TABLE X.

Comparison of the gross results of two surveys, i.e., of 1958 and 1959.

Village.	Year.	Number of persons examined	Number with micro-filaria.	Infection rate (per cent.)	Micro-filarial density.	Number of elephantiasis cases.	Percentage of ele-phantiasis cases.
I	1958	3513	806	22·9	21·5	397	11·3
	1959	3529	783	22·2	21·7	305	8·6
II	1958	1,496	237	16·4	28·0	146	9·7
	1959	1,493	121	8·4	11·3	99	6·6
III	1958	1,512	161	10·6	10·6	107	7·1
	1959	1,518	139	9·2	10·4	89	5·9
IV	1958	770	87	11·3	12·8	42	5·4
	1959	769	21	2·7	16·8	11	1·4
V	1958	1,509	352	23·3	17·6	196	12·9
	1959	1,527	321	21·0	19·1	180	11·7

Effect of mass therapy.—The infection rate did not undergo any appreciable change in Villages I and III where anti-mosquito measures alone were tried. In the comparison village (V) too, the situation was the same. But in Villages II and IV where anti-mosquito measures were supplemented by mass therapy, there was considerable reduction in the infection rate; in Village II (dieldrin plus mass therapy) it dropped from 15·4 per cent to 8·4 per cent and in Village IV (*Pistia* clearance plus mass therapy) from 11·3 per cent to 2·7 per cent. This reduction was shared by all the age-groups from 0-1 to above 50 years (see Chart 1).

(iv) CLINICAL.

As during the pre-control survey, only cases of elephantiasis were recorded during the check survey. The second survey indicated some diminution of the incidence of this condition in all the five villages, but it was more marked in Villages II and IV.

DISCUSSION.

Vector control by residual indoor dieldrin spraying.—The data presented above prove that indoor application of dieldrin at 50 mg. per sq. ft. can reduce the density of *Mansonioides annuliferus* to practically zero and maintain it at that level for

almost 6 months. The effect of dieldrin on *M. uniformis*, too, is remarkable, but is of much shorter duration than on *M. annuliferus*, i.e., about two months. But a second round of spraying at 50 mg. per sq. ft. 6 months after the first round, rendered the villages practically free from this species also for about 6 months. These observations are based not only on the routine time catches from fixed catching stations, but also on random general collections carried out from time to time in the experimental villages. Further, careful examination of breeding places within the villages showed that breeding of *M. annuliferus* and *M. uniformis* was scanty. This observation suggests that the reduction in the indoor densities of *Mansonioides* is due to an absolute reduction in their prevalence in the dieldrin-treated villages and not to any change in their resting habits, induced by the insecticide.

While the efficacy of indoor application of dieldrin for the control of the vectors is beyond any doubt, the desirability of adopting this measure is disputable in the light of the finding that it has led to a remarkable increase in the prevalence of dieldrin-resistant strains of *C. fatigans* in the villages where it has been tried. As pointed out earlier an identical experience has been reported recently from Malaya (Wharton, 1958). It may be argued that *C. fatigans* cannot transmit *W. malayi* and that any increase in the incidence of this species is immaterial from the point of view of *malayi* control. But it has to be noted that the *malayi* tract in Kerala is interspersed with several *bancrofti* foci and any expansion in the prevalence of *C. fatigans* might eventually facilitate the further spread of *bancrofti* infection as well. Again, the nuisance which *C. fatigans* is capable of causing by its bite has also to be taken into consideration. This is bound to make the measure unpopular as it has already done in the authors' experience.

Thus, in the circumstances discussed, it does not seem advisable to adopt indoor residual spraying of dieldrin in the control of *B. malayi* in Kerala.

Vector control by Pistia *clearance.*—This method has produced quite satisfactory results in Villages III and IV where it has been tried. The prevalence of the vector in these two villages was kept down at a very low level even though not to the extent achieved in the dieldrin-treated Villages I and II. Being a biological measure, the results of *Pistia* clearance depend solely on the thoroughness with which it is done.

One possibility that has to be taken into account in the choice of this method is that of the vector species of *Mansonioides* adapting themselves to weeds other than *Pistia*. Some workers (Burton, 1959, for example) have found that the larvae of *M. annuliferus* as well as *M. uniformis* do attach themselves to other aquatic weeds like *Eichornia* (Water hyacinth). In Ceylon, Antonipulle *et al.* (1958) have found egg masses, larvae and pupae of *M. uniformis* (the chief vector of *B. malayi* in Ceylon) on 12 genera of plants other than *Pistia*. They found that *Salvinia* (a water fern) and *Isachne australis* (a grass) were important host plants for the early stages of *M. uniformis*. All these reports demonstrate the adaptability of *Mansonioides*, especially of *uniformis* to different kinds of aquatic plants.

It must be pointed out here that the finding of *Mansonioides* larvae attached to a particular plant does not necessarily mean that it is a natural host plant for these mosquitoes. The true host plant is the one on which these mosquitoes habitually oviposit under natural conditions. The larvae and pupae may stray away and cling to any weed providing, for their respiration, a soft, cortical tissue containing air. Indeed, it has been possible to rear adult *Mansonioides* from larvae by substituting 'wet strength' crepe paper, for larval respiration (Laurence and Smith, 1958). Thus the finding of larvae on an aquatic weed is not by itself of much epidemiological significance.

In the coastal regions of Kerala, so far as is known, *Pistia* is the only aquatic plant on which the eggs of *Mansonioides* have been consistently seen. In the experimental villages, eggs of *Mansonioides* have been found on other plants only on two occasions even though larvae have been seen oftener on other weeds associated with *Pistia*. It is obvious that in this region, *Pistia* is yet the specific host plant for *Mansonioides*. *Pistia* clearance, therefore, continues to be a useful and dependable method of controlling *Mansonioides* breeding and thereby of preventing transmission of *B. malayi* infection in the coastal areas of Kerala.

Mass therapy as an adjuvant to vector control.—It was shown that in Villages II and IV where vector control measures were supplemented by mass therapy, a notable reduction was observed not only in the natural infection rates of the vectors, but also in the microfilaria infection rates in man. The reduction in the microfilaria infection rate, it was pointed out, was experienced by all the age-groups. Even though it was possible to give full treatment only to about 50 per cent of the population in each village, preventive therapy has been useful in reducing considerably the level of the human reservoir of the infection.

CONCLUSIONS.

(i) It is practicable to prevent transmission of *B. malayi* through measures directed solely against the transmitters, namely, *Mansonioides annuliferus* and *M. uniformis*. The two such measures tried, (i) indoor residual spraying with dieldrin in two cycles of 50 milligrams per sq. ft. each, and (ii) *Pistia* clearance all the year round, gave equally satisfactory results. However, the use of dieldrin was found to have one serious disadvantage in that it gives rise to resistant strains of *C. fatigans* which, even though not a vector of *B. malayi*, might facilitate the spread of *W. bancrofti* infection which is endemic in several urban areas situated in or contiguous with the *B. malayi* belt. Despite its efficacy, *Pistia* clearance is a comparatively tardy process which requires to be carried out throughout the year. This would appear to limit the practicability of its adoption over large areas.

(ii) Mass therapy with diethylcarbamazine by itself, is not a feasible preventive measure against *B. malayi* in Kerala, because, with the best efforts, it has not been found possible to cover more than 50 to 60 per cent of the experimental

population. However, its value as an adjuvant to a suitable antivector measure (residual adulticide spraying or *Pistia* clearance) is considerable.

(iii) Of the four measures tried out, a combination of vector control by *Pistia* clearance and mass therapy with diethylcarbamazine, gave the most satisfactory results.

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