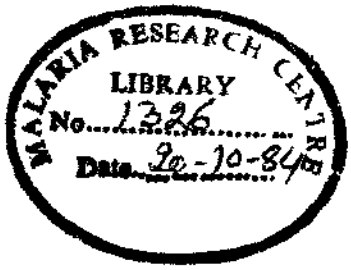
INDIAN JOURNAL OF MALARIOLOGY.

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

Editor:—Lieut.-Colonel JASWANT SINGH, M.B., Ch.B., D.P.H., D.T.M. & H., F.N.I.,
Director, Malaria Institute of India, Delhi.

Vol. X, 1956.



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3. Does it contain the amount of active material claimed?	4. What technical supervision is observed in the factory and the laboratory?

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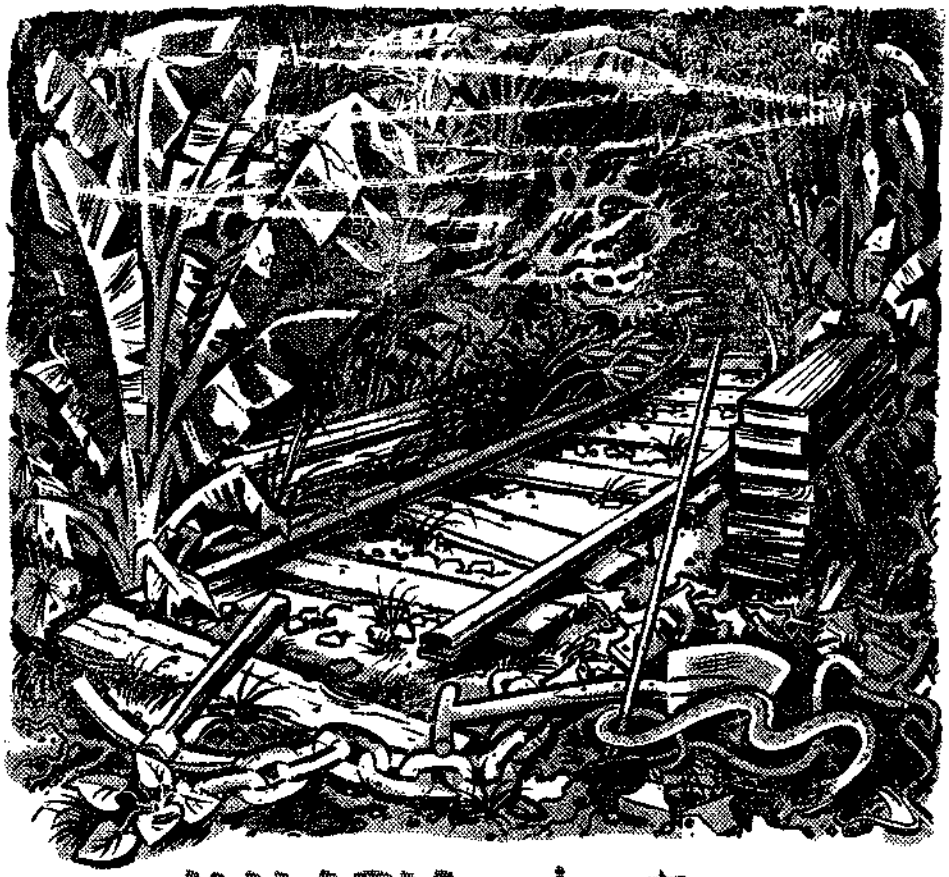

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Indian Journal of Malariology, **10**, *1*, *March 1956*.

OBITUARY.

PATRICK ALFRED BUXTON

THE Science of Medical Entomology has suffered a great loss in the sad demise at the age of 63 of Prof. Patrick Alfred Buxton, C.M.G., F.R.S., Professor of Medical Entomology, University of London, and Director of the Department of Entomology, School of Tropical Medicine and Hygiene, London. Prof. Buxton was one of the most eminent specialists on the relationship between insects and diseases and his contributions on this subject are widely known and admired.

Prof. Buxton was born in 1892 and went to the Rugby Training College in Cambridge of which he was a Fellow from 1916 to 1921. He served in Mesopotamia and North West Persia, and from 1921 to 1923 he was Entomologist to the Government of Palestine. During 1923-1924 he was leader of the expedition to Samoa. He was appointed as Director of the Department of Entomology at the London School of Hygiene and Tropical Medicine in 1925 and Professor of Medical Entomology in the University of London in 1933.

Under his inspiring personality and genius, entomologists from practically all parts of the world received training in his department which became a focal point during the last World War when most of the work on medical entomology for the allied forces was centred under his expert guidance. Prof. Buxton published a number of technical papers on a variety of arthropods, their bionomics and control and two of his well-known books are "*Animal life in deserts*" and "*The louse*". A comprehensive treatise on "*The tsetse fly*" has just been published.

His work and accomplishments will no doubt be reviewed at length by many others. This note is in token of the deep sentiments of affection with which the Indian entomologists regarded him.

J. S.

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RELATIONSHIP OF TUBERCULOSIS ON THE COURSE AND INTENSITY OF PLASMODIAL INFECTIONS IN *M. MULATTA*.

BY

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AND

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NURI strain of *P. knowlesi* (Jaswant Singh, Ray and Nair, 1953; Edeson and Davey, 1953) has been maintained, since its isolation, in the laboratories of Malaria Institute of India, by successive serial passages of the blood forms of this parasite in North Indian brown monkeys (*M. mulatta*), and so far it has undergone 233 passages in these animals. As reported earlier, the strain is highly virulent and death invariably follows in four to six days after patent infection is established. The course of parasitæmia is almost dramatic and 90 per cent or over cell infection is not infrequent.

A strain of *P. inui*, originally isolated by Sinton and Mulligan (1932), is also being maintained from the time it was isolated. During the initial phase of acute infection, there is progressive rise in parasitæmia, though not of the same order as in *P. knowlesi*. This strain produces a benign infection and is also maintained by serial passages of blood-induced infection.

In the course of routine daily examination, infection with one or the other plasmodium in a few monkeys was noticed to be of a much lower order than commonly encountered, and postmortem examination in all such cases revealed tuberculous lesions.

In order to determine whether there was any correlation between malaria and tuberculosis, or the findings were merely a coincidence, the results of 33 monkeys (19 tuberculous and 14 non-tuberculous) were carefully analysed and are presented in this paper.

RESULTS.

The parasitological patterns observed in healthy and tuberculous monkeys infected with the Nuri strain of *P. knowlesi* are shown in Table I and Graph 1.

TABLE I.

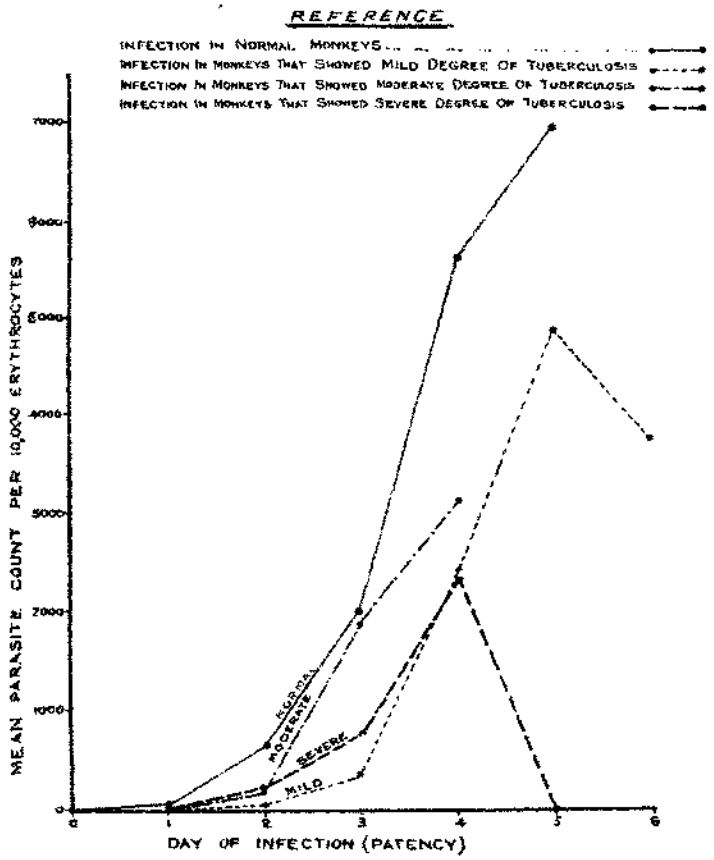
Course and intensity of P. knowlesi *(Nuri strain) infection in tuberculous and non-tuberculous monkeys.*

Monkey number.	Details.	Prepatent period (days) (from the date of inoculation).	Duration of parasitæmia (days).	Parasite count per 10,000 r.b.c.			Day of death after inoculation.
				Daily average.	Peak.		
					Days after inoculation.	Number.	
Series I.—Non-tuberculous monkeys.							
3621*	Healthy (Non-tuberculous)	2	4	3,424	6	9,250	6
3624*	,, ,,	1	4	2,000	5	9,000	5
3628*	,, ,,	2	4	2,457	6	9,000	6
5298	,, ,,	<1	5	2,485	5	8,000	5
5462	,, ,,	1	4	2,580	5	7,200	5
5596	,, ,,	<2	4	2,892	5	7,500	5
5714	,, ,,	1	4	2,366	5	6,800	5
5805	,, ,,	2	4	3,970	6	7,200	6
5830	,, ,,	<2	5	2,623	6	8,000	6
5848	,, ,,	<2	5	1,971	6	6,500	6
Series II.—Tuberculosis Stage I.							
3272	Tuberculous (mild)	1	3	175	4	500	3
4195	,, ,,	2	4	3,700	6	9,800	6
4517	,, ,,	3	5	950	8	4,800	8
5424	,, ,,	1	5	1,254	6	4,000	6
5345	,, ,,	2	4	1,603	6	8,600	6
5332	,, ,,	2	4	1,646	6	4,600	6
5379	,, ,,	2	4	1,327	6	5,600	6
Series III.—Tuberculosis Stage II.							
3280	Tuberculous (moderate)	4	3	1,589	7	4,500	7
3741	,, ,,	2	2	30	4	100	4
4801	,, ,,	6†	...	...	...	...	...
5043	,, ,,	2	4	1,099	6	4,200	6
5672	,, ,,	2	4	961	6	3,200	6
Series IV.—Tuberculosis Stage III.							
5851	Tuberculous (severe)	2	5	1,940	7	8,400	7
5385	,, ,,	2	6	55	5	200	11
5364	,, ,,	3	4	289	7	1,200	7

<=Less than. *Jaswant Singh *et al.*, 1953. †Died before becoming positive.

GRAPH 1.

Course of P. knowlesi *(Nuri strain) infection in normal and tuberculous monkeys.*



Whereas in normal infection, the prepatent period was approximately on an average of 1·6 days, the same observed in mild, moderate, and severe tuberculosis was 1·9, 3·2, and 2·3 days, respectively. There was no apparent difference in the duration of parasitæmia in the non-tuberculous and tuberculous monkeys (average 3·75 to 5) but the main variation was observed in the density of parasites (mean parasite count for the whole infection period) amongst the four groups (2,692 parasitized erythrocytes per 10,000 r.b.c. in non-tuberculous monkeys, 1,761 in mild, 920 in moderate and 761 in very severe tuberculous cases). Peak parasite count, recorded during the course of infection, showed that the density attained was inversely proportional to the degree of tuberculosis. As against an average count of 7,845 in non-tuberculous monkeys, parasitæmia in tuberculous monkeys ranged only from 3,000 to 6,233. This peak parasitæmia occurred within an average of 5·5 to 6·3 days since infecting them, irrespective of the fact whether they belonged to non-tuberculous or tuberculous group. Similarly, death of the monkeys due to *P. knowlesi* infection occurred on an average of 5·5 to 5·84 days

after inoculation in non-tuberculous monkeys as well as in those infected with mild or moderate tuberculosis. But in animals with severe lesions, the period was prolonged (on an average of 8·3 days). One of the monkeys (Number 5385) which had severe tuberculosis, showed no parasites in the peripheral blood till just prior to death.

The data with regard to non-tuberculous and tuberculous monkeys infected with *P. inui* are shown in Table II, and Graph 2. The degree of tuberculous lesions

TABLE II.

Course and intensity of P. inui *infection in tuberculous and non-tuberculous monkeys.*

Monkey Number.	Infection in non-tuberculous or tuberculous monkeys.	Prepatent period (days).	Duration of initial parasitæmia.	Parasite count per 10,000 R. B. C.			Relapse				
				Daily average.	Peak		Total observation period (after the initial infection).	Number.	Total duration of parasitæmia (days).	Average daily parasite count per 10,000 R. B. C.	Peak parasite count per 10,000 R. B. C.
					Day after inoculation (days).	Number.					
5917	Non-tuberculous	7	>70*	76	30 and 61	400	...	...	...	...	...
5103	,, ,,	7	69	5·7	18	80	60‡	1	18	4	15
5870	,, ,,	8	9	2·8	14	8	16§	1	5	<1	<1
5575	,, ,,	9	61	51	53	900	109§§	4	58	3·4	14
5784	Tuberculous (Mild to moderate lesion)	21	2	<1	13	<1	31§§§	1	6	2·8	6
5774	,, ,,	26†	...	...	...	...	...	...	...	...	...
5737	,, ,,	26	5	<1	39	<1	10	1	2	<1	<1
5736	,, ,,	26	...	...	...	...	...	...	...	...	...

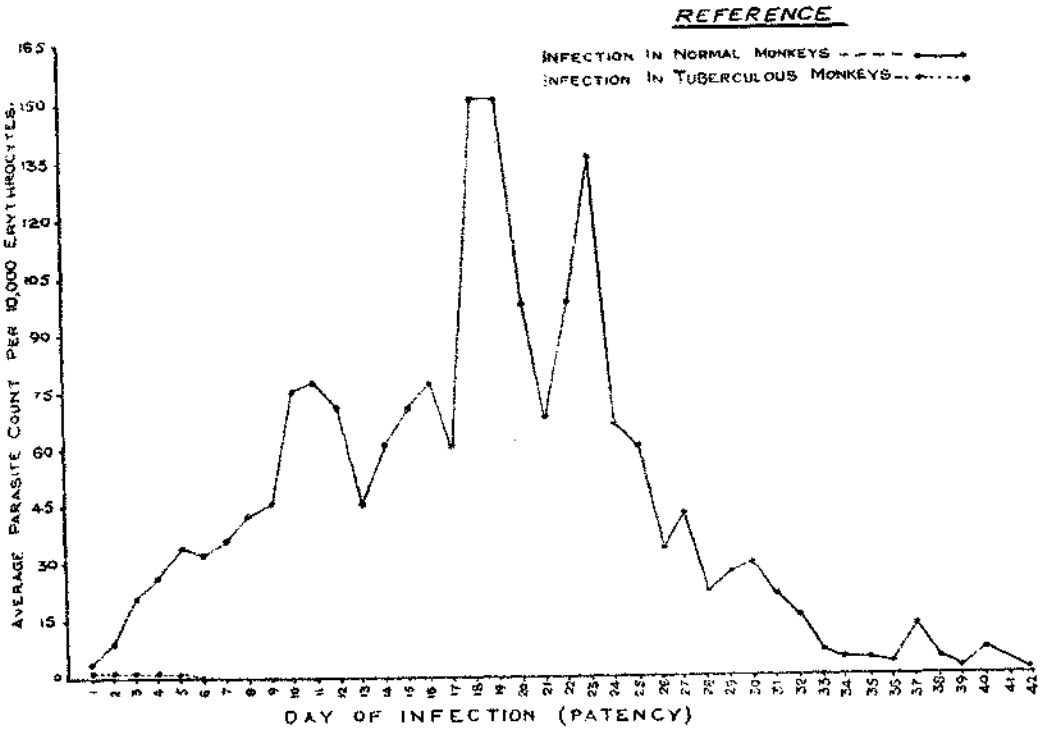
> =More than. < = Less than. *Still running positive.
§Splenectomy performed after the period. Became positive three days after. Parasitæmia continued for 59 days with a peak parasitæmia of 1,800 and average 333·5 per day.
§§Still running positive. §§§Died.
†Died before becoming positive and showed tuberculous lesion.
‡Splenectomy performed after this period. Became positive three days after. Parasitæmia continued for 25 days, with a peak parasitæmia of 2,100 and average 378·6 per day.

observed was " mild to moderate " in these series. The parasitological patterns in tuberculous monkeys, both during initial infection and relapses, were in striking contrast to those found in non-tuberculous monkeys. As against an average pre-patent period of 7·75 days in normal infection, in tuberculous cases it was more than 24·75 days. These include two monkeys that died 26 days after inoculation without becoming positive. The duration of initial parasitæmia was only 3·5 days (average) in tuberculous monkeys as against 52·25 days in non-tuberculous ones.

The daily average parasite count and peak parasitæmia were on an average 34 and 347 per 10,000 erythrocytes, respectively, in normal infection, but the same in tuberculous monkeys was even less than one in both these respects. During relapses, parasitæmia lasted on an average of 27 days in normal infections whereas in those that suffered from tuberculosis, it was only four days. Peak parasitæmia amounted to an average of 10 per 10,000 erythrocytes in non-tuberculous and three in tuberculous monkeys. The daily average count in the same way was 2·7 (average) in the former and 1·4 in the latter case.

GRAPH 2.

Course of P. Inui *infection in normal and tuberculous monkeys.*



DISCUSSION.

There is no clear cut evidence at present to indicate any antagonism between tuberculosis and malaria in vertebrate hosts. Rather, the general belief is that after an attack of malaria, a patient becomes more vulnerable to tuberculosis.

Cacciapuoti (1930) from his experimental evidences concluded that carbolized old tuberculin is valuable in bringing to light latent malaria and thus aiding diagnosis. According to Shirokogorov (1934) malaria could not be considered an activator of tuberculosis.

Makari (1947) encountered an altered tuberculin sensitivity in patients with chronic malaria (41·4 per cent in chronic malaria as compared to 23·3 per cent in the control series). This sensitivity was the greatest during the period of moderate activity of malarial infection and less marked during the periods of marked activity and of latency. In the experience of Freund *et al.* (1948) tuberculosis did not usually have a conspicuous effect on the progress of *P. knowlesi* infection. Boyd (1947) stressed that there was no known disease between which and plasmodial infection, there existed any unilateral or mutual antagonism. But on the other hand, Coggeshell and Kumm (1938) observed that tuberculosis had a somewhat retarding effect on *P. knowlesi* infection. Intraperitoneal injection of live tubercle bacilli into guinea pigs enhanced the antibody formation to various antigens subsequently introduced by the same route (Lewis and Loomis, 1924: 1925). Killed tubercle bacilli, when used as an adjunct to formalin-killed plasmodia, and combined with paraffin oil and an emulsifying agent, could produce immunity against malaria parasites without infection (Freund *et al.*, 1948; Thomson *et al.*, 1947). According to Freund (1947) tubercle bacilli augment antibody formation against non-related antigens when the latter are introduced into tuberculous animals, or tubercles caused by living or killed tubercle bacilli.

During the present studies, those that showed a few scattered minute caseating nodules on one or both lungs were taken as mild ones, whereas moderate number (12 to 14) of scattered caseating nodules of the size of pin to cut-pea indicated moderate cases. Extensive caseous pneumonic and broncho-pneumonic and generalized miliary types as well as chronic forms with dense appearance of the lungs and firm adhesions to the chest wall (Nair and Ray, 1955), constituted very severe lesions. A careful perusal of the results show that although mild and moderate degrees of tuberculosis appear to have only a mild antagonistic effect on the highly virulent *P. knowlesi* (Nuri strain) infection, advanced tuberculosis does seem to have an appreciable effect. However, it should be mentioned here that the number of animals under Series IV, showing severe tuberculous lesions, is quite small and, therefore, no attempt has been made to analyse the data statistically. But the fact remains that at least in two out of three monkeys showing advanced tuberculosis, the course of parasitæmia was considerably altered so much so that the mean peak parasitic density in these two animals was recorded to be seven per cent as against 65 to 92·5 per cent in non-tuberculous animals. This phenomenon has also been observed in *P. inui* infection. Although this plasmodium produces a benign infection and has a tendency towards chronicity like *P. malariæ* infection in human beings, the antagonism between plasmodial and tuberculous infection appeared to be much more marked in this series. Against the mean peak parasitic density of 347 per 10,000 in non-tuberculous monkeys, it was even less than 1 per 10,000 in those suffering from tuberculosis.

P. knowlesi originally isolated by Sinton and Mulligan (1932) gradually lost its former virulence (Jaswant Singh, Ray and Nair, 1949). Though the actual cause for this is not well understood, the fact remains that the strain had to be passed during that period, through a series of heavily infected tuberculous monkeys.

In the present state of our knowledge on the subject, it is difficult to offer any definite explanation for such a phenomenon, but one is inclined to put forward the following arguments:—

It has been observed that the course of *P. cynomolgi* is somewhat altered in monkeys kept on low protein diet in that the peak density is of a somewhat lower order than in infected monkeys kept on standard diet; further, the clearance of parasites from the peripheral circulation, after the crisis, is often delayed in the former group of monkeys (Ray, 1955). Protein deficiency appears to be a plausible factor under such circumstances. It is, therefore, likely that in tuberculous animals as well, such protein deficiency occurs as to inhibit the normal growth of the parasites. Further, it is possible that there may be a competition for some essential nutrients between tubercle bacilli on one hand and the plasmodia on the other. Mckee and Geiman (1946) demonstrated that deficiency of ascorbic acid in the host, like *M. mulatta*, results in the slow rise in parasitæmia, and that ascorbic acid is essential for the plasmodial growth. It is also well known that in tuberculosis there is a deficiency in ascorbic acid. In view of these observations, it could be argued that in tuberculous animals, plasmodial growth is inhibited on account of deficiency of ascorbic acid in the host.

Whatever be the nature of the inferences from the present studies, it still remains to be solved whether or not there is a fundamental relationship between tuberculosis and the malarial infection caused by different species of plasmodia and if there is a relationship, what the mutual factors are that influence the course of these two infections when they co-exist in the same vertebrate. Further studies, therefore, on this line are necessary to throw more light on the subject.

SUMMARY.

The course and intensity of *P. knowlesi* (Nuri strain) and *P. inui* infections were studied in both normal (non-tuberculous) and tuberculous monkeys (*M. mulatta*). The mean average daily parasitæmia due to *P. knowlesi* (Nuri strain) in monkeys with severe tuberculosis was much less than in the normal animals. A more positive finding was evident in *P. inui* infection even though the co-existing tuberculosis was only of a moderate degree.

Importance is stressed on the necessity for further work on this line to confirm whether a real and fundamental relationship exists between tuberculosis and malaria.

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STUDIES ON NURI STRAIN OF *P. KNOWLESI.*

Part X. Therapeutic effect of bromoguanide and its active metabolite.

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BROMOGUANIDE, the para-bromophenyl analogue of proguanil (Curd *et al.*, 1948; Bami and Guha, 1948; Ainely *et al.*, 1949), was tested previously (Jaswant Singh, Nair and Basu, 1950) against *P. cynomolgi, P. inui, P. knowlesi* (the strain originally isolated by Sinton and Mulligan, 1932) and also against *P. gallinaceum.* It was observed that it was less effective against *P. knowlesi* and *P. gallinaceum* than the former two. Subsequently Bami (1953) isolated 1-p-bromophenyl-2-4-diamino-6:6-dimethyl-1:6-dihydro 1:3:5-triazine (active metabolite of bromoguanide) from the urine of *M. mulatta mulatta* (*S. rhesus* monkeys) receiving daily doses of bromoguanide. The antimalarial action of this compound as well as the parent drug (bromoguanide) was assessed againt *P. cynomolgi* (Ray, Nair *et al.*, 1954; Nair *et al.*, 1953*a*) and it was noted that the former was approximately twice as effective as the latter. The present paper reports the findings of the results of bioassay with both the compounds against blood-induced Nuri strain of *P. knowlesi* (Jaswant Singh *et al.*, 1953; Edeson and Davey, 1953) which has been established to be extremely virulent to the simian host.

METHODS AND MATERIALS.

The general technique adopted for these investigations was similar to that reported earlier by Nair, Ray, and Jaswant Singh (1953*b*) and Nair and Ray (1955). Normal healthy *M. mulatta mulatta* were infected with the Nuri strain of *P. knowlesi* and the injected dose (intravenously) was 5×10^6 parasitized erythrocytes per kg.

body weight. Blood smears were taken twice a day, stained with J.S.B. (Jaswant Singh and Bhattacharji, 1944) and examined. Drug administration (oral) was commenced in all cases at a time when cell infection had reached 0·1 to 1 per cent, and continued thereafter daily for six days (total seven doses). The dosage schedules were calculated in terms of the base content per kg. body weight of the animal.

The criteria of assessment was the clearance of parasites from the peripheral blood by the day following the last dose (Class II effect of Shannon). The minimum dose of a compound which produced this effect was considered to be the minimal effective dose (M.E.D.). To determine the Class III effect (complete sterilization of blood-induced infection) blood smears were examined daily after the cessation of treatment for 30 days. Splenectomy was undertaken in those animals which did not relapse during the period. Thereafter they were observed for a further period of 30 days. Class III effect was established in those which remained negative throughout. Class I effect was recorded when there was deceleration in the course of parasitæmia to the extent of 50 to 75 per cent of untreated infection and the life of the animal was prolonged for at least three days after the cessation of treatment.

RESULTS.

Bromoguanide.—A total of 36 monkeys were placed under different regimes. The dosage schedules ranged from 0·05 to 10 mg. per kg. body weight of the monkey. With doses of 0·05 to 8 mg., the results were found to be inconsistent as in some the drug proved ineffective while in others Class II effect was evident irrespective of the dose (Table I). However, when the dose was raised to 10 mg./kg., Class II effect was observed in all the five monkeys. This dose was, therefore, taken as the minimal effective dose (M.E.D.). The earliest clearance of parasites in these experiments was observed in 48 hours in one which had received 0·8 mg., while in contrast the time taken for clearance in another treated with 10 mg. was 168 hours. In the rest, the period varied between the two extremes. Class III effect was observed in none as those which had indicated Class II activity earlier, relapsed some time or other.

Metabolite of bromoguanide.—Sixtythree animals were placed under treatment and the dosage schedules employed were from 0·01 to 50 mg. per kg. body weight. Some plasmodicidal effect could be traced in monkeys treated with 0·03 mg. and upwards up to 40 mg., with the exception of 0·5 and 1·8 mg. schedules in which the course of infection was similar to that observed in uninterfered *P. knowlesi* infection, and all the monkeys succumbed in spite of treatment (Table II). Out of 49 animals treated with doses ranging from 0·03 to 40 mg. doses, no effect was produced in 13 irrespective of the schedules, while in 20 there was mere deceleration in parasitæmia. Class II activity was observed in 12, and Class III in the remaining monkeys. The results obtained were thus quite inconsistent. However, in the series treated with 50 mg., clearance of parasites was recorded in three out of four by the day following conclusion of treatment. The fourth animal died during the course of treatment probably on account of toxic effects. Postmortem examination did not reveal inter-current infection.

This dose may, therefore, be considered as the M.E.D. even though it is near the toxic level.

Judging from the speed of action it is considered to be tardy as in most cases parasite clearance was not observed earlier than 72 hours, the range being 48 to 168 hours. Relapses after the initial clearance occurred between two and nine days from the completion of treatment in all, except three cases one each under 0·15, 1·00 and 2·00 mg. series.

TABLE I.

Effect of bromoguanide on blood-induced P. knowlesi *(Nuri strain) infection.*

Dosage mg. (base) per kg. body weight.	Number of monkeys employed.	Inactive (number).	Class I. Deceleration of parasites.	Class II. Clearance of parasites. Number.	Class II. Clearance of parasites. Hours (since starting drug administration) Average. / range.	Class II. Relapse. Number.	Class II. Relapse. Days (since cessation of drug) Average. / range.	Class III.
0·05	3	2	...	1	60	1	6	...
0·08	3	3	...	...	...	...	...	...
0·1	5	2	...	3*	96 84–108	2	4 2–6	...
0·2	4	1	...	3	72 60–84	3	3 3	...
0·3	2	1	...	1	92	1	7	...
0·5	3	1	...	2	72	2	2·5 2–3	...
0·8	2	1	...	1	48	1	4	...
1·0	2	...	1	1	120	1	3	...
2·0	2	...	2	...	...	...	...	...
5·0	2	2	...	...	...	...	...	...
8·0	3	2	1	...	...	...	...	...
10·0	5	...	...	5†	93·6 60–168	2	4 3–5	...
	36							

* One died during observation period.

† Two died during observation period and one soon after the last dose of the drug.

TABLE II.

Effect of the active metabolite of bromoguanide in blood-induced P. knowlesi *(Nuri strain) infection.*

Dosage mg. (base) per kg. body weight.	Number of monkeys employed.	Inactive (number).	Temporary deceleration of parasites (Number).	Clearance of parasite. Number.	Clearance of parasite. Hours (since starting drug administration) Average. / Range.	Relapse. Number.	Relapse. Days (since cessation of drug) Average. / Range.
0·01	3	3	...	...	...	...	...
0·03	3	2	1	...	...	...	...
0·05	2	...	1	1*	108	...	...
0·1	3	...	2	1*	96	...	...
0·15	3	...	1	2†	138 132–144	1	3
0·3	3	2	...	1*	168	...	...
0·5	4	4	...	...	...	...	...
1·0	5	2	1‡	2†	90 60–120	1	9
1·5	3	1	...	2*†	144 120–168	1	2
1·8	3	3	...	...	...	...	...
2·0	5	2	...	3†	84 72–96	2	5 3–7
5·0	2	1	...	1	48	1	2
10·0	2	1	1	...	...	...	...
15·0	3	...	3‡	...	...	...	...
20·0	2	1	1‡	...	...	...	...
25·0	3	...	1+1‡	1	96	1	4
30·0	3	...	3‡	...	...	...	...
35·0	4§	1	1+1‡	...	...	...	...
40·0	3	...	2	1*	84	...	...
50·0	4§	...	...	3§§	68 48–72	2	7 4–10

*Died during observation period. †Infection was cured in one.
‡Disappearance and reappearance of parasites during drug administration.
§One died during drug administration before becoming negative.
§§One died during drug administration after becoming negative.

DISCUSSION.

From the results recorded above and the previous findings of Nair, Ray and Jaswant Singh (1953*b*), the M.E.D. of bromoguanide and its active metabolite has been found to be 10 and 50 mg. and their quinine equivalent 3·0 and 0·6 respectively. The effect of these two drugs is no doubt tardy and their minimal effective dose is near the toxic level as some of the monkeys died at the time of treatment. The activity of the two drugs as well as proguanil and its active metabolite have already been assessed against *P. cynomolgi*, *P. knowlesi* (Nuri strain), *P. berghei*, and *P. gallinaceum*. The quinine equivalent as determined in the above trials are indicated below:—

Name of Drug.	Species of parasite.	Quinine (equivalent).	Reference.
Proguanil	*P. gallinaceum*	16	Jaswant Singh *et al.*, 1952.
	P. berghei	480 (for Class I effect) 240 (for Class II effect)	Krishnaswami *et al.*, 1953.
	P. cynomolgi	20	Nair *et al.*, 1953*a*.
	P. knowlesi (Nuri strain)	150	Nair *et al.*, 1953*b*.
Bromoguanide	*P. gallinaceum*	32	Unpublished.
	P. berghei	...	...
	P. cynomolgi	9·09	Nair *et al.*, 1953*b*.
	P. knowlesi	3	Present investigation.
Active metabolite of proguanil.	*P. gallinaceum*	256	Jaswant Singh *et al.*, 1954.
	P. berghei	12,000 for Class I effect, 192 for Class II effect.	Krishnaswami *et al.*, 1953.
	P. cynomolgi	3·3	Jaswant Singh *et al.*, 1956 (In press)
	P. knowlesi (Nuri strain)	0·86	Nair *et al.*, 1955.
Active metabolite of bromoguanide.	*P. gallinaceum*	512	Jaswant Singh *et al.*, 1954.
	P. berghei	...	...
	P. cynomolgi	16·7	Ray *et al.*, 1954.
	P. knowlesi (Nuri strain)	0·6	Present investigation.

The sensitivity of *P. knowlesi* (Nuri strain) and *P. cynomolgi* was more marked to proguanil than bromoguanide or the two metabolites. On the other hand, *P. gallinaceum* appeared to be more sensitive to the two metabolites than their corresponding parent drugs. Regarding the relative sensitivity of *P. berghei* to proguanil and its metabolite, the metabolite in terms of the Class II effect was 0·8 times and in terms of Class I effect 25 times as effective as proguanil. Activity of bromoguanide against *P. cynomolgi* was almost half as much as its metabolite but against *P. knowlesi* (Nuri strain) the parent drug was five times more active than its active metabolite. Proguanil was about eight times more active against *P. knowlesi* (Nuri strain) than against *P. cynomolgi* but on the contrary bromoguanide was three times more active against *P. cynomolgi* than against *P. knowlesi* (Nuri strain). This compares well with the earlier finding that bromoguanide as judged from its speed in the clearance of parasites was more effective against *P. cynomolgi* than against *P. knowlesi*, the strain isolated previously by Sinton

and Mulligan in 1932 (Jaswant Singh, Nair and Basu, 1950). In this connection, it may be mentioned that trials are in progress in human malaria with the metabolite of bromoguanide, but there is evidence to believe that in the case of bromoguanide, it is generally inferior to proguanil not only against avian (Jaswant Singh *et al.*, 1950) and simian (Nair *et al.*, 1953*a*: 1953*b*) infections but also against human infection due to *P. vivax* (Chaudhuri, 1954).

SUMMARY.

Bromoguanide (the para-bromophenyl analogue of proguanil) and its active metabolite (1-p-bromophenyl-2-4-diamino-6:6-dimethyl 1:6 dihydro 1:3:5-triazine) were tested against blood-induced *P. knowlesi* (Nuri strain) infection in a total of 99 monkeys (*M. mulatta mulatta*) according to the standard procedures and technique followed at the Malaria Institute of India, and the minimal effective dose (M.E.D.) was found to be 10 and 50 mg. base, respectively, per kg. body weight of the animal.

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

XXII. Effect of oophorectomy on the course of infection in albino rats with blood-induced infection.*

BY

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(December 27, 1955.)

CULBERTSON (1941) showed a significant rise in the resistance of young female rats to artificially induced infection of *Trypanosoma lewisi* by giving them subcutaneous injections of œstrin. He concluded that the female sex-hormone influenced the resistance of young animals to parasitic infections. Ray and Bose (1954), on the other hand, observed that injections of œstrogenic hormones did not affect the growth of *P. berghei* in laboratory mice and rats. These authors further observed that the density of parasitæmia remained at a considerably lower level in the oophorectomized animals than in the intact ones. In their opinion, the difference is due to some kind of a disturbance in the metabolic pattern of the animal host resulting from the absence of ovarian hormone, which might not be favourable for parasitic growth. Zuckerman and Yeoli (1954) showed that oophorectomy had no demonstrable effect on the susceptibility of the animal host to *P. berghei*.

It appears from the above that, there is a divergence of opinion regarding the rôle played by the ovarian hormone in the host-parasite relationship in protozoal infections. The present work was carried out with the object of further studying if and to what extent the course of infection in the oophorectomized animals precisely differed from that in the intact ones.

MATERIAL AND METHODS.

The strain of *P. berghei*† maintained in white rats at the Malaria Institute of India was used. The standard dose of inoculation was 1×10^6 parasites per animal

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

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

injected through the intraperitoneal route. Details regarding the experimental inoculation and enumeration of parasites per 10,000 erythrocytes in the daily blood smears were as described by Ramakrishnan, Satya Prakash and Krishnaswami (1953). The main criterion of assessment was the difference between the daily and peak parasitæmia per 10,000 erythrocytes in the experimental and control animals.

The experiment was conducted with a total of 19 young adult, virgin female albino-rats of approximately the same age (eight months), and weight (175 gm.). The animals were divided into four groups, as follows:—

Group I	...	...	6 animals
Group II	...	...	3 animals
Group III	...	...	4 animals
Group IV	...	...	6 animals
Total	...	...	19 animals

All the nine animals of Groups I and II were subjected to bilateral oophorectomy under general anæsthesia (nembutal administered intraperitoneally 15 mg./ 20 gm. body weight). The technique of oophorectomy was as described by Ingle and Griffith (1949). About 11 to 14 days after the oophorectomy, the three animals of Group II along with the four of Group III, were splenectomised according to the technique described also by Ingle and Griffith under nembutal anæsthesia as in the case of oophorectomy. A little over 20 days after the splenectomy, all the animals of the four groups were experimentally inoculated with parasites. Splenectomy in the animals of Group III was performed in order to determine the effect, if any, of oophorectomy on the innate immunity to infection. That spleen is responsible for enhancing the innate resistance of the host against parasite infection, has been amply stressed upon by Perla and Marmorston (1935), Culbertson (1941), Rodhain (1949; 1951), Galliard (1949), Fulchiron (1952) and others, although Baldi (1952), Fabiani and Fulchiron (1954) and Matilla *et al.* (1954) have held the opinion that spleen has not got much of a rôle to play in the innate resistance of the host. According to the latter group of authors, on the other hand, this organ is an essential element in maintaining and evolving the acquired specific immunogenic response of the host.

The six animals of Group IV, which were neither oophorectomized nor splenectomized, served as control to the remaining experimental animals.

All the animals were maintained throughout on balanced diet.

RESULTS.

Since individual variations are apt to occur frequently in a biological experiment, average figures for the daily and peak parasitæmia for the individual groups of animals were considered for the purpose of interpretation of results.

TABLE I.

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

Animal groups.	Number of animal.	Average daily parasitæmia per 10,000 erythrocytes.		Average peak parasitæmia per 10,000 erythrocytes.	Day of peak.
		First week.	Second week.		
Group I.—Oophorectomised	6	541	1,777	3,111	8th
Group II.—Oophorectomised and splenectomised ...	3	590	All died.	2,525	8th
Group III.—Splenectomised ...	4	402	All died.	1,984	8th
Group IV.—Controls ...	6	914	2,559	3,165	9th

It was observed during the course of infection that Group II and III animals died in about eight to nine days, apparently from heavy parasitæmia and anæmia. Curiously enough, the onset of anæmia was seen to occur earlier in the oophorectomized and splenectomized animals than in the controls. No quantitative determination of the actual amount of anæmia present was, however, made.

It is seen from Table I that the peak parasitæmia in all the groups of animals appeared more or less on the same day. The range of the peak parasitæmia did not significantly differ from each other, as was found by the application of the statistical test of significance for small samples (Fisher, 1934; Tippet, 1945). While considering the average daily parasitæmia it is observed that in the first week of infection it was highest in Group IV and lowest in Group III, and in the second week it was higher in Group IV by 782 parasites per 10,000 erythrocytes than in Group I. In the first week also the parasitæmia in Group IV was higher than in Group I by 373 parasites per 10,000 erythrocytes. But the above two differences of means, *viz.*, 373 and 782, did not prove to be significant by the application of "*t*" test. The difference between the mean average parasitæmia of Groups III and IV (512) was also found to be statistically insignificant.

DISCUSSION.

Within the limitations of the investigation it would appear from the results of the experiment that, unlike the male sex-hormone (Chakrabarti, 1955), absence of the female sex-hormones does not cause any modification or alteration of the course of *P. berghei* infection in rats. It is further evident from the fact that, since neither the peak nor the average daily parasitæmia in the Groups II and III animals differed significantly, the female sex-hormones do not perhaps influence the innate resistance of the animal host. The present findings are in agreement with those of Zuckerman and Yeoli (1954) referred to previously.

After the parasitæmia in the eleven surviving animals of Groups I and IV had remained latent for about 12 to 14 days, splenectomy was performed in all of them with a view to study the effect of the previous oophorectomy on the acquired specific immunity of the host because the spleen is indispensible for such immunity (Galliard and Lapierre, 1950; Ramakrishnan, Satya Prakash and Krishnaswami, 1951; Fabiani and Fulchiron, 1954). Unfortunately, 6 out of the 11 animals succumbed very early to the operational shock in some and to heavy parasitæmia in others. In the surviving five animals belonging to both the above groups, of course, no significant difference in either the peak or the average post-splenectomy parasitæmia was observed. It would appear from the available data that the female sex-hormones perhaps have no influence upon the acquired immunity of the animal host to *P. berghei* infection.

Ramakrishnan, Satya Prakash and Krishnaswami (1951), while studying the influence of sex on the different stages of infection in *P. berghei*, held the view that female rats were able to overcome the primary parasitæmia comparatively earlier than the males. Greenberg, Nadel and Coatney (1953) also supported the above observation by saying that, apart from the genetic constitution of the host, the female mice of the same strain survived the infection longer than the males. Bennison and Coatney (1948), on the contrary, observed a significantly greater susceptibility of female chicks than the males to infection with *P. gallinaceum*. Apparently from the observations of the above authors it is not quite indicative that the female sex-hormone, as such, has anything to do with the modification of the course of infection in that sex. It is possible, however, that the existence of some distinctive metabolic pattern, perhaps regulated by the influence of other endocrines like thyroid, hypophysis and adrenals, in the particular sex of animals, can be responsible for bringing in a change in the parasitic metabolism.

SUMMARY.

Bilateral oophorectomy in virgin young adult albino rats did not show any significant difference in the course of infection with *P. berghei*, either in peak or in average daily parasitæmia when compared with intact controls. Neither initial splenectomy prior to inoculation nor splenectomy during parasitic latency appeared to have altered or modified the course of infection in oophorectomized animals.

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STUDIES ON THE BIONOMICS OF *ANOPHELES FLUVIATILIS* IN MYSORE STATE, INDIA.*

II. Bionomics in Western Hill-tracts, Mysore State.

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[October 21, 1955.]

INTRODUCTION.

In view of the varied observations recorded in different parts of the country on *A. fluviatilis*, the available literature on the bionomics of this species was reviewed by Brookeworth and Sitaraman (1952). As a result of this review, these authors came to the conclusion that *Anopheles fluviatilis* was not a homogeneous species in different parts of India. They visualized two possible forms of *A. fluviatilis:* (1) an anthropophilic form, mainly resting outdoors, submontane in habitat, efficient vector and of a low density; and (2) a zoophilic form, resting mainly indoors, plains inhabiting, poor vector and high in density. They concluded that these characteristics were very significant in any discussion of geographic races of this species.

In the present paper are given some observations made on the bionomics of this species, extending over a period of three years, in the Western Hill-tracts of Hassan District, Mysore State, India.

AREA OF STUDIES.

The areas where these studies were conducted display remarkable variations in rainfall on an unusually sharp gradient. The western portions of Mysore State—the so-called 'Malnad'—receive heavy south-west monsoon precipitation, in

*The studies on which this paper is based were conducted by the Bureau of Malariology, Department of Public Health, Government of Mysore, with the support and under the auspices of the Division of Medicine and Public Health of the Rockefeller Foundation.

some places approaching 230 inches annually. These are characterized by steep hills covered with thick forests and deep, narrow valleys where paddy can be grown. To the east of these parts, there is a fairly abrupt transition to relatively dry terrain with 40 inches or less of rainfall per year. As one proceeds from west to east, rainfall decreases at the average rate of ten inches per mile. Forests are thinner, valleys are shallow and wide, and paddy is more extensively cultivated. This disparity in meteorologic, vegetative and geographic conditions results in a diversity of mosquito-breeding places.

For purposes of studying the epidemiology of 'malnad' malaria in typical situations where no insecticides had been in use, three groups of villages were selected in the low, intermediate and high rainfall areas. The factors which governed the selection of these villages were high spleen rates and abundance of suitable breeding places of the alleged vector species—*A. fluviatilis*. The average annual rainfall in the three areas was 48·23, 83·19 and 223·0 inches, respectively.

A rapid malariometric survey indicated that the area as a whole varied from low to high endemicity for malaria. The parasitæmia was very low, though it exhibited all the three species of malaria parasites. Table I depicts the three groups of selected villages according to the intensity of rainfall, physiography and habitats of *A. fluviatilis* larvæ.

TABLE I.

Rainfall, physiography and habitats of A. fluviatilis *larvæ in three selected areas.*

Selected villages.	Annual rainfall (inches)	Physiography.	Habitats of *fluviatilis* larvæ.
Bikkodu, Intitolalu Moolehole.	40–50	Gently rolling plain, supporting extensive *scrub* jungle. Large areas under coffee and paddy cultivation. South-west monsoon main source of irrigation, though tank-irrigation is also carried out to a limited extent.	1. Streams with slow current, marginal vegetation, grassy banks, partial shade and sunlight, clear water. 2. Drainage channels along margins of paddy fields. 3. See page streams with slow current.
Magalu Holageri, Kerodi, Sakłaspur Town and nearby villages.	80–100	Broad valleys. Extensive areas under coffee, cardamom and pepper and some paddy cultivated. South-west monsoon main source of irrigation.	1. Perennial streams with grassy edges. 2. Drainage channels along margins of paddy fields. 3. River banks with emergent vegetation. 4. Large tanks with gentle wave action.
Bhattaballi, Godamane, Kurovu.	Above 200	Steep rugged valleys and dense forests. Sparsely populated. Cardamom chief crop; a small amount of tea; paddy cultivation in the narrow valleys. South-west monsoon main source of irrigation.	1. Perennial streams with slow current and marginal vegetation; if current rapid, islands with overhanging trees and dense shade sometimes. Turbid water. 2. Drainage channels along margins of paddy fields, with grassy edges.

Saklaspur Town and nearby villages, corresponding to the intermediate rainfall area cited above, were, however, being sprayed with residual insecticides since December 1948 and thus served as an experimental area for these three groups of unsprayed villages.

MATERIALS AND METHODS.

Standard entomological methods were employed for anopheline surveys. Daytime collections of larvæ and adults were made between 09.30 and 12.30 hours. Night-time hand and window-trap collections were conducted between 18.00 and 06.00 hours to study the nocturnal activities of the anopheline species in the three areas. Outdoor collections were carried out for a period of five months in the low rainfall area near prolific breeding places of *A. fluviatilis*. Hand and window-trap collections were carried out at four-hour intervals on a twenty-four-hour basis in two villages near Saklaspur Town for a period of five months to study the anopheline composition during the different quarters of the day and the night. Adult captures were expressed as per man-hour or per trap-hour figures as the case may be, while larval collections were reduced to average number of larvæ per ten minutes of actual dipping to emphasize intensity.

LARVAL HABITATS.

Puri (1949) lists the larval habitats of *A. fluviatilis* as "clear water pools along edges of running streams and irrigation channels". He further states that the same larval habitats are occupied by *A. minimus*, although the latter breeds in ricefields also, thus implying that *A. fluviatilis* does not breed in ricefields. Christophers (1933) also fails to mention ricefields as a larval habitat of this species. Viswanathan (1946) lists the optimal breeding places of this species in Bombay Presidency as streams, channels, shallow earthen wells, sugarcane and betelnut garden trenches, uncultivable swampy fields and ricefields in all stages of paddy culture.

There has been ample confirmation of the stream-breeding habits of this species during these studies, but exceptions were also recorded from more than one standpoint (Table II). Before and soon after the onset of the south-west monsoon when this species had reached its peak of abundance, larvæ could be found in numerous habitats in addition to the usual breeding places cited by Puri (1949). They were taken frequently in warm—almost stagnant—side-pockets of streams, sometimes with extremely muddy water and no appreciable current. Though marginal vegetation seemed to be usually required for their shelter, at times a mass of half-submerged rootlets accomplished the same purpose. In the intermediate rainfall area, larvæ were collected in large numbers from a shallow tank with grassy edges and with a gentle wave action. A small percentage (0·4 per cent) was collected from swamps and paddy fields.

According to Covell and Harbhagwan (1939) this species resorts to shallow wells during the monsoon when the usual breeding places are flushed by heavy rains. But intensive searches to locate the alternative breeding places during the rainy season in the high and intermediate rainfall areas were fruitless. The reasons

for the virtual disappearance of the larvæ from their breeding places soon after the beginning of the monsoon, and their sudden reappearance after the cessation of rains still remain a mystery. But in the low rainfall area, where flooding is less extensive, *fluviatilis* larvæ were encountered right through the monsoon, although in depleted numbers (Table III).

Of the 9,146 *fluviatilis* larvæ collected during these studies, 7,826 (86·5 per cent) were collected from running water, while the remainder (13·5 per cent) were found either in water with no appreciable current or in stagnant water. Table II lists the numbers of *fluviatilis* larvæ collected from different habitats in the three areas under study. The seasonal prevalence of the species is depicted in Table III.

It was frequently observed during these collections that habitat of *varuna* was not "wells and clear water pools" as given by Puri (1949), but the identical habitat occupied by *fluviatilis*, i.e., various streams with abundant marginal vegetation. *Varuna* larvæ almost always exceeded *fluviatilis* larvæ in number at these sites and it was actually possible to use *varuna* as a guide to the discovery of *fluviatilis* on many occasions by making a concentrated search for *fluviatilis* where *varuna* had already been found.

TABLE II.

Optimal breeding places of A. fluviatilis *in the three rainfall areas under study.*

Habitats.	Numbers.	Percentage.
Streams... ...	... 7,826	86·5
Tanks	... 618	6·2
Channels ...	... 425	4·3
River bed ...	... 197	2·0
Tank outflow seepage	... 34	0·3
Swamps... ...	... 25	0·2
Paddy fields ...	... 19	0·2
Ponds	... 2	00·2

TABLE III.

Seasonal prevalence of A. fluviatilis *larvæ in the three rainfall areas under study.*
(Saklaspur Town and nearby villages excluded.)

Months.	Low rainfall area (Bikkodu):		Intermediate rainfall area (Yeslur):		High rainfall area (Kadmane):	
	Number collected.	Averageper 10 minutes.	Number collected.	Averageper 10 minutes.	Number collected.	Average per 10 minutes.
January ...	150	1·0	26	0·2	40	0·3
February ...	157	1·1	89	0·9	56	0·4
March	345	2·5	170	1·6	187	1·2
April	939	7·6	220	3·7	592	6·0
May	338	3·3	138	2·2	364	4·7
June	216	2·2	169	2·9	171	2·1
July	143	1·5	18	0·2	7	0·1
August	30	0·3	0	0·0	0	0·0
September ...	16	0·1	1	...	0	0·0
October ...	2	...	0	0·0	0	0·0
November ...	17	0·1	0	0·0	1	...
December ...	24	0·2	0	...	8	...

ADULT HABITS.

(*a*) *Daytime resting places.*—Observations on the daytime indoor-resting places of *A. fluviatilis* by Jaswant Singh and Jacob (1944) in North Kanara; Measham and Chaudhury (1934) in the Annamalai Hills; and Viswanathan (1950) in Bombay State indicate that the fraction of the *fluviatilis* population electing to remain indoors during the day is almost exclusively in human dwellings, with only about five per cent occupying cattlesheds. Russell and Jacob (1942) record that this species is often found on lower portions of walls, not more than five feet from the floor.

The data gathered during the present studies, on the other hand, exhibit marked deviations from the foregoing observations. In all the three rainfall areas the bulk of the collections came from cattleshed, mixed dwellings yielding the next highest catches, while in human dwellings were recorded the lowest numbers. Also, they were captured more often hanging down from the attic rather than resting on the walls below five feet from the floor. The numbers of *A. fluviatilis* collected in the three types of dwellings are summarised in Table IV.

TABLE IV.

Daytime resting places of A. fluviatilis *in three types of dwellings.*

Type of dwelling.	Low rainfall area (Bikkodu):		Intermediate rainfall area (Yeslur):		High rainfall area (Kadmane):	
	Number collected.	Percentage.	Number collected.	Percentage.	Number collected.	Percentage.
Human dwellings ...	13	6·0	0	0·0	8	10·7
Mixed dwellings ...	28	13·0	20	41·7	21	28·0
Cattlesheds ...	174	81·0	28	58·3	45	61·3
Total ...	215		48		74	

Senior White (1941:1946), Viswanathan and Ramachandra Rao (1943), Viswanathan *et al.* (1944) and Issaris *et al.* (1953) observed a significant fraction of *fluviatilis* population resting outdoors during the daytime in Satpura Ranges and Singhbhum Hills, North Kanara (Bombay) and U.P. Tarai, respectively. They found that the outdoor resting places were mainly on banks of streams where mosquitoes could find shelter under overhanging vegetation, projecting stones, culverts, etc.

With a view to discover favourite and characteristic outdoor resting places of this species, intensive searches were carried out in the low rainfall area near a stream where large numbers of *fluviatilis* larvæ were being regularly collected. In vigorous searches extending over five months along the banks of the stream, under culverts and in clefts along the bank and paddy fields, not a single *fluviatilis* was collected, though numbers of *aconitus, annularis, barbirostris, culicifacies, jeyporiensis,*

pallidus, *subpictus*, *vagus* and culicines could be collected. During the same period, 46 *fluviatilis* were taken in the routine catching stations in the area. From this, one is led to infer that in this area outdoor resting *A. fluviatilis* does not form a large part of the vector population. This was further corroborated by a window-trapping programme extending over nine months, which yielded only one *A. fluviatilis* in the traps, although significant numbers could be collected resting inside the same dwellings.

(*b*) *Biting time.*—Conflicting records exist regarding nocturnal activity of *A. fluviatilis*. Nursing *et al.* (1934) recorded in Mysore State that as many *fluviatilis* entered houses during the first half of the night as in the second. Since, however, abdominal conditions of these mosquitoes were not recorded, it is not possible to say whether this represents biting activity or not. Viswanathan and Ramachandra Rao (1943) in North Kanara (Bombay) found that females of *A. fluviatilis* entered a human shelter shortly after dusk and the majority completed feeding before midnight. They observed that 71·0 per cent entered houses for feeding during the first quarter of the night, 19·0 per cent in the second, 7·0 per cent in the third and 3·0 per cent in the last quarter. Observations of Jaswant Singh and Mohan (1951) in the Nilgiris indicated that feeding took place in all four quarters of the night, most of this activity being confined to the second and third quarters, indicating a difference from the early feeding mosquitoes in Bombay State.

In studies on the anopheline composition of two villages near Saklaspur Town during the different quarters of the day and night, all mosquitoes collected were identified on the spot and their abdominal conditions recorded. In the first collection (18·00 to 18·30 hours) no fresh-fed specimens were collected ; in the second collection (22·00 to 22·30 hours) sixty specimens with fresh blood and thirteen with partially digested blood were taken, while in the third collection between 02·00 and 02·30 hours eight specimens with fresh blood and twenty-two with partially digested blood were recorded, and in the fourth collection between 06·00 and 06·30 hours four had fresh blood and nineteen had partially digested blood. From these observations it was evident that the peak feeding interval of *A. fluviatilis* in this area was between 21·00 and 22·30 hours (81·0 per cent) though some feeding took place between 02·00 and 06·00 hours also.

In all the three rainfall areas smaller numbers of *A. fluviatilis* were collected during night collections as compared to daytime collections in the same catching stations. As in the daytime collections, more *A. fluviatilis* adults were collected in cattlesheds than in human dwellings in all the three areas.

(*c*) *Anthropophilism and zoophilism.*—Jaswant Singh and Jacob (1944) observed that in North Kanara 64·0 per cent of captured *A. fluviatilis* had fed on human blood. These authors stated further that in the foot-hills of the United Provinces the anthropophilic index was less than five per cent. In the Singhbhum Hills, Senior White (1947) found an anthropophilic index of 56·8 per cent. Viswanathan (1946) quotes further work by Jaswant Singh at Poona where 99·0 per cent of 109 *A. fluviatilis* contained blood of bovine origin. These reports indicate that anthropophilism is characteristic of *A. fluviatilis* in hill regions where vectorial capacity is high and population density is low, while zoophilism characterises plain regions where vectorship is poor and densities are high.

Our observations reveal that, contrary to the notion of a high anthropophilic index in the foot-hill regions of Peninsular India, in all the three rainfall areas under study, the anthropophilic index of *A. fluviatilis* is very low, only 22 out of 656 specimens being positive for human blood. Of these 22, 15 were from specimens found resting inside cattlesheds. This finding was of some significance, as only human and mixed dwellings were so far included in spraying programmes. The fact that a significant percentage of *fluviatilis* found resting in cattlesheds had previously fed on human beings, indicated the necessity to spray detached cattlesheds also. From these data it appears as though *A. fluviatilis* in this area is a mixture of anthropophilic and zoophilic races, the former forming a very low proportion of the population.

(*d*) *Flight range.*—There are no records of observations on the flight range of *A. fluviatilis* from their natural larval habitats. The studies of Adisubramaniam and Vedamanikkam (1943) in Wynaad indicated that *A. fluviatilis* had a short range of flight—not more than a thousand feet from human dwellings. Venkat Rao and Philip (1947) in the Hazaribagh ranges and Jeypore Hills found significant numbers breeding up to at least half a mile from human habitations and presumed this to be the usual flight range of *A. fluviatilis* in these areas.

During the present studies it was found that there was an inverse relationship between the numbers of *A. fluviatilis* larvæ collected and the distance of nearest human habitations. Maximum numbers were collected when the distance was up to two furlongs, and, when it was six furlongs, there was some breeding but not in an appreciable manner.

(*e*) *Seasonal prevalence.*—The annual peak of the adult populations in the area as a whole is in the early part of the year up to April (Table V). The most striking feature of the seasonal cycle of this species in all its stages in the high and intermediate rainfall areas is its virtual disappearance following the onset of the south-west monsoon and its sudden reappearance in December, after the rains have stopped.

(*f*) *Colonisation in the laboratory.*—All attempts to rear *A. fluviatilis* in the laboratory in cages of $3 \times 3 \times 3$ feet were unsuccessful. Larvæ were collected in large numbers from the three areas under study and were reared on yeast. Water in the larval pans was changed daily and relative humidity in the insectary was maintained at about 70·0 per cent. Larval mortality was less than 4·0 per cent, pupæ formed being transferred to emergence cages. Adults, after emergence, were fed on soaked resins and glucose solution for twenty-four hours before being offered a blood meal (both human and rabbit). Mortality among adults was very high, 30·0 per cent dying off within four or five days. Though the females took a blood meal, this was not accompanied by a corresponding ovarian development. Dissections of these specimens indicated that they had not mated. Illumination of the cages by a blue light (Mohan, 1945) did not induce swarming and mating.

(*g*) *Critical densities.*—Wherever this species is an important vector, it has exhibited a strikingly high infection rate but low prevalence. Viswanathan (1946) is of the opinion that four anthropophilic *A. fluviatilis* per ten man-hours is the threshold density for effective malaria transmission in the area.

TABLE V.

Seasonal prevalence of A. fluviatilis *in the three rainfall areas under study.*

(Daytime collections.)

Months.	Low rainfall area, Bikkodu		Intermediate rainfall area, Yeslur		High rainfall area, Kadmane	
	Actuals.	Per 10 man-hours.	Actuals.	Per 10 man-hours.	Actuals.	Per 10 man-hours.
January ...	26	5·0	9	3·0	13	4·0
February ...	28	7·0	9	3·0	17	5·0
March	24	5·0	7	2·0	23	6·0
April	20	5·0	14	5·0	19	6·0
May	9	3·0	2	0·1	0	0·0
June	20	7·0	4	2·0	0	0·0
July	25	6·0	0	0·0	0	0·0
August	13	3·0	0	0·0	0	0·0
September ...	4	1·0	0	0·0	0	0·0
October ...	0	0·0	0	0·0	0	0·0
November ...	8	2·0	0	0·0	0	0·0
December ...	38	8·0	3	0·1	2	0·6

Though no naturally infected specimens were found in the course of these studies, in view of the high zoophilic predilection of the species in the three areas, it is safe to infer that the critical density must be considerably more than four per ten man-hours.

DISCUSSION AND SUMMARY.

The various reports about the bionomics of *A. fluviatilis* in different regions indicate that the differential behaviour of this species must be a reflection of physical attributes of the environment in which it finds itself. It is well known that, when populations within a species have become geographically isolated, adaptation to local ecological conditions, accompanied by natural selection, must progress constantly, affecting not only structures but also functions and behaviour.

Though no constant morphological variation has, as yet, been detected either in adults or larvæ, nevertheless the hypothesis has been advanced about the possibility of biological races of *A. fluviatilis*, judging from different vectorial capacities and host predilections in the various parts of its range of distribution.

Viswanathan (1946) has postulated that vectorial capacity varies inversely with the abundance of the species. Another correlation is that vectorial capacity is high in foot-hill regions but low in the Deccan Plateau. It has been asserted that, in hilly regions of Peninsular India, this species is an extremely efficient vector and it may be significant in the Deccan if abundant. But it has been shown repeatedly that it need not be, wherever it occurs, the efficient vector it is in the Western Ghats. Christophers (1933), however, states that it is an important carrier wherever found.

The various reports about the feeding habits of this species have indicated that hilly regions are inhabited by anthropophilic *A. fluviatilis* with a high vectorial capacity and low density, while plain regions are characterized by zoophilic members with poor vectorial capacity and high densities. The present studies have revealed that this cannot be taken as a generalization, as it has been shown that zoophilic populations may form a considerable proportion even in hilly regions.

It might be argued that extensive residual spraying programmes have brought about an alteration in the environment of the mosquitoes and that the species may be responding to this altered ecological condition in a way that has led, through adaptation and natural selection, to the development of house-avoiding strains, more or less completely adjusted to an environment where houses are routinely sprayed with residual insecticides. While this may be true in the case of whole areas where intensive spraying is carried out (for example, Saklaspur Town and nearby villages), the same behaviour prevailing in entirely unsprayed areas also indicates that given regions, with the same stimulating forces, must be populated by genetically pure strains of mosquitoes. It adds further evidence to the fact that this species does not exclusively utilize human dwellings as daytime resting places in all hilly regions.

The disparity in the various reports about the behaviour of this species in the different regions in which it has received attention tends only to emphasize that it exists as biological variants despite morphological homogeneity. The present studies may well lend further support to such a contention. There may be populations of given species of anophelines in South India, living within a few miles of one another but so far removed by ecological barriers as to show different adaptations.

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ADDITIONS TO THE RECORDS OF THE ANOPHELINE FAUNA OF MANDYA DISTRICT, MYSORE STATE, SOUTH INDIA.

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[January 5, 1956.]

Rao and Nassiruddin (1945) while discussing malaria problem in the Irwin Canal area, now called Visweswariyya Canal area, reported the following fourteen species of anophelines as a result of intensive collections of adults and larvæ made between 1935 and 1939, in ten selected villages round about Mandya:—*aconitus*, *annularis*, *culicifacies*, *fluviatilis*, *hyrcanus*, *jamesi*, *jeyporiensis*, *pallidus*, *stephensi*, *subpictus*, *tessellatus*, *turkhudi*, *vagus* and *varuna*.

From 1939 up until 1951, though there was no continuous collection of mosquitoes and larvæ, yet collections were made in connection with certain special studies undertaken during that period. These collections revealed the presence of two additional species, viz., *A. barbirostris* and *A. splendidus* in the area.

Consequent to the use of residual insecticides for malaria control in the area, systematic collections of adult mosquitoes and larvæ were organized at weekly, fortnightly and monthly intervals in selected villages of the area. During these collections, over a period of four years (July 1951 to June 1955) specimens of *A. karwari* and *A. philippinensis* were also encountered in the area.

It is the purpose of this note to bring the anopheline records of Mandya District, Mysore State, up to date.

During the period, July 1951 to June 1955, a total of 39,286 anopheline adults belonging to eighteen species, and 52,949 larvæ of only fifteen species were

collected. Of these, the number of adults and larvæ of *A. barbirostris*, *A. karwari*, *A. philippinensis* and *A. splendidus* were as follows:—

Serial number.	Name of species.	Number of adults.	Number of larvæ.
1.	*A. barbirostris*	82	941
2.	*A. karwari*	2	0
3.	*A. philippinensis*	14	0
4.	*A. splendidus*	65	1,320

Iyengar (1926) examined extensive seepages under the bund of Sankey's tank in Malleswaram area, Bangalore City and reported *A. barbirostris*, *A. karwari*, and *A. splendidus* along with six other species of anophelines. Sweet and Rao (1934) added *A. philippinensis* and six other species to the anopheline fauna of Bangalore City, though they did not encounter *A. karwari* and *A. tessellatus*, which were recorded by Iyengar (1926). *A barbirostris*, *A. philippinensis* and *A. splendidus* were reported from Nagenahalli, Hiriyur and Mudigere areas, and *A. karwari* from Mudigere area only by Sweet (1933). Rao *et al.* (1952) recorded all these four species from Shimoga and Hassan districts.

A. barbirostris.—The adult specimens of this species were collected throughout the year excepting April and May. The larvæ were collected in all the months of the year from pools, channels, paddy fields and tanks.

A. karwari.—No larvæ of this species were recorded, but only two adult specimens were collected as late as the second fortnight of October 1954 from two villages lying close to the Bangalore-Mysore railway line.

A. philippinensis.—No larvæ of this species have yet been collected though, the adult specimens were taken from three villages situated near unbreached tanks during the months of February, April, May, July and November.

A. splendidus.—Larvæ of this species were found to be breeding in paddy fields, pools, and channels during the twelve months of the year. Except in June, in the other eleven months of the year, adult specimens were collected.

SUMMARY.

By adding *A. barbirostris*, *A. karwari*, *A. philippinensis* and *A. splendidus* to recorded list of anophelines from Visweswariyya Canal area, the list is brought up to date.

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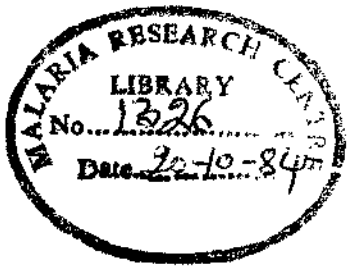

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A PRELIMINARY RECORD OF THE MEGARHINE AND CULICINE MOSQUITOES OF NEPAL WITH NOTES ON THEIR TAXONOMY (DIPTERA: CULICIDÆ).

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[January 10, 1956.]

INTRODUCTION.

No records have been published previously of the megarhine and culicine mosquitoes of Nepal. Raghavan (1953) mentions that "Kathmandu and parts of the main (Rapti) valley have numerous breeding places favourable for the vector (of *Bancroftian filariasis*) *C. fatigans*". Barraud (1934) quotes numerous records of species occurring in the Himalayan region to the east and west of Nepal, many of which may be discovered ultimately in this country.

Most of the material on which this paper is based was collected in the area of the Rapti River valley at an altitude of about 2,000 feet during the course of an entomological survey made by the staff of the World Health Organization—United States Operations Mission to Nepal—Nepal Government Malaria Control Project. A general account of this jungle foothills area, together with a sketch-map, are given in an earlier paper (Peters *et al.*, 1955) which recorded data on the anopheline fauna. Most of the specimens recorded here were collected in the vicinity of Hetaura (approximately 27°26′ N., 85°2′ E.). A few were collected near Bhimphedi which is situated at the source of the Rapti River about 14 miles north-east of Hetaura. Two species were collected in Kathmandu itself. All the collections were made in the dry season of 1954-1955, those in Hetaura between January and May. Tree-hole breeding species were obtained by adding water to the dry residue in tree-holes and removing the larvæ when they reached the 4th instar. In the dry season, water is scarce in this area and is more or less localized to residual pools in the river beds, and small swamps and seepage pools in the

jungle. These situations come to form foci of survival for numerous mosquito species so that collections in such sites, especially in the shelter of the jungle, yield, as it were, a concentrated sample of most of the locally occurring species, especially, of course, ground-pool breeders.

LIST OF SPECIES.

The following 23 species were collected:—

MEGARHININI.

Megarhinus splendens Wiedemann, 1819.

CULICINI.

Uranotænia campestris Leicester, 1908.
U. annandalei Barraud, 1926.
Ædes (*Finlaya*) *albolateralis* Theobald, 1908.
A. (*Christophersiomyia*) *annulirostris* Theobald, 1905.
A. (*Stegomyia*) *w-albus* Theobald, 1905.
A. (*Stegomyia*) *albopictus* Skuse, 1894.
Heizmannia indica Theobald, 1905.
Culex (*Lutzia*) *vorax* Edwards, 1921.
C. (*Mochthogenes*) *castrensis* var. *folialus* Brug, 1932.
C. (*Mochthogenes*) *malayi* Liecester, 1908.
C. (*Lophoceraomyia*) *plantaginis* Barraud, 1924.
C. (*Lophoceraomyia*) *infantulus* Edwards, 1922.
C. (*Culiciomyia*) *pallidothorax* Theobald, 1905.
C. (*Culex*) *bitæniorhynchus* Giles, 1901.
C. (*Culex*) *vishnui* Theobald, 1901 (*sensu lata*).
C. (*Culex*) *whitei* Barraud, 1923.
C. (*Culex*) *barraudi* Edwards, 1922.
C. (*Culex*) *gelidus* Theobald, 1901.
C. (*Culex*) *mimeticus* Noé, 1899.
C. (*Culex*) *mimulus* Edwards, 1915.
C. (*Culex*) *fatigans* Wiedemann, 1828.
C. (*Culex*) *fuscocephalus* Theobald, 1907.

FIELD NOTES.

All collections were made in the vicinity of Hetaura unless otherwise stated.

Megarhinus splendens, *Ædes albolateralis*, *A. annulirostris*, *A. w-albus*, *A. albopictus*.—These species were all reared from eggs contained in the dried residue in holes in mango trees. These eggs had survived at least seven months since the last rainy season and hatched within a day or two of the addition of water. *M. splendens* and *A. albolateralis* were together in one hole while the other three species were found in mixed batches from two other holes. *Uranotænia campestris* and *annandelei* imagines were captured at rest near two small shallow jungle seepage pools surrounded by tree roots in one case and large stones in the other.

Larvæ and pupæ of *campestris* and a pupa probably of *annandalei* were found in these pools. A single female of *Heizmannia indica* was found under a rock by one of the same jungle pools.

Larvæ of *C. vorax*, *C. plantaginis* (probably), *C. infantulus*, *C. pallidothorax*, *C. whitei*, *C. barraudi*, *C. mimulus* and *C. fatigans* were also found in various shaded jungle pools. In addition, imagines of *C. casterensis* var. *foliatus*, *C. plantaginis* and *C. infantulus* were found at rest near these pools. One pupa was found on dissection and mounting to contain an unemerged male imago of *C. malayi*.

C. mimeticus, *C. bitæniorhynchus*, *C. vishnui* (*sensu lata*) and *C. fatigans* were collected from residual pools in the main river beds, the first two from as far north as Bhimphedi (3,800 feet). *C. bitæniorhynchus* was also common in backwaters of the Bagmati River in Kathmandu where there was an abundance of green filamentous algæ. *C. fatigans* was also very common in Kathmandu where it was a serious domestic pest. It was found in vast numbers in exposed ground pools, drainage pits and domestic grease traps.

C. fuscocephalus larvæ together with those of *C. bitæniorhynchus* were found in irrigation ditches and patches of swampy ground containing shallow pools. *C. mimeticus* was occasionally found in these situations but was commonest towards Bhimphedi where *C. mimulus* was never found. Adults of *C. vishnui* (*sensu lata*), *C. gelidus*, *C. bitæniorhynchus* and *C. fatigans* were found in native dwellings and cattlesheds and in the tents of the team's compound. The two latter species were common house visitors in Kathmandu.

TAXONOMIC NOTES.

(By W. Peters).

1. (*CHRISTOPHERSIOMYIA*) *ANNULIROSTRIS*.

Larva.—The larva of this species was not described by Barraud although he stated that it was similar to that of *A.* (*C.*) *thomsoni* which is figured in his book. It is distinguished from the latter species by the lighter colour, the single lateral saddle hair and the longer pecten spines. The following descriptions of larvæ employ the terminology of Belkin (1950), the old terminology following in brackets.

Length: About 6 mm. *Colour:* Body whitish, head and siphon pale brown, very lightly chitinised. *Head:* About as long as broad; antennæ a little less than the length of the head with minute spicules from base to near apex; seta 1 (tuft) of two to three branches just before halfway, the longest branch reaching the apex of the antenna; seta 1 of head capsule (clypeal spine) bristle-like; setæ 7, 6 and 5 (A, B and C) with 6-8, 2 and about 9 plumose branches, respectively, situated as in *thomsoni*, 7 and 5 short and slender, 6 long and strong; 8 and 9 (*d* and *e*) short with 1 very plumose and 3 slender branches respectively; mentum as in *thomsoni* with 11 teeth on each side of a larger central tooth. *Thorax:* Metathoracic pleural hairs (9-12 of Belkin) similar to those of *thomsoni*. *Abdomen:* Comb with a single row of about 14 strongly pointed teeth fringed at the base; siphon with index of about $2\frac{1}{2}$ in crushed specimens, widest in the middle; acus absent; pecten (Fig. 1*a-d*) of 7-10 small spines each with one long dorsal, one smaller ventral and one or more

minute basal denticles, all teeth approximately the same size; seta 1 (tuft) of 4-7 simple branches at the middle of the siphon, its base some distance beyond the most distal pecten spine; anal segment with the saddle incomplete ventrally, smooth dorsally; seta 2 (upper caudal seta, *ucs*) with 1 long and 1 shorter simple branch; seta 3 (lower caudal seta *lcs*) single, simple and longer than 2; seta 1 (lateral seta *ls*) single, simple or slightly frayed, a little longer than the saddle; " gills " broadly spatulate, about three times as long as the saddle, the ventral pair slightly shorter than the dorsal; setæ 4 (ventral brush) with about 8 short hair tufts, the proximal 2-3 and the distal 5-branched; barred area absent.

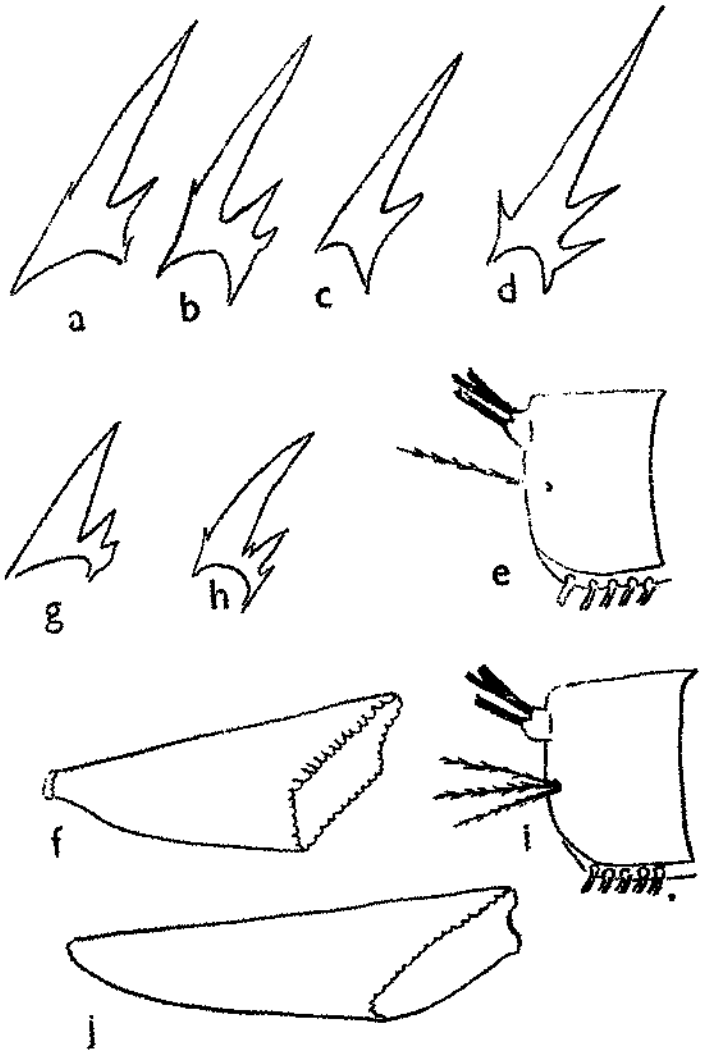
Pupa.—The pupa resembles those of the subgenus *Stegomyia* but has 2 long single or double hairs on the cephalothorax and a paddle quite rounded at the apex, slightly spiculate and with a single (occasionally double) long terminal seta. The descriptions of pupæ in this paper follow the nomenclature of Knight and Chamberlain (1948) as modified by Belkin (1952; 1954).

Cephalothorax: Trumpets (Fig. 1*f*) short and broad resembling those of *Stegomyia*, slightly darker than the general integument; no conspicuous pigment pattern; seta 1 long and single or with 2 strong branches from the base; 2, 3, 5 and 6 short with 3, 1 forked, 1 and 1 slender branches, respectively; 4 fairly long, single; 7 very long, single, simple; 8 and 9 moderately long, slender, double from the base; 10 minute with 3-4 delicate branches; 11 moderately long, single; 12 short with 5 or 6 branches from the middle. *Abdomen:* Segment I—1 plumose tuft hair with about 8 main branches; 2 short, single; 3 and 6 long, simple, single; 5 and 4 very small, single and 3-branched, respectively; 7 very small, single; 10 moderately long, double from the base. Segment II—1 short, finely dendritic; 2 short, single but longer than on I, base lateral to 1; 3 and 6 moderately long, simple, single, 6 longer than 3; 5 and 4 very small, single and double respectively; 7 a single microseta placed laterally; 10 short, single or double from the base. Segments III-VIII—0 a microseta on III-VIII; 1 short, slender, 3 branches on III, single or double on IV-VII; 2 a microseta medial to 1 on III-VII; 3 very short, double, base posterior to 4 on III, medial to 4 on IV, lateral to 4 on V, single, medial to 5 on VI and VII; 5 moderately long, single, simple on III and IV, very long on V and VI, rather short and slender on VII and VIII, may be double on IV; 4 small, single on III and IV, double on V, slightly longer and 3-6 branched on VI and VII; 6 smaller than on II, single, slender on III-VI, double and slightly plumose on VII; 7 a laterally placed single microseta on III-VI, very short, spinose, its base ventral and well anterior to 6 on VI, strong and plumose with 3 branches on VII; 8 very small, 2-4 branched on III and IV, single, longer on V-VII; 10 very short, forked on III and IV, single, longer on V-VII; 12 short, single on IV-VII, slightly longer on VI; 11 minute, single on III-VII; 14 a microseta on III-VIII. Segment IX—Paddles with strong central spine, laterally with slightly spiculate buttress to 3/5, ending evenly rounded and slightly spiculate; seta 1 (terminal seta) single, strong, simple, a little more than 1/2 the length of the paddle and sometimes with a second delicate branch from the base.

A. (CHRISTOPHERSIOMYIA) THOMSONI.

Pupa.—This species was not found in Nepal but as it is very similar to the preceding species and the pupa has not been described, a note has been introduced

FIGURE 1.



a.-f. *Aëdes* (*Christophersiomyia*) *annulirostris* (Hetaura, Nepal).
g.-j. *A.* (*C.*) *thomsoni* (Patiala, India).
Larvæ.—*a,g.* Proximal pecten spines.
b,c,d,h. Distal pecten spines (*d.* atypical).
e,i. Lateral saddle seta.
Pupa.—*f, j.* Trumpet.

here for comparison. It is based on the examination of a specimen in the B.M. collection.

The entire pupa of *thomsoni* is darker, especially the respiratory trumpets which are relatively longer and narrower than those of *annulirostris* (Fig. 1*j*). On the cephalothorax, seta 1 is very long and double or treble. Seta 8 is well developed but rather short with 5 or 6 branches arising from the base. On the abdomen, seta 1 on segment IV has 4 well developed branches in *thomsoni* but is single or double in *annulirostris*.

URANOTÆNIA CAMPESTRIS.

Larva.—The larva of this species was originally described and figured by Senior White (1927). Specimens from Hetaura differ from his description as follows:—

Head: Seta 7(*A*) with 4 slender branches, 6 and 5 (*B* and *C*) stout bristles with finely barbed edges, 4(*d*) with 2 short, very slender branches, 8(*e*) fairly long, single and slender. The base of 6 lies a little posterior to the line joining the bases of 7. *Abdomen:* Comb plate present (not shown in Senior-White's figure) and bearing 8 teeth fringed at the sides and terminating in a single sharp point. Pecten of 9 scale-like ovate teeth fringed all round; seta 1 of siphon (lateral tuft) of 12-14 simple branches; seta 1 of anal segment (*ls*) with 5 branches; edge of saddle with a fringe of well developed spines; anal papillæ twice as long as the saddle.

This larva is very similar to that of *U. macfarlanei* Edw. 1914. Specimens of the latter species in the B.M. from Singapore and Java differ from *campestris* in having a pecten of about 14 teeth which are smaller than those of *campestris*.

Pupa.—The following description is based on the examination of 3 pupal pelts with associated adults from a jungle pool near Hetaura.

Colour: Pale with a distinctive pattern of dark pigmentation. *Cephalothorax:* Trumpets (Fig. 2*a*) about 4 times as long as broad in flattened specimens, basal 2/5 darker, meatus deep and extending as a slit for half the length of the trumpet; setæ 1-4 and 7-12 with short slender branches arising from a short stem, 6-8, 5-6, 3, 7-8, 4-5, 7, 3, 6-8, 6-8 and 6-8 branches, respectively; 5 with 9 moderately long slender branches; 6 fairly long and single. *Abdomen*: Segment I—1 plumose with about 10 main branches; 2 short and single; 3 with 3 short branches arising from a short stem as do most of the branched abdominal setæ; 5 and 4 very short and dendritic; 6 with 2 fairly long branches; 7 minute, single; 10 with 5 short branches. Segment II—0 a microseta; 1-4 with 8-9, 3-4, 3 and 1 moderately long branches, respectively, the base of 4 lateral and just anterior to that of 3; 5 with 3 short slender branches; 6 with 2 fairly long slender branches; 7 a microseta; 10 with 4 very slender moderately long branches. Segments III-VIII—0 a microseta placed rather laterally on III-VIII; 1 with 9 fairly long branches on III and IV, 6 on V, 5 on VI and 4 on VII; 3 with 6 short slender branches on III, its base antero-lateral to 1, medial to 4 on IV, single or double and more medial on V, more posterior with its base between 1 and 5 on VI, displaced postero-laterally and with 5 short slender branches on VII; 4 short and forked on III, dendritic on IV, with 5 branches on V and VI, longer on VII and VIII; 5 with 9 moderately long branches their base medial to the hairless setal ring on III, longer and posterior on IV-VII; 6 with

4 short slender branches on III, 3 on IV, 2 longer branches on V, 4 on VI and VII; 7 a microseta, dorsal on III-VI, longer and more posterior on VII with 2-3 slender branches and with 4 moderately long slender branches on VIII; 8 very short and dendritic on III-VI, longer with 4 branches on VII; 10 with 3 very short branches on III, longer with its base more lateral on IV, longer on V, with 2 moderately long branches on VI and VII; 12 with 2 very slender moderately long branches on III-VII, longer on VI and VII; 11 very short, single, its base medial to 12 on III-V, lateral to 12 on VI, posterior to 12 on VII; 14 a microseta present on III-VIII. Segment X—seta 0 very short, single. Segment IX—Paddles (Fig. 2*b*) with a slight lateral buttress on the proximal 1/4 serrated on lateral and medial edges; terminal seta 1 minute.

? URANOTÆNIA ANNANDALEI

Pupa. - The following description is based on the examination of a single pupal pelt with its associated imago both of which are mounted on a slide. As the imago is a female, it is not identifiable with certainty but since only this and the preceding species, *U. campestris* were located in this area and the pupa of the latter is known and distinct it is likely that the example here described is that of *annandalei*.

Colour: Pale yellow with a distinctive pattern of brown pigment. *Cephalothorax :* Trumpets (Fig. 2*d*) rather cylindrical with a small meatus, about 5 times as long as broad in flattened specimen; setæ 1, 2, 4, 5, 7, 8 and 9 with 5-6, 4, 3, 6, 3, 5 and 3 slender short branches, respectively; 3 not identifiable with certainty; 6 fairly long, single; 10 with 2 branches, 11 single, both short and subplumose; 12 with 2 slightly longer subplumose branches. *Abdomen:* Segment I— 1 a plumose tuft with about 16 main branches; 2 short, single; 3 single moderately dendritic; 5 and 4 very short with 2-3 branches; 6 moderately long, slender and forked; 7 minute, forked; 10 moderately long, slender, branched from base. Segment II—1 submedial, dendritic; 2 moderately dendritic; 3 with 6 very short slender branches; 5 single, medial to 4; 4 with 4 minute branches; 6 long, single, slender; 7 minute, single; 10 with 2-3 moderately long slender branches. Segments III-VIII—0 a microseta on III-VIII, more medial on VIII; 1 short on III-VII with 4, 4, 3, 3 and 1 branches, respectively; 2 minute and single, its base anteromedial to 1 on III-V and just anterior to 1 on VI and VII; 3 moderately long, dendritic, its base medial to 5 on III, similar but its base lateral to 5 on IV, with 2-3 branches and its base medial to 5 on V, in the same site but single on VI and VII; 5 with 3 short branches on III, 4 long branches on IV and V, with 3 long branches on VI, single and moderately long on VII; 4 with 3 short branches on III and IV, 2-3 moderately long branches on V, single on VI and VII; 6 short and slender on III and IV, double on V and VI, very short, single and posterior on VII; 7 a microseta on III-VII, with 5 short slender branches on VIII; 8 with 3 very short branches on III and IV, 2 on V and VI, 3 on VII; 10 short and single on III, longer on IV-VII; 12 moderately long with 2 branches on III, single on IV and V, longer on VI and VII; 11 minute, single, medial to 12 on III-V, lateral to 12 on VI, antero-lateral to 12 on VII; 14 a microseta on III-VIII, more lateral on VIII. Segment X—seta 0 short and single, surface of segment slightly spiculate. Segment IX—Paddles (Fig. 2*c*) with lateral edge serrated on distal 1/3, medial edge from the base; terminal seta 1 single, minute.

? CULEX (LOPHOCERAOMYIA) PLANTAGINIS

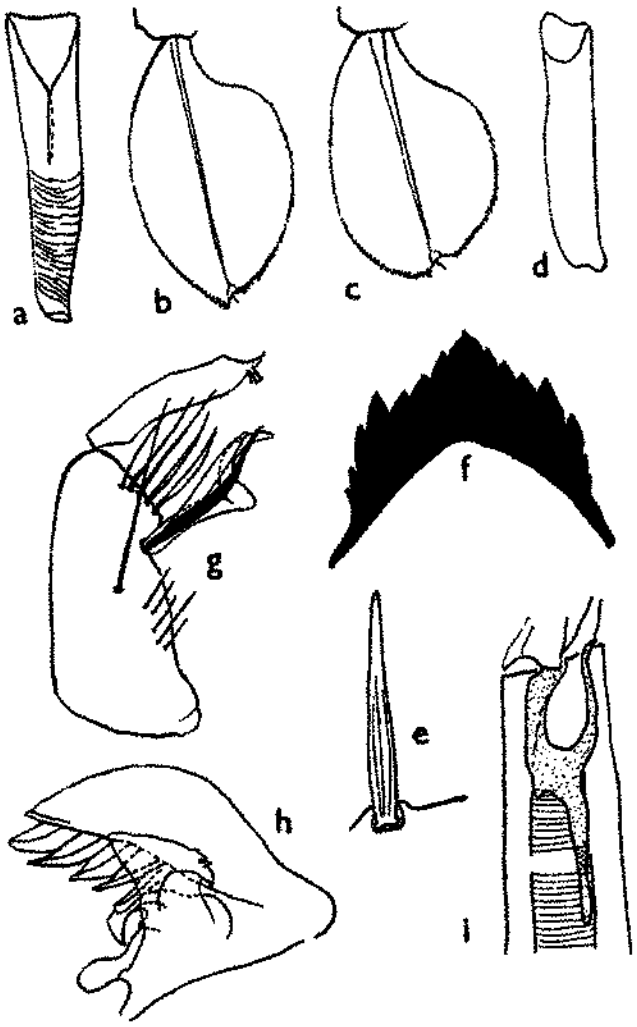
Larva.—The following description is based on the examination of 1 unassociated larval pelt and 2 whole larvæ in slide mounts. The larvæ are assumed to be those of *plantaginis* as this and *C. infantulus* are the only members of the subgenus represented otherwise in this collection, the larva and pupa of the latter being known and also represented in the collection. Mattingly (1949) stated that the larva of *plantaginis* had not been described until that date. The specimens examined here differ from all other species represented in the B.M. collection and cannot be fitted into Mattingly's key to the subgenus. It is rather similar to the larva of *C. tuberis* as described by Bohart and Ingram (1946), *infantulus* and *minutissimus* but may be differentiated by the number of branches in head seta 5(*C*) and the single minute branch of the anal segment seta 2 (*ucs*) combined with a long siphon.

Head: Rather broad, pale; seta 1 (clypeal spine) (Fig. 2*e*) short and rather stout, dark; antennæ infuscated at the extreme base and beyond the tuft (seta 1 of Belkin), markedly spiculate up to the tuft and slightly on the lateral surface beyond this; tuft at 3/5 of about 20 branches; setæ 2 and 3 (subapical spines) long, equal to 1/2 the length of the antenna, equal, reaching to the end of the longest apical spine (seta 4 of Belkin), their bases some distance proximal to the apex; setæ 7, 6 and 5 (*A*, *B* and *C*) with 6-8, 2-3 and 3-5 branches respectively; 4(*d*) tiny, single, 8(*e*) minute with 2-6 branches, 9(*f*) minute with 2-6 branches; 5 placed posteriorly and slightly medial to 6, 4 slightly anterior and well medial to 6; mentum (Fig. 2*f*) with 7 unequal teeth each side of a larger central tooth. *Abdomen:* Segment VIII—setæ 1, 3 and 5 (*A*, *B* and *C*) with 3-5, 6-10 and 4-6 branches respectively; comb a patch of 35-45 equal sized scales; siphon pale, tapering on the proximal half then parallel-sided and straight or with a slight curve caudalwards on the distal 1/3, index 9-10 in flattened specimens; pecten of about 16 spines extending to 1/3 the length of the siphon; basal spines distinctly smaller and closer together than the distal, the basal with 3 or 4 and the distal with 7 or 8 denticles extending along the whole ventral edge; 1 pair of subdorsal and lateral and about 8 unpaired subventral tufts, the proximal placed well beyond the distal end of the pecten and $1\frac{1}{2}$ times the diameter of the siphon at its middle, the distal about the same as the diameter; anal segment with complete minutely spiculated saddle bearing a lateral seta (1 of Belkin) of 2 small branches 1/3 its length; seta 2 (*ucs*) with a single very short slender branch; seta 3 (*lcs*) single, both setæ about 7 times as long as the saddle; ventral brush (4 of Belkin) with 7-8 pairs of well developed tufts; "gills" narrow and lanceolate, $1\frac{1}{2}$-2 times the saddle length.

In Mattingly's key, couplet 3 may be altered as follows to include this species:—

"3.	Head seta "C" with 5-8 branches, siphonal index less than 7 ...	*4*
	This seta with 3-5 branches, upper caudal seta with a single minute branch, siphonal index 9-10	*plantaginis*
	This seta with at most 3 branches, usually single or double (if 3 branches then upper caudal seta with several branches or siphonal index less than 9)	*6*"

FIGURE 2.



a,b. *Uranotænia campestris.*
c,d. *U. annandalei.*
e,f. *Culex (Lophoceraomyia) plantaginis* ?
g. *Culex (Mochthogenes) castrensis* var. *foliatus.*
h,i. *Culex (Culex) barraudi* (Mahdopur, India).

Larva.—*e.* Clypeal spine.
f. Mentum.
i. "Stirrup-shaped piece" of siphon

Pupa.—*a,d.* Trumpets.
b,c. Paddles.

Imago.—*g.* Coxite and style.
h. Phallosome.

C. (LOPHOCERAOMYIA) INFANTULUS.

Larva.—The larvæ from Nepal lack the dark central ring on the siphon which is present in some specimens seen in the B.M. collection from Hong Kong and elsewhere.

C. (MOCHTHOGENES) CASTRENSIS VAR. *FOLIATUS.*

Imago.—This species is represented in the collection by a single male the genitalia of which conform to Barraud's description of Jackson's specimens from Hong Kong in the B.M. collection and to the genitalia of Brug's type. In external appearance, this subspecies is identical with the type form which occurs in North Kanara (India) and Ceylon. As Brug's figure of the genitalia (1932) is inaccurate, a new figure is given here (Fig. 2*g*).

C. (CULEX) BARRAUDI.

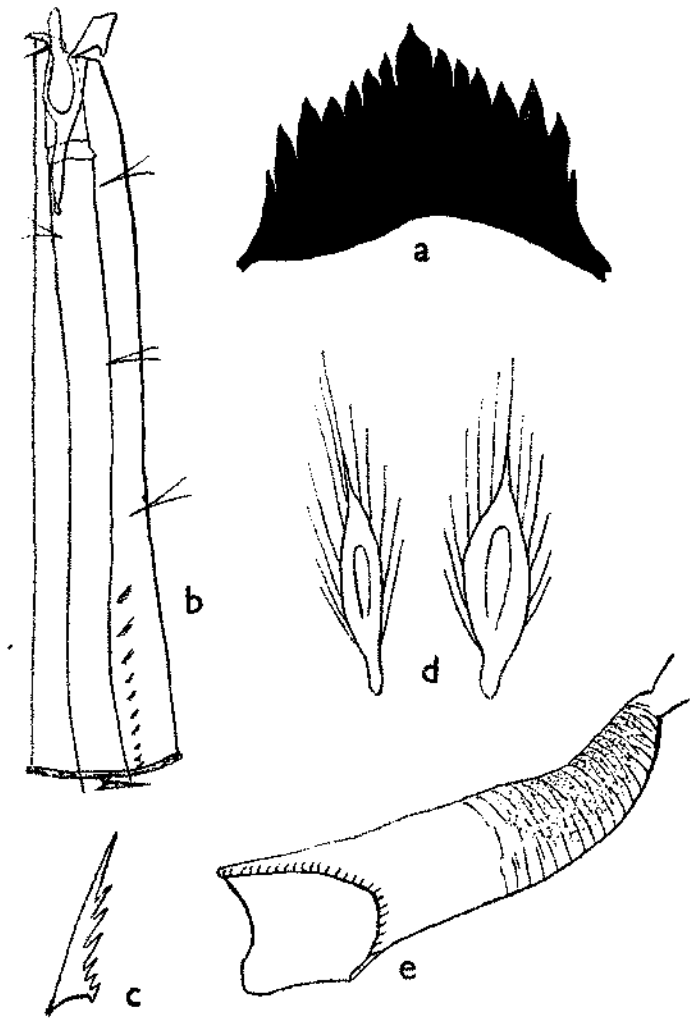
Imago.—Barraud's illustration of the phallosome plate of *C. barraudi* is misleading. The subexternal process (Baisas, 1938) is well developed and hook-like and arises from the origin of the basal process. The external process is a delicate recurved hook arising from a delicate membranous fold which itself arises from the proximal portion of the basal process (Fig. 2*h*). (The senior author is indebted to Mr. Donald Colless for pointing out this feature to him). The specimen here illustrated is from Mahdopur, India, and has 5 scoop-like median processes. The Nepal example has only 4.

Larva.—A distinctive feature of the larva is the "stirrup-shaped piece" of the siphon (Breland, 1952; Peters 1955) (Fig. 2*i*.) which is very dark in colour and has a prolongation of the ventral cornu proximally directed.

C. (CULEX) WHITEI.

Larva.—The larva of *C. whitei* has not been fully described. Barraud states that it resembles *C. barraudi, tritæniorhynchus* and *vishnui,* differing from the last in the more numerous comb teeth. It is easily differentiated from all these by the shape of the siphon and in addition, from *barraudi* by the "stirrup-shaped piece" of the latter and from *tritæniorhynchus* by the short multi-branched lateral saddle seta of the latter. The following description is based on the examination of a larval and pupal pelt with associated female imago from Nepal and the identical larval pelt in the B.M. collection associated with a male imago. In both larval pelts, several head setæ are missing. *Colour:* Head and siphon pale. *Head:* Clypeal spines (setæ 1 of Belkin) fairly long, tapering; 7 (*A*) has 7 branches, 6 and 5 (*B* and *C*) are missing, 4(*d*) is a long slender seta whose base is antero-medial to 5; 8 and 9 (*e* and *f*) have 6-7 and 4 short delicate branches, respectively; antennæ infuscated at extreme base and beyond tuft, coarsely spiculated to the tuft and just beyond, rather broad proximal to the tuft which is placed about 2/3 the way along the shaft and has about 25 branches; setæ 2 and 3 (subapical spines) based well proximally to the apex, long and equal extending to the end of the long apical seta; mentum (Fig. 3*a*) with 7 teeth each side of a larger central tooth.

FIGURE 3.



a—*e*. *Culex* (*Culex*) *whitei*.

Larva.—*a*. Mentum.
b. Siphon.
c. Pecten spine.
d. Comb scales.

Pupa.—*e*. Trumpet.

Abdomen: Segment VIII—comb a patch of about 40 scales (Fig. 3*d*) with fringe hairs few but long, the terminal ones longest and approaching the *vishnui* type; setæ 1, 3 and 5 (*A*, *B* and *C*) with 5, 6 and 5 stout branches, respectively, siphon with index about 7 in crushed specimens (Fig. 3*b*) tapering very gradually for the proximal 4/5 then very rapidly on the ventral side; pecten of 10 spines (Fig. 3*c*) with about 6 ventral denticles on the larger distal spines, extending to 1/4 the length of the siphon; 3 pairs of 2-branched subventral tufts starting beyond the pecten, their length half that of the diameter of the siphon at its middle, 1 pair of subdorsal tufts; saddle complete, minutely spiculated, especially towards the base of the long caudal setæ; seta 2 (*ucs*) with 1 long and 1 very short branch, 3 (*lcs*) single, 1 (*ls*) single and as long as the saddle; setæ 4 (ventral brush) with 6 pairs of well developed tufts; " gills " long, narrow and lanceolate, twice as long as the saddle.

Pupa.—Colour: All pale. *Cephalothorax:* Trumpets (Fig. 3*e*) about 4 times as long as broad in flattened specimen, meatus broad, about 1/4 as long as the trumpet; setæ without conspicuous features, 1, 2, 5 and 7 moderately long with 3, 2, 3 and 2 slender branches; 4 and 6 very short, double and single, respectively; 3 and 8 short, single or double and single, respectively; 9-12 a little longer, with 2, 4, 2 and 1 branch, respectively. *Abdomen:* Segment I—1 a dendritic plume hair with 7 or 8 main branches; 2, 4, 5 and 7 tiny with 1, 5, 4 and 2 branches, respectively; 3 and 6 moderately long, single and double; 10 short, single. Segment II—0 a microseta; 1 very short, dendritic; 3 moderately long, double; 2 tiny, single, its base antero-lateral to 3; 5 moderately long, treble; 4 with 3 short delicate branches, its base postero-lateral to 5; 6 long, single or with a tiny branch; 7 a microseta; 10 dorsally placed, long and single. Segments III—VIII-0 a microseta on III-VIII; 1 with 5 moderately long branches on III, 3 on IV - VII; 2 minute, single, medial to 1 on III-VII; 3 moderately long, double, its base between 1 and 5 on III, with 3 branches and placed more anteriorly on IV and V, single or double on VI, single and longer on VII; 5 single, short and slender on III, longer and treble on IV, missing on V, longer and double on VI, long and single on VII; 4 with 3 short branches on III, single on IV, missing on V, slightly longer, double on VI, moderately long, single on VII and VIII; 6 moderately long, single on III-V, double on VI, short, single on VII; 7 laterally placed microseta on III-VI, with 3 and 6 moderately long branches on VII and VIII; 8 short, single on III and IV, double on V and VI, longer with 2-4 branches on VII; 10 with 4 very short branches on III and IV, slightly longer on V, long, single on VI and VII, its base more posterior on the last; 12 with 2 short branches on III and IV, longer and single on V and VI, with 2 moderately long slender branches on VII, 11 minute double, its base medial to 12 on III, single on IV and V, its base lateral to 12 on VI, longer and double on VII; 14 a microseta on III-VIII. Segment X—seta 0 single, minute. Segement IX—Paddles smoothly rounded with slightly spiculated buttress on the proximal 2/3 of the lateral margins; setæ 1 and 2 (terminal setæ) very short and equal in length.

SUMMARY.

This is the first detailed record of Nepalese *Culicini*. The *Anophelini* have been recorded in another paper by the authors (Peters *et al.*, 1955).

Twenty-three species are recorded here. New descriptions of various stages of the following species are described:—

A. (Christophersiomyia) annulirostris	...	Larva and pupa
U. campestris ...	...	Larva and pupa
? U. annandalei ...	...	Pupa
? C. (Lophoceraomyia) plantaginis	...	Larva
C. (Culex) whitei ...	...	Larva and pupa

A note on the pupa of *A. (Christophersiomyia) thomsoni* and the larva of *C. (Lophoceraomyia) infantulus*, notes and new figures of *C. (Mochthogenes) castrensis* var. *foliatus* and *C. (Culex) barraudi* are also given.

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ADDENDUM

Following the submission of the first part of this paper for publication, additional material was collected by one of the authors (S.C.D.). This collection, made during the early part of the monsoon rains in June and July, 1955, includes six species not listed above, bringing the number of species recorded from Nepal to 29. The material consists of the following additional species:—

Orthopodomyia anopheloides Giles, 1903.
Mansonia (*Mansonioides*) *uniformis* (Theobald), 1901.
Edes (*Finlaya*) *assamensis* (Theobald), 1908.
Edes (*Stegomyia*) *vittatus* (Bigot), 1861.
Armigeres (*Armigeres*) *kuchingensis* Edwards, 1915.
Culex (*Neoculex*) *brevipalpis* (Giles), 1902.

Toxorhynchites splendens.—2 ♂♂ and 2 ♀♀ with associated larval and pupal pelts were reared from tree holes in mango and rain water in old kerosene tins (Hetaura and vicinity, June 1955). No other species were found with them, presumably having been devoured.

Orthopodomyia anopheloides.—One early instar larval pelt of this species recovered from a tree in June or July (at Chisapani, a small collection of huts in the jungle, four miles East of Hetaura) together with *Culex* (*Neoculex*) *brevipalpis* was submitted to Mr. P. F. Mattingly who commented on it as follows (personal communication):—

" On the position of the siphonal tufts this would run down to *O. andamanensis* in the key given by Knight and Mattingly (1950) although on distributional grounds it is more likely to be *O. anopheloides*. *O. andamanensis* was recorded by Barraud (1934) from the Darjeeling District but it has not been possible to confirm this record from specimens ".

Mansonia (*Mansonioides*) *uniformis.*—4 ♀♀ were collected from a house at Nayagoo (or Kamira, about eight miles East of Hetaura on the Karra River). No collections of water with floating *Pistia* suitable for their breeding could be found within the vicinity of the village.

Aëdes (Finlaya) assamensis.—A single ♀ was collected from a house at Nayagoo. This species is difficult to separate with certainty in the female from *Æ. feegradei* Barraud (1934), the main difference being the absence of a median line of white scales on the head in the latter species. The specimen in this collection is somewhat rubbed and moulded but does appear to correspond to the specimens of *assamensis* in the British Museum collection. *Æ. feegradei* is reported so far only from Burma whereas *assamensi* is common in Assam and East Bengal.

Aëdes (Stegomyia) vittatus.—2 ♂♂ and 3 ♀♀ with associated larval and pupal pelts were reared from water in a wooden tray in the main bazaar of Pokhara (one mile East of Hetaura, June or July).

Armigeres (Armigeres) kuchingensis.—This species is difficult to distinguish in the female from *A. obturbans* (Walker), 1860. Two females were taken, one biting the collector and another hovering over him in a tent during the day at Hetaura (June or July) and a third female was found resting in a house at Nayagoo. All three specimens were somewhat damaged in transit, but in all the abdominal sternites appear lighter than is usual in *obturbans*, one resembling the type form and two the var. *durhami* as defined by Barraud (1934) of *A. kuchingensis*.

Culex (Neoculex) brevipalpis.—Two larval pelts from specimens found in a tree hole (species unknown) together with an *Orthopodomyia* pelt at Chisapani are clearly of this species which has a very long distinctive siphon and is common all over the area.

REFERENCE.

KNIGHT, K. L. and MATTINGLY, P. F. (1950) ... The orthopodomyia anopheloides sub group of mosquitoes (Diptera: Culicidæ) *Proc. Ent. Soc.*, (*Washington*), **52**, pp. 1-20

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HISTORY OF THE CONCEPT OF " RELATIVE IMMUNITY " OR " PREMUNITION " CORRELATED TO LATENT INFECTION*.

BY

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AND

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SUMMARY.

I. INFECTION AND INFECTIOUS DISEASE

It is necessary to define with precision the expressions " infection " and " infectious disease ", which are often employed one for the other.

Before the era of Pasteur, the word infection meant " corruption produced in a body by substances or deleterious miasms which are introduced into it " (Littre, 1863-1872). After Pasteur's discovery of the existence of a new influence of Nature, the influence of microbes, the word infection has assumed a more precise meaning. The infection, in the passive sense, corresponds to a state of an organism invaded by a microbe†; in the active sense it means the act of contamination‡. The occupation by the microbe can be either harmful or indifferent or even useful. We shall revert later to this distinction.

*Thanks are due to Mr. K. Gopalakrishnan, Assistant Director-General, Posts and Telegraphs, Delhi, for translating the original paper written in French into English.—*Editor.*

†The invasion of the organism by a non-microbial parasite is an infestation.

‡A primary infection is the state of an organism invaded for the first time by a microbe.

The infection can thus be defined: the life in common, disturbed or peaceful, of a microbe and of another living being*.

In this association the microbe is a parasite: It draws its nourishment from its host. Depending on its own virulence and the resistance of the host—qualities which vary according to the respective species to which the one or the other partner belongs—the disease manifestations are limited. As stated by J. Bordet (1927), "The virulence of the microbe is its immunity *vis a vis* of the organism, the immunity of the organism is the virulence of the microbe. There are some microbes which suddenly, at the first contact with their host, behave as mortal enemies: to kill or to be killed. They are called pathogenic microbes. On the contrary there are 'good' microbes which living at little cost, not only do not trouble the organic economy but serve the host: a live vaccine like the B.C.G., living germs like the lactose ferment, yeasts, are useful by reason of the immunity which they provoke against the pathogenic organisms. The yeasts of wine and drosophile render to each other mutual services.

On the other hand when a pathogenic microbe has succeeded in implanting itself in a susceptible organism, it proliferates and invades the tissues most often only with delay†. For some time the host does not suffer, its presence remains unsuspected, "latent". This period of the commencement, when the infection incubates, is called for this reason the incubation period. It is "the time which lapses between the entry of the virus and the appearance of the symptoms" (*Pasteur*, **45**, p. 574). It can thus also be called latent period prior to the disease.

But it may happen that the state of equilibrium is broken. The microbe multiplies rapidly and disturbs the function of the host. The host reacts: inflammation, pain and fever. The conflict becomes patent; the crisis which explodes, manifests itself by two concomitant phenomena; parasitic attack and clinical attack. Infection rages in infectious disease.

For the purpose, we think it would be desirable that in the medical dictionaries, the definition of the word 'infection' and that of 'infectious disease' should be fairly differentiated. Garnier and Delamare (1945) give the following definition of the term 'infection': "Invasion of the organism by a pathogenic germ". Dorland (1938) on the other hand defines 'infection': "Invasion of the tissues of the body by pathogenic organisms in such a way that injury followed by reactive phenomena results". We think that a wider meaning must be given to the word 'infection' by suppressing from its definition the adjective 'pathogenic', since there are some infections which do not make the host suffer and that in certain cases the microbe renders service to its host. We will say, therefore, the infection is the harbouring of a microbe by a host; the infectious disease is the series of disturbances of vital functions which reveal a conflict between the host and an aggressive microbe.

*It is not possible to give a scientific definition of microbes conforming to the taxonomic rules of Botany or Zoology. It could be simply stated, by way of simple classification, in inspired conceptions of our Master Emile Roux: "A microbe is a unicellular being, so small that it can be seen separately at any period of its life only with the aid of a microscope (optical or electronic)". According to this criterion, the microbes comprise bactæria, spirochætes, ricketttsia, virus, protozoa, and some inferior fungi.

†The observation and experience indicate that most of the infections miscarry. One can think that in Nature the number of seeds lost must be infinite.

The period of violent fight which marks the crisis of the infectious disease is terminated by the death or the survival of the host. If the organism succumbs, its death leads to that of the microbe unless it is one of the bacteria capable of forming spores which can survive long in an inert state in the external surroundings (the carbuncled bacteria and the "*champs maudits*").

If the host resists the crisis, its survival is accompanied by one or the other of the following phenomena: the microbe is exterminated or else the host and the microbe both survive.

In the first case, the recovery from the clinical attack and the termination of the parasitic attack are simultaneous. The rapidity of the decisive result has given to the diseases of this category, the name of acute infectious diseases.

In the second case when the outcome of the fight between the organism and its parasite remains indecisive, when both survive, it is stated that the disease has become chronic. After clinicl recovery, the infection becomes latent as during the incubation. It runs the period which, for this reason, is called the latent metacritique period.

We shall see later, after the infection establishes itself without the explosion of a clinical attack of first invasion, the host is saved from the stormy crisis which marks the perilous moment of the infectious disease: the infection is latent from the beginning.

II. LATENT INFECTION.

Two categories of infectious diseases can thus be distinguished according to the existence of a single period or two periods of latent infection in the evolution of the disease. The severe cyclical diseases have only a single latent period, the incubation or the pre-disease period; the chronic diseases have two latent periods, the pre-disease (the primary latent infection of the Germans) and the post-disease period (*sekundare latency*).

The term 'latent' was introduced in the French language in the fourteenth century (Oresme) in the sense 'hidden'. During the nineteenth century, in the medical dictionaries the word 'latent' is found to be used or employed to describe viruses which do not manifest their presence in the body by any sign (Nysten, 1858; Thomas, 1877; Littre and Robin, 1878; Roth, 1878). Following the discovery of Pasteur over six decades ago, the expression 'latent microbe' has been used (Nielly, 1889).

We are employing the word 'latent' in the classical sense, 'hidden' (Littre, 1863-1872). To say that an infection is latent, is meant simply that it is hidden and does not reveal its presence by any visible sign. Latent infection can become patent and cause parasitic relapse. But other infections can remain indefinitely, without ever emerging from its clandestine nature. The notion of latency is in no way connected with the idea of an eventual relapse.

We will see in the following chapter that the infection can be even latent from the outset, establish itself from the time of infection and exist as such as long as the host is not deparasitized.

In brief, an infection is said to be latent when it is not manifested by any perceptible symptom or sign, when it does not fall directly under this sense. The host is inhabited by harmless organisms which multiply themselves without any manifestation in the host.

The opposite of latent infection is patent infection. It is also called manifest infection.

Every infection, as we have seen, begins after contamination with a latent phase. Trousseau (quoted by Bernheim, H., 1877) was already saying towards 1850, with a foresight, " Pathogenic germs can remain silent during a certain period, they can thus hide themselves for some days, months, years, for favourable conditions for their evolution to manifest their presence ".

In 1889, M. Nielly wrote, " All the infectious diseases have a period of first appearance prior to which is the period or the phase of latency, which is utilized by the infectious germs to multiply themselves in the host: it is the incubation period, the duration of which is variable following the nosologic species " (Nielly, 1889).

In normal conditions, a period of latent pre-disease phase presents for each disease a mean value which the epidemiological studies have determined and on which the sanitary regulations base their prophylactic measures. But the existence of some important variations, for the same disease, must be emphasized.

Thus the incubation period of malaria, is two to three weeks in general, but can exceed many months. Some colonials or North Africans are often seen to present their first attack of malaria in France, a long time after their departure from the malarious country. In Holland, malaria which manifests itself in spring has been, most often, acquired in the preceding autumn.

Incubation period of trypanosomiasis in dogs, infected in the laboratory, is from eight to ten days. We have, however, seen it to last five, seven and eight months.

The incubation of rabies varies in dogs between extreme figures of eight days to one year. Among certain animals, some observers affirm that it can last three years. On the contrary, the fixed virus, freshly made, inoculated into the brain of a rabbit, evolves regularly with an incubation of five days. In vampire, the latency of virus is of very long duration. The usual long incubation period of rabies in man (40 days on an average) permits the preventive Pasteurian treatment after being bitten by rabied dog. The incubation of the North African bovine Theileriosis, in the case of natural infection by a tick, is often less than a week. Following transfusion of blood during the acute attack, its incubation is regularly 17 days. But virus, after the acute attack, can give rise to an incubation period of several months (Sergent, Ed., *et al.*, 1945).

The pre-disease latency can also be as much the attribute of microbes of severe diseases, as those of mirobes of chronic disease. As regards the acute diseases, for example, the Schick reaction shows that a large number of subjects who have never suffered from diphtheria symptoms, possess immunity against this disease. The infection which has conferred this immunity to them, thus remains hidden. Same is the case of Dick reaction which reveals that many persons have been immunized against scarlet fever without giving rise to manifest symptoms.

The latent post-disease period of chronic diseases, during which the microbes multiply themselves only in small numbers, hidden in different organs or circulating in blood, is sometimes of a very long duration. Observations show that parasites of certain malarias, as well as anaplasma, subsist during the entire life of their hosts. Same is the case with spirochætes of recurrent fevers. Those of the North African-Spanish recurrent fevers, remained dormant for some years (in one case three years) in the brain of experimentally inoculated guinea pigs (Sergent, Andre, 1938*a*; 1938*b*:1942 and Sergent, Ed., 1945), J. Bordet writes, " Various microbes have the property of maintaining themselves for a long time in small numbers in an apparently cured person, without giving rise to marked disease, being quite susceptible, at a given moment, to multiply in large number and thus to provoke new symptoms at long intervals. It is latent parasitism (Bordet, 1927). The evolution of the infectious diseases is often quite irregular. Sometimes relapses manifest themselves after long latent periods; it is known for example that prognosis of syphilis is uncertain and that in this disease the unforeseen can always happen. Some hosts which are believed to be definitely purged of pathogenic organism, sometimes retain them very long, hidden in obscure parts of the body, without any symptom or sign to disclose their presence, but it often happens afterwards that they flare up afresh. In monkeys, inoculated with syphilis, the spirochætes persist for long in the bone marrow, even when the cure appears complete " (Bordet, 1939).

Many synonyms for the expression ' latent infection ' have been employed: hidden infection (Italian *nascosta*), occult (expression used particularly by Vallee, 1946), secret, clandestine, obscure, attenuated, sleeping, dormant, torpid, silent, inapparent, abortive, mitigated, sub-clinical (English expression). By a curious mixture of words, some authors say " insensible development of a disease " (" not manifesting itself and which is noiseless "—Littre, 1863-1872) and others say " dumb infection ".

In reality, latent infection remains occult only where the investigatory and diagnostic methods used are imperfect.

Claudes Bernard upholds that " latent life "–either of vegetable grains or rotifers—was not a feeble life or a reduced life, but an arrested life—a life in a state of absolute chemical indifference (cited by Rostand, 1954).

It is a fact that during its entire post-disease latent life, which is often equal to more than the half or the totality of the duration of the life of its host, the residing microbe remains virulent. Inoculated in new hosts, it infects them, just as the normal strain does. On the other hand, the gravity of the acute attack has not, often, any relationship with the length of the incubation. Thus in rabies, in bovine Theilerions, the attacks appearing after a long incubation are fatal, like attacks coming after a short incubation.

The expression of latent infection must be applied only to microbes living in intimacy with the host organism. Such is not the case with pathogenic microbes which are often found on the integuments or on the mucous surfaces of old patients or even of healthy persons. It is known that the staphylococcus is very widely spread on the skin which subsists for long in the upper respiratory passages of convalescents from diphtheria, pneumonia or influenza, along with the diphtheria bacillus, pneumonococcus and the influenza virus. The same is the case even

with the typhoid bacillus in the intestines or the gal-bladder of convalescents, and the cholera vibriœ in the intestines of old patients or of apparently healthy persons. In reality, the natural cavities in which these microbes live, constitute a sphere external to the organism. The latter only live in these situations. The host cannot act on them through phagocytes or antibodies. There is no real association between the organism and the microbe, hence no infection, but only a carrier state of germs " external " to the host (Sergent, Ed., 1948).

The affirmation of the existence of a latent infection requires the evidence of the presence of microbes. Numerous techniques are employed to disclose them. The improvement of the technique progressively diminishes the number of unknown infections. Morbid signs or anatomical lesions which are subjective symptoms, are often revealed to us by some devices like auscultation, radioscopy, etc. The microbiological methods—microscopic finding of the germ, culture methods*, inoculation to susceptible animals (isodiagnosis of Sergent, Et., 1920) (Sergent, Et., 1920; Sergent, Et. and Sergent Ed., 1921*b*; 1921*c*), artificial provocation of relapses (splenectomy† etc.), serological reactions—can provide the final proof of the origin and the nature of the disease (Sergent Ed. and Parrot, L., 1935*a*).

III. IMMEDIATELY LATENT INFECTION.

Whether it is a question of acute infectious disease or of chronic infectious disease, the clinical attack of the first invasion can be wanting; it happens, in fact, that there has been multiplication of microbe after its introduction in the host without any visible reaction of the latter. Roger (1920) says, " the morbid reaction is not fatal ". Pasteur (1882) wrote, " It is very probable that many cases of silent rabies must have escaped the observation "; and Laveran (1907) wrote, " the hæmatozoa of malaria can remain latent among persons who have never presented symptoms of malaria, as well as among those who have had one or several attacks of fever ". Bordet (1920), speaking of latent microbism, distinguishes among the carriers of germs " those who harbour the virus *without ever having been ill,* and those who conserve it after having overcome the infection ".

We have used, from 1910, the adverbial expression " immediately " (at the outset) to characterize the establishment of a latent infection, without crisis, in a host (Sergent, Et. and Sergent, Ed., 1910:1921*a*:1921*b*:1921*c*; Sergent, Ed. and Beguet, 1914; Sergent, Ed. and Sergent, Et., 1918:1921; Sergent, Et., Sergent, Ed. and Catanei, 1923*a*:1923*b*; Sergent, Ed., Sergent, Et. and Catanei, 1934; Sergent, Ed. and Parrot, 1935*b*; Sergent, Ed., 1937*a*:1938*a*:1947:1948). The immediately latent infection is defined as " infection which is installed silently

*For example, some researches pursued at many places in the world have demonstrated that among many of the bovines, healthy in appearance, the existence of avirulent trypanosomes, which the direct examination of the blood would not reveal (*Trypanosoma theileri*), could be revealed by the process of culture in ordinary bouillon medium.

†The spleen, major portion of the phagocyte system, appears as the principal organ of resistance and, consequently, of premunition, in many infections, particularly in the diseases due to hæmocytozoa or related microbes (malaria, piroplasmosis, bartonellosis, etc.). The ablation of the spleen results in the break down of premunition and the return to activity, transitionary or permanent, of the latent post-disease infections which are revealed in the above manner [Parrot *et al.* (1930), *C. R. Soc. Biol.*, **104**, p. 866].

without patent clinical attacks. In the chronic infectious diseases, the pre-disease latent phase and the post-disease latent phase are joined together and are intermingled ".

These ' immediately latent infections ', asymptomatic, in nature, are observed, as we have stated, both in the acute disease groups as well as in chronic diseases. Among the acute disease, the fact is demonstrated, for diphtheria by the Shick reaction, and for the scarlet fever by the Dick reaction. As regards the chronic diseases, the most common type of immediately infection is that of tuberculosis infection which is revealed only by the tuberculin and the B.C.G. reactions. The diseases due to protozoa also furnish a multitude of examples of infections of the same character.

The immediately which have been latent from commencement from the time of introduction into the host, *i.e.*, which have not provoked acute attack of first invasion, do not produce any relapses. In effect, by definition, a parasitic attack which explodes months after the primary inoculation, would be an acute attack of first invasion appearing after a long incubation and not a relapse. An infection which is latent from the commencement, remains so as long as it lasts.

Some immediately latent infections are transmissible in serial passage with the same character of occult microbism, not determined by clinical signs, and revealed only by the microbiological methods. Thus the small piroplasm of the ox, central anaplasma, which provokes in its host a parasitic attack, without clinical attack, is transmitted indefinitely in the laboratory, by inoculation from infected bovine to healthy bovine, without there being an exaltation of the virulence of germs at any time and appearance of general disorders. The infection remains latent in all the passages (Sergent, Ed. and Parrtot, 1935*a*).

The existence of an unsuspected immediately latent infection is often revealed fortuitously, as in the following example reported by Jessie Marmorston:—

" The intraperitoneal inoculation, to mice aged three weeks, of the blood of an adult mouse strongly infected with *Eperythrozoon coccoides*, does not provoke any apparent illness. But if the spleen of these young mice is removed, a large number of *Eperythrozoon coccoides* are seen to appear in 24 or 48 hours following splenectomy in the erythrocytes and plasma" (Marmorston, 1935).

The remarkable fact, in the group of acute diseases, is that the immediately latent infections give an immunity as old as patent infections (scarlet fever, diphtheria, typhoid fever) and, in the group of chronic diseases, the immediately latent infections procure a premunition as vigorously as the latent infections consequent to an acute attack*.

The immediately latent infection (In Italian: *Infezione latente immediate*) which, it is stated, would correspond to a miscarried disease, has received sometimes other names.

In 1928, H. Reiter proposed the expression " dumb infection " for describing the latent infections which establish themselves after the first attack, without being succeeded by a manifest infection and an accute attack. The " dumb infection " is thus a synonym for immediately latent infection (Reiter, 1928).

*Vaccination against some piroplasmosis with the living virus-vaccines, such as we have practised at the Pasteur Institute of Algeria, show precisely to profit the possibility of conferring the premunition by an immediately latent infection.

In 1936, J. Fortner stated that mice and parakeets inoculated with psittacosis could harbour the virus without ever having presented clinical symptoms. It is this which the author calls a " silent infection ", a synonym also of immediately latent infection " (Fortner, 1936).

In 1919, Charles Nicolle and Charles Lebailly have proposed " the name of inapparent infections for a type of silent infections, in which an acute disease grows in the animal without previous disease undergoing its periods of incubation, of infectious condition (septicæmia and virulence), then of recovery, without any sign of warning " (Nicolle and Lebailly, 1919).

In 1925, Charles Nicolle gives the following definition (*Arch. Inst. Pasteur Tunis*, **14**, 2, p. 211).—

" The *inapparent infection* is an acute disease*, a septicæmia which has its incubation, evolution, recovery, and which allows, later, an immunity more or less durable. It distinguishes itself from the ordinary type of infection by the absence of clinical symptoms, but it is connected by all forms of moderate seriousness and it is only the mildest type. The inapparent infection has nothing to do, on the contrary, with the latent infection. The *latent infection* is a sub-acute or chronic state in which the carrier conserves for short or long periods, without any suffering, the germ of a disease from which it could have suffered previously and which (germ) may or may not be capable of recovery from virulence for the host itself or to transmit the disease to other individuals ".

The definitions given in 1919 and 1925, by Charles Nicolle, of the " inapparent infection ", agree exactly with the notion of " immediately latent infection " which we have given in 1910 and precisely in 1914, 1918, 1921 and 1923. But Charles Nicolle has nowhere suggested the expression " immediately latent infection "; he always said simply " latent infection ". If one considers, in detail, the characters which differentiate, according to Charles Nicolle, the " inapparent " from the " latent " in general, one can make the following comments.—

(*a*) The first character would reside in the evolution which would be peculiar to an inapparent infection: no clinical symptoms, incubation, with septicæmia, evolution, recovery, ensuring immunity. But it is exactly the evolution which has been pointed out since long in the " immediately " latent infection. Here is an example, among many others observed in the laboratory, from which one can conclude as follows:—

A calf is inoculated with *Theileria mutans*; it does not present clinical attack of Theileria—no fever, no morbid symptoms—but the daily examination of blood reveals, after 65 days of incubation, a parasitic attack of 25 days' duration, with a maximum of 175 parasitized erythrocytes per 1,000, after which the parasites disappear from the blood to reappear rarely from time to time. Thus it is seen that infection persists (Sergent, Ed., *et al.*, 1927). This dissociation between the virulence and the symptoms, by which inapparence is defined and which has already been given previously the characteristic of "immediately latent infection", is noticed here again clearly; in the complete absence of any clinical attack, regular display of a pure parasitic attack, comprising an incubation, a period of infectious state, a period of decline of parasitæmia. As regards " immunity following inapparent infection ", it is well known, as we have referred to above, that immediately latent infections can bring about immunity in acute diseases (scarlet fever, diphtheria), or premunition in chronic diseases (tuberculosis and malaria).

(*b*) A second character would separate, according to Charles Nicolle, the inapparent infection from the latent infection: " in the latent infections the carrier *conserves the germ of a disease from which it has suffered previously* ". This second differential character cannot be retained because very often the carriers of latent germs have never been ill: they have an *immediately latent infection*, without acute attack of first invasion. One could cite innumerable cases according to those of Pasteur, Laveran and J. Bordet, reported above.

In fact the expression " inapparent infection " is only a synonym, *proposed later*, for "immediately latent infection "; on the other hand the term " latent " presents an advantage over the term " inapparent ", that of being more comprehensive. One can certainly call " inapparent " a latent infection like tuberculosis when the skin reaction to tuberculosis has revealed the presence of the causal microbe without showing it. But a latent infection cannot be called " inapparent " if, thanks to laboratory techniques (for example, the examination of blood in hæmatozoosis), the microbes can be seen. *To become visible* is the proper meaning of the word " to appear " (Littre, *Acad. French*). Consequently the meaning of the word " latent " is wider than that of " inapparent ".

There was, therefore, no necessity to substitute for the expression " immediately latent infection " the appellation " inapparent infection ", which conveys no new idea and over which the first had priority (Sergent, Ed., 1947).

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*One can remark the contradiction which exists, in the phrase: " The inapparent infection is an acute disease ", since an inapparent infection is a silent infection, without morbid symptoms, while, by definition, the disease is an alteration of the health (Littre).

In short, immediately latent infection is distinguished as follows: The microbe invades the host silently, the progress and decrease of the occult parasitæmia unfolds itself following the same rhythm as in a manifest infectious disease: incubation, mutiplication, static period, regression, but without provoking any morbid symptom; on his part, the host of the beneficent silent microbe, without the risk of crisis, acquires a state of immunity (Sergent, Ed., 1948).

According to the progress in techniques of disclosure, new diseases, where cases of immediately latent infections are frequent, are being discovered.

IV. ANTI-INFECTIOUS IMMUNITY—STERILIZING OR NON-STERILIZING.

In the medical sense, the term immunity describes the resistance which, in natural conditions, a host resists an infectious disease; in brief, according to etymology: the exemption from disease*. It is considered that immunity can be inborn that is to say, inherited or acquired during the period following an infectious disease. But under the name of acquired immunity, two categories of refractory state are confused, which can be clearly characterized today, and are thus distinguished. This distinction does not present only the superiority of speculative nature of analysing the problems in order to resolve them better, as Descartes desired it. It offers still an immediate practical interest for the preventible and therapeutic medicine; it enables to explain certain post-attacks, and to prevent possibilities of modalities (methods) and limits of prophylactic vaccination (Sergent, Ed., 1936; 1937*a*; Sergent Ed. and Parrot, 1935*a*).

It is from the reports of resistance acquired in the course of infections which provoke it that the two modalities can be distinguished: (1) post-infectious immunity, succeeding the acute infection and persisting long after the clinical and microbiological recovery; *it is the real sterilizing immunity* which manifests itself habitually after acute cyclical infectious diseases (variola, scarlet fever, etc.); (2) a co-infectious immunity contemporaneous with the infection and stopping when the latter has disappeared; it is a *non-sterilizing immunity* which has received the name of relative immunity. It is common to diseases which, after an episode more or less acute of primary invasion, or even perhaps outright, comprise a long metacritic stage of chronic latent infection (Parrot, 1955).

The expression 'relative immunity' is found in the writings of Pasteur, in April 1881, with reference to the immunity acquired against, which is "variable with the intensity of the virulence of the virus with which it attacks" (Pasteur, 1881*b*).

It is Albert Plehn who was the first to use, in 1901, the expression *relative immunity* in the sense of non-sterilizing immunity when he published his study of

*We are greatly obliged to Professor A. Baudoin for the following indications: the word "immunity" (=freedom from attacks) has come into the French language in the 13th century, and is found, in its medical sense, in the dictionary (Littre), by 1863. Warlomont speaks of variolic "immunity" in a communication made on June 24, 1865, to the Academy of Medicine of Belgium (*Bulletin*, Volume 8, page 503) which cites a report of Depaul appearing in the *Bulletin de l'Academie de Medecine*, Volume 31, (1866), page 367. The word "immunity" is also found under the pen of P. Ficord, in 1864 (*Letters sur la syphilis*, 3rd edition, (1863), p. 48. Bailliere Publications, Paris).

malaria in the natives of Cameroon (Plehn, 1901). His clinical observations led him to describe under this name a verified condition among the Blacks. They suffer from free attack of malaria only following particularly intense and repeated infections; they present ordinarily only a benign malaria, often completely abortive. A. Plehn distinguishes clearly this relative immunity from " the absolute immunity which the measles, typhoid, plague, variola, etc., confer " (Plehn, 1902). In the blood of the natives in a condition of relative immunity, parasites are not found on microscopic examination (*Latenz periode*). The immigrant Europeans when they submit themselves to a systematic quinine treatment, acquire a relative immunity against malaria similar to that which the natives show.

The experimental demonstration of the existence of a form of non-sterilizing immunity, and the explanation of its real nature, was given in the first instance by Th. von Wasielewski. The experiments which he has reviewed are not many but their plan is judicious, and the commentaries are clear.

He tackled the question, in 1901, in a short passage of a study on bird malaria, appearing in a periodical, and, much later, in 1908, in the chapter, devoted to malaria, in a book on pathogenic protozoa, where it is found, at the end of a sentence, the expression *relative immunity*, already employed by Plehn in 1901, and which has had, since, a deserved usage.

By reason of their importance, we reproduce below, the long passages from these two publications.

1. RESEARCHES ON BIRD MALARIA, 1901 (WASIELEWSKI, 1901).

" Inoculation of finchs and of canaries with haemomceba from Germany has given results different from those which Koch had obtained with a strain from Italy* and Ruge with German strain†. After a period of acute infection, a very chronic infection was almost always observed, with very rare parasites, more easily revealed by inoculation of blood to healthy birds than by microscopic examination. In some animals under experiment, parasites in the blood could be found even 11 months after inoculation, that is to say, for the majority of them, up to death. Cases of disease of short duration, followed by complete recovery and immunity, as Koch has described after the inoculation of Italian strain, have not been observed. On the other hand, animals in a condition of chronic infection, apparently free from parasites, when re-inoculated, do not suffer from acute parasitic attacks; at the most some parasites are seen ".

2. RESISTANCE OF INOCULATED ANIMALS AGAINST MALARIA PARASITES, 1908 (WASIELEWSKI, 1908).

" The small number of birds which had recovered, seven in six years, unfortunately do not permit the study of their immunity.

" I have been able to study the effect of re-inoculation only on a small number of animals, chronic patients, which, at moments, did not show any parasite in their blood. Twelve animals died of chronic malaria, after a long time, it is true, for the most part earlier than the healthy control animals. Also, almost always, at the end of five or six days, occasional parasites could be seen in the blood, their parasites had been absent during the weeks before the re-inoculation. Only in one case, parasites appeared on the 60th day after re-inoculation. Just as the chronic evolution of the first infection had already led to the presence of a greater resistance in all these animals, in the same way it can be deduced from these experiments that the chronic sick animals do not suffer gravely from the re-inoculation of high doses and that they succumb to it less quickly than the healthy control animals. This conclusion is in contradiction with the observation of Ross, who states that, through mosquito bites he could infect strongly four animals which had up till then showed only a few parasites. As long as it could never be demonstrated, through a large number of experiments, that the reinfection done through sporozoites has an effect essentially different from that of blood inoculation. I could assume that the number of persisting parasites, in the blood of four sparrows, underwent variations irrespective of mosquito bites. My results published previously and confirmatory observations

Zeitschrift f. Hyg., **32**, (1899).

†*Centralbl. f. Bakt.*, *I*, **29**, No. 5 (1901).

repeated since long are in favour, it appears to me, of the idea that some resisting hosts can overcome the infection but, apart from rare exceptions, parasites persist living in their blood during a relatively long time. If, afterwards, re-inoculation in such hosts is proceeded with, one need not wait to see the appearance of same morbid symptoms in them as in the completely healthy animals. In the first place these animals possess manifestly superior resistance on the average; secondly, chronic infection has the effect of provoking the development of all the means of defence of the host against the multiplication of parasites, and they enter into action from the time of the introduction of the new infection. The re-union of these factors explains the difference in the result of the re-inoculation from that obtained in non-infected animals. But, considering that the previously infected birds are not capable of offering shelter to a new parasitic attack—whether the old parasites multiply afresh due to the provocation offered by the new inoculation—or whether the newly introduced parasites multiply themselves, even if it is to a more modest degree than normal—it is permissible in my opinion to speak all the more of relative immunity. The manner in which the immunity establishes itself to protozoal infections is still nowhere classified. In this difficult terrain, we must still surrender all claim to exact researches ".

The experimental study of relative immunity in *P. relictum* malaria, inaugurated by Wasielewski, was taken up again and developed by Edmond Sergent and Etienne Sergent. They undertook in 1901, in Algeria, the verification of the discovery by Ronald Ross of the rôle of the mosquitoes in the transmission of malaria and established, at the same time as epidemiological and prophylactic studies of human malaria in the country side of North Africa, and the laboratory researches, which are continuing in 1955, on the experimental infection of canaries by *Plasmodium relictum* and that of pigeons by *Hæmoproteus columbæ*. From 1901, they were employing the expression relative immunity " in the researches concerning the resistance acquired by the previously infected.

They are endeavouring to prepare from attenuated living virus vaccines, capable of giving to the birds an immediate latent infection, without passing through the stage, always dangerous, of an acute attack. It is this which they called in 1910, to give an " immediately relative immunity" (Sergent, Et. and Sergent, Ed., 1910). They are obtaining a certain number of good results with a virus vaccine made of sporozoites drawn from the salivary glands of mosquitoes and submitted to aging,—and with slightly infected blood, previously raised in the bird during incubation—latent period. But the best results, which are very regular, are given by restricted quinine treatment, administered to canaries before or after their experimental inoculation (*see* Section VII).

In 1917 (Sergent, Et. and Hempl, 1917), in 1918 (Sergent, Ed. and Sergent, Et., 1918), in 1921 (Sergent, Ed. and Sergent, Et., 1921; Sergent, Et. and Sergent, Ed., 1921*a*:1921*b*:1921*c*), Edmond Sergent and Etienne Sergent reported from confirmatory facts relating to the resistance to re-infections conferred by latent *P. relictum* infection, as long as they lasted, and on the susceptibility which reappears at the time the bird is deparasitized.

In 1923, Etienne Sergent published through his disciple S. Mazza some observations, pursued in his laboratory, on 1,700 canaries inoculated with a strain of *P. relictum* maintained for a large number of years. Among them 70 per cent survived the acute attack and presented a metacritic latent infection. Among the survivors, the blood of a bird inoculated four years and three months previously was still infective; but two other birds inoculated three years and three months previously were deparasitized (isodiagnostic negative) and, re-inoculated, presented an infection as grave as that of the controls (Mazza, 1923).

Finally, in 1952, Edmond Sergent and Etienne Sergent summed up the general results of 47 years of experiences on the relative immunity against *P. relictum* malaria, pursued in nearly 6,000 canaries (Sergent, Ed., 1950; Sergent, Ed. and Sergent, Et., 1950:1952). Six hundred and forty-nine canaries inoculated with *P. relictum* were observed during all their life periods (mean longevity of the canary was seven years). Three kinds of techniques were employed: microscopic examination of the peripheral blood and of the organs; inoculation of new canaries with a fifth of the total volume of blood; inoculation of the total mass of blood, with the product of ground internal organs (it is the most severe test which gives the best results whether positive or negative). Out of the total, 417 cases of latent infection were observed among canaries inoculated

for a very long time, sometimes for several years (61, 66 and 70 months in three cases). The parasitic recovery can occur very soon (before ten months in one canary). Seventy-seven canaries which had survived the acute attack were re-inoculated after varied intervals ranging from four to six years. All have shown a clear resistance. The 78th canary re-inoculated three years and ten months after the primary inoculation, was again susceptible. The refractory state persisted as long as the latent metacritic infection lasted, that is to say, often up to the ripe old age of the bird. The parasite had not lost its virulence for the host which sheltered it; the relative immunity is entirely the effect of organic reactions of the infected host. Resistance conferred by pre-munition is not re-inforced by repeated re-inoculations.

Edmond Sergent and Etienne Sergent observed the same phenomena of relative immunity connected with the existence of a latent infection in *Hæmoproteus* malaria of pigeons (1914) and in the trypanosomiasis of dromedaries of North Africa (1919).

In 1914, they reported history of two pigeons which they had injected with *Hæmoproteus columbæ* malaria by the bite of infected *Lynchia maura* (whose rôle they have shown in the transmission of this disease). These two pigeons retained the latent infection during four years. After a further period of four years, during which the pigeons did not show any *Hæmoproteus* in their blood, they were re-inoculated: both were infective. They had not thus retained any resistance and had become again susceptible after clinical and parasitic recovery from their primary infection (Sergent, Ed. and Beguet, 1914).

In 1919, Edmond Sergent and Etienne Sergent showed that the 'debab', trypanosomiasis of North African dromedaries, presented equally a latent metacritic period conferring a relative immunity during which they are refractory to super-infections (Sergent, Ed., Sergent, Et. and Lheritier, 1919).

In 1912, J. Moldovan also concluded from his observations that canaries infected with chronic *P. relictum* malaria resist a super-infection, but that deparasitized canaries become susceptible once again.

In one experiment, five canaries carrying occasional parasites in their blood were re-inoculated at the same time as a canary completely cured of its first infection. When the chronically infected bird did not present severe infection, and continued to show only very rare parasites in their blood, the cured canary had a very grave infection, just like the controlled ones (Moldovan, 1912).

These are thus some experiments carried out on avian malaria which have shown for the first time, through the method of experimental inoculation, that, in some infectious diseases, the relative resistance, acquired after the clinical cure, depends on the survival of latent parasites.

Some researches of the same type have been carried out later by numerous investigators studying other malarias of birds and monkeys.

During the decades after 1910, some investigators of different countries have placed on evidence, in a growing number of infectious diseases, and, among them, in grave and widespread diseases, like syphilis and tuberculosis, the absence of a real and sterilizing immunity. They comprise only a relative immunity connected with the existence of a latent infection.

The parasites which cause diseases with relative immunity, belong to all the microbial groups: bacteria (tuberculosis, brucellosis, etc.), spirochaetal (syphilis, relapsing fevers), ricketsiosis (exanthematic typhus, animal ricketsiosis), viral (herpes, poliomyelitis, psittacosis, etc.), protozoal (malaria, piroplasmosis, trypanosomiasis, coccidiosis, etc.), fungal (mycosis) and even of non-microbial parasites (helminthiosis). Some infectious diseases with latent periods and relative immunity in the vertebrates have been observed in insects and in plants.

V. SYNONYMS OF NON-STERILIZING "RELATIVE IMMUNITY".

The idea of an acquired anti-infectious resistance, coinciding with the existence of a latent infection and stopping with it, has been explained by terms other than that of "relative immunity".

Paul Ehrlich was content to say: " non-sterilizing immunity ", an expression employed after 1910, by some authors: E. Martini, Martin Mayer, Friedrich Fulleborn. It is mentioned by H. Schlossberger (Schlossberger, 1929).

IMMUNITY FROM INFECTION OR SUPER-INFECTION.

Immunity from infection.—W. Kolle and R. Prigge described, in 1929, the immunity which is acquired against tuberculosis under the name of *Infektionsimmunitat*. This form of immunity consists of a state of resistance against re-infection so long as the bacteria introduced by the first infection are present in the host: it disappears when these bacteria are completely eliminated (Kolle and Prigge, 1929).

Immunity from infection.—Schlossberger (1929).

Immunity from infection.—In 1934, E. Martini employed this term with reference to malaria (Martini, 1934).

Immunity from infection (*Durchseuchungsimmunitat*) which developes itself in the field of " dumb infection " (Reiter, 1925).

Immunity from infection (*Infection-immunity*).—Topley and Wilson described, in 1929, the " infection-immunity " which they observed in the relapsing fever, in syphilis, in tuberculosis, during the latent infection period.

Immunity from super-infection.—R. Debre and Bonnet described under the name of super-infection the resistance to the new infections related to the presence of aggressive microbes in the host (tuberculosis, syphilis), and under the name of immunity from re-infection that which survives the cessation of the disease, as in small-pox, for example (1927).

Immunity from super-infection.—W. Gingrich, in 1932, concluded from his experiments that a latent or chronic infection of *P. relictum* or *P. elongatum* or *P. cathemerium* is accompanied by an immunity against a super-infection by the same strain of parasites (Gingrich, 1932).

Immunity from super-infection.—H. W. Mulligan and J. Sinton proved in 1933 that a chronic or latent infection by *P. knowlesi*, or by a strain of *P. inui* var. *cynomolgi*, appeared to confer immunity against the clinical effects of a super-infection by the same strain of parasites (*Mulligan* and Sinton, 1933).

Resistance to infection (*Durchseuchungsresistenz*).—Petruschky, 1928 (cited by R. Kraus, 1928).

Tolerance of infection.—Yorke.

IMMUNITY-TOLERANCE.

The term " relative tolerance " is already found in the writings of Kelsch and Kiener, 1889 (Sergent, Ed., 1950).

Proposed by F. Mesnil (Laveran and Mesnil, 1912) (*See* also Sergent, Et., Sergent, Ed. and Catanei, 1923*b*).

Plehn used the same term.

PARTIAL IMMUNITY.

H. Schlossberger mentioned this expression in 1929 (Schlossberger, 1929).

J. Bordet employed in 1920 (Bordet, 1920:1939), in 1927 (Bordet, 1927), in 1934 (Bordet, 1934), in 1939 (Bordet, 1920:1939) the term partial immunity. " It can be imagined that an infected organism cannot succeed in recovery but defends itself, however, with sufficient vigour to modify the evolution of the disease by impeding in a certain measure the microbial invasion. This is precisely what is proved in most of the chronic diseases such as tuberculosis, syphilis. Morbid evolution transforms itself in the sense of low multiplication of the virus or of a more marked tendency to localization " (Bordet, 1934).

Latent immunity.—H. Schlossberger, 1929.

Concomitant immunity.

Failing immunity (immunite labile).—Cl. Schilling.

Immunity of depression (*Depressions immunitat*).—Morgenroth.

In the mice, the inoculation of a streptococcus, which is slightly virulent, provokes a chronic infection which puts them under the shelter of a super-infection by a virulent streptococcus. Cited by Schlossberger (1929).

IMMUNITY OF COMPENSATION, WASSERMANN, 1929.

When a state of equilibrium is established between the means of defence of the *organism* and the aggressive means of the pathogenic germ, it is a question of a sort of "*compensation*" between the forces of the microbes and those of its host (Cited by Schlossberger, 1929).

Immunity of lymphatic vessels (*Lymphbahnimmunitat*, Weleminsky. Cited by R. Kraus, 1928).

In South Africa, the expression "dirty beast" has been used since long to describe the bovines which, having recovered from the first attack of piroplasmosis and of trypanosomiasis, resist super-infections but continue to be a reservoir of virus. The same expression "dirty" is used, says E. Martini, for the dogs which have *recovered* from piroplasmosis (Martini, 1952).

A. Le Dantec uses, with reference to malaria, the term "allergy" to explain the idea of immunity. This denomination cannot be retained, since it has been created by Pirquet and continues to be employed by writers to define the following phenomenon: An organism which has received a foreign substance, living or not, "reaching otherwise" to the first penetration of this substance and to a subsequent penetration. The change of reaction is more or less due to the former. This can be either a greater sensitiveness to a second introduction of the substance, in the case of anaphylaxis, or, on the contrary, a reduced reaction, in the case of relative immunity (tuberculosis, brucellosis). The term "allergy" cannot, therefore, be used to describe relative immunity itself (Sergent, Ed., 1929).

VI. PREMUNITION.

The different expressions used for describing a non-sterilizing immunity, correlative of a latent infection, have a disadvantage of giving rise to a confusion with real immunity and serving very badly the requirements of every day usage. Can it be said, for example, of a bovine chronically infected with piroplasmosis that it is "relatively immunized" or "tolerantly immunized"? Can it be said of an anti-tubercular vaccine, like the B.C.G. of Calmette and Guerin, that it immunizes, "tolerantly" or "relatively"? It would be either to commit a frightful barbarism or throw a kind of suspicion on the protective value of B.C.G., through a fault of an ambiguous adverb. Moreover, when one speaks of tolerance, and expresses only a part of the phenomenon, the habit of the infective organism, one suppresses the other essential characteristics, that is, the resistance to super-infections. As we have said in 1927, in order that science remains a "well-qualified language", and for reasons of convenience, it is necessary to signify by a single and clear term, exact and precise, the complex idea of "relative immunity" and to indicate the differences, which are considerably all told, which separate it from the immunity properly called "sterilizing" (Segrent, Ed., 1905).

That is why, based on our researches concerning malaria, piroplasmosis, trypanosomiasis, brucellosis *B. melitensis*, spirochetosis, we proposed, in 1924, with our colleagues L. Parrot and A. Donatien, the term "premunition" to define the kind of immunity which is associated with a state of latent infection (Sergent, Parrot and Donatien, 1924; Sergent, Parrot, Donatien and Lestoquard, 1931; Sergent, Ed., 1936:1937*a*). "*Premunir*" (to premunish) means "*munir par precaution, fortifier d'advance, metre en garde contre*" (to fortify by precaution, to fortify in advance, to put and guard against"), according to its etymology: *prae*, before, *munire* (from *moenia*, walls of defence). There is without doubt a relationship, according to M. Breal and A Bailly† between the words *munus*, obligation (from

*Edm. Sergent, L. Parrot, A. Donatien and F. Lestoquard (1927), *Arch. Inst. Pasteur d'Algerie*, **5**, 4, pp. 469-474.

†*Dict. Etymologique Latin*, 6th Edition (1906), Hechette Publications, Paris.

which comes *immunitās*, exemption from obligation), and *moenia* (from which comes *premunir*, i.e., to strengthen beforehand). L. Cledat* indicated that the Latin *munire*, is related to *murus*, wall. The common Latin root being *moi*, wall, according to R. Grandsaignes d'Hauterive.†

From the point of view of vocabulary, the word "premunition" is the technical term of the state corresponding to the word "immunity"; the same word "premunition" is also the name indicating the function corresponding to the word "immunization". The verb "premunish" corresponds to the verb "immunise". It is said of a virus vaccine that it is "premunishing" or "premunitive", as is said of a vaccine that it is immunizing (Sergent, Ed., 1935).

Since 1924, our master, Dr. E. Roux, has approved immediately the term "premunition". He has seen some clear examples of it during his researches with E. Metchnikoff on syphilis inoculated to anthropoids. The non-living vaccine did not confer any immunity and the attenuated virus vaccines procured a certain resistance.

A. Calmette writes: "We have voluntarily adopted (since 1924) the word "premunition" to describe more explicitly the special form of immunity connected with the persistence of living germs in the organs of the immunized host (Calmette, 1936).

Similary, H. Vallee has employed since the beginning the term premunition (Vallee *et al.*, 1946).

The word "premunition" is being used more and more in foreign countries as in France§.

E. Martini has used since 1935 the words "*praemunition*" and "*præmunieren*" to describe the *immunitas non sterilisans* of Ehrlich (Martini, 1952). "*Praemunition*" and "*praemunieren*" are the terms which he employed in a lecture delivered in 1941 to the Military Academy of Medicine (Martini, 1941). Since then he has written "*praemunition*" and "*praemunieren*". It is the spelling adopted by German authors, such as Franz Doflein and Eduard Reichenow (Doflein and Reichenow, 1949). See also Pflugfelder (1950) and our note (Sergent, Et. and Sergent, Ed., 1921*c*). In English, it is written "premunition" and "to premunish" (for example, Swellengrebel, 1940); in Italian, "premunizione"; in Spanish, "premunicion"; in Portuguese, "premunicao"; in Czechoslovakian, "premunice" (Sergent, Ed., 1950).

It is better to state precisely that a parasite which lives in a latent state in its host, which is in the metacritic period, provokes, only by its presence, the main-

**Dict. Etymologique de la Langue Francaise*, Hachette Publications, Paris.

†*Dict. des Racines des Langues Europeenes* (1949), Larousse Publications, Paris.

§In 1935, Charles Nicolle tried, without success, to put into circulation two barbarisms he had coined: "*premunite*" and "*premunisation*" (premunity and premunisation), to which he had given the same definition as the definition of "premunition" which he had proposed eleven years before, in 1924. (Charles Nicolle.—*Responsibilities de la Medecine*, 1935, p. 111, F. Alcan Publishers, Pais; *Rev. d'Immunol.*, **1**, 3, May 1935, p. 269; *Arch. Inst. Pasteur Tunis*, **24**, 3-4, June 1935, p. 513).

tenance of measures of defence against the possible aggression of another similar parasite. It is inoculated, to a host with a latent previous infection, of a virulent microbe of the same species, this virulent microbe does not re-infect during latency. The host, already immunized by the latent parasite, overcomes the aggression. We have expressed in 1934 this idea in writing, " *la place reste au premier occupant* ". " This is what could be called the law of precedence " (Sergent, Ed., 1934:1949; Sergent, Ed., Sergent, Et. and Catanei, A., 1934; Sergent, Ed., Parrot, L. and Donatien, A., 1935; Sergent, Ed. and Parrot, L., 1935*a*) (Pampana, 1944).

There are some degrees in the extent of acquired immunity. In this regard, three categories of premunition can be distinguished: it can be racial, specific or generic. Premunition is said to be racial or homologous when a microbial strain confers very solid resistance only against itself. It is called specific or heterologous when a microbial strain confers also a resistance against other strains of the same species. Laveran and Mesnil have based on this idea of the specificity of the resistance to the reinoculations, their methods of identification of trypanosomes through " crossed inoculations " (Sergent, Ed. and Parrot, L., 1935*a*). A strain is often seen eliciting complete protection against itself (strain specific premunition) and eliciting protection also, but less strongly, against other strains of the same species (specific premunition). For example, premunition acquired against a given strain of *P. vivax* does not always entirely protect the individual premunished against other strains of *P. vivax* (Sergent, Ed. and Parrot, L., 1935*a*); the premunition acquired against a strain of trypanosome is least marked against another strain of the same species (Sergent, Ed. and Parrot, L., 1935*a*). Finally, there is a generic premunition, that is to say, a resistance conferred by one species against another species of the same genus. For example, *Anaplasma central*, a species of low virulence, which has been isolated by Arnold Theiler from a South African bovine host, premunishes not only against itself but also against another species of the same genus, *Anaplasma marginal*, which is extremely virulent.

Can the infectious diseases which have provoked, during a phase of chronic infection, the phenomena of premunition, confer sometimes to the organism a real immunity after recovery? (Sergent, Ed. and Parrot, 1935*a*).

Sinton has employed the term " residual immunity " to describe the immunity which, in his opinion, can persist for a variable time after the disappearance of the parasite from the host (Commission du Paludisme de la Societe des Nations, 1940; Sinton, 1938).

Louis Parrot thinks that the existence of this residual immunity is not proved and that one can also think of a residual premunition. In effect, he writes:

" The peremptory demonstration of the existence of premunition in a subject which resists a re-inoculation, results from the disclosure of the malaria infection which determines it. But it happens that for want of opportune employment of all the laboratory methods (direct microscopic examination of blood, splenectomy, isodiagnostic procedure, tests of infection and of re-infection) necessary for revealing it, the latent infection passses unnoticed. One can thus believe that the infected subject has completely recovered and, if he resists further a homologous re-inoculation, concludes erroneously that the premunition has succeeded in it a state of properly called immunity..... In fact, no investigator has still brought, to our knowledge, the irrefutable proof of a real secondary or " residual " immunity in malaria, according to the expression of J. A. Sinton (Sinton, 1938), following premunition. Moreover, this supposed secondary immunity lasts for a short period—generally a few months, it is said—in contrast with real immunity known to be of long persistence. In admitting that it exists, that is to say, that a short period of resistance without active infection takes place between the microbiological recovery of the infective subject and the return of its sensibility to homologous re-inoculations, one is inclined to think that it corresponds simply to the time necessary for the organism to

eliminate, through the role played by phagocytosis and the losses, cytoplasmic, nuclear and pigmentary, of a parasitism recently disappeared. And since the refractory state depends in such a case on the actual presence of antigenic elements in the body of the host, it is still a question of premunition—of a residual premunition" (Parrot, 1955).

Etienne Sergent has shown that, in the *P. relictum* malaria of sparrows, the parasites, which provoke the premunition in the vertebrate host, do not confer any premunition in the invertebrate host: a malarial infection, which progresses to the sporozoite stage in a mosquito, does not prevent the normal evolution, in the same mosquito, of other gametocytes, freshly ingested, at least 16 days later. The infected *Culex* mosquitoes are not premunished against a fresh infection from the ingested parasites four months later. The *Culex* mosquitoes can harbour even three broods of *P. relictum* of different ages at the same time (Sergent, Et., 1940).

If an overall view of the problem of anti-infectious immunity is taken, it is proved that the nature of immunity, either sterilizing or non-sterilizing, conferred by an attack of infectious disease, depends on the specific character of the pathogenic microbes: their compatibility with the host which they attack. The life of certain pathogenic microbes (for example those which cause the following diseases: measles, scarlet fever, small pox, chicken pox, whooping cough, diphtheria and anthrax) is incompatible with the life of the infected host; they kill or they are killed. The complete and rapid victory of the organism is the fruit of the powerful effort of the means of defence, which result in an intense production of antibodies. The antibodies, soluble in the organic fluids, spread themselves in the entire body. The modification [*Umstimmung* of the Germans (Schlossberger, 1929)] which the body has undergone is such, in this category of infections, that it continues, for a more or less long time, to elaborate the antibodies; it thus remains durable against any new aggression of microbes of the same species: it is the " active immunity ", which is above all humoral in quality. " The active immunity is only the continuation of the processes of recovery ", writes Jules Bordet.

After recovery, there is thus, for some time, overproduction by the host of antibodies which remain unused. The blood of the convalescents or, better, the serum of the animals hyper-immunized in the laboratory, injected to a patient attacked with the same disease, brings to his means of defence the reinforcement of antibodies all prepared. This force of resistance is called " passive immunity ". It is on the basis of serotherapy (Sergent, Ed., 1948).

When the host triumphs, recovery follows immediately after the termination of the acute attack; there is no latent metacritic infection.

On the contrary, the life of other pathogenic microbes (for example those that cause malaria, syphilis, tuberculosis, brucellosis, spirochætosis, trypanosomiasis, piroplasmosis) is compatible with that of the attacked organism: the attack of first invasion, which does not kill the host, results in a sort of compromise, of " *modus vivendi* ", between the microbe and its host, which happen to tolerate each other (relative immunity). A vital equilibrium is established between the microbe and its host. The parasitism then becomes a sort of symbiosis, the parasite survives in a moderated form; the latent metacritic infection is installed and ends only after a long time.

There is necessity to point out two inconveniences of latent metacritic infections: it can revive when the host is enfeebled and causes relapses; moreover, the

carriers of latent germs constitute a reservoir of virus which can be the point of departure, often misunderstood, of epidemics or of epizooites.

As regards the host himself, the recovery, not being the consequence of a prompt and energetic reaction of the organism, is not accompanied by the formation of abundant antibodies. There is no efficacious serotherapy*. When the latent metacritic infection terminates, when the host is definitely deparasitized, the relative immunity disappears: the host becomes again susceptible, and a re-inoculation can provoke a re-infection.

Briefly, relative immunity is a co-infective refractory state and real immunity a post-infective refractory state†.

In conclusion, distinctive characters of sterilizing true immunity and of premunition or relative immunity, can be summed up in the following tabular statement (Sergent, Ed., Sergent, Et. and Catanei, A., 1934).—

True immunity.	Premunition.
Complete resistance acquired by a host which, clinically cured, is, at the same time, deparasitized. Prevents re-infections.	Relative resistance acquired by a host which, clinically cured, remains a carrier of latent germs. Prevents superinfections even though the host remains a carrier of germs, but does not prevent re-infections from the time it ceases to be a carrier.
Survives the disappearance of the parasites.	Correlative to the visible or latent presence of parasites.
Establishes itself after recovery.	Ceases with recovery.
Is conferred by killed vaccines.	Is conferred by living virus vaccines.
Is accompanied by the formation of abundant antibodies after recovery. Hence possibility of serotherapy.	Does not form abundant antibodies after recovery. No serotherapy with the serum of the recovered.
Conferred by acute diseases.	Conferred by chronic infections.

We have thus proposed, with Parrot and Donatien, the following terms:—

Name of state.	IMMUNITY.	PREMUNITION.
	Post-infectious refractory state.	Co-infectious refractory state.
Name of action	IMMUNIZATION. Act by which the host is put in a state of resistance to a reinfection, as if it had recovered from a spontaneous attack of the concerned disease.	PREMUNITION. Act by which resistance to superinfection is obtained, through a chronic infection provoked, and well tolerated.
	TO IMMUNIZE. IMMUNIZED. IMMUNIZING.	TO PREMUNISH. PREMUNISHED. PREMUNISHING OR PREMUNITIVE.

*There still exists a third category of infectious diseases: those which neither confer immunity nor premunition. They are due to pyogenic bacteria: for example, pneumococcus, streptococcus, staphylococcus, gonococcus.

†Sergent, Ed., Sergent, Et., Parrot, L. and Donatien, A.—*Trans. Roy. Soc. Trop. Med. Hyg.*, **27**, 3, (November 1933), pp. 227-280.

In short, premunition (or relative immunity) has the following essential characteristics:—

—Tolerance of the infected host with regard to the infecting virus (tolerance manifesting itself by a state of chronic latent infection, which is not necessarily a morbid state);

—Resistance of this infected host to any new infection by the same virus (resistance preventing superinfections during latent infection, but not the re-infections after recovery from latent infection).

We will repeat with L. Parrot: " In order to give a concrete example of the fundamental differences which separate premunition from true immunity, it can be stated: one no more contracts scarlet fever—a disease conferring true immunity—when one has had it; one no more contracts malaria—disease of premunition—when one has it and as long as one has it*.

VII. VACCINATION AGAINST DISEASES OF PREMUNITION.

We have seen at the beginning that, the techniques of discovery of latent infections, in the hosts having survived the first attack of an infectious disease, are being progressively perfected. One is no more satisfied with the microscopic finding of the parasite in the blood or the organs. Some experimental inoculations are added to it: on the one hand, inoculation to a new susceptible animal of the largest possible quantity of blood and of portions of organs of the subject under observation—that is to say, the isodiagnostic or proof of infection; on the other hand, inoculation to the host under observation of microbes of the same species which has caused the primary infection, that is to say, the proof of immunity (Sergent, Et. and Sergent, Ed., 1921*b*:1921*c*).

The existence of latent infection periods, conferring premunition, is discovered, in an ever growing number of infectious diseases.

Can the Pasteurian methods of vaccination be utilized for the prophylaxis of diseases producing premunition? When it is a question of infections entering the category of diseases producing premunition, to vaccinate, is, by definition, to give a latent infection. To describe the protective vaccine, the inoculation of which will create a tolerated parasitism, guaranteeing a permanent defence against an abrupt attack of a parasite coming from outside, the Pasteurian term of virus-vaccine is more suitable. In a note on the attenuation of virus, Pasteur wrote, in 1881: " To seek to lessen the virulence through rational means, is to base on the experimentation the hope of preparing with active virus, easy to culture in the body of man or of animals, virus vaccines of restrained development, capable of preventing the deadly effects of the active virus " (Pasteur, 1881*a*). The term " vaccine " is reserved for the preparations the inoculation of which gives true sterilizing immunity.

The first characteristic of a virus vaccine is of its being a living virus, since it must create carriers of germs, as well as sterilizing immunity, which characterizes the other category of infectious diseases, and can be caused by the inoculation of killed microbes or of anatoxins (Sergent, Ed., 1929).

Arch. Inst. Pasteur d'Algerie*, **21, 2, June, 1934, p. 265.

The question that arises is of taking the minimum risks for the host to whom a living virus vaccine is inoculated. The object is consequently to secure for him an immediately latent infection which will save him from the dangerous stage of acute attack of first invasion. This will be "Acclimatization without risks".

The immediately premunition can be obtained by different procedures.

We will pass in review, in chronological order, the experimental findings of protective virus vaccines, known up to the present time.

Malaria.—Sporozoites, extracted from salivary glands of mosquitoes and grown old *in vitro*, have constituted a protective attenuated virus. Inoculated to canaries, they have given them a latent *P. relictum* infection which has protected from commencement (Sergent, Ed. and Sergent, Et., 1910).

The same protective vaccination has been obtained (1923) by the inoculation to birds of a virus-vaccine, composed of a small number of living sporozoites raised previously, at the ultimate period of sporogony, in the salivary glands and in the rest of the body of the *Culex*. The optimum dose consisted of between 3/4th and 2/3rd of the ground bodies of *Culex* mosquitoes normally infected (Sergent, Et. and Sergent, Ed., 1910; Sergent, Ed. and Sergent, Et., 1918:1921; Sergent Et., Sergent, Ed. and Catanei, A., 1923*a*:1923*b*).

Previously raised blood of canaries during incubation (procritic latent stage) has conferred to canaries an immediately premunition (Sergent, Et. and Sergent, Ed., 1921*a*).

If one compares between two kinds of anti-malarial vaccines under consideration, prepared against a microbe which undergoes a double evolution, *i.e.*, (1) sporogonic in mosquitoes, and (2) schizogonic in bird, it is established that better results have been obtained with the sporozoites previously raised in the host of the invertebrate than with the schizonts put into the blood of the vertebrate.

A quite different behaviour has been published, in February 1921, by Edmond Sergent and Etienne Sergent. Their experimental study of avian malaria has shown them that quinine and its derivatives acted on *P. relictum* as in human malaria, and they set up the method of experiment, on malaria of canaries, synthetic antimalarials, which have been utilized later by Schuleman for his discovery of Atebrin and Plasmoquine. During the course of these experiments of remedies, they established that one could obtain the protective vaccination of canaries against *P. relictum* by means of attenuated virus through a non-sterilizing drug therapy. For example:—

In ten birds, the preventive treatment by quinine, given in daily dose of 0·7 mg. during a period equal to that of the acute phase in the control birds (from 10 to 30 days), has prevented the invasion of peripheral blood by the parasites during that period. The blood infection visible to the microscope remains very feeble or nil. In the latter case, it was latent from commencement. The same doses of quinine, injected afterwards every alternate day, have continued to hold in check the virus, even when the bird undergoes several re-inoculations. The relative immunity in the quininized hosts, which makes them resist the super-inoculations, was present as long as they took quinine. When the treatment was stopped, the infection took the upper hand and susceptibility returned.

On the contrary, the non-quininized controls presented an acute infection, during the course of which 30 per cent of them succumbed. The parasitic invasion of their peripheral blood was intense from 9th to 14th day after the inoculation.

The advantage of this procedure of vaccination by an attenuated virus, thanks to a non-sterilizing treatment, is thus for the individual, a premunition, which is often established from commencement. The whole advantage consists in that the treated birds, in not having any latent infection, will not also be dangerous reservoirs of virus unlike the untreated controls, to intense blood infections (Sergent, Et. and Sergent, Ed. 1921*b*: 1921*c*).

The experimental findings on *P. relictum* malaria show finally that the immediately latent infections give a premunition as solid as the latent infections following an acute attack (Sergent, Ed., Sergent, Et., and Catanei, A., 1934).

As regards human malaria, A. Plehn has remarked, in 1901, during his clinical study of malaria in the Blacks in certain parts of Cameroon, where the immigrant Europeans who take quinine regularly in prophylactic doses, acquired a premunition which appears similar to that which the natives naturally presented (Plehn, 1901). Much later, in 1933, S. P. James, based on clinical observations similar to those of Plehn, had adopted through the Malaria Commission of the League of Nations the following proposal: It is preferable not to institute, in subjects destined to live in a country endemic to malaria, an energetic treatment capable of deparasitizing the patient completely. In fact it is desirable that, clinically recovered, these patients retain a protecting latent infection. The Malaria Commission concluded thus: " It would be extremely unwise, in certain malarious countries (Dark Africa for example), to intervene very radically in the natural processes, thanks to which the natives are immunized against the disease ". The Malaria Commission " recommends to the malarious countries to restrain themselves, except the collective places under special conditions, to base their medical and sanitary services on the principle that, in matter of fight against malaria, it is necessary to aim at attenuating the seriousness of the disease and to reduce mortality rather than to have recourse to radical measures which are imposed when the complete elimination of the parasite in a given region is sought " (Commission du Paludisme de la Societe des Nations, 1933).

This technique consists, in short, of using a protective vaccination by means of an attenuated virus through a non-sterilizing drug cure, a procedure the efficacy of which has been demonstrated by experiments on *P. relictum* malaria.

Tuberculosis.—The experiments of Calmette and Guerin, commenced in 1906 on bovines, demonstrated for the first time that a single mild bacillary infection determines in general an infection which remains benign and confers a manifest resistance to subsequent re-infections. Calmette arrived at this conclusion that, the durable tolerance, of cattle facing the tuberculosis infection, is a function of the presence in the organism of the animals of living bacilli (Calmette, 1936). At the same time he verified the fact, already established by different investigators, that, tuberculous bacilli which are dead or the protoplasm of which has undergone profound alteration, have only a very feeble or no vaccinating power: They are not, therefore, utilizable in practice for the protective anti-tuberculosis vaccination (Calmette, 1911). Calmette succeeded later, with his collaborator, C. Guerin, in attenuating the virulence of bacilli of bovine strain by cultivating them for 13 years continuously in a culture medium constituted essentially from the bile of ox. These bilious bacilli had lost all pathogenic power, and gave

from the outset a protection against infection by pathogenic bacilli. The application to some human beings of the protective vaccination through the bilious virus-vaccine of Calmette and Guerin (B.C.G. vaccine), generally applied in France and in Algeria since 1924, has responded magnificiently according to expected results.

Syphilis.—P. Ricord has already stated, in 1863, that syphilis "does not double itself" (Ricord, 1863). The critical proof of the existence of a premunition in syphilis has been brought home by the experimental researches of E. Metchnikoff and E. Roux, undertaken in Paris, on anthropoid monkeys. They showed that the killed treponemes conferred no resistance to the monkeys, but that the living microbes protected them against virulent inoculation (Metchnikoff and Roux, 1904:1905:1906).

E. Metchnikoff and E. Roux have proved that one could attenuate the syphilis virus by making it undergo several passages through the bodies of inferior monkeys (*Rhesus*). They have found also an attenuated virus in the human beings, probably contaminated by monkeys. Same experimental results have been confirmed in syphilis by their pupil Paul Salmon (Salmon, 1907) and in anthropoids by A. Neisser (Neisser, 1904).

Since long, same conclusions have been drawn from numerous experiments on syphilis inoculated to rabbits, and to white mice. Uniform views, on premunition in syphilis, are explained by J. Bordet (Bordet, 1927:1934) and C. Bruck (Bruck, 1930).

Brucellosis.—The veterinarians knew for a long time that the first abortion caused by Bang's bacillus in cattle protected them from subsequent abortions. Th. Zammit and Edmond Sergent had observed that same is the case in goats, reservoir of virus of undulant fever. The bacteriologists have thus sought to vaccinate cows and goats against *Brucella abortus* and *B. melitensis*. The vaccines killed by ether or by other processes are found to be ineffective. Living vaccines, attenuated by different methods, are employed. Zammit, for attenuating a strain of *B. abortus*, treated it by a filtrate of a one-month-old culture and passed through Chamberland filter (1932). W. E. Cotton, J. N. Buck and H. E. Smith have been experimenting since 1915 on avirulent living vaccines, the first of which was numbered S. 19. M. Lisbonne and G. Roman have been preparing with the avirulent Strain 112 of *B. abortus*, a vaccine which they have perfected during 1939 to 1943.

Recurrent fevers.—The spirochætes, which pass through invisible phases in man and in vector insects (Sergent, Ed. and Foley, H., 1908:1910:1914*a*:1914*b*: 1914*c*:1939; Sergent, Ed., Gillot, V. and Voley, H., 1911*a*:1911*b*), show long periods of metacritic latency (Catanei, 1923; Sergent, Andre, 1938*b*:1942; Sergent, Ed., 1945). The recurrent fevers have not given place to the use of protective vaccines. They recall to mind, the big deadly epidemics which broke out, like those which ravaged Eastern Europe after the First World War. Edmond Sergent, V. Gillot and H. Foley have shown that "monkeys, infected with spirochætosis, and treated by arsenobensol, present an immunity very early, against a latter virulent inoculation (Sergent, Ed., Gillot, V. and Foley, G., 1911*a*).

Trypanosomiasis.—Laveran and Mesnil wrote in 1912: "There is place to distinguish between the clinical recovery (return of the animal to health) and

microbiological recovery, which consists in the complete disappearance of the invading microbe. The animal can appear recovered or cured when the microbes persist in the host. There is then the state of mutual tolerance between the host and the parasite ". The authors do not cite experiments of vaccination (Laveran and Mesnil, 1912).

In 1921, Edmond Sergent and Etienne Sergent proposed a method of protective vaccination, by means of an attenuated virus by a non-sterilizing drug cure, in camels of the mounted troops of Sahara, against the "*debab*", trypanosomiasis which is the principal disease of the North African camels. One could inoculate small doses of virus to grazing animals, in whom they remain for some weeks or some months, and avert the acute attack by an appropriate medication, for example with an emetic containing atoxyl,—in a manner to confer premunition from commencement, without risks (Sergent, Ed., 1949).

Piroplasmosis.—*Piroplasmosis* of different animal species offers remarkable types of premunition diseases, presenting a good deal of resemblance with malaria. They constitute a wide field of application of protective vaccinations (Sergent, Ed., Donatien, A., Parrot, L. and Lestoquard, F., 1945).

In the course of their deep researches on *Piroplasmosis* of North African cattle (1912-1945), Sergent, Donatien, Parrot and Lestoquard used, for vaccination against *Piroplasma bigeminum* and against *Babesiellosis*, small doses of virus vaccines the virulence of which is measured beforehand. Their protecting vaccine against *Theileriosis* in bovines (*Theileria dispar*) of the Mediterranean Basin presents a particular characteristic. It is a strain, called "Kouba", which is naturally very benign, from the time of its isolation, when *Theileriosis* is remarkable for its usual seriousness. The "Kouba" strain has, therefore, been adopted as the virus-vaccine. Another anti-piroplasmic virus-vaccine presents the characteristic of conferring a generic premunition, and not only specific premunition. It is *Anaplasma centrale* isolated in Nature by Arnold Theiler, which is avirulent from commencement and which acts as a vaccine against another species of the same genus, *Anaplasma marginale*, a highly pathogenic microbe. This strain of *Anaplasma centrale* is preserved at the Pasteur Institute of Algeria, in conformity with the wishes of Arnold Theiler, and is kept at the disposal of research workers or administrations which may need it.

Typhus exanthematic.—The hypothesis which H. Zinsser gave out in 1934 for explaining the Brill disease, of a very long latent infection of typhus, is generally admitted. As shown by A. Donatien and F. Lestoquard for the rickettsiosis of animals, the typhus and other human rickettsiosis are the diseases having long latent infection and premunition. See, on this subject, the thesis of G. Parrot, 1937 (*Arch. Inst. Pasteur d'Algerie*, **15**, June 1937, pp. 188-213).

H. Zinsser prepared, in 1930, with Castaneda, during experiments on guinea pigs, an efficacious vaccine against typhus (European strain), by inoculating to them a vaccine composed of *Rickettsia* killed by formol, obtained from tissues of infected guinea-pigs (Zinsser and Castaneda, 1931).

G. Blanc, M. Noury and M. Baltazard prepared in 1935 a protective living anti-typhus virus-vaccine, attenuated by bile, constituted by a suspension of

Rickettsia obtained from organs of guinea-pigs infected with murin typhus (Blanc, Noury and Baltazard, 1935:1936).

Since 1938, they are delivering a bilious virus-vaccine prepared from fleas infected by rats with murin typhus (Blanc and Baltazard, 1941).

Paul Durand and Paul Giroud are preparing, since 1939-40, a non-living anti-typhus vaccine with a suspension of *Rickettsia* killed by formol obtained from the lungs of animals infected through the respiratory passage with exanthematic typhus (Durand and Giroud, 1940).

H. R. Cox, whose first attempts date back to 1938-39, has also produced a non-living anti-typhus vaccine, constituted by a suspension of *Rickettsia* cultivated in embryo of chicken and killed by phenol and formol (Cox, 1941).

The non-living Durand-Giroud vaccine and the non-living Cox vaccine have the common characteristic of conferring a real resistance, but of short duration; about six months in man. This brevity of acquired resistance comes, no doubt, when the typhus is not capable of producing a sterilizing immunity, but of premunition. Louis Parrot explained, through his hypothesis of residual premunition (Parrot, 1955) the fact that a non-living vaccine can confer a state of resistance of short duration against typhus. After inoculation of the non-living anti-typhus vaccine, a certain time is necessary for the host to re-absorb the balance of existing antigens in the killed *Rickettsia*. The reaction of the host of this residue of antigens places in it a state of premunition. It can be supposed that in contaminated environment, hosts vaccinated by non-living vaccines which, due to this fact, resist for some months natural contagions,contract during this period of time, in Nature, a latent infection which procures them a real premunition.

Ultravirus.—Vaccination against rabies, the first viral disease to be known, offers the only example of a vaccine which is inoculated during the incubation of the disease which threatens the host, that is to say, during the procritic latent infection. The virus-vaccine, in the original method of Pasteur and his collaborators Roux, Chamberland and Theillier, was the spinal marrow of the rabbits infected by rabies, the virus of which was attenuated by desiccation (Pasteur, 1881*c*:1933). Other virus-vaccines are prepared from brains treated by antiseptics, in particular by carbolic acid.

The works of numerous investigators have made known, since several years, a large number of diseases of man, animals insects and vegetables, due to virus. These investigations have also brought to light more and more existence, in these virus diseases, of stages of prolonged latent infection coinciding with a stage of resistance, of premunition—herpes, influenza, poliomyelitis, psittacosis, etc. Some experiments of vaccination are in progress in several countries against poliomyelitis, either with living virus vaccines (G. Blanc), or with non-living vaccines (Salk, P. Lepine). Joseph Fortner and Pfaffenberg have been able to vaccinate some mice against psittacosis with a living virus-vaccine, which sometimes gives an immediately latent infection; they have seen that a vaccine in which the virus was killed by formol, conferred only a very feeble resistance.

The subjects premunished by a virus-vaccine which give them a latent infection are, by definition, carriers of germs, reservoir of virus. They can, therefore, under certain circumstances, be the cause of epidemics or epizootics. The ideal is

consequently to prepare virus-vaccines, which, though living ones, are not contagious under natural conditions. This ideal can be realized in several ways. Thus the B.C.G., the proof of it is seen, is an attenuated strain by a definite method, and cannot become virulent. Another example, is the hæmocytozoon *Theileria dispar*, agent of the most deadly of cattle piroplasmosis in the sub-tropics, which is transmitted by the bite of the tick *Hyalomma mauritanicum*, in which is effected the sexual development of the parasite. But we have isolated strains from cases of natural infection, which after several passages from bovine to bovine effected by inoculation of blood from sick animals, have lost the property of producing gametocytes. They do not perpetuate themselves any more than by schizogony in the blood of the successively inoculated hosts. Consequently, these strains constitute obvious virus-vaccines which procure premunition in animals which are carriers of schizonts, but not of gametocytes, and, therefore, are not capable of infecting the ticks (Sergent, Ed., Donatien, A., Parrot L. and Lestoquard, F., 1945).

It is observed in the premunished subjects that when infection comes afresh, all the intermediate stages between a fresh attack and " latency from commencement " of the infection take place. Ordinarily, the reaction caused by the superinfection is benign and marked by the suppression of clinical attack, particularly of the febrile attack.* One can know the parasitic attack only if it is revealed by microscopy, or by culture methods, or by experimental inoculation to new and receptive hosts. If we do not utilize these laboratory techniques, the parasitic attack would pass unnoticed. The perfection of techniques of examination reduces progressively the threshold below which the latent infection remains occult.

We designate: *accès de prémunis* (attack among premunished subjects) as averted attacks which a new infection provokes in the hosts vaccinated by a first attack or by a virus-vaccine (Sergent, Ed., 1937*b*; Sergent, Ed. and Parrot, L., 1938).

It is permissible to think that, according to the cases, very feeble and very short attack which a new microbial infection can provoke in a premunished subject, is due either to the awakening of latent parasites owing to disturbances caused by re-inoculation, or to the moderate and transitory multiplication of the aggressor parasites held in check.

In brief, the essential character of the attack among premunished subjects is to be less serious than the attack of primary infection. Even if the refractory state to superinfections conferred by the protective vaccination is not absolute, but only relative, the result of the vaccination is not less useful.

Pasteur has written, in April 1881: " To preserve from attacks of virulent diseases, it is not indispensable to place economy in conditions of absolute immunity, but only relative " (Pasteur, 1881*b*).

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* In the recurrent fever, the attack of the premunished subjects which were infected afresh present a special aspect: the relapses, which constitute the essential characteristic of the disease, are lacking. Edmond Sergent and H. Foley have observed that six individuals who had been attacked in 1908 with recurrent fever, have shown, six months later, a recurrence of the disease during new epidemics. These recurrences have assumed a particular clinical type, characterized by the appearance of only one attack without relapse, when in the first attacks of recurrent fevers the acute attack is usually followed by one, two, three or exceptionally four relapses (Sergent, Ed. and Foley, H., 1914*b*; Sergent, Ed., 1934-1949).

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OBITUARY.

JOHN ALEXANDER SINTON, V.C., O.B.E., M.D., D.SC., F.R.S.

THE Indian malariologists, more particularly the staff of the Malaria Institute of India, express their profound sorrow on the sad demise of Brigadier J. A. Sinton, the first Director of the Malaria Institute of India and the Founder Editor of the *Records of the Malaria Survey of India*, now *Indian Journal of Malariology*, following so close upon the award of Manson Medal by the Royal Society of Tropical Medicine and Hygiene for his contributions towards the building of the Institute in the early stages, researches in malariology and founding of the *Records*.

The passing away of Brigadier Sinton, especially at a time when the long and arduous research work on malaria in India to which he had made such marvellous contribution has begun to bear fruit and the sapling of Malaria Institute of India which he had laid with his hands has grown up to be a magnificent tree bearing the huge burden of a National Malaria Control Programme, adds great poignancy to the sad event. His demise has left a very wide gap in the field of malariology which it may take long to fill in. His tireless energy, unstinted devotion to the cause of medical science and able guidance even after retirement, have been a source of great inspiration to all workers, especially those who had the privilege of working with him some time or other.

Brigadier Sinton associated himself with malaria research in India soon after the conclusion of World War I and made very valuable and voluminous contributions to the malaria literature during the 15 years he was in India. His last article "Some lacunae in our knowledge of the malaria parasite" published in the Silver Jubilee Number of *Indian Journal of Malariology* last Decemeber, is an index of his dedication to the subject even at that age.

The following appreciation by Sir Gordon Covell, who had succeeded Brigadier J. A. Sinton as Director, Malaria Survey of India (now known as Malaria Institute of India), appeared in the *British Medical Journal* dated April 7, 1956:—

"Brigadier J. A. Sinton who had the unique distinction of being the only holder of the Victoria Cross who was also a Fellow of the Royal Society, died on March 25 at his home at Cookstown, Northern Ireland, at the age of 71.

John Alexander Sinton was born on December 2, 1884, in British Columbia. His parents came from Ulster and he was educated at the Royal Belfast Academical Institution and at Queen's College, Belfast, where he was an exhibitioner. He

graduated M.B., Ch.B., with first-class honours at the Royal University of Ireland in 1908, and after holding house appointments at the Royal Victoria Hospital at Belfast he became Riddell demonstrator in pathology at Queen's University and clinical pathologist to the Ulster Eye, Throat, and Ear Hospital and to the Mater Infirmorum Hospital. In 1911 he took the Diploma of Tropical Medicine at Liverpool and entered the Indian Medical Service in the same year, taking the first place in the entrance examination. During the first world war he served in Mesopotamia with an Indian cavalry regiment and was awarded the Victoria Cross for most conspicuous bravery and devotion to duty during an action at Sheikh Sa'ed in 1916. Although shot through both arms and through the side he refused to go to hospital and remained as long as daylight lasted attending to his duties under very heavy fire. The citation records that in three previous actions he had displayed the utmost bravery. He was also mentioned in dispatches on four occasions and awarded the Russian Order of St. George. In 1919, Queen's University of Belfast conferred the honorary degree of M.D. on him in recognition of his early academic distinctions and of his valour in the field. Sinton was promoted brevet major in 1919, and after the armistice he saw further active service in Afghanistan and Waziristan, being again mentioned twice in dispatches and appointed O.B.E. in 1921.

On reversion to civil employment in 1921, Sinton entered the Medical Research Department of the Indian Medical Service. He was in charge of the Quinine and Malaria Inquiry from 1921 to 1930 and was Director of the Malaria Survey of India from its foundation in 1927 until 1936. During this period he was a member of the Malaria Commission of the League of Nations and made several epidemiological surveys on its behalf. When he returned to England in 1937, he became Manson Fellow of the London School of Hygiene and Tropical Medecine and Adviser on Malaria to the Ministry of Health, and while holding these posts he took part in researches at the Horton Malaria Laboratory. On the outbreak of the second world war he was recalled to active service and after a brief period in India was successively Consultant Malariologist to the East African Forces, the Middle East Forces, and the War Office. He finally retired towards the end of 1943 with the honorary rank of brigadier and returned to Ulster, where he had an estate at Cookstown. He took an active part in public affairs, being a justice of the peace and High Sheriff for Tyrone in 1953.

Sinton published more than 200 scientific papers, many of them in collaboration with others, but all of them recording studies in which his was the moving spirit as regards design and direction. Most of them dealt with various aspects of malariology—chemotherapy, immunology, parasitology, laboratory and survey techniques, or sociological effects. He also published a series of 36 papers on Indian species of *phlebotomus*, on which he was a leading authority.

His name was first brought into prominence by his chemotherapeutic studies at the Malaria Treatment Centre in Kasauli, where the properties of antimalarial drugs were investigated for the first time on a rational basis. His demonstration that pamaquin combined with quinine brought about a striking reduction in the

relapse rate of *vivax* malaria was a finding of fundamental importance in subsequent researches on the 8-amino-quinolines. His collaboration with H. W. Mulligan in studies on monkey malaria formed the subject of series of important papers dealing with the mechanism of immunity. He wrote also a number of papers on the host-parasite relationships of human and simian malaria, on techniques of examination and enumeration of parasites, on their systematic position, and on their reaction to drugs, as well as miscellaneous papers on other protozoa. His tireless energy is well exemplified in his *Bibliography of Malaria in India*, which contains references to 2,200 publications, and by his well-known work entitled *What Malaria Costs India*, which might indeed have been more appropriately called "What Malaria Costs the World", so comprehensive is its scope and documentation.

It was for his work on malaria and kala-azar that Sinton was elected F.R.S. in 1946, but this was only one of the many honours bestowed on him. He was awarded the Arnott Memorial Medal of the Irish Medical Schools and Graduates Association in 1917; the Chalmers Memorial Medal of the Royal Society of Tropical Medicine and Hygiene in 1928; the Bisset-Hawkins Medal of the Royal College of Physicians of London in 1944; the Robert Campbell Memorial Medal of the Ulster Medical Society (of which he was an honorary fellow) in 1946; and the Mary Kingsley Medal of the Liverpool School of Tropical Medicine in 1949. Only a few days before his death the Royal Society of Tropical Medicine and Hygiene had decided to award him the Manson Medal. In 1927 Queen's University, Belfast, conferred on him the honorary degree of D.Sc., and in 1952 he was elected Pro-Chancellor of the University.

The reputation which the Malaria Survey of India (now the Malaria Institute of India) earned in its early years as a research organization was due chiefly to the able and energetic direction of Sinton, its first Director. Under his leadership it became a scientific and training institution of world-wide repute. He was a source of inspiration to all who came in contact with him, and his personal charm and unfailing kindness will long be remembered by his many friends.

In 1923, he married Eadith Seymour Steuart, only daughter of Mr. E. S. Martin. She survives him, together with a daughter of the marriage".

J.S.

Indian Journal of Malariology, **10**, 2, June 1956.

OBITUARY.

SIR MALCOLM WATSON, M.D., D.P.H., LL.D., F.R.F.P.S.

THE passing away of Sir Malcolm Watson at the age of 82 on December 28, 1955, is deeply mourned by all who knew him and his note-worthy contributions to tropical medicine. He was a pioneer and outstanding exponent of malaria control in many parts of the world.

Sir Malcolm joined the Malayan Medical Service in 1900 at the age of 27. The prospects of prosperity from the new and rapidly expanding rubber industry were being seriously jeopardised by devastating malaria epidemics. Ross had just then demonstrated the transmission of malaria through mosquitoes. Watson in Malaya was one of the earliest pioneers to control malaria by preventing the breeding of mosquitoes. The results of his malaria control will remain classic.

Sir Malcolm had a broad outlook, an adaptability and willingness to discover techniques of civil engineering suited to control mosquito breeding. Within a few years, the malaria peril to expanding rubber industry was brought under control under his supervision. The previously pest-stricken towns of Malaya were transformed to health resorts.

In 1908, Sir Malcolm left Government service and settled in estate practice and became available as a public health consultant in malaria control and hygiene. This was probably the only unique instance of a preventive medicine expert who practised his speciality in private practice. He returned to London from Malaya in 1928. He was the first Director of the Malaria Department of the Ross Institute, and later became the Director of the Institute when it amalgamated with the London School of Hygiene in 1933. In the capacity of the Director of the Department of Tropical Hygiene of the school, he visited India, Ceylon, Belgian Congo, Rhodesia and several countries in Europe and South America and initiated in some of the areas the application of his principles and techniques of malaria control. He retired in 1942. He was a well-known author of several books and many scientific publications and was a member of numerous committees and the recipient of several honours.

In the passing away of Sir Malcolm, malariology has lost a stalwart leader whose memory will continue to inspire posterity.

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STUDIES ON NURI STRAIN OF *P. KNOWLESI*.

Part XI. Comparative studies on quinine and chloroquine administered intravenously.

BY

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[March 15, 1956.]

In an earlier publication, Jaswant Singh, Ray *et al.* (1952) reported that in fairly heavy *P. cynomolgi* infection in *M. mulatta mulatta* (rhesus monkeys), parenteral use of chloroquine showed the best results. Comparison was made against quinine, NAB, quinarsol, and mepacrine, all administered intravenously.

In the present paper, the authors have recorded their observations on the relative merits of quinine and chloroquine, administered parenterally, in *M. mulatta mulatta* suffering from severe infection with a virulent strain of *P. knowlesi*.

MATERIALS AND METHODS.

Host.—Two hundred and one healthy *M. mulatta* weighing between three and six kg. were selected for the investigation.

Parasite.—A newly isolated (Nuri) strain of *P. knowlesi* (Jaswant Singh, Ray and Nair, 1953; Edeson and Davey, 1953) was used for the studies. The strain is extremely virulent, causes a rapidly developing infection which ultimately leads to 100 per cent mortality. Death usually follows between four and six days once patent infection is established. Prior to death, cell infection attained is usually between 70 and 96 per cent. Haemoglobinuria is more frequent at the terminal stage.

Antimalarials.—The antimalarials used were quinine bihydrochloride, chloroquine diphosphate injectible (resochin) and chloroquine sulphate injectible (nivaquine). The dosage regimes adopted ranged from 1·42 to 10 mg. base in the case of chloroquine and 8·6 to 34·4 mg. base in the case of quinine. The recommended doses of these two drugs for human cases of an average weight of 70 kg. correspond to 5·71 and 8·6 mg./kg. respectively.

Route and frequency of administration.—The drugs were injected intravenously and only one such dose was administered to each animal.

Degree of infection.—Treatment was begun at various stages of parasitæmia, indicating the degrees of infection as shown below:—

(I)	Group I	...	Cell infection 10 to 19 per cent (mild).
(II)	Group II	...	,, ,, 20 to 39 per cent (moderate).
(III)	Group III	...	,, ,, 40 to 59 per cent (heavy).
(IV)	Group IV	...	,, ,, 60 to 79 per cent (moderately heavy)
(V)	Group V	...	,, ,, 80 per cent or over (extremely heavy)

The results have been categorized groupwise. Criteria for assessment were based on (*a*) prevention of the death of the animals; (*b*) complete clearance of parasites from the peripheral circulation; (*c*) speed at which such clearance was effected; (*d*) complete sterilization of blood-induced infection.

RESULTS.

Mild infection (10-19 per cent) cell infection.—With 1·42 mg. dosage, resochin proved ineffective whereas in two out of three monkeys, parasite clearance occurred between two and four days when treated with nivaquine. But recrudescences were observed in 2-5 days after initial clearance of parasitæmia in both of them. In the next higher dosage (2.5 mg.), complete parasite clearance was observed in all the three animals under resochin treatment, and in two out of three under nivaquine. However, excluding the one that died during observation period, recrudescences were common in all the remaining four. In doses of 5 and 7·5 mg., parasite clearance occurred in all animals under resochin or nivaquine. However, complete sterilization was not observed in any.

The results observed in the group treated with quinine were inconsistent. Out of 15 animals treated with doses ranging from 8·6 to 34·4 mg., parasite clearance occurred only in two, one under 8·6 mg. and the other under 25·8 mg. regime. Apart from slight deceleration in a few, the course of infection proceeded unhampered in the rest. The results are shown in Table I.

Moderate infection (20 to 39 per cent cell infection).—Under the lowest dosage regime, parasite clearance occurred in one out of three animals in each group treated with resochin or nivaquine, whereas with 2·5 mg., such clearance occurred in two out of three under resochin and none under nivaquine. In these dosages, therefore, the result was somewhat inconsistent. Under the schedules 5 and 7·5 mg., parasite clearance was observed in all the animals. However, recrudescences were frequent except in three out of the total 15 treated with these doses (Table II).

In none of the animals treated with quinine, there was complete clearance from the peripheral circulation.

TABLE I.

Effect of single intravenous administration of resochin, nivaquine and quinine against Nuri strain of P. knowlesi *at* 10 *to* 19 *per cent cell infection.*

Drug.	Dosage mg./kg. (base).	Ineffective.		Effective.			Number of deaths due to toxic effect.
		Number of animals.	Days of death (days).	Number of animals.	Parasite clearance (days).	Relapse (days)	
Resochin ...	1·42	3	3,4,6,	...	...	...	...
Nivaquine	1·42	1	6	2	2,4	2,5	...
Resochin ...	2·5	...	...	3	3,3,4	1,2,2	...
Nivaquine	2·5	1	9	3	3,4*	6	...
Resochin ...	5·0	...	...	3	3,3,5	4,4,6	...
Nivaquine	5·0	...	...	3	3,4,4	2,4,10	...
Resochin ...	7·5	...	...	3	2,3,4	3,6,6	...
Nivaquine	7·5	...	...	3	3,4,4†	2	...
Quinine ...	8·6	2	2,4	1	4*	...	...
Quinine ...	17·2	6	1,2,3,3,4,8	...	...	...	...
Quinine ...	25·8	2	3,4	1	2	3	...
Quinine ...	34·4	...	...	...	...	...	3

*One died of intercurrent disease during observation period.
†Two died due to anæmia during observation period.

TABLE II.

Effect of a single intravenous administration of resochin, nivaquine and quinine against Nuri strain of P. knowlesi *at* 20 *to* 39 *per cent cell infection.*

Drug.	Dosage mg./kg. (base).	Ineffective.		Effective.			Number of deaths due to toxic effect.
		Number of animals.	Days of death (days).	Number of animals.	Parasite clearance (days).	Relapse (days)	
Resochin ...	1·42	2	2,2	1	3	2	...
Nivaquine	1·42	2	6,8	1	3	2	...
Resochin ...	2·5	1	1	2	2,3	4,6	...
Nivaquine	2·5	3	6,6,8	...	...	...	...
Resochin ..	5·0	...	...	3	2,4,4*	2,4	...
Nivaquine	5·0	...	...	3	3,3,4‡	6,7	...
Resochin ...	5·7	...	...	3	2,3*,4†	1	...
Resochin ...	7·5	...	...	3	3,3*,4†	...	...
Nivaquine	7·5	...	...	3	2,3,4	6,9,9	...
Quinine ...	8·6	3	1,1,1	...	...	...	...
,, ...	17·2	3	2,3,4	...	...	...	...
,, ...	25·8	1	3	...	...	...	2
,, ...	34·4	1	4	...	...	...	2

*Infection was cured in one.
†One/two died of intercurrent disease during the observation period.
‡One died due to anæmia during observation period.

In many of the animals (treated with chloroquine or quinine) in which complete parasite clearance was not attained, there was some degree of deceleration in the course of parasitæmia and thus the life was prolonged by three to five days.

Heavy infection (40 *to* 59 *per cent cell infection*).—In five out of six animals treated with 1·42 mg. of resochin or nivaquine, there was no effect. In the remaining one there was clearance after four days but recrudescences occurred soon after. In the remaining group under schedules 2·5 to 7·5 mg., the effect was partial. In majority of cases, parasite clearance was attained but in every group there was some which did not react satisfactorily. None of the animals treated with quinine responded well as parasite clearance was not attained in any. In most cases in which parasite clearance was not observed, life was prolonged by one to three days. Details are given in Table III.

TABLE III.

Effect of a single intravenous administration of resochin, nivaquine and quinine against Nuri strain of P. knowlesi *at* 40 *to* 59 *per cent cell infection.*

Drug.	Dosage mg./kg. (base).	Ineffective.		Effective.			Number of deaths due to toxic effect.
		Number of animals.	Days of death (days).	Number of animals.	Parasite clearance (days).	Relapse (days)	
Resochin ...	1·42	3	1,2,2	...	...	...	...
Nivaquine	1·42	2	3,1	1	4	1	...
Resochin ...	2·5	...	...	3	3,3,4	2,3,4	..
Nivaquine	2·5	1	3	2	3,3*	1	...
Resochin ...	5·0	1	1	2	3,4	4,5	..
Nivaquine	5·0	1	2	2	2,5†	...	..
Resochin ...	5·7	1	2	2	2,3†	8	..
.. ...	7·5	1	3	2	3,6	1,4	
Nivaquine	7·5	1	2	2	3,3	7,8	...
Quinine ...	8·6	3	1,1,1	...	...	...	..
.. ..	17·2	3	1,2,1	...	...	...	...
..	25·8	2	2,3	...	...	...	1
.. ...	34·4	...	...	...	...	...	3

*One died due to anaemia during observation period.
†One/two died of intercurrent disease during the obsrevation period.

Moderately heavy infection (60 *to* 79 *per cent cell infection*).—Dosage schedules of 1·42 and 2·5 mg. of chloroquine preparations were totally ineffective and the course of parasitæmia progressed unhampered. All animals died during the

normal scheduled period. The effect of these preparations in 5 and 7·5 mg. was partial, similar to that observed under the previous groups of animals (heavy infection). Ten mg. dose of resochin proved toxic to two out of three monkeys. Quinine was found to be totally ineffective. The results are tabulated in Table IV.

Only in a few cases, life was prolonged by one to five days in animals in which complete parasite clearance was not attained.

TABLE IV.

Effect of single intravenous administration of resochin, nivaquine and quinine against Nuri strain of P. knowlesi *at* 60 *to* 79 *per cent cell infection.*

Drug.	Dosage mg./kg. (base).	Ineffective.		Effective.			Number of deaths due to toxic effect.
		Number of animals.	Days of death (days).	Number of animals.	Parasite clearance (days).	Relapse (days)	
Resochin ...	1·42	3	1,1,1	...	...	...	...
Nivaquine	1·42	3	0,0,0	...	...	...	...
Resochin ...	2·5	3	0,2,6	...	...	...	...
Nivaquine	2·5	3	3,0,2	...	...	...	...
Resochin ...	5·0	3	0,0,2	...	...	...	...
Nivaquine	5·0	1	2	2	4,6*	...	...
Resochin ...	5·7	2	0,1	1	4†	...	...
,, ...	7·5	1	5	2	5,6	4,4	...
Nivaquine	7·5	3	3,2,2	...	...	...	...
Resochin ...	10	1	1	...	...	...	2
Quinine ...	8·6	3	1,1,1	...	...	...	...
,, ...	17·2	3	1,1,1	...	...	...	...
,, ...	25·8	3	0,0,1	...	...	...	...
,,	34·1	...	...	...	...	...	3

*Both died of anaemia during observation period.
†Died of intercurrent disease during observation period.

Extremely heavy infection (80 *per cent cell infection or over*).—The results are set out in Table V. Parasite clearance was attained only in two out of 30 animals treated with chloroquine preparations, and even then the results were inconsistent. Both died subsequently. The course of infection took the normal pattern in the rest of the 28 monkeys and all died more or less within the scheduled period. The results observed, after quinine therapy, showed equally poor results.

TABLE V.

Effect of single intravenous administration of resochin, nivaquine and quinine against Nuri strain of P. knowlesi *at* 80 *and over* 80 *per cent cell infection.*

Drug.	Dosage mg./kg. (base).	Ineffective.		Effective.			Number of deaths due to toxic effect.
		Number of animals.	Days of death (days).	Number of animals.	Parasite clearance (days).	Relapse (days)	
Resochin ...	1·42	3	0,0,0	...	...	...	...
Nivaquine	1·42	3	0,0,0	...	...	...	...
Resochin ...	2·5	3	0,0,0	...	...	...	...
Nivaquine	2·5	2	0,0	1	4*	...	..
Resochin ...	5·0	3	0,0,0	...	...	..	...
Nivaquine	5·0	3	0,1,1	...	...	...	...
Resochin ...	5·7	2	0,3	1	3†	...	...
.. ...	7·5	3	0,0,0	...	...	...	...
Nivaquine	7·5	3	1,2,2	...	...	...	...
Resochin ...	10·0	...	...	...	...	...	3
Quinine ...	8·6	3	1,1,1	...	...	...	...
.. ...	17·2	3	1,1,1	...	...	...	..
.. ..	25·8	3	1,1,1	...	...	...	...
.. ..	34·4	...	...	...	...	...	3

*Died of intercurrent disease during the observation period.
†Died of anæmia during the observation period.

The course of infection progressed rapidly in most cases though in a small number treated with the higher doses of chloroquine salts, life was prolonged by a day or two.

DISCUSSION.

During the early stages of the infection (mild or moderate) a dose of 1·42 mg. of resochin or nivaquine proved ineffective in most cases whereas 2·5 mg. showed inconsistent results. However, in most cases in which parasite clearance was not attained, there was deceleration in the course of parasitæmia and the life of the animals was prolonged. Resochin appeared better in these dosages than nivaquine. Doses of 5 and 7·5 mg. were uniformly effective in the clearance of parasites from the peripheral blood although recrudescences were frequent.

When the infection was heavy, doses of 1·42 and 2·5 mg. were usually ineffective while the higher dosage schedules showed somewhat better results as two out of three animals under each group responded well, and complete parasite clearance was attained in them, while in the other there was deceleration in the course of parasitæmia and the life of the animals was prolonged.

In moderately heavy infection, the lower doses proved totally ineffective as neither there was any clearance nor deceleration in the course of parasitæmia. In the higher dosage schedules, only in a few animals complete parasite clearance was attained while in others the pattern of infection was similar to that seen in untreated animals.

In extremely heavy infection, all the six dosage schedules proved ineffective.

From these, it would be obvious that 1·42 mg. of chloroquine, administered parenterally as a single dose, was ineffective even in mild infection whereas in 2·5 mg. the result was not consistent. However, the two higher dosage schedules (5 and 7·5 mg.) proved more or less uniformly successful in mild and moderate infection. Therefore, the dose of 5 mg./kg. should be considered as the minimum effective dose for single parenteral therapy in *P. knowlesi* infection during the early stages (up to about 40 per cent cell infection) of the infection.

Subsequently, when the infection had gone up to 59 per cent cell infection, even the highest dosage regime did not show satisfactory results in all cases. Beyond 60 per cent, practically all dosage schedules were ineffective.

In 2·5 mg. dose, out of six monkeys each treated with resochin and nivaquine against mild and moderate infections, only in one there was no effect in the resochin series as against four in the nivaquine series. Excepting this, no appreciable difference in action was observed in the relative merits of the two drugs.

The results observed after parenteral use of quinine, administered as a single dose, were highly disappointing. Irrespective of the stage of infection or the dosage schedule used, complete parasite clearance was not attained in 58 out of 60 animals. Only in those animals in which the infection was mild, there was some deceleration in the course of parasitæmia, thereby prolonging the life of the animals by two to three days. During the subsequent stages of infection, the drug failed to alter the course of events. In most cases, doses higher than 25·2 mg. proved somewhat toxic to the animals.

Thus the overall picture appears to be that in *P. knowlesi* malaria, a single injection of quinine, irrespective of the stage of infection, was totally ineffective in doses ranging from 8·6 to 34·4 mg./kg. Moreover, the highest dose was not well tolerated by the animals. But when animals were treated with chloroquine preparations, infection could be controlled practically in all cases with a single intravenous injection of 5 mg. or more up to the stage when cell infection had reached about 40 per cent. Beyond that and up to about 60 per cent infection, the results were inconsistent whereas above 60 per cent the drug was totally ineffective. From these studies, it should be clearly evident that in the single dose parenteral therapy, chloroquine is definitely more effective than quinine in *P. knowlesi* infection.

In heavy *P. cynomolgi* infection, Jaswant Singh, Ray *et al.* (1953) observed that the highest density attained was about 30 per cent, and a single dose 1·4 mg.

of chloroquine, administered parenterally, broughtabout a reduction in parasitæmia by about 78 per cent, by 98 per cent with 5·7 mg. and by 99·5 per cent with 11·4 mg. as against reduction by about 67 per cent with 8·6 mg. of quinine. Further, in all animals there was complete clearance of parasites from the peripheral circulation. Thus, though the maximum parasite density attained in this infection was observed to be 30 per cent, doses of 1·4 mg. of chloroquine or 8·6 mg. of quinine were effective in the clearance of parasites from the peripheral circulation. This is in contrast to the observations made in *P. knowlesi* infection in which these doses proved totally ineffective even in mild infection. This variation in reaction to the same doses of the drugs in the same host, could be interpreted to be due to the different behaviouristic pattern of the two species of plasmodia.

But, in the final analysis, it may be said that whether in *P. cynomolgi* or *P. knowlesi* (up to a certain stage of cell infection) infection, parenteral administration of chloroquine proved superior to quinine, which is still considered by many as the only remedy in serious cases of *falciparum* malaria. However, recent studies in such infection have shown that treatment with chloroquine, administered parenterally, is highly satisfactory (Spicknall *et al.*, 1949; Scott, 1950; Jelliffe and Jelliffe, 1953; Mohr, 1953; Laing, 1955; Harris, 1955; World Health Organization, 1955). However, no report is available on the comparative studies on parenteral chloroquine vis-a-vis quinine in serious cases of *falciparum* malaria. Therefore, the final judgement regarding the superiority of one over the other must be held in abeyance unless such investigations are taken up.

One other interesting feature of the present studies is that no drug by itself was effective once the cell infection had reached 60 per cent or over. These findings confirm the observations reported by Ray (1954) who reported that under such circumstances, life of the animals could be saved by the concurrent administration of antimalarials along with blood transfusion. In human malaria, *Fairley* (1947) had observed that when cell infection reached 20 per cent or over in *falciparum* malaria, the course of infection was invariably fatal. This further indicates the need of transfusion of blood in serious cases of malaria, besides parenteral administration of drugs like chloroquine or quinine.

SUMMARY.

A single dose intravenous injection of resochin and nivaquine in 1·4 to 10 mg. per kg. (base) doses, and quinine in 8·6 to 34·4 mg. doses, was administered to 201 monkeys to test their efficacy against various degrees of parasitæmia ranging above 10 per cent cell infection in Nuri strain of *P. knowlesi*.

Irrespective of the stages of infection, quinine was ineffective in all the doses. Resochin and nivaquine in 5 and 7·5 mg. doses proved uniformly successful in mild and moderate infections (up to 40 per cent cell infection). Between 40 and 60 per cent cell infection, the effect of the two drugs even in the highest doses was not satisfactory. Beyond 69 per cent, all dosage schedules were ineffective.

Five mg./kg. dose is considered the minimal effective dose of resochin and nivaquine for single intravenous therapy in *P. knowlesi* (Nuri strain) infection.

ACKNOWLEDGEMENT.

The authors wish to express their thanks to Messrs. Bayer & Co. and to Messrs. May & Baker, for the free supply of resochin and nivaquine, respectively, required for these investigations.

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STUDIES ON NURI STRAIN OF *P. KNOWLESI*.

Part XII. Bleeding and coagulation time and thrombocyte count in trophozoite-induced infection.

BY

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[March 15, 1956.]

In a previous report, Menon and Nair (1955) recorded the total erythrocyte and leucocyte counts, hæmoglobin concentrations, saline fragility of cells and body temperature of *M. mulatta mulatta* prior and subsequent to inoculating them with blood forms of Nuri strain of *P. knowlesi*. Subsequently, Menon and Nair (1956) studied the changes in erythrocyte sedimentation rate during malarial attacks caused by the same species of parasite. The present paper records, on a similar line, the bleeding and coagulation time and thrombocyte count in normal and infected *M. mulatta mulatta*.

METHODS AND MATERIALS.

Forty-three *M. mulatta mulatta* were used in this experiment and they weighed between three and eight kg. Blood was drawn for the purpose of investigation in the mornings during the summer months (April to July, 1955) in fasting condition.

In most of the cases, the bleeding and coagulation time and thrombocyte counts were made once before infecting them. After that they were inoculated intravenously, 0·1 to five million parasitized erythrocytes per kg. body weight of the animal, from donors harbouring heavy infection with Nuri strain of *P. knowlesi*. During the prepatent period, these observations were made for one to three days and thereafter during patency, once daily in the majority of the cases till their death.

Procedure adopted for thrombocyte count was similar to that for erythrocyte count by the direct method (Napier and Das Gupta, 1942).

Bleeding time was determined by Duke's method (Napier and Das Gupta, 1942). Blood was obtained from the ear lobe, and at every ten or fifteen seconds blots were made of the blood which flowed out on strips of soft filter paper until the entire bleeding stopped.

Coagulation time was determined according to Lee and White (modified) method (Napier and Das Gupta, 1942). Five c.c. blood was withdrawn from the dorsal leg vein into a dry syringe. The time when the blood entered the syringe was noted. One c.c. of blood each was put into three small paraffined test tubes (1/4 inch in diameter) kept ready in a water bath at 37° C. These tubes were taken out in rotation from the bath, at intervals of half a minute or even less, and the contents were tilted to see whether the blood had set. This was continued until the tubes could be inverted without displacing the clot. The time from the entry of blood into the syringe until it was found to have set, was noted. The average of the three readings constituted actual coagulation time.

Blood smears were made at the time of drawing blood and examined for malaria parasites after staining with J.S.B. (Jaswant Singh and Bhattacharji, 1944). Parasite density was determined during patency and expressed as the number per 10,000 erythrocytes.

The means of the bleeding and clotting times and of thrombocyte counts were calculated for the different periods (i.e. preinoculation, prepatent and different days of patency) and their standard errors worked out. Finally whether the findings obtained during the prepatent and patent periods were significantly different or not from the preinoculation figures, were determined according to Fisher's "*t*" test.

RESULTS.

Details of the findings are given in Table I. The mean morning parasite count on the first day of patency was 114 ± 66. The count gradually went up on the subsequent days, and on the fifth day it was 3675 ± 1044·7.

Bleeding time.—Bleeding time recorded during the whole investigation ranged from 15 to 110 seconds. The mean observed during the pre-inoculation period was 48·1 ± 2·7 seconds. General reduction in this timing was observed during the different days of infection and ultimately on the last day of infection, it was only 31·2 ± 8·2 seconds. Statistical analysis shows that this reduction observed on the different days, except during the prepatent and fourth day of patency, was significant when compared to the findings during the normal stage. However, there is no clear evidence to show that there is significant reduction in the bleeding time on the successive days after inoculation.

Coagulation time.—The mean coagulation time before inoculation of the monkeys was observed to be 235·1 ± 8·7 seconds. This time, for all days after inoculation (156 to 180 seconds), was significantly less than the normal but here again the difference in the mean between the successive days was not significant at all.

Thrombocyte count.—Normal platelet count was observed to be 93,600 ± 4,200. During the prepatent and patent periods of infection, the average count ranged from 87,200 to 1,00,900 but the differences between these means and the preinoculation mean were not statistically significant.

TABLE I.

Bleeding and coagulation time and platelet count of M. mulatta mulatta *prior and subsequent to inoculating them with Nuri strain of* P. knowlesi.

Investigation period	Number of observations	Morning parasites count per 10,000 erythrocytes (Mean)	Bleeding time (seconds)		Coagulation time (seconds)		Platelet count (thousands)	
			Range	Mean	Range	Mean	Range	Mean
Preinoculation	42	...	25–110	48·1±2·7	155–378	235·1±8·7	40–170	93·6±4·2
Prepatent	99	...	20–135	41·9±1·8	111–330	†179·6±3·9	10–190	94·5±2·5
Patent								
First day	30	114±66	20–60	†*35·7±2·1	98–228	†156.1±17·9	50–120	99·7±10·1
Second day	13	694·5±361·6	20–85	†38±2·3	93–242	161·5±6·6	50–200	87·2±4·9
Third day	38	1824·2±75·4	15–70	†33·1±2·0	76–270	156·3±6·3	30–220	100·9±6·3
Fourth day	13	2025±418·2	25–60	*40·9±3·1	81–218	164·4±11	50–150	89·1±8·8
Fifth day	10	3875±1044·7	20–75	†31·2±8·2	81–205	156·5±13·8	60–170	89±15·5

*Difference significant as compared with the previous day mean.
†Difference significant as compared with the preinoculation mean.

DISCUSSION.

In man, normal bleeding time is one to three minutes and coagulation time, three to six minutes (Napier and Das Gupta, 1942). Thrombocyte count is considered to be 2,50,000 to 3,50,000 (Todd, Sanford and Wells, 1953). The mean bleeding time of 48·1 ±2·7 seconds and thrombocyte count of 93,600 ±4,200 per c.mm. recorded in non-infected *M. mulatta mulatta* in this investigation, are definitely less as compared to the human standard. In man, thrombocyte count is generally less in the direct count and more during winter and when one ascends to higher altitudes (Todd, Sanford and Wells, 1953). With regard to the present investigation, the counts were made in Delhi during hot summer months by following the direct method. Perhaps these factors are partly responsible for the low counts obtained by the authors.

There is not much published information on the changes brought about in the bleeding and clotting time and platelet count during malarial attacks. Mikeladse (1924) observed that bleeding time in this disease was little altered. Maslova (1924) noted reduction of the coagulation time and decrease in platelet count during malarial paroxysm. The present investigation, however, indicates that in *P. knowlesi* (Nuri strain) infection, there is no alteration in the thrombocyte count but there is slight but significant reduction in the bleeding, and coagulation time. In the latter case, the reduction was observed both during prepatent and patent period, but in the former it was noticed only during patent infection. Only very great variations in thrombocyte count have clinical significance. Great

reduction in thrombocyte is generally associated with prolongation in bleeding time. Shortening of coagulation time may be due to deficiency of antiprothrombin and is significant only in relation to possible thrombosis. In malaria, thrombosis of the capillaries of brain has been reported by Thomson and Annecke (1926); Thayer (1899); Dudgeon and Clarke (1917); Spitz (1946) and Marchiafava and Bignami (1894). According to Paisseau and Lemaire (1916), similar lesions occur in adrenals. Heart (Markal, 1946); liver (Dudgeon and Clarke, 1917) and spleen (Barker, 1895) also have been reported as the seat of such pathological lesions in malaria. But evidence of thrombosis is not very convincing in the adrenals, heart and liver capillaries according to Maegraith (1948); in spleen according to Craig (1909) and Deaderik (1911); and in brain capillaries according to Gaskell and Miller (1920) and Rigdon (1944). The present observations regarding reduction in bleeding and coagulation time in *P. knowlesi* (Nuri strain) infection, though may be taken as factors helpful in intravascular thrombosis, the same does not support fully the formation of this pathological lesion as the above haematological changes were not found to progress proportionately to the degree of infection in the animals. It is difficult, therefore, to decide from the present findings also whether real thrombosis could occur in *P. knowlesi* infection. Again in *P. knowlesi* infection, with the appearance of parasites in the circulation, massive precipitation and agglutination of the circulating red cells, has been recorded (Knisely *et al.*, 1945). This changes the blood into a thick sludge and this, in turn, decreases the rate of blood flow. Whether shortening of bleeding and clotting time in *P. knowlesi* infection has anything to do with the sludge formation in *P. knowlesi*, is not understood. The above two aspects (thrombosis and sludge formation), however, deserve further clarification.

SUMMARY.

Bleeding and coagulation time and thrombocyte count were studied in *M. mulatta mulatta* (*S. rhesus* monkeys) both before and subsequent to inoculating them with blood forms of Nuri strain of *P. knowlesi*.

While no change was noted in the thrombocyte count during the entire period of investigation, there was slight but significant reduction in the bleeding and coagulation time during the period of infection.

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STUDIES ON NURI STRAIN OF *P. KNOWLESI*.

Part XIII. Blood sugar in monkeys (*M. mulatta mulatta*) with *P. knowlesi* (Nuri strain) infection.

BY

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[May 3, 1956.]

SINTON and Kehar (1931) reported that blood sugar increased during the acute stage in human malaria (*P. vivax*, and *P. falciparum* infection). But a large number of workers failed to observe any such increase (Ruge, 1935; Petersen, 1926; Flosi, 1944). However, in *P. knowlesi* infection in *M. mulatta mulatta* (rhesus monkeys), it was shown by Fulton (1939) that there was hypoglycæmia. There being an element of doubt as to the level of blood sugar in malarial infection, attempts have been made during the present experimental investigations to correlate the degree of parasitæmia and the level of blood sugar in *M. mulatta* infected with a virulent strain (Nuri) of *P. knowlesi* (Jaswant Singh, Ray and Nair, 1953; Edeson and Davey, 1953).

MATERIALS AND METHODS.

Host.—Fifteen tuberculin negative *M. mulatta mulatta* monkeys weighing between 4 and 9.5 kg. were utilized for these investigations. The diet provided to the animals consisted of grams (1·25 oz.), wheat flour in the form of chappatis (0·50 oz.), and vegetable or fruits (2·00 oz.). The total caloric value was between 760 and 800.

Parasite.—The monkeys were inoculated intravenously (through the dorsal leg vein) with an effective dose of 5×10^6 parasitized cells (Nuri strain of *P. knowlesi*). As a rule, the dose proves fatal to the animals in five to six days from the date of inoculation (0-day). The cell infection reaches between 60 and 96 per cent.

Blood smears were collected every four hours and in certain cases only once a day. The degree of parasitæmia was assessed by counting the number of parasitized cells per 10,000 erythrocytes. Ehrlich eyepiece was used for the purpose. Smears were stained with J. S. B.

ESTIMATION OF BLOOD SUGAR.

One to two mg. of neutral potassium oxalate per c.c. of blood was used as an anticoagulent. Techniques for obtaining protein-free blood filtrate and for the estimation of blood sugar were after Folin and Wu (1919:1920). The proteins of the whole blood were removed by precipitation with tungstic acid and filtered. The protein-free filtrate was heated with alkaline copper solution using Folin-Wu tubes to prevent reoxidisation. The cuprous oxide formed was treated with a phosphomolybdic acid solution, a blue colour being obtained which was compared with that of a standard. For photometric measurement, instead of visual colorimeter, the Klett summerson photo-electric colorimeter was used.

A standard corresponding to a 200 mg. per cent blood sugar was used. The concentration of sugar in mg. per cent in the unknown blood sample being obtained by the calculation $\frac{200}{\text{reading of standard}} \times$ reading of the unknown.

GLUCOSE ADMINISTRATION.

When glucose was administered, it was in the form of 25 per cent dextrose (The National Laboratory, Amritsar) solution introduced through the dorsal leg vein.

Before undertaking investigations on infected animals, observations were made first on the blood sugar level in normal *M. mulatta mulatta*. As such, experiments and results were categorized under two groups.

I. Studies on normal *M. mulatta*.
II. Studies on infected *M. mulatta*.

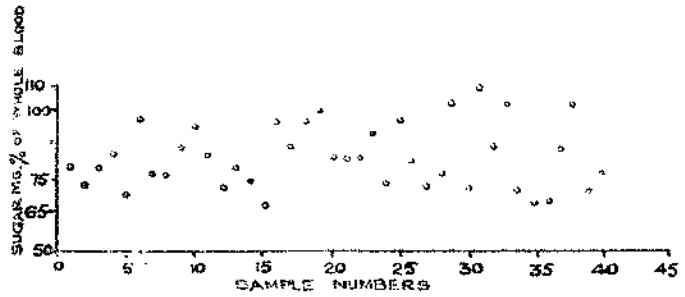
EXPERIMENTS AND RESULTS.

I. STUDIES IN NORMAL *M. MULATTA MULATTA*.

(*a*) *Determination of fasting blood sugar values.*—Blood sugar was estimated in 40 fasting blood samples obtained from ten different normal monkeys on two to three consecutive days. The distribution of fasting blood sugar values is represented in Chart 1. The highest value obtained was 109 mg. per cent and the lowest was 64·8 mg. per cent. The maximum number of values lay between 75 and 100 mg. per cent.

CHART 1.

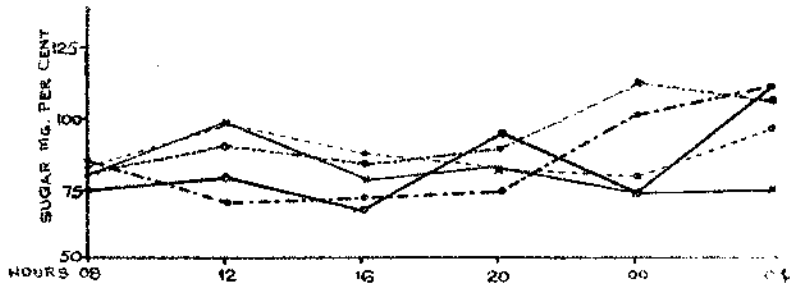
Fasting blood sugar values in normal M. mulatta *monkeys.*



(*B*) *Determination of blood sugar values at 4-hourly intervals.*—Blood samples were collected at interval of four hours from five normal monkeys kept on standard diet, and the amount of sugar determined. The values obtained in different monkeys at different hours are represented in Chart 2. A total of 30 samples were involved in these studies. At 08·00 hours, the blood sugar values from all the five monkeys were between 69·4 and 83·3 mg. per cent and these values may be taken as fasting blood sugar values as the morning meals were given at about 09·00 hours.

CHART 2.

Four-hourly determination of blood sugar in five monkeys (Normal).



At 12·00 hours, the blood sugar values ranged between 64·8 and 94·4 mg. per cent. At 16·00 hours just prior to the second meal, the range varied from 62 to 84·2 mg. per cent. At 20·00 hours, the blood sugar values fluctuated between 75 and 93·5 mg. per cent. At 00·00 hours, the values were between 72 and 111 mg. per cent whereas at 04·00 hours, the blood sugar in the monkeys ranged between 71·7 and 111 mg. per cent. Thus, it would be observed that blood sugar in the monkeys did not drop lower than 62 mg. per cent nor go higher up than 111 mg. per cent during the 24 hours observation.

(*C*) *Effect of a single intravenous infection of glucose on the blood sugar level.*—Two dosage schedules were adopted. One batch consisting of five monkeys were injected with 0·25 gm./kg., while the other batch of five received 0·5 gm./kg. Samples of blood were collected one, three and five hours after injection, in addition to the fasting samples obtained earlier. A total of 40 blood samples were dealt with. Results have been shown in Charts 3 and 4.

Determination of blood sugar in 5 normal monkeys 1 hour, 3 hours, 5 hours after intravenous glucose injection (single injection).

CHART 3.
(0·25 gm./kg. glucose)

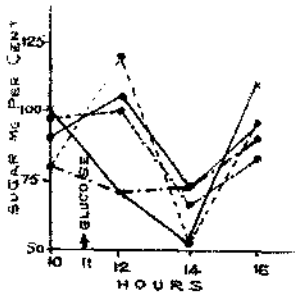
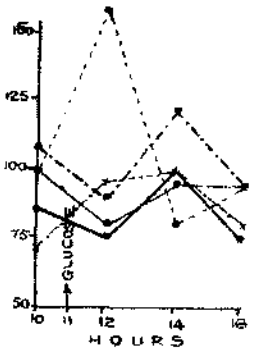


CHART 4.
(0·5 gm./kg. glucose)



In the first batch, three animals showed an appreciable increase of blood sugar level one hour after the injection. The range varied from 100 to 123 as compared to 80 to 100 mg. per cent of fasting sugar level. But in the other two, there was no rise. Samples collected three hours after glucose administration, showed an appreciable degree of fall in all the five animals. The level was observed to be between 50 and 75 mg. per cent. The readings recorded five hours after the injection, showed that the level had come within the normal range.

In the second batch, all the five animals receiving 0·5 gm./kg. of glucose showed a high level of blood sugar which was maintained at 79 to 124 mg. per cent in the first and third hour samples. The fasting level was recorded to be 69·4 to 103 mg. per cent. In samples, collected five hours after injection, the level had reached within the normal 24-hours range.

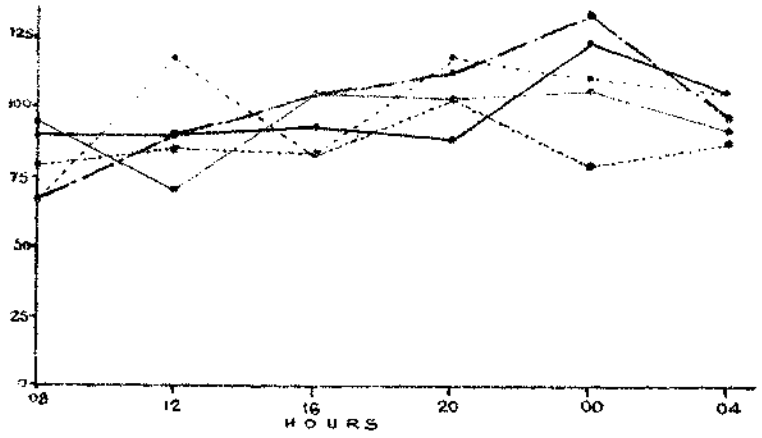
(*D*) *Effect of 4-hourly intravenous administration of 0·5 gm./kg. of glucose on the blood sugar level.*—

(*i*) *For a period of 24 hours.*—Five animals were placed under this group and blood samples were collected at interval of four hours each time just prior to glucose injection. The dose of 0·25 gm./kg. was discarded as the rise in blood sugar level was not uniform. Altogether 20 samples were involved. The level ranged between 68·6 and 137 mg. per cent (Chart 5) as compared to the data (62 to 111 mg. per cent) observed in 4-hourly samples in animals which had received no glucose (Chart 2).

(*ii*) *For a period of six days.*—In this series, 34 samples were examined from one monkey. A dose of 0·5 gm. /kg. of glucose was administered every four hours.

CHART 5.

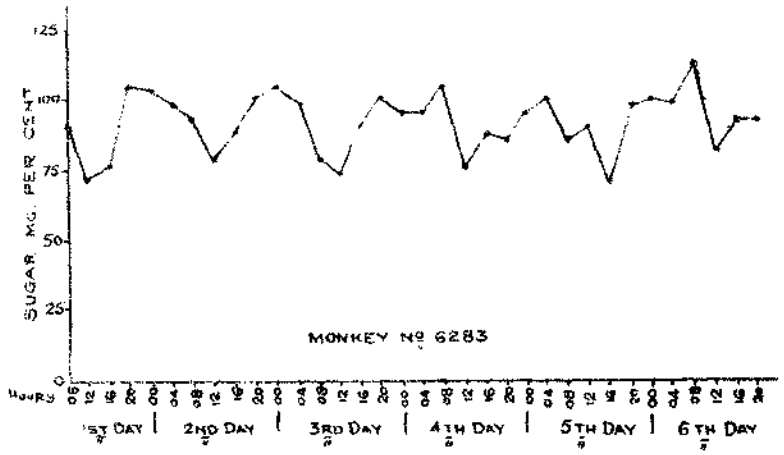
Four-hourly determination of blood sugar with 4-hourly injection of glucose 0·5 gm/kg. i.v. in 5 monkeys.



Side by side, blood sugar was estimated at four-hourly intervals. Throughout this period, the lowest blood sugar level was 70 mg. per cent and the highest was 110 mg. per cent. Majority of the values remained above 90 mg. per cent. The results are presented in Chart 5*A*.

CHART 5A.

Effect of intravenous glucose on the blood sugar level in a normal monkey.



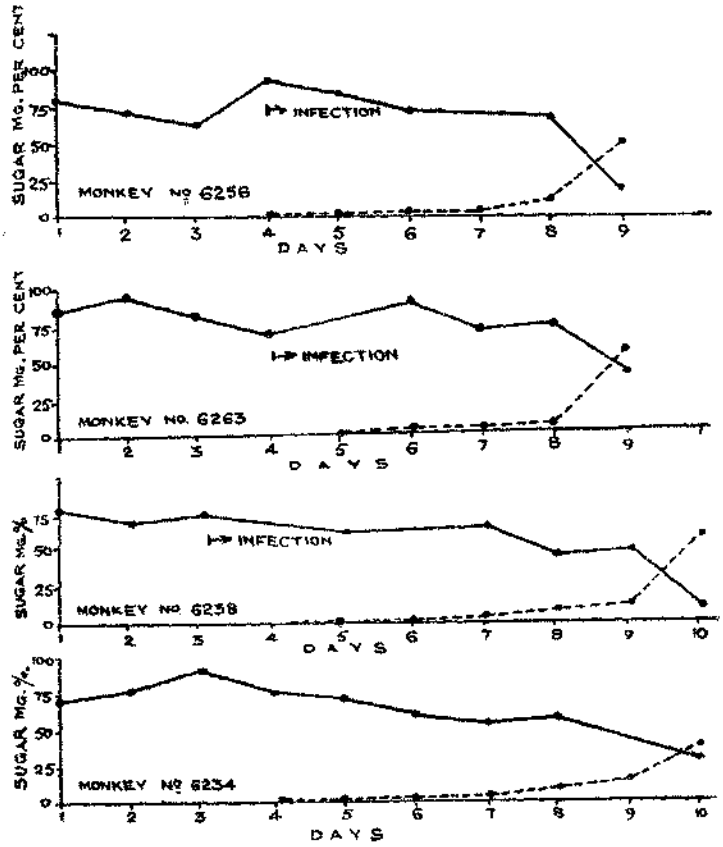
(II) STUDIES IN BLOOD SUGAR IN INFECTED MONKEYS.

(*A*) *Estimation of fasting blood sugar.*—Observations were taken up in four monkeys and fasting blood sugar estimated on three to four consecutive days prior

to infective inoculation. Subsequently, estimation on fasting level was carried out daily throughout the course of infection, except on five occasions when it was missed once for each of three out of four monkeys. Altogether 33 samples were involved. Side by side, records were kept on the degree of parasitæmia. The blood sugar level and the data on parasite count have been shown in Chart 6.

CHART 6.

Fasting blood sugar on consecutive days in P. knowlesi *infection.*



Prior to infection, the fasting level was recorded to be between 65 and 85 mg. per cent which lie well within the normal limits. During the course of infection, it was observed that in three out of four subjects the level was maintained within the normal level during the first four days. However, in the fourth animal, the readings showed a progressive downward trend. The fifth and sixth day readings in all the four animals indicated a precipitous fall which varied between 12·7 and 40 mg. per cent.

Record of parasite count showed that although during the first four days the count was comparatively low (15 to 25 per cent), on the fifth and sixth days

after inoculation (when the animals survived that long), cell infection had gone up to 68 to 80 per cent.

(*B*) *Estimation of 4-hourly blood sugar.*—The studies were undertaken in two monkeys and blood sugar estimated in 60 samples collected prior and subsequent to infective inoculation. Parasite count was carried out throughout the course of infection.

It was observed that the course of parasitæmia and blood sugar level was inversely proportional, particularly at the terminal stage of infection.

In one animal (6282), the degree of parasitæmia upto Day 3 was between 1 and 10 per cent, and the blood sugar level was maintained between 70 and 105 mg. On Days 4 and 5, the parasite level gradually increased from 45 per cent on Day 4 to 80 per cent on Day 5. During this period, there was a progressive fall in blood sugar and the lowest level attained was about 12 mg. per cent at 20·00 hours on the fifth day. Subsequently, there was a slight rise a few hours before death.

In the second monkey (6285), the parasite count ranged between 0 and 12 per cent up to Day 4, and the blood sugar level was maintained between 70 and 110 mg. per cent. Up to 16·00 hours on Day 5, the blood sugar ranged between 72 and 125 mg. per cent when parasitæmia gradually increased from 5 to 15 per cent. Subsequently, as the cell infection increased rapidly to 65 per cent there was a precipitous fall in blood sugar level to 15 mg. per cent. The blood sugar level and data on parasite count have been shown in Chart 7.

(*C*) *Studies on the effect of 4-hourly intravenous glucose administration on the blood sugar level during the course of infection.*—Glucose administration (0·5 gm./kg.) was begun every four hours, in two monkeys soon after infective inoculation. Fifty-six blood sugar determinations were made. It was observed that up to 16·00 hours on Day 4, in one monkey the level of blood sugar ranged between 70 and 140 mg. per cent. The cell infection attained by that time was 70 per cent. But at 20·00 hours on the same day, when the cell infection had reached 80 per cent after the last sporulation, the blood sugar level attained was still 105 mg. per cent. As the animal died soon after, further observations could not be continued. In the other monkey, the blood sugar level was between 70 and 105 per cent during the first four days. The maximum cell infection at the end of the fourth day was recorded to be 5 per cent. On the fifth day when cell infection had attained 75 per cent, the blood sugar level (at 00·00 hours) was recorded to be 95 mg. per cent. However, at 20·00 hours on the sixth day, the cell infection attained was 90 per cent, the blood sugar level had reached 23 mg. per cent, though the lowest level attained on the same day was 5 mg. per cent (12 noon, prior to sporulation). The results are presented in Chart 8.

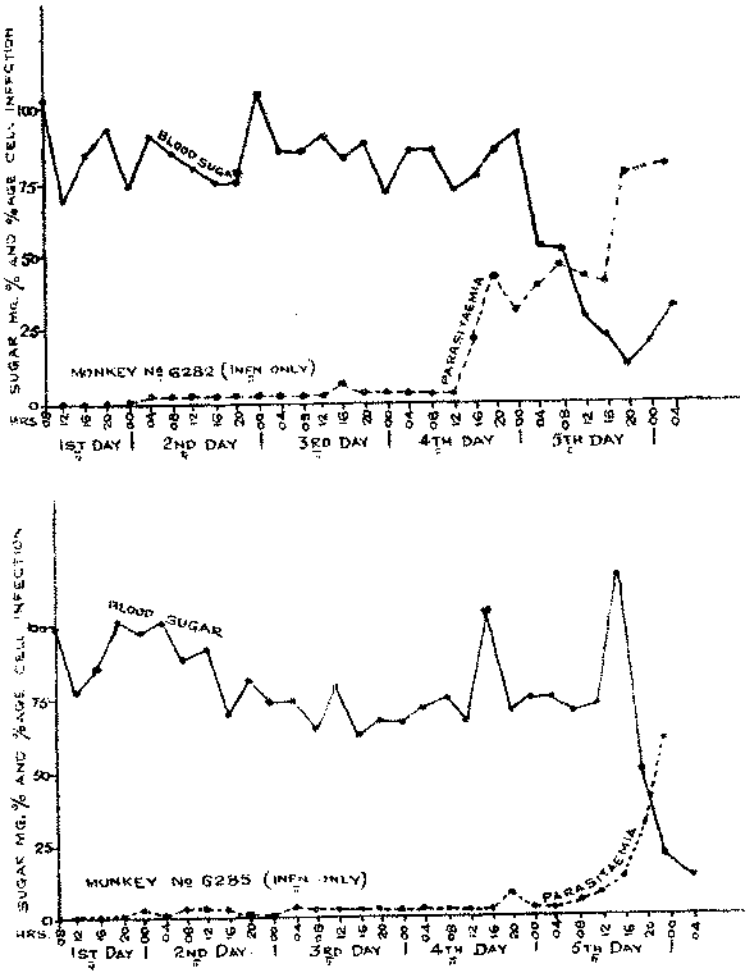
HISTOPATHOLOGICAL FINDINGS.

Pieces of liver were removed from all infected animals soon after death to study the changes in the organ.

The salient microscopic features in all cases were varied degree of centrilobular necrosis, fatty changes of the parenchyma cells and dilatation or rupture of the central hepatic veins. The sinusoids were found to be invariably packed with

CHART 7.

Blood sugar values and parasitaemia in P. knowlesi *infection.*



parasitized cells, cell debris, pigments, leucocytes and swollen kupffer cells containing varying amounts of phagocytosed materials.

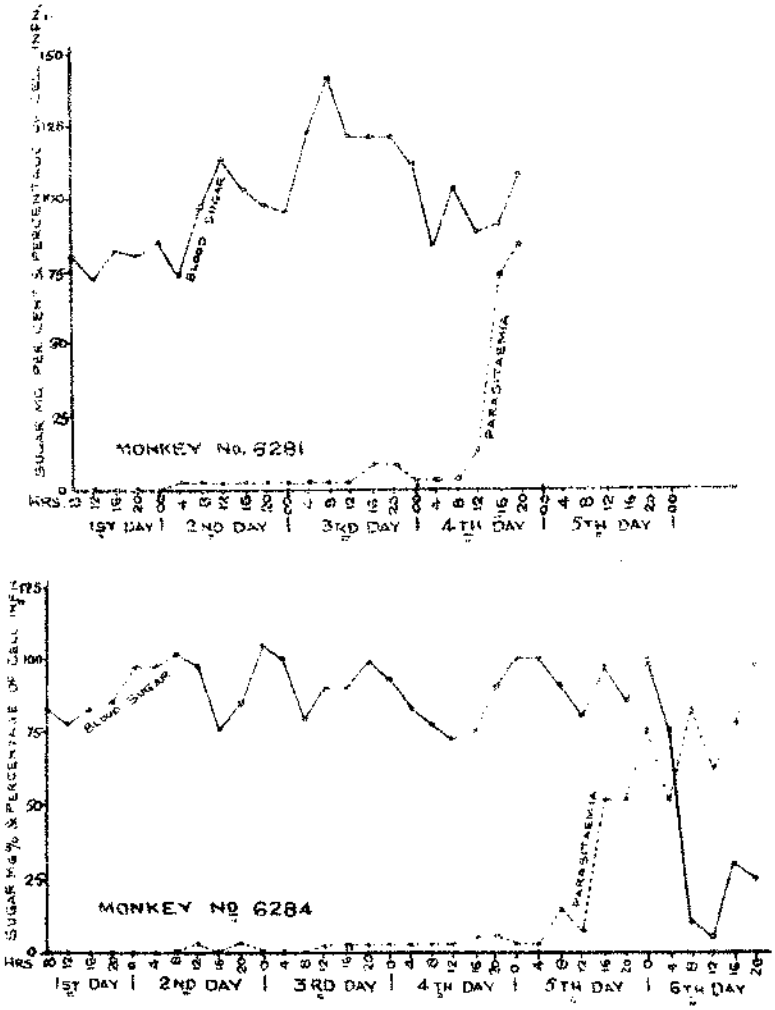
Details of parenchyma changes in infected animals, treated with glucose and those which were not, will be reported elsewhere.

DISCUSSION.

The fasting blood sugar level in 40 samples obtained from 10 normal *M. mulatta mulatta* was observed to be between 64·8 and 109 mg. per cent though the maximum number of values lay between 75 and 100 mg. per cent. Blood sugar level estimated every four hours, showed a range between 62 and 111 mg. per cent.

CHART 8.

Effect of intravenous glucose on the blood sugar level and parasitæmia in P. knowlesi infection in monkeys.



In *P. knowlesi* infection, which runs a progressively rapid and fatal course, the cell infection attains as high a level as 90 per cent or over within a period of five to six days after infective inoculation. The daily fasting blood sugar level in these monkeys, prior and four days subsequent to infective inoculation, was found to be within normal range in three out of four monkeys. However, a progressively downward trend was recorded in the fourth animal from the day after inoculation. On the fifth and sixth days, the fall in blood sugar was precipitous in all animals and the level attained at the final stage of infection (sixth day) varied between 12·7 and 40 mg. per cent.

The blood sugar level estimated from samples obtained every four hours from monkeys infected with *P. knowlesi* showed a striking inverse correlation with the degree of parasitæmia during the terminal stage of the infection. It was indicated earlier that the blood sugar level from 4-hourly samples in normal animals fluctuated between 62 and 111 mg. per cent. In infected animals, it ranged between 70 and 110 mg. per cent, that is well within normal limits during the first three to four days after infection. But, on the fifth and sixth days, there was a rapid and progressive fall in blood sugar and the level reached much below the normal limits (12 to 15 mg. per cent).

Thus the overall picture appears to be that up to 30 to 40 per cent cell infection, the blood sugar level is well maintained within the normal limits. Thereafter, hypoglycæmia sets in, and once established, it becomes progressive till the death of the animal. Working with a strain of *P. knowlesi* originally isolated by Sinton and Mulligan (1932), Fulton (1939) reported similar fall in blood sugar but in his series the extent of hypoglycæmia was not as severe as observed during the present investigation. This could be due to the difference in the strains of plasmodia used. Marvin and Rigdon (1945) also reported similar fall in blood sugar during the terminal stage of *P. lophuræ* infection in ducks.

During the course of these investigations, it was observed that at the terminal stage of the infection (fifth and sixth day) the intake of food was very little specially on the last day. That the fall of blood sugar noticed in the previous investigations was not merely due to dietary deficiency, particularly of carbohydrate, was revealed in the subsequent studies in which estimation of blood sugar was undertaken in normal and infected animals after administration of glucose.

In normal animals, a single intravenous dose of 0·25 gm./kg. of glucose did not present a uniform picture whereas with 0·5 gm./kg. the blood sugar level was found to be maintained at a higher level than normal (79 to 124 mg.) in all animals up to the first three hours. Thereafter the level fell within the normal limits. Four-hourly blood sugar estimation in normal animals receiving 0·5 gm. of glucose every four hours, showed a high level maintenance between 68·6 and 137 mg. per cent as compared to 62 to 111 mg. per cent observed in normal animals which did not receive any glucose treatment as indicated earlier.

The findings in infected animals treated with 0·5 gm. intravenous glucose, administered every four hours, were somewhat interesting. Up to fourth day, the blood sugar level estimated every four hours showed a high level maintenance, similar to that seen in uninfected animals. The same was observed on Day 5 in respect of one animal (the other having died at the end of the fourth day), even though the parasite level had reached about 80 per cent cell infection. But subsequently there was a meteoric fall in the sugar level, the lowest value recorded being 5 mg. per cent in spite of glucose administration. The animal died at the end of the sixth day with about 96 per cent cell infection.

From these observations, it was evident that the blood sugar level was maintained at a higher level in these series of animals during the first five days (in spite of progressive infection) as compared to those infected animals in earlier series which had no glucose treatment. But, at the terminal stage there was a rapid fall in blood sugar level in spite of glucose administration.

It is, therefore, interesting to note that in all cases of *P. knowlesi* infection, there was a rapid fall in blood sugar level irrespective whether the animals were treated with glucose or not. But hypoglycæmia set in somewhat earlier in those animals which had not received any glucose injections.

In this connection, it may be mentioned that in an earlier investigation, Ray (1954) had reported that necrosis of the liver parenchyma round the central veins was invariably found in *P. knowlesi* infection and that centrilobular necrosis was a dominant feature when the cell infection had attained 70 per cent or over. Under such conditions, practically all lobules were involved and in almost all cases the necrosis had spread well beyond the midzonal region. During the present investigations, liver specimens were examined and somewhat similar necrotic changes of the liver were observed.

At the terminal stage of infection when the parasite count is high and there is gross damage to the liver parenchyma, the blood sugar level goes down far below the normal standards.

As to the factor or factors responsible for the fall of blood sugar, it would be argued that hypoglycæmia was on account of increasing demand for sugar by the rapidly developing parasites. According to Fulton (1939) the cause of hypoglycæmia in *P. knowlesi* infection could probably be due to one or several factors like loss of storage capacity of the liver, reduced carbohydrate intake or to demands made on the sugar. Maier and Coggeshall (1941) were of the opinion that glucose is an important factor in the metabolism of plasmodia. Were the demand made on the blood sugar by the rapidly increasing parasitæmia the only factor, it would be difficult to explain the picture observed during the first three to four days of the infection. During this period, the density of plasmodia has been found to be increasing progressively up to 30 to 40 per cent cell infection and yet the blood sugar level fluctuated within limits observed in normal animals, instead of slowly going down as should be expected. Again, in spite of 4-hourly glucose treatment, there was still a sudden fall in the sugar level at the final stage of infection. Further, it may be noted here that unlike *P. lophuræ* infection (Marvin and Rigdon, 1945), parasitæmia in *P. knowlesi* infection malaria increases rapidly in spite of the terminal hypoglycæmia.

On the other hand, Marvin and Rigdon (1945) interpreted that severe hypoglycæmic condition observed in *P. lophuræ* infection in ducks was as a result of anoxia which brings about liver damages. It had already been indicated that gross liver damage was invariably present at the terminal phase of *P. knowlesi* infection. The abrupt fall in blood sugar level seemed to coincide with such liver changes. Although centrilobular necrosis was reported even at a stage when cell infection had attained 30 to 50 per cent cell infection, the degree of damage was not severe as seen at a stage when cell infection had reached 70 per cent (Ray, 1954). Since liver can maintain normal functions until the lesion is very extensive (Himsworth, 1950), it is reasonable to conceive that blood sugar level was well maintained in infected animals during the earlier stages of infection when the damage was not so severe. But during the terminal phase when cell infection is high and therefore tissue anoxia is intense, the liver damage should naturally be very extensive. Such gross damage had been invariably encountered and at this period a sudden fall in blood sugar level had been observed in the series with

or without glucose treatment. These findings would, therefore, lend support to the view that hypoglycæmia noticed in *knowlesi* malaria was perhaps due largely to severe damage to the liver. Demand on blood sugar by the parasites could be a contributory factor.

As such severe damage is rarely encountered in ordinary cases of human malaria; severe hopoglycæmia has not been reported. On the contrary the findings had been rather of a controversial nature. According to Sinton and Kehar (1931), there was a transient rise in blood sugar level during paroxysm. This has been attributed to anoxia of the adrenal and thus excess of the secretion. But a few others like Rudolf *et al.* (1927) (as quoted by Maegraith, 1948) reported hypoglycæmia during a paroxysm, though the level returned to normal shortly after.

It may, therefore, be concluded that in ordinary cases of malaria there could be a transient change in the blood sugar level, particularly during a paroxysm. But in general, the level is perhaps maintained more or less at a normal range similar to that seen in early stage of *P. knowlesi* infection. But in severe infection, when liver is likely to be damaged severely, there would be a state of hypoglycæmia as has been observed during the present studies. The cause of hypoglycæmia would appear to be due to rapid utilization of available blood sugar by growing parasites and also on account of the inability of the liver to store and produce sufficient amount of sugar. Low glycogen content of the liver in association with *P. knowlesi* hypoglycæmia has already been recorded (Fulton, 1939). The low glycogen content would perhaps be due to the necrotic changes.

SUMMARY.

1. Blood sugar estimation was carried out in normal monkeys as well as in those infected with *P. knowlesi*.

2. Fasting blood sugar in normal monkeys showed a range of 64·8 to 109 mg. per cent. In infected monkeys, the fasting level was usually well maintained in most subjects up to the third or fourth day of infection. Subsequently, when the cell infection was severe there was a precipitous fall in blood sugar level.

3. Blood sugar, estimated every four hours in normal animals, showed a range between 62 and 111 mg. per cent. In infected animals, the level was found to be more or less within the normal range up to about Day 4. Thereafter, at the terminal stage there was a sudden fall in the blood sugar level which coincided with high parasitæmia and gross damage to the liver.

4. Blood sugar level in normal monkeys, receiving a single 0·5 gm. glucose injection, showed a high level maintenance up to the first three hours; thereafter there was a progressive fall till the level returned to the normal range.

In infected monkeys, receiving 4-hourly intravenous glucose (0·5 gm./kg.), higher blood sugar level was observed up to the beginning of Day 5, even though the parasitæmia was fairly high. But subsequently there was precipitous fall in blood sugar level.

As in all infected animals severe liver damage has been observed at the terminal stage, and as the fall in blood sugar is precipitous usually at this period, it is conjectured that fall in blood sugar is mainly due to liver damage. Excessive demand for glucose by rapidly developing parasites could be a contributory factor.

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J.S.B. STAIN—SIMPLIFIED METHOD OF PREPARATION.

BY

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[May 3, 1956.]

J.S.B. STAIN (Jaswant Singh and Bhattacharji, 1944) was introduced as a simple water soluble stain, the preparation of which was subsequently slightly modified by Jaswant Singh *et al.* (1953). In the original and modified techniques for the preparation of J.S.B. Solution I (polychromed methylene blue solution), emphasis has been laid on certain colour changes in the solution to reach the end point while the time required for this oxidation of the solution varied from three to four hours. Unexperienced workers found it sometimes difficult to decide as to when the end point was achieved, and thus the stain was either over-oxidised or under-oxidised. This, naturally, led to lack of uniformity in the staining properties of the stain. An attempt has been made to overcome these difficulties by a more simplified technique in the preparation of J.S.B. Solution I, details of which are given below:—

METHOD OF PREPARATION.

Methylene blue, medicinal (0·5 gm.) is dissolved in water (500 ml.), and 1 per cent sulphuric acid (3 ml.) is added gradually with stirring to ensure thorough mixing. Potassium dichromate (0·5 gm.) is then added which forms a purple precipitate. Disodium hydrogen phosphate dihydrate (3·5 gm.) is added next, and after stirring the solution for some time, the precipitate appears to get dissolved. This solution is boiled in a flask with a reflux condenser for one hour when the blue colour of the solution deepens. This solution is ready for immediate use as J.S.B. Stain Solution I in the usual manner (Jaswant Singh *et al.*, 1953).

J.S.B. Solution I may be prepared in concentrated form as well. For this purpose, the chemical ingredients are dissolved in water (150 ml.) and the

mixture allowed to boil in a beaker approximately for one hour. When the solution is reduced to nearly 25 ml., the concentrated stain is stored in glass stoppered bottles. This can be diluted with distilled water to make it up to 500 ml. and allowed to mature for about three days prior to use.

J.S.B. Solution I thus prepared has been found to have all the advantages as the original preparation. The staining properties have also been found to be uniformly good. This simplified technique in the preparation of Solution I has resulted in some additional advantages, such as:—

1. The time required for polychroming of methylene blue is only one hour.
2. No strict supervision is required to note the colour changes, particularly regarding the end point. Thus, it can be prepared under field conditions more easily.
3. The stain solution needs no filtration prior to use.
4. The dilute preparation requires no maturation prior to use.

The stain has been regularly used in these laboratories for the last one year with extremely satisfactory results.

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J. S. B. STAIN—A REVIEW.

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[May 4, 1956.]

INTRODUCTION.

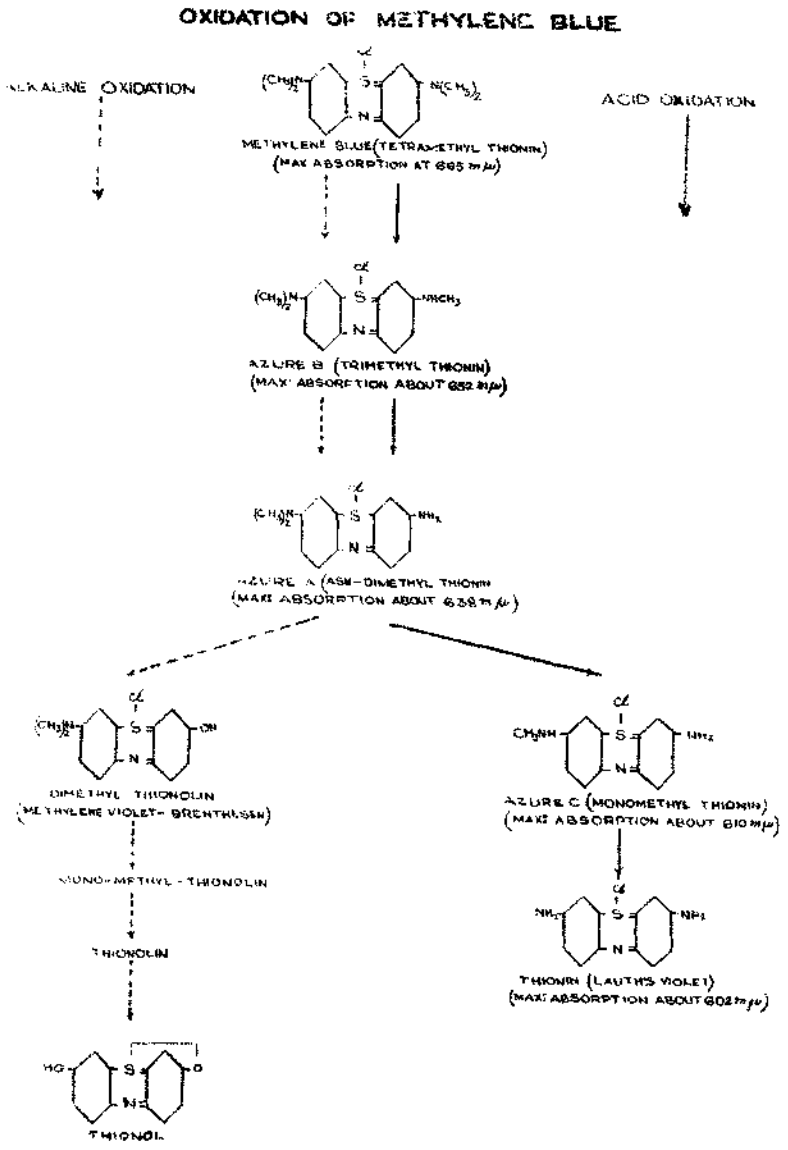
STAINING of malaria parasites in blood films was first evolved by Romanowsky (1891) by combining eosin and methylene blue. It was later found that a Romanowsky stain was not an empirical mixture of the two dyes but also contained other products derived from oxidation processes of methylene blue (Nocht, 1898). Such a methylene blue mixture was called " polychrome methylene blue " and its major components were demethylated products like Azure *A* and *B* (Kehrmann, 1906; Bernthsen, 1906; MacNeal, 1906:1925), Azure *C* (Holmes and French, 1926), methylene violet, thionin etc. (Fig. 1).

However, isolation of these azures in chemically pure state has not been easily possible and as such actual merits of these individual dyes have remained obscure or at least controversial. Trade names of the type Azure I and Azure II represented variable mixtures of methylene blue, Azures *A* and *B*, and a standard product as such was not easily available for the purpose (Conn, 1940).

The original idea of Romanowsky formed the basis of a blood stain introduced by Nocht (1898). In this case, polychroming of methylene blue was achieved by oxidation with dilute alkali and this oxidised material later mixed with eosin. Jenner (1899) introduced the idea of dissolving the precipitate, formed by mixing methylene blue and eosin in methyl alcohol. However, he did not use polychrome methylene blue and this product was unsatisfactory for staining. It was left to Reuter (1901) and Leishmann (1901) to combine the basic techniques of Nocht's stain and Jenner's stain. According to this, polychrome methylene blue was combined with eosin and the precipitate formed was collected and dissolved in methyl alcohol. This stain was only diluted at the time of application. Wright's stain (1902) is a minor modification of the Leishmann stain. Giemsa stain (1904) also consists of alkaline oxidation products of methylene blue combined with eosin and methylene blue, but the composition and purity have been better standardized. Tetrachrome stain introduced by MacNeal (1922:1925) unlike Leishmann stain is a straight mixture of various dyes like Azure *A*., methylene violet etc., and as

such much less satisfactory for the purpose. Amongst all the stains based upon alkaline oxidation of methylene blue (Fig. 1), Leishmann stain (Wright stain being very nearly the same) and Giemsa stain were previously in general use depending upon individual preference. These stains were undoubtedly expensive, less stable especially in the tropics, and above all the quality varied from product to product in many cases.

FIG. 1.



Polychroming of methylene blue is also possible through acid oxidation (Fig. 1). Holmes and French (1926) observed that acid oxidation products of methylene blue were more homogeneous, stable and possessed better staining properties, when compared to stains containing alkali oxidised methylene blue. The latter type of stains are usualy heterogeneous mixtures containing hydrolysed products and azures which are susceptible to rapid loss of staining quality due to further oxidation (Lillie, 1943).

Simeons (1942) and Lillie (1943) oxidized methylene blue with potassium dichromate but like the alkali-oxidised product, the polychrome methylene blue is combined with eosin and the product dissolved in methyl alcohol. Although Lillie's modification was meant to overcome the handicap of inconsistent results with Giemsa stain due to variations in composition, its preparation was also complex and costly. Field (1940) introduced a rapid stain which employed separate solution of eosin and alkaline azure mixture but its use was only limited to thick smears. With the above background, and considering the fact that Azure C, a powerful nuclear stain, is only produced during acid oxidation of methylene blue (Holmes and French, 1926), Jaswant Singh and Bhattacharji (1944) evolved the J.S.B. stain which has successfully overcome many handicaps associated with blood stains already in use. As a rapid soluble stain, it essentially consists of an aqueous polychrome methylene blue solution (J.S.B. Solution I) obtained by the acid oxidation of the dye, and a simple aqueous eosin solution (J.S.B. Solution II). The composition of oxidised methylene blue solution (J.S.B. Solution I) is adjusted by the quantity of potassium dichromate and acid employed and the time of heating. Modifications in the dichromate acid oxidation of methylene blue have been studied by Manwell and Feiglson (1948), Cole and Sewell (1953), Jaswant Singh *et al.* (1953) and Bami and Nair (1955), but there has been no basic change excepting slight experimental variations.

J.S.B. STAIN—PREPARATION AND MODIFICATIONS.

Original method.—J.S.B. stain consists of, (*i*) Solution I (polychrome methylene blue), (*ii*) Solution II (soluble eosin) and (*iii*) acidic wash water.

For preparing Solution I, medicinal methylene blue (0·5 gm.) is first dissolved thoroughly in water (500 c.c.). To this, one per cent sulphuric acid by volume (3 ml.) is added, and after mixing them thoroughly, potassium dichromate (0·5 gm.) is finally added to the mixture. This results in the formation of a heavy amorphous purple coloured precipitate of methylene-blue-chromate. The resultant mixture is heated in an autoclave at a temperature of 100-109° C. and a pressure up to 5 lbs. is maintained for three hours. At the end of this period, the solution turns blue which indicates sufficient polychroming. If the colour shows a greenish tinge, further heating for another hour or so is required. If the temperature is allowed to rise above 110° C., oxidisation of methylene blue may have been carried too far and the solution turns violet purple which is undesirable. After boiling the solution as indicated above, the solution is allowed to cool at room temperature and one per cent potassium hydroxide solution (10 c.c.) is added drop by drop gradually, taking precaution to shake the flask constantly all the time. After the total amount of alkali has been added, content of the flask is shaken for

15 minutes when the precipitate will be found to dissolve gradually. The final solution turns deep blue with a violet iridescence and is left at room temperature for 48 hours to mature. Afterwards, the solution is filtered through a rapid filter paper and the stain stored in a bottle.

Solution II is readily prepared by dissolving one gramme of water soluble eosin in 500 ml. of water. A freshly prepared eosin solution, though can be used immediately, may not yield as satisfactory results as the one which has turned deep red after some use.

According to the original publication, the recommended wash water is an acidulated tap water with a *p*H 6·2 to 6·6. Tap water in Delhi has approximately *p*H of 7·6 (indicator bromothymol-blue). To reduce this to *p*H of 6·2 to 6·6., it is necessary to add 50 mg. of sodium hydrogen phosphate or 5 drops of 5 per cent acetic or citric acid solution for 100 c.c. of water.

Preparation of stain Solution I in powder form.—With the popularity of J.S.B. stain, it became necessary to send large quantities to various centres in India and abroad. It was thought that if J.S.B. Solution I could be reconstituted at the time of use, considerable expense and trouble could be avoided. With this object in view, Jaswant Singh, Ray and Nair (1953) evolved preparation of the stain in powder form. According to this method, the original ingredients (medicinal methylene blue, dilute sulphuric acid and potassium dichromate) are mixed thoroughly in water and heated in an autoclave. It was also found that autoclaving was not essential and the mixture could be boiled with direct heat or even water bath depending on the facilities available to the worker. To prevent excessive evaporation, a reflux condenser or a long glass tubing fitted to the centre of the flask has been recommended. The period of heating under such circumstances may vary. As such, they have emphasized that the end point of boiling depends on the appearance of permanent deep blue colour with slight violet iridescence, which does not turn greenish on cooling. In the latter event, the stain solution is boiled again for some more time. The question of number of hours for which the solution should be heated was later critically investigated by Bami and Nair (1955) and it was observed that products heated for 6 - 7 hours under above conditions, were most satisfactory. The indication that the solution has been heated properly can be had by filtering a few c.cm. of the boiling solution. The filterate must maintain its blue colour even after cooling it. The same can also be ascertained by drawing the boiling solution in a capillary tube and finding whether the blue colour of the solution is retained, when the tube is cooled. After the solution has turned deep blue, the flask is cooled to the room temperature and the precipitate filtered. The precipitate on the filter paper is dried at room temperature in a vacuum desiccator. The dried precipitate is carefully collected and powdered thoroughly with 1·75 gm. of disodium hydrogen phosphate dihydrate (Na_2 HPO_4, $2H_2O$) in a glass mortar and stocked in small specimen tubes. Whenever required, the powder in the specimen tube is dissolved in distilled water (450 ml.) and used after allowing it to mature for at least four days. The yield of the powder (without disodium hydrogen phosphate) from different batches, varied between 0·35 gm. and 0·5 gm. When only small quantities of the stain are required, it is preferable to mix 1/5th of the stain powder

with 0·35 gm. of disodium hydrogen phosphate and dissolve in about 100 c.c. distilled water. The dried powder keeps well. In experienced hands, the above filtrate (after the removal of precipitate) with addition of 2·5 gm. of disodium hydrogen phosphate, and allowing to mature for about two weeks, can also be used as Solution I, though less satisfactory.

Preparation of stain J.S.B. I solution in modified liquid form.—According to this method, methylene blue (0·5 gm.) is dissolved thoroughly in 500 c.c. water in a flask. Sulphuric acid (one per cent. 3 ml.) is added drop by drop to the solution and then mixed with potassium dichromate (0·5 gm.). Addition of potassium dichromate leads to the formation of some precipitate and at this step, 3·5 gm. of disodium hydrogen dihydrate is added. The resulting mixture is boiled for only half an hour. The stain is allowed to cool at room temperature and is ready for use as J.S.B. Solution I (Jaswant Singh and Misra, 1956).

Preparation of stain J.S.B. Solution I in concentrated form.—For the preparation of J.S.B. Solution I in a concentrated form, the ingredients (medicinal methylene blue 0·5 gm., one per cent sulphuric acid 50 minim; potasium dichromate 0·5 gm. and disodium hydrogen phosphate 3·5 gm.) are dissolved in 150 c.c. water in a beaker and the mixture allowed to boil for approximately one hour. When the solution has been reduced to nearly 25 ml., the concentrated stain is stored in glass stoppered bottle and diluted with distilled water to make it up to 500 ml. It is allowed to mature for three days prior to its use (Jaswant Singh and Misra, 1956).

MODIFICATION IN THE PREPARATION OF WASH WATER.

Buffered wash water has been recommended instead of acidulated water for the washing of blood smears (Manwell and Feiglson, 1948; Jaswant Singh, Ray and Nair, 1953). This is prepared either by dissolving disodium hydrogen phosphate dihydrate (0·417 gm.) and potassium acid phosphate (0.752 gm.) in distilled water (2 litre), or by dissolving 0·22 and 0·74 gm., respectively, of the above two in 1.000 ml. distilled water. To maintain the initial *p*H, it is preferable to keep the buffered water in glass bottles. It is easier to weigh the salts according to the second formula, and at the same time the buffered water keeps the proper *p*H for a longer period. The use of buffered acid water has given more uniform results.

3. CHEMICAL COMPOSITION AND STABILITY.

Polychromed methylene blue is known to consist of a mixture of methylene blue and its oxidation products depending upon the extent and nature of oxidation technique employed.

From Fig. I, it will be evident that trimethyl thionin (Azure *B*) and asm-dimethyl-thionin (Azure *A*)) are formed as primary products in both acidic and alkaline oxidation of tetra methyl thionin (methylene blue) (Kehrmann, 1906; Bernthsen, 1906; MacNeal, 1906:1925). Further extensive oxidation of methylene blue under acidic conditions leads to the formation of monomethyl thionin, called Azure *C* (Holmes and French, 1926) and finally the parent compound thionin

itself (Fig. 1). The alkaline hydrolysis finds a different route and leads to the formation of thionol. J.S.B. stain essentially consists of Solution I, an aqueous polychrome methylene blue solution prepared by dichromate acid oxidation of methylene blue, and a simple aqueous eosin solution (*See* method of preparation). Although several minor modifications in the dichromate-acid-oxidation of J.S.B. Solution I have been successfully employed (Jaswant Singh *et al.*, 1953; Manwell and Feiglson, 1948; Cole and Sewell, 1953), the original method of oxidation has continued to give highly satisfactory results. Therefore, the chemical studies on the composition, quality, and stability of J.S.B. Solution I were confined by Bami and Nair (1955) to the product obtained by original technique of oxidation.

Bami and Nair (1955) prepared J.S.B. Solution I with varying time of oxidation and the final *p*H of the solution was adjusted by the addition of 2·5 gm., 5 gm., 10 gm. and 15 gm. of disodium hydrogen phosphate dihydrate.

From the results, it was evident that original method for the preparation of J.S.B. Solution I was very satisfactory, provided the partial polychroming of methylene blue is controlled by heating the mixture for 6 to 7 hours, and the final solution buffered to a *p*H of 7·75 to 7·8 with disodium hydrogen phosphate dihydrate. An azure absorption reading of 0·21 ±0·03, according to the method of Bami and Nair (1955), correctly determines the extent of oxidation. Longer periods of heating and excess of alkaline buffer salt have resulted in poor quality products (Table I). Basic dyes like the thionin derivatives display intense staining properties with increasing *p*H (Haynes, 1928) but it seems that staining qualities of polychromed methylene blue in the case of J.S.B. stain are optimum around *p*H 7·8. It had been observed previously that Azure *C*, a good nuclear stain and exclusively produced during the acid oxidation of methylene blue (Fig. 1), gave the best performance at *p*H of 7.7. to 8 (Holmes and French, 1926).

After over five months of storage under drastic tropical conditions, J.S.B. Solution I maintained at an alkaline *p*H underwent general precipitation and/or decomposition of the azures. Basic thiazine dyes, stored at an alkaline *p*H, are known to have precipitated from their solution on storage (Haynes, 1928; Holmes and French, 1926), which caused weakening of the stain solution and faulty staining. However, it has been observed that if J.S.B. Solution I is kept in a cooler place (air-conditioned rooms during severe summer months), the keeping qualities improve very considerably. In the present experiment, no doubt, the stain solutions have been maintained in very adverse conditions but even then they have lasted for five months (Bami and Nair, 1955).

Spectrophotometric analysis of the medicinal methylene blue gave maximum absorption at 650-655 millimicrons when compared to that of 665 millimicrons in the case of pure methylene blue sample (Stotz *et al.*, 1950) indicating the presence of Azure *B* as a major contaminent (Lillie, 1943). Absorption curves for freshly prepared and 10-weeks-old J.B.S. stain Solution I showed flat tops (maximum absorption between 600-640 millimicrons) which lie between the peak absorption of thionin (590 millimicrons) and methylene blue (650-655 millimicrons) and also covered the maximum absorption wave lengths of Azures *A*, *B* and *C* (Fig. 1 and Graph 1).

Height of the J.S.B. Solution I curve is lower than that of thionin and methylene blue showing it to be a mixture of several of these products. On the

TABLE I.

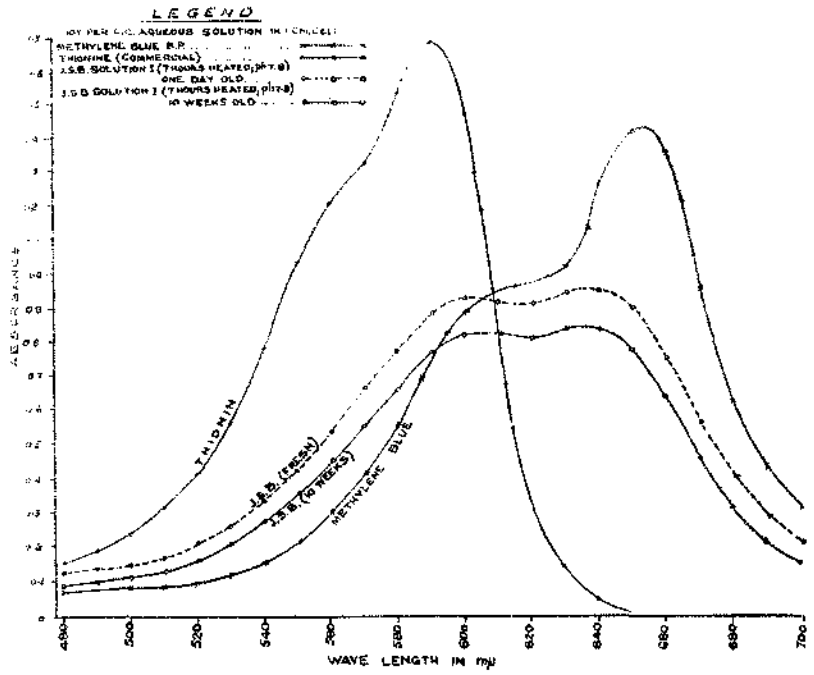
Effect of tropical storage conditions on J.S.B. Stain Solution I with different pH.
(Average of five months performance.)

Serial number.	Disodium hydrogen phosphate dihydrate added after dichromate-acid oxidation.	THREE HOURS HEATED:			FOUR HOURS HEATED:			FIVE HOURS HEATED:			SIX HOURS HEATED:			SEVEN HOURS HEATED:			EIGHT HOURS HEATED:		
		Staining quality.	*p*H	Mean absorption.	Staining quality.	*p*H	Mean absorption.	Staining quality.	*p*H	Mean absorption.	Staining quality.	*p*H	Mean absorption.	Staining quality.	*p*H	Mean absorption.	Staining quality.	*p*H	Mean absorption.
1.	2·5 gm.	Good	7·4	·09±·02	Good	7·4	·15±·03	Good	7·5	·18±·03	Good	7·5	·23±·03	Good	7·65	·26±·03	Good	7·6	·30±·03
2.	6 gm.	Good	7·65	·09±·02	Good	7·7	·13±·02	Good	7·7	·17±·02	Very good	7·75	·21±·03	Very good	7·8	·24±·03	Fair	7·85	·28±·03
3.	10 gm.	...	...	...	Good	7·85	·15±·02	Good	7·85	·19±·02	Fair	7·85	·23±·02	Fair	7·85	·27±·02	Poor	7·9	·32±·03
4.	15 gm.	...	...	...	Good	8·05	·12±·02	Fair	8·05	·16±·02	Poor	8·05	·20±·02	Poor	8·1	·26±·02	Poor	8·1	·32±·02

Mean absorption = Absorption values for other extracted azures.
Thin smears of *P. cynomolgy* were used for the purpose.

other hand, 10-weeks-old product appears to be a little diluted, perhaps due to some precipitation of the dissolved dyes (Graph 1).

GRAPH 1.

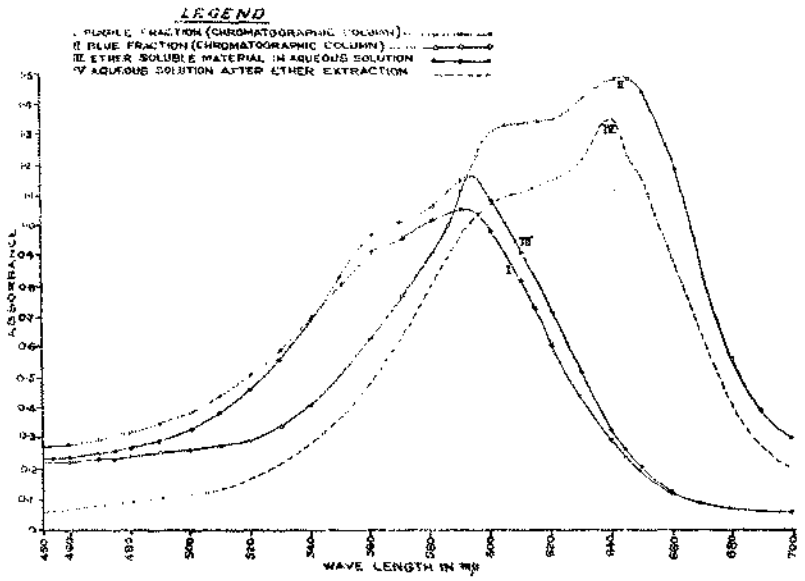


Spectrophotometric analysis of ether soluble azure fraction of J.S.B. Solution I gave maximum absorption at 590 millimicrons, but the nature of absorption curve was not exactly similar to that of thionin (Graph 2) while further chemical considerations led to the conclusion that ether soluble fraction contained not only thionin but also materials closely similar to it, such as Azure *C*, (Bami and Nair, 1955). The stain solution left after ether extraction offered an absorption curve, indicative of methylene blue slightly contaminated with other products like Azure *A*, and *B* (Graph 2). Absorption curves of two main fractions obtained through paper chromatography of J.S.B. Solution I (Graph 2) were also identical to those obtained in the case of separation by ether extraction (Bami and Nair, 1955). Spectrophotometric studies with azures have so far not given constant results (Stotz *et al.*, 1950; Ball and Jackson, 1953) and as such the conclusions are based on the available data and the present experimental findings.

TECHNIQUE OF STAINING.

Solutions I and II and wash water should be kept in wide mouth stoppered jars 1½ inches diameter by 2½ inches height.

GRAPH 2.



ORIGINAL TECHNIQUE (JASWANT SINGH AND BHATTACHARJI, 1944).

(*a*) *Thick smear alone.*—

1. Immerse the slide in Solution I for 10 seconds;
2. Wash in jar containing acidulated water (*p*H 6·2 to 6·6) for two seconds;
3. Stain in Solution II for one second;
4. Wash in the same jar (2) for five seconds;
5. Immerse in Solution I again for ten seconds;
6. Wash as above for two seconds or till the smear gives pink background (2);
7. Dry and examine.

The total time taken is only 39 seconds. This can be shortened to 10 seconds, but with old or very thick smears, it is preferable to follow the above timing.

(*b*) *Thick and thin smears taken on the same slide.*—

1. Fix the thin smear by dipping that part of the slide in a jar containing methyl alcohol for a second or two;
2. Dry thoroughly, preferably by waving the slide in the air;
3. Immerse the whole slide in Solution I for 30 seconds;
4. Wash in jar containing acidulated tap water (*p*H 6·2 to 5·6);
5. Stain in Solution II for one second;
6. Wash in the same jar (4) for four seconds;

7. Immerse in Solution I again for 30 seconds (3);
8. Wash as above for 10 seconds or till the smear gives pink background (4);
9. Dry and examine.

The total time taken for staining is 1 minute and 20 seconds.

MODIFIED TECHNIQUE (JASWANT SINGH, RAY AND NAIR, 1953).

(*a*) *Thick and thin smears taken on the same slide.*—

1. The thin film is fixed by dipping that part for a second in a jar containing methyl alcohol.
2. After thoroughly drying, immerse the whole slide in a jar containing Solution II (eosin) for one or two seconds.
3. Excess of eosin stain is removed from the smear by dipping the slide in a jar containing the buffered wash water.
4. It is then immediately transferred to Solution I and kept for 40 to 45 seconds;
5. After this, it is finally washed by dipping in the same wash water for three to four seconds;
6. Dry and examine.

Thus the whole staining period has been reduced to less than a minute instead of 1 minute and 20 seconds;

(*b*) *Thick smears alone:*—

If only thick films are to be stained, a dip for 10 or 15 seconds in Stain I after staining in Solution II is considered sufficient to give good results.

One jar full of wash water is invariably enough for one day's use without changing, and will suffice to stain approximately 50 blood smears.

When large number of blood smears have to be stained, special metal racks, holding 20 to 100 slides and troughs of suitable sizes can be used to save time.

USES OF J. S. B. STAIN.

Various workers have also reported that stipplings in certain plasmodia are better demonstrated with J.S.B. Stain than Giemsa. Ramakrishnan and Satya Prakash (1950) reported that stipplings in cells infected with *P. berghei* were demonstrable to a great extent in smears stained by J.S.B. than Giemsa. Nair (1950) demonstrated stipplings in *P. falciparum* with greater ease than with Leishman or Giemsa. Subsequently, Jaswant Singh *et al.* (1953) reported stipplings in the Nuri strain of *P. knowlesi* hitherto not reported in this species of plasmodium with ordinary methods, when the smears were stained with J.S.B. Stain. It can be used for staining oöcysts in the midgut of mosquitoes and sporozoites in the salivary glands (Jaswant Singh and David, 1949).

It can also be used for staining all the blood elements where the leucocytes are stained well and thus it aids in differential leucocyte count.

It can be used for staining organisms, other than plasmodia also. It gives excellent results in staining Leishmania (Craig, 1948; Manwell and Feiglson, 1948) and trypanosomes (Craig, 1948; Nair and Basu, 1950). Subsequently, the stain was advocated for staining organisms such as toxoplasma, hæmoproteus (Ray, 1949); microfilaria (Raghavan and Krishnan, 1949); hepatazoon (Nair and Basu, 1950); pasteurella, spirochaetes and anthrax (Nair and Basu, 1950; Dass, 1953).

ADVANTAGES OF J. S. B. STAIN.

One spectacular achievement of this stain is the remarkable shortening of the staining time in that a thick and thin film on the same slide can be stained within a minute or so. Later, due to the recent modifications, the staining time has been further reduced to less than a minute. Preparations treated with J.S.B. Stain compare favourably with those stained with any standard preparation like Leishman or Giemsa.

After working extensively with J.S.B. Stain, Manwell (1945) reported that the stain has the following advantages:—

" 1. It is easily prepared from ordinary medicinal methylene blue and eosin.
2. It is relatively inexpensive.
3. It keeps well under different climatic conditions.
4. It is extremely fast (for thin smears, eight seconds; thick films, thirty seconds).
5. It is equally good for both thick and thin smears which may be stained together on the same slide.
6. Solutions used for staining do not have to be made up each time since they deteriorate little on standing.
7. Blood cells and parasites are clearly and brilliantly differentiated.
8. Results depend less on the *p*H of the diluting agent than with any other Romanowsky stains (especially Giemsa) ".

He further indicated that " all the common blood parasites, for which Giemsa or Wright's stains are usually used, can be demonstrated equally well by the method just outlined ". In conclusion, he mentioned that " the J.S.B. Stain, recently introduced by Singh and Bhattacharji has been tested and been found superior in most respects to any of the other commonly used processes for the staining of blood and blood protozoa ".

Russell *et al.* (1946) also confirmed the findings of Manwell (1945) and in a subsequent publication, Russell (1952) described only two stains for studies on malaria parasites, namely, Giemsa and J.S.B.

At present the stain is being widely used in India and in many institutions and laboratories abroad. According to Belding (1952), " Among the various modifications and refinements of the rapid methods of Romanowsky stains, J.S.B. method of staining has been the most popular ".

The latest simplified technique of preparing the stain Solution I has brought in some more advantages in that:

1. The time required for its preparation can be even reduced up to one hour.
2. No strict supervision is required to note the colour changes, particularly for the end point.
3. The stain can be prepared just as well under field conditions as in any well equipped laboratory.
4. The stain needs no filtration and
5. Requires no maturation.
6. Jaswant Singh, Ray and Nair (1953) worked out the cost of this stain and found that to stain 100 slides, the cost of J.S.B. is approximately 0·22 annas and Giemsa 11 annas, thus J.S.B. stain is about 50 times cheaper than Giemsa.

SUMMARY.

J.S.B. Stain (Jaswant Singh and Bhattacharji, 1944) was introduced as a rapid, efficient and economical water soluble stain for malaria parasites during the Second World War when the need for a good rapid stain was most pressing. The advantages of J.S.B. Stain were soon recognized by workers in the field all over the world and its popularity continued to grow from year to year. J.S.B. Stain has a proven merit for not only staining malaria parasites but a number of other organisms as discussed in the text. Studies on its chemical composition and standardization of techniques of preparation and staining have increased its range of usefulness. Economy in cost, coupled with rapid and excellent staining qualities, has greatly popularized the product.

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Indian Journal of Malariology, **10**, 2, June 1956.

THERAPEUTIC EFFECT OF SULPHADIAZINE AND DIHYDROTRIAZINES AGAINST BLOOD-INDUCED *P. CYNOMOLGI* INFECTION.

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[May 1, 1956.]

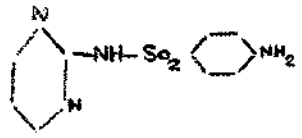
In the previous communications, antimalarial activity of quinine, chloroquine, avloclor, nivaquine, amodiaquin (Jaswant Singh, Nair and Ray, 1953); proguanil, bromoguanide, pyrimethamine (Nair, Ray and Jaswant Singh, 1953); M. 3349, mepacrine, and the active metabolite of bromoguanide (Ray *et al.*, 1954) against blood-induced *P. cynomolgi* infection was reported. In this paper, the comparative efficacy of three other drugs, sulphadiazine, supazine (sulphonamide substituted dihydrotriazine) and the active dihydrotriazine metabolite of proguanil, has been recorded.

MATERIALS AND METHODS.

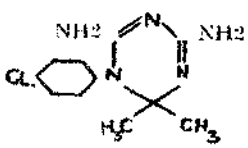
Host.—Sixty-five healthy *M. mulatta mulatta* (*S. rhesus* monkeys) were utilized for the test.

Parasite.—*P. cynomolgi* strain (Sinton and Mulligan, 1932) maintained at the Institute by serial sub-passages from monkey to monkey, was used for the investigation. The dose of inoculum was standardized at five million parasitized erythrocytes per kg. body weight of the animal as indicated in earlier publications (Jaswant Singh, Nair and Ray, 1953). The inoculum was injected intravenously. Daily parasite count was recorded in terms of 10,000 erythrocytes. Up to the cessation of treatment, blood smears for parasite enumeration were obtained twice a day (morning and evening), but subsequently only morning smears were collected. The stain used for staining the smears was J.S.B. (Jaswant Singh and Bhattacharji, 1944).

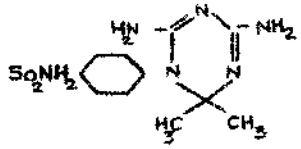
Drug.—The chemical structure of the three drugs tested is given below:—



Sulphadiazine



Active dihydrotriazine metabolite of proguanil



Sulphadiazine substituted dihydrotriazine

The active dihydrotriazine metabolite of proguanil [1-(3:4-dichlorophenyl)-2:4-diamino-6:6-dimethyl-1:6-dihydrotriazine] and the sulphonamide substituted dihydrotriazine (1-p-sulphonamidophenyl-1:6-dihydro-2:4-diamino-6:6-dimethyl-1:3:5-triazine) used for the tests were from the samples synthesized at the Institute. Sulphadiazine used was the M. & B. product. The dosages of these drugs were all in terms of the base of the drug per kg. body weight of the animal. Drugs were started at about 0·1 per cent cell infection and continued once a day thereafter up to a total of seven doses. The route of administration was oral in all cases.

Criteria of activity.—The general practice of classifying the activity as Class I, II and III at the Institute was adopted in this case also. Mere deceleration of parasites to about 25 per cent of the normal infection was classified as Class I effect. Clas II effect of Shannon (Wiselogle, 1946) was indicated if complete clearance of parasites from the peripheral blood was obtained by about 24 hours after the last dose of the drug. On the other hand, complete absence of parasites for a month preceding, and for a similar period after splenectomy performed four weeks subsequent to the cessation of treatment, marked Class III effect. Contrary to all these, if infection ran more or less parallel to the pattern observed in the infected 'control' monkeys, the particular dosage of the drug was considered ineffective. The minimum dose of a particular drug that gave rise to Class II effect, in a total of five monkeys so treated, was taken as the minimal effective dose (M.E.D.).

RESULTS.

Sulphadiazine. Dosage regimes ranging from 0·05 to 20 mg. were tried. The details of the results obtained are recorded in Table I. It was observed that a dose of 0·05 mg. was not sufficient to produce Class II effect in any of the monkeys. With 0·1 mg., Class II effect was observed only in one out of three monkeys. With 0·15 mg. dose, clearance of parasites was obtained in all the five monkeys tried by the day following the cessation of treatment. This dosage, therefore, represented the M.E.D. of sulphadiazine. Between 0·25 and 20 mg. doses, Class III effect was observed in about 28 per cent of the monkeys, and in the rest Class II effect.

Sulphonamide substituted dihydrotriazine.—The results are set out in Table II. With 10 and 15 mg. doses, Class II effect was observed only in one-third of the total number of animals treated. The rest showed only Class I effect. However, a dose of 20 mg. or above was able to show Class II effect in about fifty per cent of the monkeys and Class III effect in the rest. Hence the minimum dose of 20 mg. is taken as the M.E.D. of sulphonamide substituted dihydrotriazine.

TABLE I.

Effect of sulphadiazine against blood-induced P. cynomolgi.

Dosage mg./kg. (base).	Number of monkeys employed.	Results.							
		Inactive (Number).	Class I effect (Number).	Class II effect (Number).	Class III effect (Number).	Parasite clearance (hours).		Relapse (Days).	
						Average.	Range.	Average.	Range.
0·05	2	1	1	...	...	...	...	...	...
0·1	3	1	1	1	...	168	168	16	16
0·15*	5	...	...	5	...	110	84–142	10·75†	8–13
0·25	5	...	...	4	1	127·2	96–156	11·7†	11–12
0·5	4	...	...	3	1	146·4	96–168	11†	4–18
1·0	2	...	...	2	...	120	120–132	‡	...
2·5	2	...	...	...	2	144	144	...	...
15	2	...	...	2	...	108	96–120	11	2–20
20	3	...	...	2	1	112	108–120	20†	20

*M.E.D. (Minimal effective dose). †One died during the observation period.
‡Both died during observation period.

TABLE II.

Effect of sulphonamide substituted dihydrotriazine against blood-induced P. cynomolgi *infection.*

Dosage mg./kg. (base).	Number of monkeys employed.	Results.							
		Inactive (Number).	Class I effect (Number).	Class II effect (Number).	Class III effect (Number).	Parasite clearance Hours.		Relapse (days).	
						Average.	Range.	Average.	Range.
10·0	3	...	2	1	...	48	48	...	...
15·0	3	...	2	1	...	72	72	...	...
20·0*	5	...	...	3	2	115	48–154	4‡	4
25·0	3	...	...	1†	2	92	48–132	...	...
30·0	3	...	...	1†	2	96	72–108	...	...

*Minimal effective dose (M.E.D.). †One monkey died during observation period.
‡Two monkeys died during observation period.

Active metabolite of proguanil.—Though Class II effect could be observed in a few cases with as low a dosage as 0·3 mg., only a higher dose of 6 mg. and above could be depended upon for the complete clearance of parasites by about the day following the completion of treatment. Besides, complete sterilization of the infection (Class III effect), in this dose, was also attained in about 55 per cent of the monkeys. Six mg. dosage is, therefore, considered to be the M.E.D. of the drug. Details of the results are shown in Table III.

TABLE III.

Effect of the active metabolite of proguanil against blood-induced P. cynomolgi *infection.*

Dosage mg./kg. base).	Number of monkeys employed.	RESULTS.							
		Inactive (Number.	Class I effect (Number).	Class II effect (Number).	Class III effect (Number).	Parasite clearance (hours).		Relapse (days).	
						Average.	Range.	Average.	Range.
0·3	2	...	1	1	...	72	72	16	16
0·5	2	...	2	...	...	...	...	...	...
1·0	2	...	1	1	...	132	132	3	3
5·0	3	...	1	1	1	61	60–72	13	13
6·0*	5	...	...	3	2	93·6	60–120	12†	12
8·0	3	...	...	1	2	108	108	‡	...
10·0	3	...	...	1	2	128	96–128	‡	...

*M.E.D. (Minimal effective dose). †Two monkeys died during the observation period.
‡One monkey died during the observation period.

DISCUSSION.

The minimum effective dose of quinine against *P. cynomolgi* was observed to be 20 mg. (Jaswant Singh, Nair and Ray, 1953) and therefore the quinine equivalent of sulphadiazine was calculated to be 133·3, of supazine 1, and that of the active metabolite of proguanil 3·3.

The M.E.D of proguanil was previously recorded as 1 mg. (Nair, Ray and Jaswant Singh, 1953). From this it is clear that the active metabolite of proguanil, the M.E.D. of which was observed to be 6 mg., is about six times less active as that of the parent drug. Schmidt *et al.* (1952) made similar observations and reported that proguanil is two to four times as active as its metabolite against the same species of parasite.

Another interesting feature observed was that although sulphadiazine was highly effective against *P. cynomolgi* and proved to be many times superior to quinine, the sulphadiazine substituted dihydrotriazine had only the same effect as that of quinine. In an earlier investigation, supazine was found to be even less

effective against *P. gallinaceum*, its quinine equivalent being only 0·125 to 0·25 (Nair, Misra *et al.*, 1955).

Against Nuri strain of *P. knowlesi*, the quinine equivalent (Q.E.) of sulphadiazine was calculated as 37.5, that of supazine was established to be 0·105 (Ray and Nair, 1955) and the active metabolite (dihydrotriazine) of proguanil as 0·86 (Nair, Bami and Ray, 1955).

Thus it would be evident that *P. cynomolgi* is more sensitive to these groups of compounds than the Nuri strain of *P. knowlesi*. This difference in reaction in the same host to the same drugs could obviously be due to the biological difference of the two species. Perhaps the metabolic reaction of *P. knowlesi* is interfered with less effectively by the above drugs than that of *P. cynomolgi*. It may also be assumed that the metabolic pathway employed by *P. cynomolgi* is probably more adversely effected by these drugs than that followed by *P. knowlesi*. Finally, whether this observed difference in the response to drugs can be due to the differences in the absorption of the drugs by the two species of plasmodia, cannot also be ruled out.

SUMMARY.

Sulphadiazine, sulphonamide substituted dihydrotriazine (supazine) and the active dihydrotriazine metabolite of proguanil were tested against blood-induced *P. cynomolgi*, and their minimal effective doses (M.E.D.) were determined as 0·15, 20 and 6 mg. base per kg. body weight of the monkey respectively.

Sulphadiazine was found to be 133 times superior to the sulphonamide substituted dihydrotriazine (supazine) and 40 times superior to proguanil metabolite.

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

Part II. Attempts at experimental transmission and determination of the sites of infection in birds and mosquitoes.

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THE first part of the studies (David and Nair, 1955) described the presence of a naturally occurring cryptic infection of trypanosomes in domestic sparrows which was detectable only by feeding *Culex fatigans* mosquitoes and dissecting them later. The strain of parasite has since been maintained in laboratory bred *C. fatigans* mosquitoes by feeding them on (clean) sparrows experimentally inoculated with developmental forms of the flagellate obtained from infected mosquitoes.

A wide variety of birds as well as monkeys and albino rats were shown to be susceptible to the trypanosomes. But, in all these animals, however, the infection was cryptic from the beginning and was only recoverable from mosquitoes fed on them.

The present report deals with experiments to locate the site of infection in birds and mosquitoes and to transfer the infection from bird to bird without the intervention of mosquitoes.

MATERIALS AND METHODS.

Laboratory bred *Culex fatigans* were used for the investigation. Experimental feeding of mosquitoes was mostly carried out at night by confining the infected birds in a wire mesh, and placing it in a cage containing the mosquitoes.

The birds utilised in the studies were procured from a local dealer. The majority of them were sparrows and bulbuls (nightingales). *C. fatigans* mosquitoes were fed on each of the birds and dissected after an interval of 8 to 14 days. Only the ones, which did not infect the mosquitoes, were labelled as "clean" birds and utilised for the experiments. The rest were rejected.

Aseptic precautions were observed in all the manipulations. All inoculations were made either intraperitoneally or intravenously. Organ suspensions were made by grinding the organ in sterile normal saline by using a pestle and mortar.

EXPERIMENTAL DETAILS.

The source of trypanosome infection in the mosquitoes.—One hundred laboratory bred *C. fatigans* female mosquitoes were fed for 15 consecutive days on a sample of raisin upon which a batch of infected mosquitoes had been previously maintained. At the end of this period, none of the mosquitoes when dissected showed any evidence of infection with trypanosomes. On the other hand, in thirty per cent of similar mosquitoes that had fed on a trypanosome infected sparrow (Number 1), showed developing trypanosomes. The experiment was repeated with identical results. This showed that the only source of trypanosome in the mosquitoes was the cryptic infection in birds.

Search for trypanosomes in the skin capillaries.—The skins of heavily infected nightingale (Number 1) and sparrow (Number 3) were scraped with the sharp edge of a knife held at right angle to the surface of the skin. Impression smears were made of the scraped surfaces on slides and stained with J.S.B. stain. On examination, these did not show any trypanosomes. It was apparent that the organisms were not to be found in the skin capillaries.

Search for trypanosomes in the peripheral blood soon after experimental inoculation.—Six clean sparrows (Numbers 4 to 9) were used for the experiment. Each of these was inoculated intravenously (right wing vein) with viable parasites obtained from experimentally infected mosquitoes. Immediately following inoculation, blood was withdrawn from the left wing vein of each of these birds at intervals of one to six minutes and examined. No parasite could be found in the stained blood smears. This perhaps indicated that the trypanosomes were not present in the peripheral blood immediately or soon after inoculation.

Blood-induced infection.—Five sparrows (Numbers 19 to 23), three nightingales (Numbers 3 to 5) and one seven sister (Number 1) were each inoculated with 0·075 to 0·75 c.c. of blood from experimentally infected birds. During an interval of four to eighty-four days after the inoculation, many batches of mosquitoes were fed on the birds. The mosquitoes were dissected three to twenty-seven days subsequent to the feed. The results are set out in Table I. The blood-induced infection was established in six out of the nine inoculated birds.

Duration of infectivity of birds to mosquitoes.—Sparrow 24 was experimentally inoculated with trypanosomes from experimentally infected mosquitoes. Eleven batches of clean mosquitoes were fed on the bird successively from six to twelve hours after the inoculation and thereafter at varying intervals up to eleven days.

TABLE I.

Transmission by blood inoculation.

DETAILS OF THE DONAR BIRDS:			RECIPIENT BIRDS:		DETAILS OF BLOOD INOCULATION:					PERCENTAGE POSTIVE:		
Species	Number.	Nature of infection (Percentage).*	Species.	Number.	Route.	Quantity (c.c.).	Interval between inoculation and feeding of mosquitoes on the recipient birds (days).	Interval between feeding and dissection (days).	Number dissected.	Hindgut.	Foregut.	Total.
Sparrow ...	10	33	Sparrow	19	Intraperitoneal	0·125	11	11	61	...	...	...
,, ...	10	33	,,	...	,,	0·125	13	3–12	31	...	...	...
Bulbul Nightingale)	2	29	,,	20	,,	0·5	6	4–9	57	...	...	...
Sparrow ...	13	100	,,	21	,,	0·14	5	9–27	145	0·6	...	0·6
,, ...	13	100	,,	22	,,	0·45	6	9	102	0·6	...	0·6
,, ...	13	100	,,	22	,,	0·45	19	5–20	70	...	...	...
,, ...	13	100	,,	23	,,	0·075	10	10–12	97	1	...	1
,, ...	11	8·3	Bulbul (Nightingale)	3	Intravenous	0·4	4	13–16	40	...	5	5
,, ...	11	8·3	,,	3	,,	0·4	83	3–11	131	0·5	1	5·4
,, ...	11	8·3	,,	4	Interaperitoneal	0·4	5	3–15	37	5·4	...	1·5
,, ...	11	8·3	,,	4	,,	0·4	84	3–8	49	2	...	2
,, ...	12	100	,,	5	,,	0·75	7	9–13	106	0.9	...	0·9
Seven sister	10	33	Seven sister	1	,,	0·125	13	8	52	...	...	...
,, ,,	10	33	,,	1	,,	6·125	19	16	135	...	...	...

*Percentage of the mosquitoes that showed infection out of the lot that were fed on the donar bird immediately after subinoculation to the recipient birds.

Similarly nine other batches of mosquitoes were fed at different intervals on a nightingale (Number 6) commencing from the twentieth day of experimental inoculation of the bird to the eighty-fourth day. Later, on dissection of the mosquitoes, infection was detected in all the batches except the ones that had fed within 12 hours after the experimental inoculation. The experimental details are given in Table II. The results indicated that trypanosomes, experimentally inoculated into birds, were not in the circulating blood during 6 to 12 hours after the inoculation, but were present in the blood thereafter for as long a period as 84 days.

Table II.

Estimation of the duration of infectivity period of the infected birds.

Interval between inoculation and mosquito feeding:		Results of dissection of mosquitoes 8 to 33 days after blood feed:			
Bird number.	Interval	Number dissected.	Percentage positive.		
			Hindgut.	Foregut.	Total.
Sparrow number 24 ...	6–12 hours	22	...	...	...
,, ,, ...	13–16 ,,	145	0·7	...	0·7
,, ,, ...	30–36 ,,	36	2·8	...	2·8
,, ,, ...	78–84 ,,	34	3·0	64·9	67·9
,, ,, ...	3 Days	5	...	40·0	40·0
,, ,, ...	4 ,,	99	3·0	11·1	14·1
,, ,, ...	5 ,,	179	...	19·5	20·1*
,, ,, ...	7 ,,	13	...	47·1	53·8†
,, ,, ...	8 ,,	23	34·8	30·4	65·2
,, ,, ...	9 ,,	47	4·3	47·3	51·6
,, ,, ...	11 ,,	66	3·0	1·5	4·5
Nightingale number 6 ...	20 ,,	43	2·4	16·2	34·9‡
,, ,, ...	22 ,,	40	2·5	22·5	50·0§
,, ,, ...	23 ,,	24	...	20·8	25·0§§
,, ,, ...	32 ,,	26	...	15·4	15·4
,, ,, ...	54 ,,	68	...	11·8	11·8
,, ,, ...	60 ,,	13	...	7·7	7·7
,, ,, ...	62 ,,	50	8·3	...	8·3
,, ,, ...	67 ,,	25	...	60	60
,, ,, ...	84 ,,	19	...	2·0	2·0

*Midgut infection 0·6 per cent. †Midgut infection 0·7 per cent.
‡Midgut infection 4·7 per cent and gland infection 11·6 per cent.
§Midgut infection 2·5 per cent and gland infection 22·5 per cent.
§§Gland infection 4·2 per cent.

Infectivity of organs of birds.—A saline suspension was prepared of brain, bonemarrow, liver, spleen and heart obtained from an experimentally inoculated myna (a kind of starling). A clean nightingale (Number 7) was inoculated intraperitoneally with the suspension. Emulsions were prepared separately of

liver, heart, brain, bonemarrow and lung from experimentally infected sparrows (Numbers 14 to 18). Clean birds were inoculated with organ suspension of either liver (Sparrows numbers 27 and 28), or heart (Sparrow number 26), or lung (Sparrow number 29 and myna number 2) or bonemarrow (Nightingale number 8) or a mixed suspension of brain, bonemarrow, liver and spleen (Sparrow number 25). Commencing from the third day after the inoculation up to the thirtieth day, several batches of mosquitoes were fed on each of the inoculated birds. The mosquitoes were subsequently dissected between the sixth and fourteenth day of the feed. It was found that mosquitoes fed on the nightingale (Number 7) which was inoculated with mixed suspension of brain, bonemarrow, liver, spleen and heart, and the sparrow (Number 29) inoculated with lung suspension did not show any infection of trypanosome. All the other birds inoculated with suspension of different organs, were infected, though only cryptically, as mosquitoes fed on them contained trypanosomes. Experimental details are given in Table III.

TABLE III.

Transmission by tissue inoculation.

Details of the donar birds.			Details of recipient birds.		Details of tissue inoculation.			Interval between tissue inoculation and feeding of mosquitoes (days).	Interval between feeding and dissection. (days).	Number dissected.	Percentage positive.		
Species.	Number.	Nature of infection (percentage).	Species.	Number.	Organ.	Route.	Quantity (c.c.).				Hindgut.	Foregut.	Total.
Myna	1	100	Nightingale	7	Brain, Bone marrow Liver, Spleen, Heart	Intraperitoneal	1	8	10–14	63	...	...	...
Sparrow	14	38·6	Sparrow	25	,,	,,	0·6	3	6–10	106	0·72	0·18	0·9
,,	15	24	,,	26	Heart	,,	0·5	5	6–10	89	1·1	...	1·1
,,	16	45·7	,,	27	Liver	,,	0·4	7	9–16	69	...	7·2	7·2
,,	17	24	,,	28	,,	,,	0·5	5	6–13	125	0·8	...	0·8
,,	18	70	,,	29	Lungs	,,	0·025	8	10	91	...	...	...
,,	18	70	Myna	2	Lungs	,,	0·025	10	8	70	...	1·4	1·4
,,	18	70	Nightingale	8	Bone-marrow	,,	0·025	9	11–13	71	...	...	...
,,	18	70	,,	8	,,	,,	0·025	30	10–12	119	4·1	...	4·1

Attempts at mosquito transmission of the infection.—Eleven and thirteen infected mosquitoes respectively were fed on two clean sparrows (Numbers 30 and 31). Ten days later, a large number of mosquitoes were fed on these potentially infected

sparrows. These mosquitoes in turn were dissected 10 days subsequent to their blood feed. Not even a single one out of these was found infected. The details are given in Table IV.

TABLE IV.

Results of the attempted transfer of trypanosome infection to sparrows by the bites of infected Culex fatigans.

Number of the clean sparrow.	Results of dissection of the infected mosquitoes within 12 hours after feeding on the two birds referred to in Column 1.			Results of dissection of mosquitoes 10 days after their blood feed from potentially infected sparrows referred to in Column 2.		
	Number fed.	Number dissected.	Percentage positive for trypanosomes.	Number dissected.	Interval between feeding and dissection (days).	Percentage positive for trypanosomes.
30	11	11	36·3	112	6–11	*Nil*
31	13	13	15·4	68	10–12	*Nil*

To determine whether mosquitoes could transmit trypanosomes during the act of feeding, some starved mosquitoes, fed on an infected bird several days before, were released into a barraud cage which contained a watch glass with a few drops of 10 per cent solution of glucose in water. The following day, 17 of the mosquitoes were found to have fed on the glucose. On dissection, three of them showed trypanosomes in the salivary glands. The glucose in the watch glass, however, did not reveal any flagellates.

TABLE V.

Infectivity of the developmental forms of the trypanosomes found in the different organs of the mosquitoes.

Sparrow number.	Source of the parasite in the mosquito.	Interval between inoculation and feeding of mosquitoes (days).	Interval between feeding and dissection (days).	Number dissected.	Percentage positive.
32	Hindgut ...	10	12–14	95	12·6
33	,, ...	10	10–11	76	19
34	Foregut ...	10	8–14	65	9·2
35	,, ...	10	9–13	105	22·9
36	Rectal papillae ...	10	8–11	92	2·2
37	,, ...	10	7–13	60	8·3
38	Salivary gland ...	10	10–12	87	21·8
39	,, ,, ...	10	11–13	84	5·9
40	Midgut	10	12–14	164	1·2
41	,, ...	10	7–14	36	8·3

TABLE VI.

Results of dissection of mosquitoes performed between seven hours and 72 days after their feeding on birds infected with trypanosomes.

Serial number of donar sparrow and nature of infection.	Interval between blood feed and dissection.	Number dissected.	Percentage positive.			
			Midgut.	Hindgut.	Foregut.	Total.
43. Heavy infection	7–12 Hours	13	15.4*	...	...	15·4
	13–18 ,,	6	...	...	...	...
	19–24 ,,	9	33·3*	...	...	33·3
	25–36 ,,	26	42·3†	...	...	42·3
	37–60 ,,	25	44‡	...	...	44
	61–84 ,,	10	10‡	...	...	10
	85–108 ,,	4	...	50	...	50
44. Heavy infection	5 Days	8	...	...	12·5	12·5
	10 ,,	6	...	...	16·7	16·7
	15 ,,	11	...	...	9·1	9·1
	20 ,,	6	...	...	50	66·6‖
	22 ,,	5	...	...	40	40
	25 ,,	2	...	...	50	50
	30 ,,	6	...	...	33·3	66·6‖‖
	35 ,,	7	...	...	14·3	14·3
	40 ,,	3	...	...	33·3	33·3
	45 ,,	9	...	...	11·1	11·1
	50 ,,	2	...	...	50·0	50·0
	55 ,,	6	...	...	...	...
	72 ,,	4	...	...	50	50

*Long adult form in half-digested blood. Could not be traced after staining.

†Stomach contents injected into a sparrow and it became positive as revealed by subsequent mosquito feeding.

‡Small adult forms.

‖16.6 per cent gland and midgut infection.

‖‖33.3 per cent gland infection.

Infectivity of trypanosomes, found in the mosquitoes, to birds.—Eight sparrows were inoculated intraperitoneally with the developmental forms obtained from hindgut (Numbers 32 and 33), foregut (Numbers 34 and 35), rectal papillæ (Numbers 36

and 37) and salivary glands (Numbers 38 and 39) of infected mosquitoes. In addition, two more (Numbers 40 and 41) were inoculated intraperitoneally with partially-digested blood obtained from the midgut of mosquitoes, sixty hours after feeding on infected sparrows. Ten days after inoculation, batches of mosquitoes were fed on these birds. These mosquitoes were dissected after an interval of 7 to 14 days and it was observed, as indicated in Table V, that all the batches had become positive for developmental stages of the parasites.

Duration of infection in mosquitoes.—A batch of mosquitoes was fed on a heavily infected sparrow (Number 43). They were divided into seven groups and dissected periodically, the first group between 7 and 12 hours and the last between 85 and 108 hours. Excepting the group that was dissected between 13 and 18 hours after the feed, all the rest showed infection to the extent of 10 to 50 per cent.

TABLE VII.

Frequency of development of trypanosomes at the different sites in the mosquitoes.

Month.	Total positive (number).	Percentage:					Combinations.
		Salivary gland.	Foregut.	Midgut.	Hindgut.	Rectal papillae.	
January ...	69	...	84·4	...	8·7	...	2·9
February ...	36	2·8	75	...	22·2	...	...
March ...	21	...	57·1	...	42·9	...	...
April ...	33	45·5	48·5	3	3	...	...
May ...	23	13·7	69·7	1·3	4·3	...	8·7
June ...	31	12·8	67·8	9·7	...	...	9·7
July ...	46	...	97·8	...	2·2	...	...
August ...	27	...	86·2	11·8	...	...	...
September	40	...	92·5	2·5	5	...	...
October ...	108	...	83·8	4·6	11·6	...	...
November	61	...	78·8	...	14·8	4·8	1·6
December	23	...	78·3	...	21·7	...	...
All months	518	4·5	78·5	2·0	10·2	2·3	2·5

In another series, large number of mosquitoes were fed on heavily infected sparrow (Number 44) as judged by dissection of mosquitoes after their infective bite. Dissection of small groups of mosquitoes out of this was commenced on the fifth day and continued up to seventy-second day (Table VI). Except the group dissected on the fifty-fifth day, all the rest showed infection. The negative results obtained on the fifty-fifth day were perhaps only a chance occurrance as dissection

of more number of mosquitoes on that day might have, in all probability, produced a different result.

Distribution of trypanosomes in the mosquito.—The sites of infection observed in mosquitoes dissected during the course of one full year, have been recorded in Table VII. Out of all positive infections, a maximum of 78.5 per cent was found in the foregut. The other sites in the decreasing order of frequency were hindgut, salivary glands, multiple sites in various combinations, such as (*a*) foregut and salivary glands; (*b*) midgut and salivary glands; (*c*) foregut and hindgut; (*d*) midgut and hindgut; (*e*) foregut and midgut; (*f*) hindgut and rectal papillæ, and finally midgut.

A perusal of the figures recorded for the different months indicates that the salivary gland infection was confined almost exclusively to summer months and the midgut to summer and autumn. Infection in foregut was observed throughout the year but a greater proportion occurred in autumn and winter. Hindgut infection was less frequent than that of the foregut, and excepting a few infections observed in the months of April, May, July and September, the incidence was the greatest between the months October and March.

DISCUSSION.

Jaswant Singh *et al.* (1950) eliminated raisins, saline and stain as a possible source of trypanosomes in a specimen of *C. fatigans* that had fed on a sparrow. They came to the conclusion that, although the flagellates could not be demonstrated in the blood smear from the sparrow, the bird was the source of the trypanosomes. In the present studies also, raisin, normally used for the maintenance feed of mosquitoes, was eliminated as a possible source of trypanosomes.

All attempts to find trypanosomes in blood and organ smears, and contact preparations of scraped skin proved unfruitful. The parasites were not traceable in the blood, even a minute after inoculation of heavy doses of the developmental forms obtained from the mosquitoes. However, the finding that the infected birds were able to impart infection consistently to *Culex fatigans* fed on them (David and Nair, 1955), is an indication that trypanosomes must be present in the peripheral blood of the birds, though in small sub-microscopical numbers. That such an assumption is correct, is also evident from the fact that out of nine clean birds (five sparrows, three nightingales, and one seven sister) which received blood inoculation from other infected birds, six proved to have taken up the infection as judged by xenodiagnosis.

It appears that the vertebrate host (birds) can impart infection to mosquitoes as nearly as 13 to 16 hours after they are experimentally inoculated, and that the birds once infected remain so even throughout their life.

In a few birds that received very heavy doses of trypanosome inoculation, paralysis of the legs was observed, but with this exception, hundreds of birds that got infected, remained healthy over several months of observation. This may, perhaps, be interpreted that infection with this strain of trypanosome is normally non-pathogenic to birds. It is to be presumed that the parasite in vertebrate host

inhabits the internal organs such as liver, bonemarrow, lungs, heart, etc., as sub-inoculation of organ emulsion of these tissues, produced satifactory infection in the recipient birds (Table III). The possibility that the infection was transmitted by the blood in the organ, cannot, however, be excluded.

The mode of transmission of the trypanosome from bird to bird is not understood. It is presumed by some workers (Manwell, 1955) that this may be through some species of bird mites. That *C. fatigans* is probably not the real intermediate host, is indicated by the fact that experiments designed to transmit the infection through natural bite of infected mosquitoes (Table 4), gave negative results.

It was found that the trypanosomes develop in the digestive tract of the mosquitoes, and on rare occasions they reach also the salivary glands. Foregut appears to be the site comparatively more favourable for development than the rest of the places. During cold months of the year, the development appears to be rather retarded and there was a tendency for these forms to remain longer in the hindgut of the mosquitoes (Table VII).

The cluster forms, on account of their bigger size, are incapable of being injected into the vertebrate host through the agency of proboscis. Thus for all practical purposes, the mosquitoes appear to act only as a cultural medium. Obviously the development of these forms, is not inimical to the mosquito as the life span of the infected mosquitoes was not found shortened as compared to the life of the normal mosquitoes when kept under identical conditions in the laboratory.

It is generally believed that only metacyclic forms, found in the insect host, can infect the vertebrate. But in the present experiment, all the stages, irrespective of the sites from where they were obtained and the interval between the infective blood feed and the artificial inoculation of clean birds, gave rise to successful infection in the latter.

Developing trypanosomes were found in mosquitoes as early as seven hours and as late as seventy-second day after their infective blood feed. This would indicate that infection can remain in the mosquito, when once infected, throughout its life span.

SUMMARY.

Observations made during the studies on the experimental transmission of trypanosomes, that exist as a natural cryptic infection in sparrows, showed that:—

(1) Transmission was possible to healthy birds by inoculation of both blood and tissue of infected birds.

(2) Infection could not be transferred through the natural bites of infected *Culex fatigans*.

(3) Infection was found in the digestive tract as well as the salivary glands of *Culex fatigans* but in a great majority of cases, the preferred seat of infection was in the foregut.

(4) The developmental forms found in the mosquitoes were infective at all stages when artificially inoculated into the avian hosts.

(5) In the case of both birds and mosquitoes, when once they got infected, the infection was found to last long, perhaps even throughout their life span.

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A NOTE ON RESISTANCE OF BED BUGS TO D.D.T. IN BOMBAY STATE.

BY

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[February 13, 1956.]

Reports have been received from several parts of Bombay State in which D.D.T. has been used extensively as an indoor residual spray for malaria control that the insecticide no longer has any effect on bed bugs. It was the general experience that during the first year of spraying people felt an immense relief from bed bugs which contributed much to the popularity of D.D.T. Sooner or later, in the subsequent rounds the popularity declined because its effects on bed bugs were no longer as good as before. The result has been that the refusals to get the houses sprayed have been on the increase, and though till now the refusals have not had any adverse effect on malaria control they are a serious hindrance to the successful working of the malaria control schemes. Such ineffectiveness of D.D.T. has been reported in Bombay State from Dharwar (1946:1951), Kanara (1946:1952) Poona City (1950:1952), Sawantwadi and Ratnagiri District (1949:1951) and Kolaba District (1953:1954). The two figures in brackets show, first the year in which indoor residual spraying was started and second the year in which public complaints of ineffectiveness of D.D.T. commenced. Generally speaking, the complaints are most strong in the humid and warm districts situated below the Western Ghats, particularly the whole of Kolaba District and Savantwadi Taluka in Ratnagiri District. Scientific proof of this ineffectiveness was so far not available. During a recent visit to Kolaba District, one of the authors (T.R.R.) actually noticed heavy deposits of D.D.T. and numerous live colonies of bed bugs on the same door frames in houses sprayed with a 75 per cent water wettable powder.

2. Among the possible causes for this situation are:—

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

(*b*) Change in behaviour of the bed bug 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.

In this note, only the question of development of resistance will be dealt with. There is little information on the other factors.

3. One of the serious drawbacks for the studies is that information is not available on the natural degree of susceptibility to D.D.T. of bed bugs before D.D.T. was widely used in the State and it is difficult to obtain it now. Fortunately, a few careful measurements of the dosage-mortality relationships of bed bugs had been made in 1952 in Poona City at a time when complaints of ineffectiveness of D.D.T. were just beginning to be voiced. Exactly similar experiments were carried out in 1955 with results indicative of development of a further and very strong degree of resistance between 1952 and 1955. A comparative series of tests by the same methods has also been made on bed bugs in Chiplun, Ratnagiri District, a town in which D.D.T. has not been used as an antimalaria measure. Chiplun is some 80 miles away from the nearest sprayed town. The experiments are described here.

4. Bed bugs (*Cimex hemipterus*) used in these experiments were all collected from the human dwellings. Though a laboratory colony is being built up, it was thought advisable (for comparative purposes) to use wild caught bed bugs because they had been used in 1952 also. No bug was used unless it was a fully developed adult and had a length of at least 4 mm. Bugs from Poona City were used in Experiments 1 to 3 and those from Chiplun in Experiment 4.

Experiment 1, 1952.—Talcum powders containing 1, 2 and 5 per cent of D.D.T. (Technical) were prepared. A quantity of the powder (containing about 45 mg. of D.D.T. Technical) was placed in petri dishes (3 inches diameter), the bottom of which was lined by filter paper. The filter paper was turned over several times so that it acquired a thin coat of the dust on either surface. Ten bugs were transferred to each dish and exposed to the powder for 2, 4, 6, 8 and 10 minutes. Six replications of each combination of each dosage and time of exposures were made. The bugs usually crawl on the D.D.T. dusts and pick up the dust on all parts of the body. The quantity of dust placed in the dish is not important so long as an adequate quantity is used to cover thinly and evenly the entire bottom of the dish. But the concentration of D.D.T. and, to a lesser extent, the period of exposure are important because they together determine the actual quantity of D.D.T. picked up and retained on the body. The bugs were then transferred to clean petri dishes lined with fresh filter paper, by two transfers so that no D.D.T., other than what the bug had actually picked up, was transferred to the recovery dish. The bugs were examined twentyfour hours later and the mortality noted. Controls without any treatment were also similarly studied. The results are summarized in Table I. It is seen that 50 per cent mortality could not be obtained by

one per cent concentration with any of the exposures used, but it could be obtained with two minutes exposure to five per cent or four minutes exposure to two per cent.

TABLE I.

Mortality at the end of 24 hours in bed bugs exposed to powders of D.D.T. in talcum, Poona City, September-October, 1952. (10 bugs in each replication).

Concentration of D.D.T. per cent.	2 MINUTES EXPOSURE.			4 MINUTES EXPOSURE.			6 MINUTES EXPOSURE.			8 MINUTES EXPOSURE.			10 MINUTES EXPOSURE.		
	Number of replications.	Per cent mortality.		Number of replications.	Per cent mortality.		Number of replications.	Per cent mortality.		Number of replications.	Per cent mortality.		Number of replications.	Per cent mortality.	
		Actual.	Adjusted.*		Actual.	Adjusted.*		Actual.	Adjusted.*		Actual.	Adjusted.*		Actual.	Adjusted.
Control (Dust only without D.D.T.)	6	8·3	...	6	8·3	...	6	8·3	...	6	8·3	...	6	8·3	...
1 per cent	6	33·3	26·9	6	36·7	30·8	6	38·3	32·7	6	41·7	35·8	6	48·3	41·4
2 per cent	6	48·3	43·4	6	55·0	51·1	6	61·7	58·3	6	63·3	60·1	6	66·7	63·7
5 per cent	6	55·0	51·1	6	56·7	52·9	6	65·0	61·8	6	68·3	65·7	6	78·3	76·4

*By Abbott's formula.

TABLE II.

Mortality at the end of 24 hours in bed bugs exposed to powders of D.D.T. in talcum, Poona City, November-December 1955. (10 bugs in each replication).

Concentration of D.D.T. per cent.	2 MINUTES EXPOSURE.			4 MINUTES EXPOSURE.			6 MINUTES EXPOSURE.			8 MINUTES EXPOSURE.			10 MINUTES EXPOSURE.		
	Number of replications.	Per cent mortality.		Number of replications.	Per cent mortality.		Number of replications.	Per cent mortality.		Number of replications.	Per cent mortality.		Number of replications.	Per cent mortality.	
		Actual.	Adjusted.*		Actual.	Adjusted.*		Actual.	Adjusted.*		Actual.	Adjusted.*		Actual.	Adjusted.*
Control (Talcum without D.D.T.)	3	10	...	3	10	...	3	13·3	...	3	16·6	...	3	6·6	...
1 per cent	3	6·6	0	3	6·6	0	3	23·3	11·5	3	16·6	0	3	0	0
2 per cent	3	6·6	0	3	3·3	0	3	10·0	0	3	13·3	0	3	6·6	0
5 per cent	3	10	0	3	10	0	3	3·23	11·5	3	13·3	0	3	3·3	0

*By Abbott's formula,

Experiment 2, 1955.—Experiments in 1955 were carried out in exactly the same manner as in 1952, except that 6 inches petri dishes were used and also a uniform quantity (1 gm.) of the powder was employed in each dish. As the total quantity of the powder used was not really as important as the actual percentage of D.D.T. Technical in the powder, the experiments are quite comparable. The opportunity for the bug to cover itself with D.D.T. was exactly the same as in the experiments of 1952. The results are set out in Table II.

It will be noticed that in 1955, except for insignificant mortalities in two tests with an exposure of six minutes, no mortalities (after applying Abbott's formula) were obtained in any other tests including in those with exposures of ten minutes.

Experiment 3, 1955.—As it was noticed that even a ten minute exposure to five per cent did not lead to any mortality, higher concentrations of D.D.T. Technical were used, viz., 25, 50, 75 and 100 per cent, and the time of exposure increased to 30 minutes. Three replications of each concentration were made. The results are set out below:—

Dosage.	Average mortality (Per cent).	Mortality adjusted after applying Abbott's formula. (Per cent).
Blank	3·3	...
Control (talc only) without D.D.T. ...	13·3	...
D.D.T. 25 per cent	26·6	15·8
50 per cent	60·0	54·2
75 per cent	53·3	45·9
100 per cent (Technical D.D.T. only)	13·3	6·6

It is rather surprising that even an exposure of 30 minutes to 75 per cent gave a mortality of only 45 per cent and there was a mortality of only 6·6 per cent when the bugs were exposed to pure technical D.D.T. In the latter case, the result is obviously due to the fact that pure D.D.T. does not stick to the body of the bugs as readily as when mixed with talc but one should have expected a much higher kill.

Experiment 4, 1956.—The bed bugs collected in Chiplun (unsprayed area) were tested under exactly similar conditions as in Experiment 2, and the results are summarised in Table III. Obviously the bugs in this town can still be killed by D.D.T. though the mortalities are somewhat lower than those obtained at the same dosages and exposures in Poona in 1952.

5. These results are definitely suggestive of a reduction in the susceptibility of bed bugs to D.D.T. between 1952 and 1955 in Poona City where extensive D.D.T. indoor residual spraying twice a year has been in practice for over five years. Combined with the observation that in most areas where bed bug nuisance is now prevalent it had been effectively controlled by the first few rounds of spraying

two to four years ago, there seems to be no doubt that the poor kill obtained is due to the development of resistance. It is significant that in Chiplun Town situated in Ratnagiri District, the bed bugs still show a fairly high degree of susceptibility. Ratnagiri District, it should be pointed out, is below the Western Ghats and is about 250 miles long and approximately 50 miles wide and except for two small sections in the northern and southern extremities, there is no malaria prevalence and therefore no D.D.T. spraying has been undertaken. The nearest sprayed towns from Chiplun are 80 miles away and resistance to D.D.T. has been noticed in them even after only two years of spraying, i.e., four rounds of 100 to 112 mg./sq. ft. each. Passive transportation of bugs which undoubtedly has taken place to some extent from the sprayed to unsprayed sections has obviously not been of a high order to affect the susceptibility of the bug populations in unsprayed areas.

TABLE III.

Mortality at the end of 24 hours in bed bugs exposed to powders of D.D.T. in talcum, Chiplun Town, (unsprayed area), January 1956. (10 bugs in each replication).

Concentration of D.D.T. per cent.	2 MINUTES EXPOSURE.			4 MINUTES EXPOSURE.			6 MINUTES EXPOSURE.			8 MINUTES EXPOSURE.			10 MINUTES EXPOSURE.		
	Number of replications.	Per cent mortality.		Number of replications.	Per cent mortality.		Number of replications.	Per cent mortality.		Number of replications.	Per cent mortality.		Number of replications.	Per cent mortality.	
		Actual.	Adjusted.*		Actual.	Adjusted.*		Actual.	Adjusted.*		Actual.	Adjusted.*		Actual.	Adjusted.*
Control (Talcum without D.D.T.)	3	0	...	3	0	...	3	0	...	3	0	...	3	0	...
0·25 per cent	3	13·3	13·3	3	13·3	13·3	3	16·6	16·6	3	16·6	16·6	3	20·0	20·0
0·50 per cent	3	16·6	16·6	3	23·3	23·3	3	26·6	26·6	3	27·7	27·7	3	26·6	26·6
1 per cent	3	13·3	13·3	3	23·3	23·3	3	30·0	30·0	3	33·3	33·3	3	30·0	30·0
Control (Talcum without D.D.T.) Second series.	3	10	...	3	0	...	3	0	...	3	0	...	3	0	...
2 per cent	3	27·3	19·2	3	26·6	26·6	3	33·3	33·3	3	33·3	33·3	3	33·3	33·3
5 per cent	3	33·3	25·9	3	33·3	33·3	3	43·3	43·3	3	46·6	46·6	3	66·6	66·6

*By Abbott's formula.

6. While the ineffectiveness of D.D.T. against bed bugs, as judged from complaints received from people, has appeared both in the districts in which D.D.T. in the form of an emulsion was used and in those where it was used as a water wettable powder, such ineffectiveness has always taken much longer to appear in the former than the latter. It is, however, significant that good control of bed

bugs has been obtained recently by using Dieldrin or B.H.C. in some houses in which they had become a nuisance. How effective these insecticides will continue to be after a more extended use, is not yet known.

7. The methods adopted for the tests in these studies now reported are certainly capable of improvement. Dusts have been used now solely with a view to get data which would be comparable to those obtained in the 1952 experiments. More refined methods, such as the use of impregnated filter papers and the topical application of the insecticide, are being tested for adoption in future. But the methods now used and reported are sensitive enough and the results obtained are not vitiated in any manner.

SUMMARY.

Bed bugs (*Cimex hemipterus*) are found to have developed a high degree of resistance to D.D.T. between 1952 and 1955 in Poona City, India, in which D.D.T. has been sprayed as an indoor residual spray for five years. While 50 per cent mortality was obtained in 1952 with two minutes exposure to a five per cent D.D.T. dust in Talcum, and with four minutes exposure to a two per cent dust, no mortalities could be obtained even with a ten minutes exposure to a five per cent dust in 1955. It is significant that the bed bugs in Chiplun Town, Ratnagiri District, which is not sprayed and is some 80 miles away from the nearest sprayed area, still show a good degree of susceptibility.

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MALARIA CONTROL IN THE COLONISATION SCHEME, KASHIPUR, DISTRICT NAINI TAL, U. P. (1949—1954).

BY

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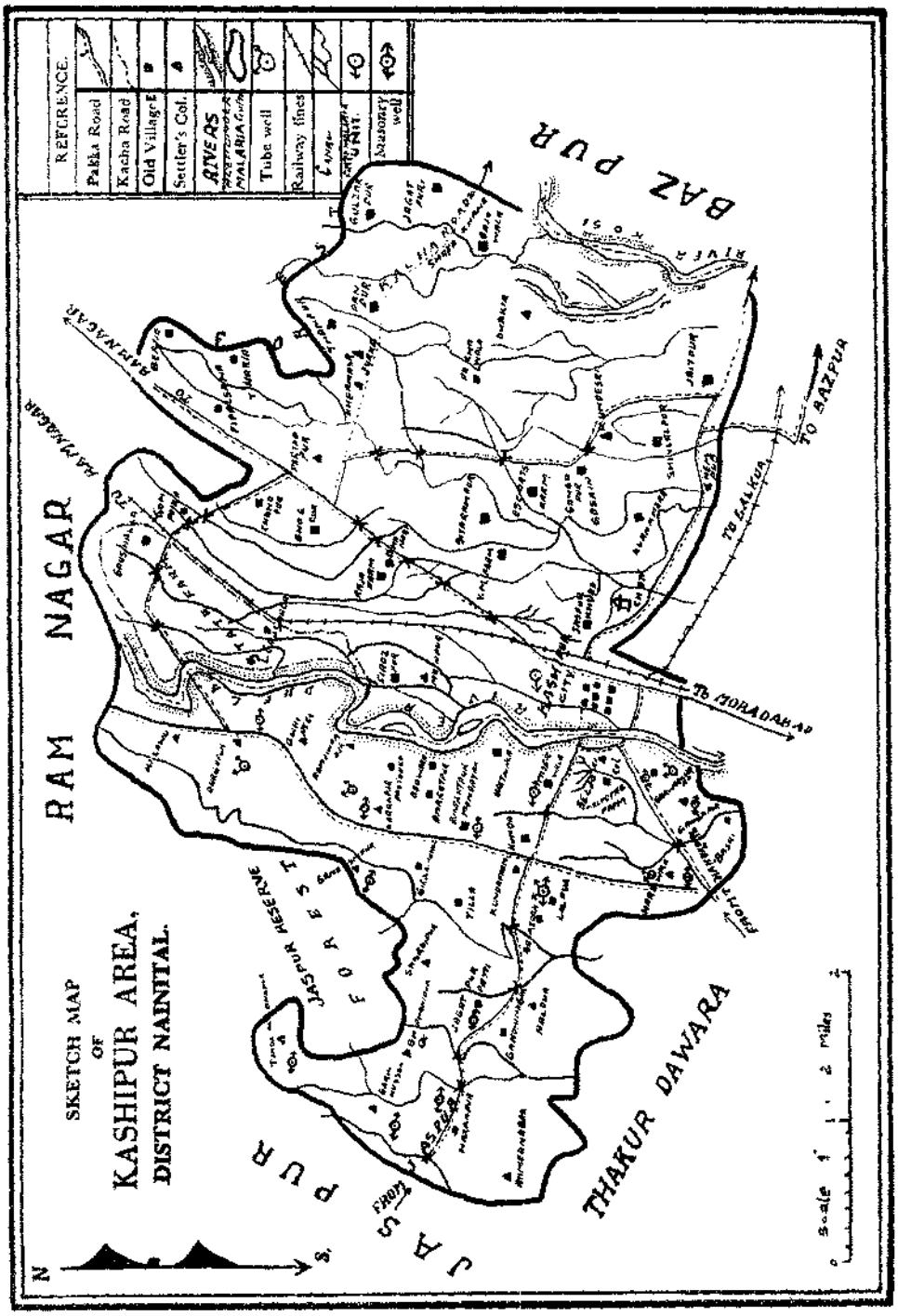
INTRODUCTION.

The history of development of the Kashipur Tarai, District Naini Tal, dates back many years and the story is rather depressing. Previously several attempts were made to develop the tract, but all efforts ended in failure, chiefly because no proper attention was paid towards the control of malaria which was the hub of the problem of this area. The question for development of Tarai was again taken up at the end of World War II and after the partition of the country when Government faced the problem to colonise the ex-servicemen and the displaced persons from West Pakistan.

DESCRIPTION OF THE AREA.

The area selected for development was 60 sq. miles having 33 villages with a population of 2,108 when the unit was established in 1949. As the conditions improved, a number of villages and farms sprung up making a total of 84 with a population of 9,800. The River Dhela running from north to south divides the area into two nearly equal halves. The area under operation lies in the south-west corner of Naini Tal District in Kashipur Tehsil (Map 1). It had been very unhealthy since a long time and the population was on the decrease.


SKETCH MAP
OF
KASHIPUR AREA,
DISTRICT NAINITAL.
REFERENCE.
Pakka Road
Kacha Road
Old Village
Settler's Col.
Tube well
Railway lines
Masonry well
RAM NAGAR
BAZ PUR
JASPUR
THAKUR DAWARA
KASHIPUR CITY
To MORADABAD
To BAZPUR
To LALKUA
JASPUR RESERVE FOREST
RAMNAGAR
N
S.

The Tarai in Naini Tal District is that strip of submontane tract of land which extends at the foot of the Himalayas between the waterless Bhabar on the north and the cultivated plain of Rohilkhand on the south.

The country in general is a tract of forest and swamps with scattered patches of cultivated land. Its general appearance is that of plain, sloping gently towards the south-east, the average fall being 12 feet in a mile.

SOIL.

The soil is moist and alluvial with negligible sign of rock formation. It is practically a virgin soil and naturally is very fertile. Good crops are grown with little effort and without manure.

DRAINAGE.

The drainage system resembles the reticulation of a leaf. The streamlets on the edge of moist country unite to form a larger channel, which in turn feeds the arterial line of drainage eventually to join a bigger stream. Most of the rivers are subject to heavy floods during rains.

IRRIGATION.

Streams have been used from the past for irrigation purposes. This system has rendered the whole area into immense swamps. Rainwater has also been used for irrigation in some places. These faulty methods made the area water-logged and gave rise to severe form of malaria. In order to remedy these evil systems, canals were introduced but invariably they became defunct due to neglect and lack of proper maintenance, creating a number of seepages and providing conditions more favourable for breeding of mosquitoes. The other sources of irrigation are wells, nalas and a few masonry wells. Since canal system of irrigation in a marshy tract is not desirable, tubewells have now been provided by the Colonisation Department.

SUB-SOIL WATER LEVEL.

The sub-soil water level ranges from 8 to 12 feet resulting in dampness of the soil and a large number of water collections forming potential breeding places.

METEOROLOGY.

The chief characteristic of the climate of this area is the great variation between day and night temperatures, due to moist nature of the soil. With the excessive moisture of the soil and great heat of summer, the climate becomes enervating. These conditions of temperature and humidity, combined with the general water-logged condition of the area, are favourable both for breeding of mosquitoes and consequently for malaria.

The average annual rainfall in the area ranges from 40 to 50 inches. Maximum rains are recorded in the months of July, August and September. Rainy season starts earlier in the Tarai than in the plains. The maximum and minimum temperatures recorded in the area are 117°F. and 33°F. in June and January respectively. The relative humidity varies from 21 to 92 per cent.

PEOPLE AND SOCIO-ECONOMIC CONDITIONS.

The population mainly consists of two classes of people, the indigenous or Deshi and non-indigenous Tharus and Bhuksas. The majority of adults and children in the Deshi population have anæmic looks and protuberant bellies. The Tharus and Bhuksas being good cultivators, are economically better off than the Deshis and also acquire tolerance sooner than the Deshi people. In addition to these two types of people, a third type of migratory population from the neighbouring hills visits Tarai in winter months to earn their livelihood.

The people of the area are mostly agriculturists. Paddy, sugarcane and wheat are the chief crops. Houses are usually made of mud walls without any provision for light and ventilation. Cattlesheds are either situated inside the houses or close-by. The houses of Tharus and Bhuksas are better built having better arrangements for light and ventilation. Cattlesheds are usually built at some distances from the houses and the surroundings are comparatively clean.

MALARIA CONTROL.

For purposes of malaria control, the entire area has been divided into two sectors, one on the east and the other on the west of the River Dhela. Each sector is under the charge of a Malaria Inspector who is responsible for D.D.T. spray and maintenance of records. He is also responsible for the administration of paludrine tablets, both prophylactic and curative. The overall supervision is exercised by the Antimalaria Officer.

ADULTICIDAL MEASURE.

Systematic indoor residual spraying of all the human dwellings and cattlesheds with D.D.T., 2·5 per cent watery suspension, prepared with gum acacia and gelatine, was used at the rate of 2 c.c. per sq. ft. giving a deposit of 50 mg. per sq. ft. In the beginning, the sprays were done every six weeks till 1950. The frequency of the spray was reduced to four times in a year as the conditions improved and the insecticide was changed to 50 per cent D.D.T. wettable powder. First spray was carried in March-April, second in June-July and two sprays at six-weekly interval thereafter. The villages situated on the forest fringes received double dose, i.e., 100 mg. per sq. foot., at an interval of six weeks up to 1953. During the year 1954 only three sprayings were carried out.

Stirrup pumps used for spraying purposes are fitted with double nozzles having an aperature of 1/32 inch.

PALUDRINE PROPHYLAXIS AND TREATMENT.

Paludrine tablets of 100 mg. each were distributed among the rural population, Government employees and labour groups working in the area. The regime followed was three tablets of 100 mg. each per adult in a single dose and proportionately smaller doses to the children of lower age groups. The prophylactic scheme continued throughout the entire period except in the months of December and January till 1950. From 1951, the prophylactic distribution was restricted to Government organised institutions, Government employees and particularly to the newly included villages falling on the fringes of the forest. During the year 1954, the prophylactic distribution of paludrine was discontinued.

For curative purposes paludrine was administered to microscopically positive cases. The regime followed was three tablets of 100 mg. each per day for ten days followed by the usual prophylactic dose of three tablets of 100 mg. each, once a week per adult, and proportionately smaller doses were given to the children of lower age groups.

DEGREE OF MALARIA.

Spleen rate.—Children of two to ten years of age were examined standing for splenic enlargement. The examinations were carried in premonsoon and postmonsoon periods and the spleen rates are recorded in Table I. The child spleen rate which was 59·8 per cent in July 1949 was reduced to 3·07 per cent in November 1954.

TABLE I.

Child spleen rate.

Years.	Number examined.	Spleen rate per cent.
1949	159	59·8
1950	568	10·7
1951	1,141	2·8
1952	1,020	2·9
1953	1,433	3·07
1954	1,143	3·07

Parasite rate.—Both thick and thin films were taken and the results of examinations are recorded in Table II. From the figures given in the Table, it is evident that child parasite rate has also reduced considerably, i.e., from 8·5 per cent in 1949 to 1·7 per cent in 1954.

TABLE II.

Child parasite rate.

Years.	Number examined.	Parasite rate per cent.
1949	149	8·5
1950	631	0·32
1951	1,206	0·91
1952	1,629	1·6
1953	1,433	1·3
1954	1,143	1·7

It will be observed from Table III that the only infection detected in 1949 and 1950 was *P. vivax* but from 1951 *P. falciparum* was the predominating infection.

TABLE III.

Percentage species prevalence.

Infection.	1949	1950	1951	1952	1953	1954
P. vivax ...	100	100	45·5	37	44·4	43·1
P. falciparum ...	0·0	0·0	54·5	59·3	55·6	56·9
P. malariæ ...	0·0	0·0	0·0	0·0	0·0	0·0
Mixed ...	0·0	0·0	0·0	3·7	0·0	0·0

MALARIA MORBIDITY.

Percentage of malaria cases to total cases, attended at the Kashipur Civil Dispensary from 1949-1954, is shown in Table IV:—

Malaria morbidity figures shown in Table IV show a fall from 19·6 per cent in 1949 to 13·9 per cent in 1954. It is also observed that September to November are the peak months for malaria.

ENTOMOLOGICAL OBSERVATIONS.

Altogether 24 catching stations, 12 in sprayed and 12 in unsprayed area were established from which mosquito catches were made. The collections were made regularly every week in the early hours of morning and the time employed for catches was half an hour. The collections were made from cattlesheds, human dwellings and mixed dwellings. The different species of mosquitoes found in

TABLE IV.

Percentage of malaria cases to total cases.

Months.	Percentage of malaria cases to total cases.					
	1949	1950	1951	1952	1953	1954
January ...	...	17·9	7·87	16·8	10·06	9·4
February ...	...	2·4	9·38	...	9·7	7·3
March	...	12·04	11·03	15·8	9·1	8·3
April	...	10·3	9·29	12·3	9·09	7·8
May	...	10·8	7·41	12·1	8·8	7·9
June .. .	18·4	13·06	11·34	11·8	10·6	7·4
July	16·3	8·7	15·24	11·2	8·8	12·9
August	15·4	12·9	11·88	16·5	20·06	13·4
September ..	12·5	16·1	13·46	21·2	23·7	24·1
October ..	27·7	21·1	20·29	22·5	20·3	26·8
November ..	21·9	13·2	21·44	19·7	17·03	26·5
December ..	20·9	7·1	20·48	15·1	11·9	9·2

the area are *A. culicifacies, A. fluviatilis, A. subpictus, A. annularis, A. pallidus, A. splendidus, A. vagus* and *A. barbirostris.* In addition to the species already referred to, the following species were also found in small number from 1949 to 1952. *A. aconitus, A. varuna, A. stephensi, A. maculatus, A. gigas,* and *A. minimus.*

The number of mosquitoes available for dissection during the years 1949 to 1954 was 1928, which included *A. culicifacies* 1586, *A. fluviatilis* 275 and *A. minimus* 67 but without positive findings.

The probable vector species of the area are *A. culicifacies* and *A. fluviatilis.*

COST OF MALARIA CONTROL MEASURES.

Temporary labour is engaged at the time of D.D.T. spray which is trained in D.D.T. spraying technique before actual operation started. The cost of D.D.T. spraying and paludrine prophylaxis is given in Table V.

DISCUSSIONS.

The control measures, as described above, in this area have proved very encouraging and helpful in view of the fact that all the developmental schemes and industrial organisations which have been started here, are flourishing without any fear of malaria. The settlers, who were afraid of malaria in the beginning,

TABLE V.

Cost of malaria control measures.

Years.	Cost of D.D.T. sprays.			Cost of paludrine and supervision.	
	Number of sprays.	Total cost of spraying.	Per capita cost of yearly spraying.	Total cost of prophylaxis paludrine.	Per capita cost.
		Rs. as. p.	Rs. as. p.	Rs. as. p.	Rs. as. p.
1949	3	2,558 8 0	0 10 9	2,932 8 0	0 12 10
1950	6	6,101 12 0	1 2 0	5,172 8 0	1 1 11
1951	4	4,687 8 0	0 12 6	1,669 3 0	0 6 4
1952	4	6,406 0 0	0 11 8	301 12 0	0 1 0
1953	4	7,239 0 0	0 12 8	504 11 0	0 1 11
1954	3	7,842 7 0	0 10 6	*Nil*	*Nil*

remained comparatively free from the disease. The daily growing population and gradual fall in malaria is significant.

As a result of successful antimalaria measures, Kashipur has grown to a very populous and flourishing town. A power-house has been constructed and the energy has helped in developing many small cottage industries. The town has been electrified and Ramnagar, a notified area about 17 miles north from Kashipur, is also being electrified. The rural area which was notorious for its unhealthiness due to malaria, is now humming with activities and life. Jungles and swamps have been cleared and almost every available land has been brought under plough. The population of Kashipur Town as well as of the area has increased four times. The economic condition of the people has also improved, tubewells and pumping sets have been installed to provide irrigation as an insurance against drought. The malaria morbidity rate has considerably reduced, i.e., from 19·4 per cent in 1949 to 13·9 per cent in 1954, along with remarkable progress in food production from one million maunds in 1949 to 2·9 million maunds in 1954. Health and medical facilities have been extended to the entire area in the form of mobile dispensary. Means of communications have improved both inside and outside the area by construction of pucca roads. Direct communications have also been established with Naini Tal, Moradabad, Bareilly and Ramnagar. Primary schools and cooperative stores have been opened all over the area.

SUMMARY.

1. The report under review deals with the progress under malaria control measures from June 1949 to 1954.

2. The hydrographical and meteorological features of the Kashipur Tarai, measuring about 125 sq. miles situated in Tehsil Kashipur, District Nainital, and their effect on malaria, have been described.

3. Low economic status of the people predisposing them to infection of malaria and hyperendemic conditions, as judged from spleen rate that prevailed before antimalaria operations started and present conditions, have been indicated.

4. D.D.T. spraying and paludrine prophylaxis and treatment, method of collecting malariometric data and per capita cost of control measures, have also been described.

ACKNOWLEDGEMENT.

The authors are thankful to Dr. K. C. Rastogi and Shri H. M. L. Srivastava, Entomologist, for their assistance in drafting the report, and also to Shri S. R. Hussain and G. S. Raizada, Malaria Inspectors of Kashipur Antimalaria Unit, for their help in the compilation of the data.

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Indian Journal of Malariology, **10**, 2, *June 1956*.

ANNOUNCEMENT.

INDIAN COUNCIL OF MEDICAL RESEARCH.

Colonel Amir Chand Trust Prizes for Medical Research.

LIEUT.-COLONEL AMIR CHAND, ex-Principal, Lady Hardinge Medical College, New Delhi, has made a donation of Rs. 50,000/- to the Indian Council of Medical Research for the purpose of awarding prizes for the best published research work in the field of medical sciences. The Governing Body of the Council has constituted a Trust called the, "Colonel Amir Chand Trust" for the administration and management of the Fund.

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

THREE of the prizes are known as, "BASANTI DEVI AMIR CHAND PRIZE" and the other THREE, "SHAKUNTLA DEVI AMIR CHAND PRIZE".

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

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

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

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

IT has been decided to award during 1956 six prizes of the value of Rs. 300/- each for the best research papers in medical science published by workers during the year 1955 (1st January to the 31st December, 1955).

THE AWARD of the prizes will be announced at the annual meetings of the Scientific Advisory Board and the Advisory Committees of the Indian Council of Medical Research, to be held at Mysore in November/December, 1956.

THE CANDIDATES are required to submit 10 REPRINTS of their papers published during 1955. These should be sent to the DIRECTOR, INDIAN COUNCIL OF MEDICAL RESEARCH, 'P' BLOCK, RAISINA ROAD, NEW DELHI, so as to reach him NOT LATER THAN THE 1ST AUGUST, 1956.

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

Indian Journal of Malariology, **10**, 3, September 1956.

A NATURAL TRYPANOSOME SP.? INFECTION IN INDIAN QUAIL (*COTURNIX COMMUNIS*)—STUDY OF THE MORPHOLOGY IN DIFFERENT AVIAN HOSTS.

BY

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[May 3, 1956.]

THE occurrence of a natural trypanosome infection in Indian quail (*Coturnix communis*) and the susceptibility of chicks, parrots, sparrows and grey partridges to this infection, has already been reported by Basu, Nair and David (1955). They had observed that infected blood from quails, inoculated to chicks, parrots etc., developed the infection in these hosts. The present paper describes the morphology, the course of infection and the pathogenicity of this trypanosome in different avian hosts.

MATERIALS AND METHODS.

Host.—The course of trypanosome infection was studied in three different avian hosts, viz., sparrows (*Passer domesticus Linnaeus*), parrots (*Palaeornis torquates*) and chicks. All the birds used in this investigation, excepting the laboratory hatched chicks, were obtained from the local bird market. Birds, other than chicks, were examined daily at least for a week prior to experimentation, to determine that their peripheral blood was free from any natural trypanosome infection.

Parasite.—The same strain of trypanosome obtained originally from quails blood (Basu, Nair and David, 1955) was transferred to different avian hosts. Fifty quails (*Coturnix communis*) were studied for a period of one month and during the entire period only one isolated trypanosome was seen in the blood smear of

one of the birds. This served as the material for the study of the parasite in the natural host, *Coturnix communis*.

All blood smears were fixed with methyl alcohol and stained with J.S.B. stain. The intensity of infection was determined by counting daily the number of trypanosomes per 10,000 erythrocytes. An Ehrlich's eye-piece was used to facilitate the counting. For the study of the morphology of the trypanosomes, dimensions were determined with the help of a micrometer scale. The figures given are mostly the averages of a large number of observations.

The drawings of trypanosome in the text of this paper, were made with a camera lucida under a standard magnification of 1625. Organ smears from quails showing no trypanosome in the blood, and from chicks and parrots during the ascending phase of trypanosome infection, were prepared, but they revealed no trypansomes.

Insect hosts.—Eighty-six *C. fatigans* mosquitoes, which had fed either on heavily infected parrots or on quail, were dissected between 3 and 24 days of feeding but failed to show any developmental forms of the parasite in them. Similarly 93 *Aedes aegypti* mosquitoes which had fed on naturally infected quail or heavily infected chicks, or parrots (as judged by the presence of trypanosomes in their peripheral blood), were dissected between 4 and 14 days after feeding, but in these also no development of trypanosomes could be seen.

EXPERIMENTAL DETAILS.

I. TRYPANOSOME INFECTION IN CHICKS.

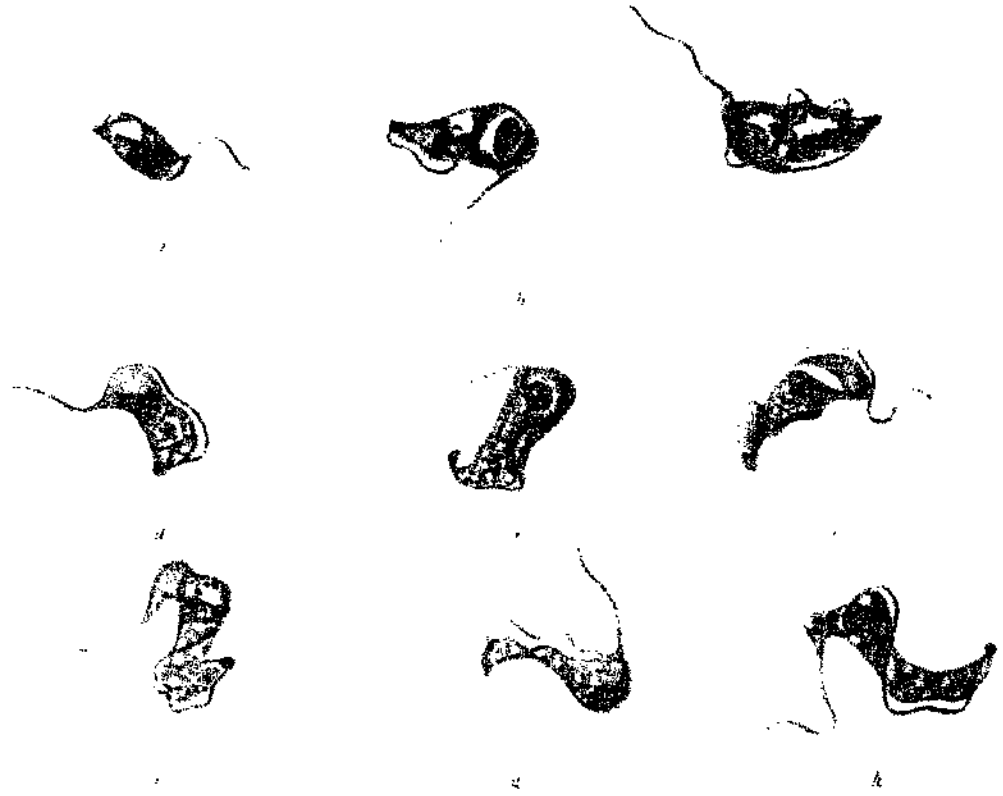
(i) Course of infection.—Two chicks (Number 349 and 686) received 0·2 c.c. of blood intravenously from Quail 8 which did not show any trypanosome in the peripheral blood. In both the chicks, the infection became patent within seven to ten days of inoculation. In Chick 349, trypanosomes were seen in the peripheral blood for the first four consecutive days of the patency, thereafter the flagellates were seen intermittently in the peripheral blood for another 15 days. The infection in this bird did not go higher than four trypanosomes per 10,000 erythrocytes. Organ smears, prepared after sacrificing the bird, did not show any trypanosome. In Chick 686, the trypanosomes were seen in the blood smears for ten consecutive days. Thereafter, the peripheral blood became negative and remained so for the following ten days when this bird died. Organ smears did not show any trypanosome. The course of infection in Chick 686 is presented in Chart 1.

(ii) Morphology of the trypanosomes.—In stained blood smears, the trypanosomes are markedly polymorphic consisting of three forms, namely, small (11 to 15μ) (Plate I, Fig. *a*), medium (15 to 20μ) (Plate I, Fig. *b*) and large (above 20μ) (Plate I, Fig. *c*), the large forms were found very frequently.

Shape.—The body of the trypanosome has a sinuous fusiform shape. The anterior end is gradually tapering and pointed, the posterior end is abruptly pointed.

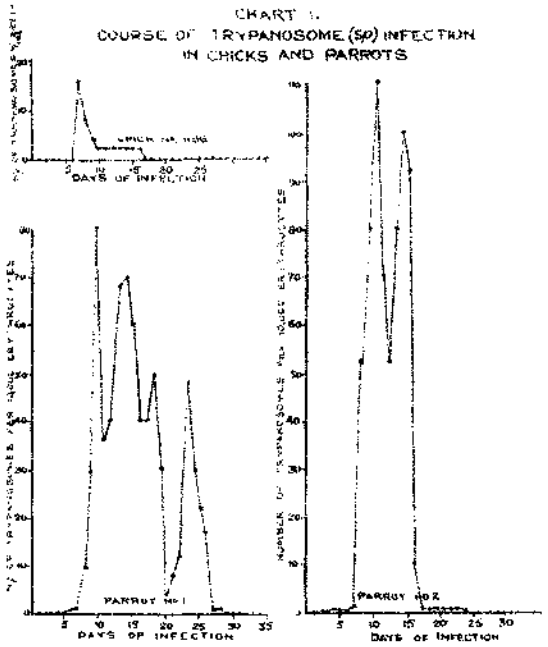
PLATE I.

TRYPANOSOME IN BIRDS.



0 10 20 30 40μ

a, *c*. Trypanosome in chick.
b, *d*, *f*. Trypanosome in sparrow.
g, *h*. Trypanosome in parrot.
i. Trypanosome in quail.



Cytoplasm. The cytoplasm is granular in appearance. In many specimens, up to six or more vacuoles are observed posterior to the nucleus. Vacuoles occur also in the cytoplasm anterior to the nucleus but they are not so common. In the small forms, the protoplasmic body is only sparingly vacuolated. The number of vacuoles are generally more with the larger forms of the trypanosome.

Nucleus.—The nucleus is situated in the anterior half of the body and stains violet-red in colour in contrast to the deep blue of the cytoplasm. Usually, the nucleus is elongated in the majority of the trypanosomes—the long axis of the nucleus lying across the long axis of the flagellate. In others, it is more or less oval. The chromatin granules are grouped into one or two deeper staining masses within the nucleus but no constant pattern is noticed. A prominent halo, either completely or partially surrounding the nucleus, is seen very often.

Kinetoplast. The kinetoplasts are round or oval situated either at the posterior extremity or a short distance away from it. The size of the kinetoplast is slightly variable in its dimension.

Blepharoplast.—The blepharoplast is not observed in any of the specimens and the axoneme appears to rise directly from the kinetoplast.

Flagellum.—The flagellum appears to arise directly from the kinetoplast and runs along the border of the undulating membrane and finally ends in a distinct free flagellum. The flagellum is longer in the larger forms.

Undulating membrane.—The undulating membrane is narrow and has three to four small folds. The membrane is completely hyaline in structure.

Dimension.—The average dimension of each of the three forms of trypanosome in chicks, are given in Table I.

TABLE I.

Trypanosome dimensions in chicks.

Particulars.	Small forms (μ).	Medium forms (μ)	Large forms (μ)
1. Body (length × width)	8·5×4·25	10·8×5·4	14·8×5·2
2. Nucleus (length × width)	1·7×1·7	3·9×1·8	2·8×1·9
3. Kinetoplast diameter	0·6	0·7	0·72
4. Length of the free flagellum ...	5·1	7·4	8·2
5. Total length of the body including the flagellum	13·6	18·2	23

2. TRYPANOSOME INFECTION IN SPARROW.

(i) Course of infection.—One sparrow was inoculated intravenously with 0·2 c.c. of blood from Chick 686 referred to above, showing trypanosome in the blood. The peripheral blood of the sparrow became positive 12 days after the inoculation, and remained so for four consecutive days. The bird died thereafter but the organs did show no trypanosome. The intensity of infection did not exceed 14 trypanosomes per 10,000 erythrocytes on any day during the patency.

(ii) Morphology.—The morphology is essentially the same as described already. Only the medium and large forms of trypanosomes occur in this host. The undulating membrance has three to five folds (Plate I, Fig. *d, e, f*).

The average dimensions of each of the two forms of trypanosomes are given in Table II.

TABLE II.

Trypanosomes dimension in sparrow.

Particulars.	Medium forms (μ)	Large forms (μ).
1. Body (Length × width)	13×5·3	16·9×4·5
2. Nucleus (length×width)	3×1·7	3·2×1·6
3. Kinetoplast diameter	0·8	0·8
4. Length of the free flagellum	6·4	8·5
5. Total length of the body including the flagellum ...	19·4	25·4

3. Trypanosome infection in parrots.

(i) *Course of infection.*- Two parrots (Number 1 and 2) received intravenous inoculations of 0·5 c.c. of pooled blood from two quails which did not show any trypanosome in the peripheral blood on microscopic examination. Both the parrots became positive for trypanosome within four to six days of inoculation. The infection remained patent for about three weeks in both. The blood of both the parrots remaind free from the flagellate during a subsequent period of observation for two months. Fresh parrots sub-inoculated with blood, obtained from Parrots 1 and 2 during the post-patent period, did not develop any infection. The course of trypanosome infection in the parrots is presented in Chart 1.

(ii) *Morphology.*—The morphology is essentially the same as observed in chicks. Only the medium and large forms of trypanosome are seen. The undulating membrane has three to five folds (Plate I, Fig. *g*, *h*), some greyish brown granules are seen in the cytoplasm. The average dimensions of each of the two forms of trypanosome are given in Table III.

4. Trypanosome infection in quails.

Only on a single occasion, a trypanosome was seen in a blood smear of a quail. The morphological features are essentially the same, but the cytoplasm contains some greyish brown granules (Plate I, Fig. *i*). The dimensions of the trypanosome are given in Table IV.

Table III.

Trypanosome dimensions in parrots.

Particulars.	Medium forms (μ).	Large forms (μ).
1. Body (length × width)	12·3×5·4	17·5×4·7
2. Nucleus (length × width)	3·4×1·7	3×1·8
3. Kinetoplast diameter	0·9	0·8
4. Length of the free flagellum	7	10
5. Total length of the body including the flagellum	19·3	27·5

Table IV.

Trypanosome dimensions in quail.

Particulars	Dimensions (μ)
1. Body (length × width)	15·3×3·4
2. Nucleus (length × width)	3·2×1·7
3. Kinetoplast diameter	0·8
4. Length of the free flagellum	8·5
5. Total length of the body including the flagellum ...	23·8

DISCUSSION.

A large number of species of birds have been found to be infected with trypanosomes (Knowles, 1928; Hewitt, 1940; Garnham, 1954; Manwell, 1954: 1955), but the available literatures, both in the natural host and in other susceptible hosts, are rather limited. Bird trypanosome morphology, with figures and measurements, have been worked out in black birds and thrushes (Coles, 1914). A comparison of the dimensions of the trypanosome in this investigation shows that it is much smaller than the species found in black birds and thrushes, and probably belongs to a different species.

Out of the large number of quails examined over a long period, only a single trypanosome was seen in the peripheral blood of one of them. This shows that the natural infections in quails are difficult to detect by routine blood examination. Susceptible chicks and parrots, when inoculated with blood obtained from different quails, do not always develop the infection showing thereby that all quails do not harbour the infection. Conversely, quails inoculated with blood containing trypanosome (e. g., chick and parrot blood, showing trypanosome), failed to develop patent trypanosomiasis, showing thereby that a good amount of acquired immunity is present in quails. This suggests that most of the quails, available in the local areas, are infected in the nestling stages and the infection dies out in most birds as they become older. That young birds in the nest, only a few days old, may be found infected with trypanosomes, has long been established (Wenyon, 1926).

The fact that this trypanosome is inoculable to a number of different avian hosts, shows that it is similar in this respect to *T. paddae* (Thiroux, 1905) of the Java sparrow (*Munia oryzivorae*) and *T. loxiæ* of *Loxia curvirostra* (Nöller, 1920). The parrots are most susceptible to this trypanosomiasis compared to chicks and sparrows, but even in them the infection is of a benign nature and apparently non-pathogenic, as judged from the fact that none of the parrots died of this infection.

Marked polymorphism has been noticed in this trypanosome and the findings show that the trypanosome attains a larger form in the more susceptible hosts. The readiness with which this trypanosome can be seen in the peripheral blood and the failures to obtain the infection in the Culex mosquitoes, prove this species to be very different from another species of trypanosome (sp.) found in sparrows in which the infection remains cryptic in the avian hosts, but easily demonstrable in the invertebrate host (*C. fatigans*) (David and Nair, 1955).

No dividing forms were seen in the blood smears nor could they be found in the tissue smears from chicks and parrots obtained during the period of ascending infection. So no indication has been obtained as to where the parasites are localized and where multiplication occurs. Limited transmission experiments show that *C. fatigans* or *Aedes ægypti* may not serve as the invertebrate hosts.

SUMMARY.

The morphology and dimensions of a trypanosome (sp.) occurring as a natural infection in *Coturnix communis* (quail) are described as it appears in different avian

host. The course of this trypanosome infection in chicks and parrots is also described. Limited experiments tend to show that *C. fatigans* and *Aedes aegypti* may not act as the insect vectors.

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Indian Journal of Malariology, **10**, 3, *September 1956*.

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

XXIII. Isolation of and observations on a "Milk-resistant" strain.

BY

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[May 3, 1956.]

A PHYSIOLOGICAL difference in the requirements of para-aminobenzoic acid by a sulphadiazine resistant strain of *P. berghei* was demonstrated by Jaswant Singh *et al.* (1954). They could not observe any growth inhibition of the strain in mice fed exclusively with milk, while growth of normal strains was found to be inhibited in similarly fed rats and mice (Macgraith *et al.*, 1952; Ramakrishnan *et al.*, 1953; Hawking, 1953).

The ease with which *P. berghei* can become resistant to sulphadiazine and consequently indifferent to the absence of para-aminobenzoic acid, has been reported by Krishnaswami *et al.* (1954) and Jaswant Singh *et al.* (1954).

Therefore, it would appear that a normal population of *P. berghei* is heterogeneous to the extent that some members are present to whom para-aminobenzoic acid is not an essential nutrient. If this were so, it should be possible to select such individuals from a population in hosts fed exclusively with milk as it has been shown that milk is deficient in the amino acid (Hawking, 1953) and such "milk-resistant" individuals should be refractory to treatment with sulphadiazine as well. This paper reports the result of an experimental approach to such a study.

MATERIAL AND METHODS.

The albino rats and mice used in the investigation were from the colonies maintained at the Institute. They were approximately twenty and sixteen weeks old respectively.

The control animals were fed on the normal colony diet (Jaswant Singh *et al.*, 1954) and the experimental animals were fed exclusively on Klim as described by Ramakrishnan *et al.* (1953). The milk diet was commenced on the day of inoculation and continued till the end of the observation. The experimental animals were housed in special metabolic cages to eliminate corpophagy.

The strain of parasites was the same as used in the previous experiments and maintained in albino rats. Three sets of observations were carried out and the numbers of serial passage of the normal strain at the time of the experiments were 207, 233 and 237 resepectively.

The dose of inoculation was one million parasites per animal as in the previous studies. In the case of the experiments to assess the presence and degree of resistance to sulphadiazine, the dose of inoculation was $5x10^6$ parasites per mouse. All inoculations were made through the intraperitoneal route.

The procedure for counting the daily parasitæmia, administration of sulphadiazine and the criteria for assessment of the presence and degree of resistance to the drug, were as described previously (Krishnaswami *et al.*, 1954).

The method followed to select parasites which were indifferent to the absence of para-aminobenzoic acid in the milk diet, was essentially the same as that followed to select and grow sulphadiazine-resistant individuals described by Krishnaswami *et al.* (1954). The serial transfer was made quickly in succession from animal to animal. In the initial stages when parasitæmia was not patent in the peripheral blood, known quantities of blood from the donor were inoculated. But when the parasites became patent, one million parasites constituted the inoculum.

RESULTS.

Three experiments were carried out, two in albino rats and one in albino mice, to establish the possibility of isolating and growing from the normal population of *P. berghei*, individuals that apparently are indifferent to the absence of para-aminobenzoic acid in the milieu.

Table I gives the protocol of the first experiment in rats. The daily parasitæmia in Rat 2512, the first in the series to be put on the milk diet, was very low till the 18th day following inoculation. Between day 19 and 22, the parasites were patent in comparatively larger numbers indicating thereby a survival of milk-resistant individuals. In the third serial passage in Rat 2575, the parasitæmia between day 7 and 12 was comparatively high and from the fourth serial passage onwards the growth inhibition observable in the earlier period, became progressively less.

Table II contains the protocol of the second experiment. Once again it was evident that selection from a normal population of members, indifferent to the absence of para-aminobenzoic acid, was rapid. In Rat 2800 of the second serial passage, the parasites were patent in sufficient numbers in the blood between day 7 and 13 following inoculation. From the third serial passage onwards, the parasitæmia became progressively higher.

TABLE I

Protocol of milk-resistant strain in rats.

Experiment 1.

Rat number.	Donor rat number	Dose of infection in c.c. of blood or million of parasites	PARASITES PER 10,000 ERYTHROCYTES ON DAY(S) SUBSEQUENT TO INOCULATION.																													
			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	37
2512	2502*	1 million	2	P	P	4	2	P	P	P	P	P	N	N	N	N	N	N	N	N	2 ↓	40	50	65	3	N	N	N	N	N	N	E.O.
2533	2512	0·5 c.c.	N	P	N	1	8	N	N	N	P ↓	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	E.O.	
2575	2533	0·7 c.c.	N	N	N	N	...	P	28	98	596 ↓	528	340	80	N	N	N	N	N	N	8	N	N	E.O.								
2584	2575	1 million	P	32	48	108	320	296	780	800	820 ↓	600	308	280 ↓	68	P	N	N	N	E.O.												
2592	2584	1 ,,	P	56	72	160	380	196	370	648	428	396	206	228 ↓	N	N	N	E.O.														
2632	2592	1 ,,	6	8	38	380 ↓	480	696	800	896	1600	280	128	28	P	P	N	N	N	E.O.												
2639	2632	1 ,,	N	N	6	92	590	1260	1828	1628	880	68	22 ↓	P	N	N	N	N	N	N	N	E.O.										
2647	2639	1 ,,	N	N	N	N	48	128	1040	1996	2000	2000	1800	1328	308	220	E.O.															

*The donor rat was fed with colony diet and harboured normal parasites.
↓ The day of serial inoculation.

P. = Positive. N. = Negative.
E.O. = End of observation.

TABLE II.

Protocol of milk-resistant strain in rats.

Experiment 2.

Rat number.	Donor rat number.	Dose of infection in c.c. of blood or million of parasites.	Parasites per 10,000 erythrocytes on day(s) subsequent to inoculation.																												
			1	2	3	4	5	6	7	8	9	10	11	12	13	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
2791	2732*	1 million	P	1	2	4	3 ↓	P	2	P	P	P	P	N	N	N E.O.															
2800	2791	0·7 c.c.	N	P	P	P	P	P	64 ↓	80	380	796	896	520	80	P E.O.															
2815	2800	0·6 c.c.	3	28	78	320	1400 ↓	2000	2000	1200 E.O.																					
2826	2815	1 million	4	45	360 ↓	510	1050	1680	2000	1920 E.O.																					
2832	2826	1 ,,	P	4	40	300	450 ↓	1110	1820 E.O.																						
2840	2832	1 ,,	3	40	240	760	1680	2000 E.O.																							

* The donor rat was fed with colony diet and harboured normal strain of parasites

P = Positive. N = Negative. ↓ The day of serial inoculation. E.O. = End of observation.

Table III contains the protocol of the third experiment, where the previous observations in rats were confirmed in mice. Here again the selection of a "milk-resistant" strain appeared to be easy and from Mouse 1918 with the third serial transfer, the parasitæmia became progressively higher.

Having established that a "milk-resistant" strain of *P. berghei* can be isolated and grown selectively, it remained to be examined if such a strain would also exhibit resistance to sulphadiazine. Such a "cross resistance" was to be expected as the strain appeared to possess an innate capacity to thrive in the absence of para-aminobenzoic acid. Table IV depicts the protocol of the experiment in mice to determine the susceptibility of the milk-resistant strain to sulphadiazine. It was found that the "milk-resistant" strain was not affected by 0·12 mg. of sulphadiazine which clears the peripheral blood of parasites in animals infected with the normal strain; even three times this dose was found to be ineffective. It should be remembered that the milk-resistant strain had been isolated by serial subinoculation of parasites surviving in hosts fed exclusively on milk and had not been exposed to the drug at any stage previously. This is perhaps the first instance where a strain, without previous exposure to a drug, exhibited resistance to it in the laboratory.

DISCUSSION.

The effect of milk diet to host animals on the growth of *P. berghei* would appear to be complex. Growth inhibition of parasites, due to milk diet of hosts, has not been a constant observation by different workers. While Maegraith *et al.* (1952), Ramakrishnan *et al.* (1953) and Hawking (1953) recorded that the proliferation of *P. berghei* was inhibited if the vertebrate host was fed purely on milk, Schneider and Montezin (1953) did not observe any such inhibition of the same plasmodium in mice fed exclusively on milk. Likewise, Galliard *et al.* (1954) could not draw any conclusion with reference to milk, consistently inhibiting parasite growth. The population of a given strain of *P. berghei* not being homogeneous, but containing varying proportions of individuals differing in their sensitivity to absence of para-aminobenzoic acid, appears to be a possible explanation for such apparent inconsistencies. If such an explanation is tenable, then it is conceivable that the strain used by Schneider and Montezin (1953) consisted predominantly of individuals insensitive to the absence of para-aminobenzoic acid.

It is apparent from the results of the studies reported here that the growth inhibition of *P. berghei* resulting from an exclusive milk diet to host animals can be overcome by rapid serial passages of the parasites through a series of animals fed similarly. Here again the explanation would appear to be that a population of a normal strain of parasites contains individuals which are not sensitive to the absence of para-aminobenzoic acid, a factor deficient in milk. It is obvious that such individuals are given a chance to selectively breed during the serial passages through animals fed on milk. That the strains developed by this technique are not sensitive to the absence of para-aminobenzoic acid, is confirmed by its comparative refractoriness to sulphadiazine.

TABLE III.

Protocol of milk-resistant strain in mice.

Experiment 3.

Mouse number.	Donor number.	Dose of infection in c.c. of blood or million parasites.	Parasites per 10,000 erythrocytes on days subsequent to infection																				
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1851*	Rat 2081*	1 million	P	28	92	72	22	44	30	68	760	1260	1280	2000 E.O.									
1852	Mouse 1851	1 ,,	P	P	P	P	P	P	N	N	N ↓												
1865	Mouse 1852	0·2 c.c.	N	N	N	N	N	N	N	N	N	2	6	20	300 ↓	350	120	80	48	32	P	2	46 E.O.
1918	Mouse 1865	1 million	N	3	18	85	180	240 ↓	410	270	370	840	210	105 E.O.									
1939	Mouse 1918	1 ,,	2	30	180	440 ↓	315	530	1360	2000	2000 E.O.												
1947	Mouse 1939	1 ,,	300	1120	390	760 ↓	2000	2000 E.O.															
1966	Mouse 1947	1 ,,	12	225	280	320	620 ↓	1050	1610 E.O.														
1967	Mouse 1966	1 ,,	6	250 ↓	270	310	320	815	780	710 E.O.													
1968	Mouse 1967	1 ,,	2	10	110	180	350	375															

*The donor rat as well as the recipient mouse were fed with colony diet and harboured the parent strain of parasites.
P = Positive. N = Negative. ↓ The day of serial inoculation. E.O. = end of observation.

TABLE IV.

Protocol of "milk-resistant" strain exhibiting resistance to sulphadiazine.

The dose of inoculum was 5 million parasites each from Rat 2592 infected with the "milk-resistant" strain.

Mouse number.	Parasites per 10,000 erythrocytes on day (s) subsequent to inoculation.															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1837	P	8	68	486*	496	560	1080	1800	2000	2000	2000	2000	2000	2000	2000	2000 E.O.
1838	P	12	120	628*	580	360	896	1968	1996	2000	2000	2000	2000	2000	2000	E.O.
1839	N	18	163	596†	428	448	1080	1868	2000	2000	2000	2000	2000	2000	2000	E.O.
1840	N	28	178	668†	388	328	260	680	1280	1980	2000	2000	2000	2000	2000	E.O.

P=Positive. N=Negative.

*†Sulphadiazine solution was administered intraperitoneally once in doses of 0·12 mg./20 gm. body weight (*) and 0·36 mg./20 gm. body weight (†) respectively.

E.O.=end of observation.

A natural variation of parasites in their response to certain inhibitory factors is not entirely unknown. Such a natural variation of strains is discussed by Covell *et al.* (1955) who present observations that certain strains of *P. vivax* as well as *P. falciparum* differ in their response to the same doses of antimalarials. The fact that paludrine resistant strains of several species of plasmodia can be selectively bred with ease in the laboratory as well as in the field (Krishnaswami *et al.*, 1954), would appear to confirm the hypothesis that a given population (strain) of parasites probably contains individuals of varying sensitivity to the drug; the African strain referred to by Covell *et al.* (1955) probably contained predominantly individuals less sensitive than others to paludrine.

Resistance of plasmodia to chemotherapeutic agents would thus appear to be more a natural character present in some individuals of a population, which are isolated by selective inbreeding, rather than an adaptation to adverse environments of susceptible individuals. It is for consideration whether the term 'acquired' commonly in use in respect of drug resistance in the field of chemotherapy, is rational.

SUMMARY.

The normal strain of *P. berghei* would appear to be a mixture of two strains, one suppressed by milk diet and the other resistant to milk.

A 'milk-resistant' strain of *P. berghei* was isolated from the standard laboratory strain by serial passage through albino rats and mice fed exclusively on milk.

The strain thus developed, showed a natural resistance to sulphadiazine.

The possibility of drug resistance of plasmodia being an inherent and natural and not an acquired character, is discussed.

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OBSERVATIONS ON ASSOCIATION OF AQUATIC VEGETATION WITH ANOPHELINE-BREEDING WITHIN DAMODAR-EDEN CANAL AREA OF WEST BENGAL.

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INTRODUCTION.

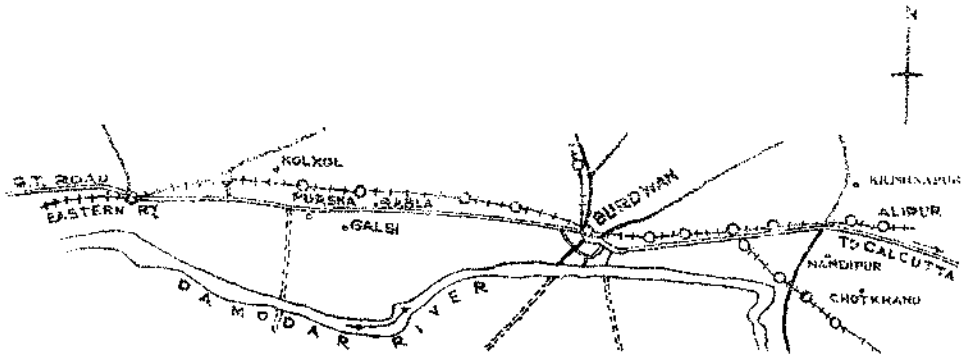
The relationship between aquatic vegetation and anopheline-breeding has long been recognised but detailed studies in this direction have not been extensive in India. Sen's (1941:1954) account of aquatic plants in the ecology of anopheline mosquitoes, the preference of anophelines to some plant associations and the changes in the fauna accompanying changes in the flora in tanks, is the only detailed study from this region available to the authors.

In the present investigation undertaken during 1954, a detailed account of relationship of aquatic vegetation with anophelines within the Damodar-Eden Canal area, is presented. Aquatic vegetation is divided into a few broad plant types (Hess and Hall, 1945) and arranged with respect to their relation with incidence of anopheline-breeding, and the changes in the plant covers are discussed in realtion to changes in the intensity of breeding. An attempt has been made to isolate the association of individual plants with individual anopheline species. A list of plants, found in the breeding pools and in some or other way associated with anophelines, is appended. A detailed list of aquatic plants will appear elsewhere.

This work forms a part of investigations concerned with breeding and ecology of possible vector species of malaria within Damodar-Eden Canal area of West Bengal. For this study, the following places, within the Burdwan District,

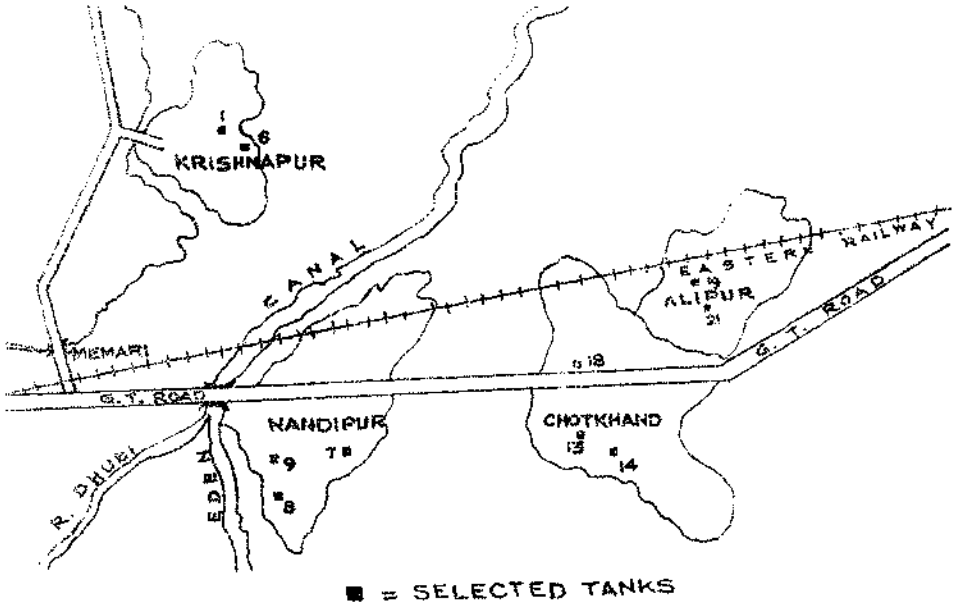
were selected. Irrigated areas: Galsi and Babla (upper end), Krishnapur and Nandipur (lower end); Unirrigated areas: Kolkol and Pursha (upper end), Chotkhand and Alipur (lower end) (Maps I(*a*), I(*b*), I(*c*). The areas lying west of Burdwan Town are termed as upper end and those eastwards as lower end.

MAP 1(a).



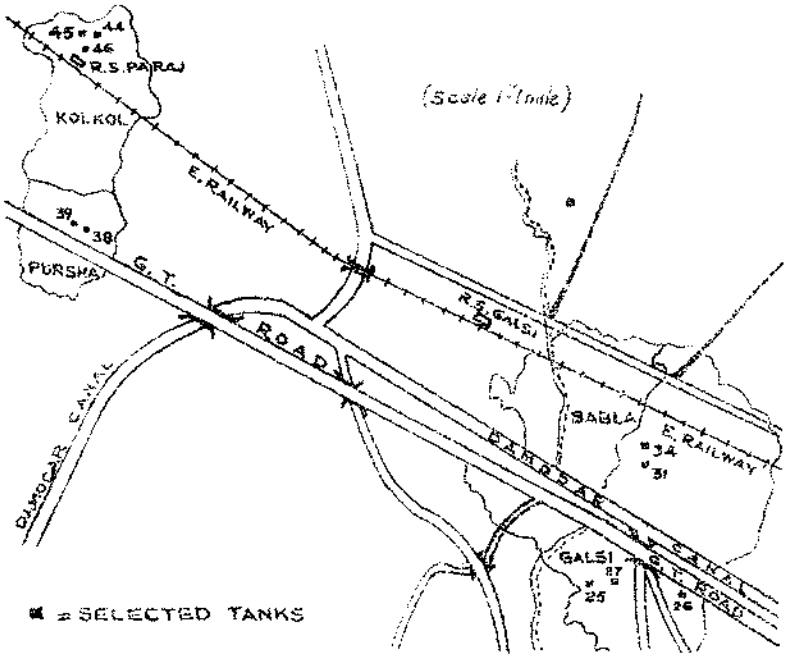
MAP 1. *a*) Location of villages in the Burdwan District, West Bengal, India.

MAP 1(b).



MAP 1. *b*) Location of villages in Eden Canal area.

MAP 1(c).



MAP 1. (c) Location of villages in Damodar Canal area.

VEGETATION OF BREEDING PONDS.

1. *Tanks, ponds and dobas**.—The anopheline species commonly breed wherever water collects but a higher incidence of their production is met with in presence of vegetation (Hinman, 1938). The various breeding pools show different combinations and associations of flora and it is possible to group this flora in a few plant types. The significance of each type with respect to breeding is of great importance, particularly to cyclic fluctuations and its intimate connection with water-level management for malaria control. The various plant types are (*c.f.* Hess and Hall, 1945; Penfound, 1953):—

(*i*) *Floating leaf.*—Has a high colonization potential since it can also extend breeding area into deep water. The floating parts can rise and fall with water-level. Main constituents are: *Nymphaea, Nelumbium, Limnanthemum*—occurring alone or in association (Plate II, Fig. 1).

*A *doba* is a small collection of water which may dry up during winter, a depressed land made deeper and regular in shape.

(*i*) *Flexuous.*—Typified by marginal emergent belt of leafy grasses, *Limnophila*, *Polygonum*, stray patches of floating leaf type, *Pistia*, *Aponogeton*, *Sagittaria*, *Utricularia* and other leafy erect shoots. The flexuous portions can rise and fall to some extent with water-level and provide favourable habitat for larvæ. This accounts for their high potential for anopheline production (Plate II, Fig. 2).

(*ii*) *Floating mat.*—Most species (*Jussiaea*, *Ipomaea*, *Alternanthera*, *Hygrorhiza*, etc.) are rooted in shallow water near the shores and extend later on water surface. The much branched stem forming a mat provides a high breeding potential (Plate II, Fig. 3).

(*iii*) *Carpet.*—*Pistia*, *Eichornia* or *Salvinia* when occurring as a complete carpet without any water surface, brings down breeding to minimum since water surface is choked-up. But if due to external agencies the mat is broken up here and there, it provides the best breeding place (Plate II, Fig. 4).

(*iv*) *Erect leafy.*—Here leaves are produced only on those portions which are above the water surface and these aerial portions only create "intersection values" [for a detailed discussion of intersection values, reference may be made to Hess and Hall (1943), *J. Nat. Mal. Soc.*, **2**, pp. 93-98]. Mainly includes marginal grasses, *Limnophila* and *Scirpus*.

(*v*) *Submerged.*—*Chara*, *Vallisneria*, *Ceratophyllum*, *Potomogeton*, *Aponogeton*, *Hydrilla* etc. when occurring purely as submerged flora is greatly handicapped due to lack of emergent vegetation which makes it less useful to larvæ. As a secondary associate of emergent flora, it is of some benefit to larvæ.

(*vi*) *Pleuston.*—Minute plants floating free at the water surface such as *Lemna*, *Azolla*-mat. Larval potential is very low or nil.

(*vii*) *Microscopical.*—Has a very low potential for breeding, mostly of a red scum formed either by decaying blue-green algæ or diatoms or both. When formed by unicellular green algæ, larval potential is slightly significant.

The relative potential importance of each plant type, in relation to anopheline production, is illustrated in Chart 1 and Table I. The total number of larvæ given is for the period July-December, 1954. Larvæ were collected once a week in each tank and each sample represented total for 25 standard dips with a frying pan having a diameter of about seven inches (also for data given in Table IV).

It is of interest to note that the floating leaf type provides the most congenial habitat for breeding, with flexuous and mat type coming next in precedence. These are the main plant types that occur in tanks, ponds, etc. How far the individual probable vector species follow this sequence, is shown in Table II.

It appears that *A. pallidus* has two maxima: in floating leaf and flexuous, whereas all other species follow the normal sequence outlined for the larvæ in general (Table I) except that in *A. philippinensis* (basing these results on the meagre collection) the maxima is in floating mat.

PLATE 11.

FIG. 1. Floating leaf vegetation. GL 31, Rabla.

FIG. 2. Clematis vegetation, NH. 13, Chatkhand.

FIG. 3. Floating mat vegetation. GL 11, Kolkol.

FIG. 4. Carpet vegetation. GL 45, Kolkol.

PLATE 15.

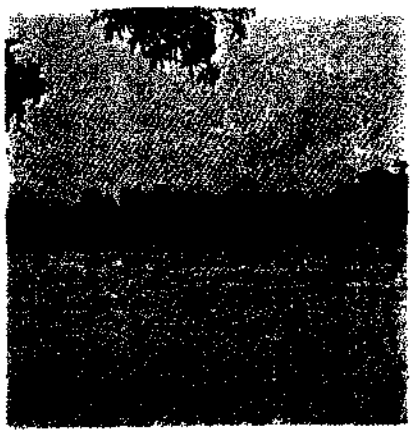


FIG. 1. Depressed land at Galsi showing floating leaf vegetation.

FIG. 2. Paddy field at Krishnapur showing a very high water table and floating vegetation.

FIG. 3. GL 45 in August note the scum over the surface and narrow marginal flora.

FIG. 4. GL 45 in December with complete carpet of *Azolla*, patches of *Pistia* in the foreground.

CHART 1.

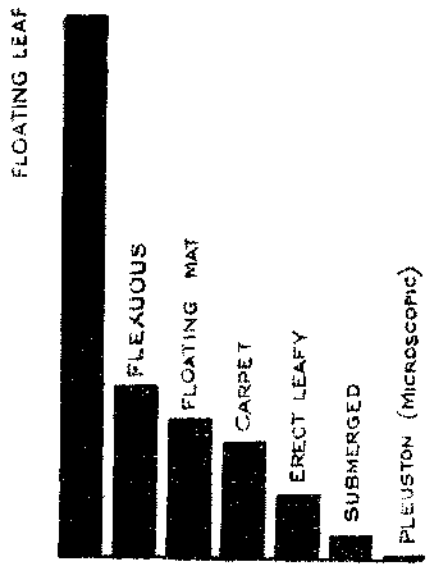


CHART 1. Relative potential importance of various plant types in anopheline production.

TABLE I.

Relative potential importance of various plant types in anopheline production.

Plant type.	Anopheline production potential.	Total number of larvae collected.
Floating leaf ...	Very high	793
Flexuous	High	252
Floating mat ...	High	205
Carpet	Low	173
Erect leafy	Very low	93
Submerged ...	Very low	38
Pleuston	Nil	0 - 5

TABLE II.

Relative incidence of probable vector species in plant types.

Plant type.	Species of anopheles.			
	philippinensis.	*culicifacies.*	*pallidus.*	*annularis.*
Floating leaf	0	7	31	118
Flexuous ...	1	0	37	25
Floating mat	2	0	7	8
Carpet ...	0	1	30	8
Erect leafy ...	0	1	3	6
Submerged ...	0	0	0	1

The various tanks selected for this study are grouped into these plant types (Table III), with the dominant flora, for the period July-December, 1954.

TABLE III.

Classification of selected tanks according to different plant types.

Plant type	Tank number and locality	Dominant flora	Surroundings: Shady (S), Semiexposed (SE), Exposed (E)
Floating leaf	ML 7—Nandipur	*Nymphaea, Limnanthemum, Hydrilla*	SE
	ML 19—Alipur	*Limnanthemum, Utricularia*	S
	GL 25—Galsi	*Limnanthemum, Pistia, Salvinia, Euglena*	SE
	26—Galsi	*Limnanthemum, Jussiaea, Cladophora*	SE
	31—Babla	*Nelumbium, Pistia*	S
	34—Babla	*Limnanthemum, Scirpus, Spirogyra*	SE
	38—Pursha	*Nymphaea*	SE
Flexuous	ML 6—Krishnapur	*Eichornia, Polygonum, Alternanthera*	SE
	8—Nandipur	*Polygonum, Limnanthemum, Sesbania*, grass	SE
	13—Chotkhand	*Polygonum, Jussiaea, Pistia, Aponogeton, Scirpus*	SE
	14—Chotkhand	*Aponogeton, Limnanthemum, Limnophila, Utricularia*, grass.	E
Floating mat	ML 1—Krishnapur	*Ipomaea aquatica, Salvinia, Scirpus, Alternanthera*	SE
	9—Nandipur	*Eichornia, I. aquatica, Jussiaea*	SE
	GL 44—Kolkol	*Hygrorhiza, Jussiaea, I. aquatica*	SE
Carpet	ML 21—Alipur	*Pistia*	SE
	GL 45—Kolkol	*Pistia, Azolla*	E
Erect leafy	GL 27—Galsi	*Utricularia, Cassia*, grass	S
	46—Kolkol	Grasses, red scum	SE
Submerged	ML 18—Chotkhand	*Alisma*	S
	GL 39—Pursha	*Cryptocoryne* (?)	SE
Pleuston	General—		
	—Krishnapur	*Lemna*	S
	—Silampur	*Lemna*	SE

2. *Paddy fields, depressed lands and fallow lands.*—The plants that occur in these breeding places are conveniently divided into three categories: terrestrial, semiaquatic and aquatic. In paddy fields, mostly terrestrial or semiaquatic plants grow and the truly aquatic flora is restricted to such species as *Utricularia, Sagittaria, Aponogeton, Lemna* and *Azolla*. However, the fields lying next to irrigated canals

and inundated by them show often truly aquatic flora of the floating type, e.g., at Krishnapur (Plate III, Fig. 2) or the choked-up fields at Burdwan with the species: *Azolla*, *Salvinia*, *Hydrilla*, *Ceratophyllum*, *Limnophila*, *Najas*, *Jussiaea* etc.

A correlation between the growth of algæ and the water table is observed in the paddy fields; as water recedes, the algæ diminish, then finally when water dries up, there appear such algal species as *Protosiphon*, *Botrydium* etc., both often associated with *Riccia*. When the fields dry up completely, moulds of perennating algæ can be detected here and there. At a number of places, if undisturbed by other factors, the algal activity goes hand in hand with the incidence of larvæ, since algæ constitute their main food. Yet it may be noted that the breeding of anophelines as a whole is meagre in paddy fields.

Larval food supply being nearly similar in quality and comparable with that in fallow land, is evidently not responsible for this great discrepancy in the number of larvæ in paddy fields; nor could this be due to activity of larval enemies because they are numerous where larvæ are most abundant and least where larvæ are practically absent. In Californian ricefields, this discrepancy is connected with the presence of blue-green algæ and scum by Purdy (1920) and Gerhardt (1954). But during the present investigation, blue-green algæ accounted for a similar state of affairs only at Nandipur (Kachroo, 1956).

In the depressed lands, the aquatic flora, both submerged and floating (rooted and free floating) find a convenient refuge and often floating type dominates (Plate III, Fig. 1). The marginal flora is mainly of marshy plants and to some degree of aquatic species, the central of floating type. It is, however, during rains that nearly all plants near the banks are swallowed-in. This class of habit includes such species as , *Cassia*, *Polygonum*, *Ricinus*, a host of Cyperaceae and Graminaceae and a number of trailing herbs. At this period of the year, the vegetation approaches that of a pond.

In the fallow lands, the vegetation is mainly of marshy plants: *Aeschynomene*, *Sesbania*, *Neptunia*, *Enhydra*, *Acanthus*, and the terrestrial ones; the floating being scarce (since the chances of prolongation of life in these circumstances are far less as compared to those of the depressed lands which are permanent water collections). Having this advantage, therefore, the incidence of larvæ in the latter is much more than in the paddy fields or the fallow lands. It is not surprising that the depressed lands have the same breeding potential as that of the *dobas* or the ponds.

With respect to association of paddy fields with anopheline production, it may be noted that they play a very insignificant role in this direction (Table IV). It is not improbable that much cultivation might bring down the production of probable vector species in this region. To realize the same it would be necessary and desirable to see that the nearby depressed and fallow lands are dried up and filled with earth.

3. *Changes in vegetational cover and its bearing on breeding.*—An elucidation of the relation between the larval incidence and the relative growth of the vegetational cover is provided by a silty water tank at Kolkol (GL 45), and a depressed land with clear water, at Alipur.

TABLE IV.

Incidence of larval species in paddy fields during July-December, 1954.

Species of anopheline.	Number of larvae collected.	
	Irrigated areas.	Unirrigated areas.
philippinensis	1	0
culicifacies	2	0
annularis	3	7
pallidus	2	14
ramsayi	0	0

In September-October, this tank (GL 45) showed a belt about 1 – 4 ft. wide and in November about 3 – 5 ft. wide; constituted mainly (in order of dominance) of *Pistia, Limnanthemum, Ipomaea aquatica, Lemna, Jussiaea, Azolla, Marsilea, Spirogyra,* few grasses and a scum probably formed by decaying diatoms and covering about a quarter of the water surface (Plate III, Fig. 3). During November, there was rapid increase in growth of *Azolla,* finally covering the whole water surface in December, with *Pistia* here and there (Plate III, Fig. 4) and nearly suffocating *Limnanthemum* to extermination. This change in vegetational set-up shows a close correlation with larval incidence—which is higher when the vegetation was marginal and suddenly fell to a minimum when the whole water surface was choked by *Azolla* (Chart 2). This sharp decline is obvious since due to complete carpet, the water surface remained choked-up resulting in scarcity of food and greatly increasing the incidence of other animalcules which might feed on

CHART 2.

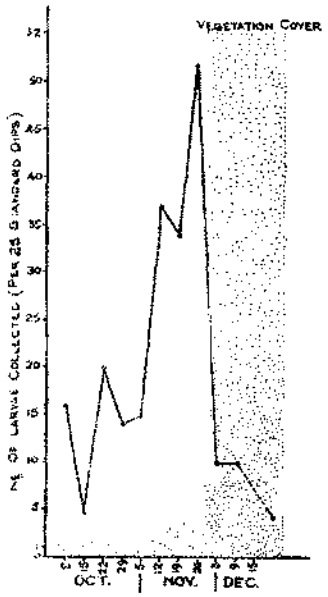


CHART 2. Relation between the growth of vegetational cover and the incidence of larvae in GL 15.

larvæ. This complete carpet would further hinder oviposition by female mosquitoes and later emergence of adults. A visit to the tank at the end of December, 1954 and during January, 1955, showed a remarkable change in vegetation—from *Azolla* mat to *Pistia* carpet—and still more decline in larval production. A similar experience was gained from a *doba* at Krishnapur during November, 1954, with a complete mat of *Lemna* (on all occasions the incidence of larvæ per 25 standard dips with a frying pan was zero). However, it may be noted that when the carpet is broken, it is not uncommon to find abundant breeding.

Whereas this is the position where flora tends towards carpet formation, a contrasting picture is presented by a place where the dominant emergent-cum-submergent flora tends towards a condition of devegetation. Thus, at Alipur, in a depressed land it was noted that great incidence of larvæ is associated with gregarious growth of emergent vegetation of *Scirpus, Limnophila, Polygonum, Utricularia, Acanthus illicifolius* and *grasses*. With the dominance of submerged flora, the incidence of larvæ decreases and finally with the loss of vegetation the larvæ dwindle to just a few (Chart 3). A glance at Chart 3 shows a significant fall in the curve on October 29. On this day the entity of the depressed land was intact, the grasses were on their last stage of extermination, marginal vegetation had diminished and algæ rare. The common species *Myriophyllum* and *Limnophila* could hardly compensate for the emergent flora and are, therefore, of little value for breeding. After this, land was broken up into patches due to drying-up of water, and the larvæ had to be collected from patches with some flora. This explains the rise in the curve. After a few weeks, when the patches also showed devegetation-phase (except for a few *Polygonums* in a small patch of water), the incidence of larvæ again declined.

CHART 3.

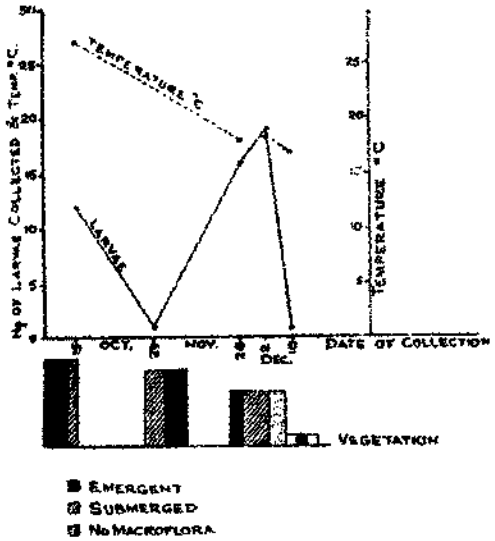


CHART 3. Relation between incidence of larvæ and various phases of vegetation at depressed land, Alipur.

PLANTS ASSOCIATED WITH ANOPHELINE PRODUCTION.

Observations of Earle (1926) regarding *A. albimanus* might apply to larvæ in general which can breed in almost any situation, except on dry land. Whereas such a statement might equally well embrace the situation for *A. culicifacies*, the situation for *A. philippinensis* is a bit different (Table V). It does not usually occur in neglected places and this goes hand in hand with the fact that its larvæ nearly always occur in clear-water pools. It is, therefore, imperative that it should lean heavily on vegetational habitat.

TABLE V.

Breeding places of A. philippinensis *and* A. culicifacies.

	Nature of breeding places.	Ground water pools etc.														Condition of water.		Area.	
		Exposed without vegetation.			Partly shaded, with vegetation.					Exposed, with vegetation.									
		Rain water pools.	Hoof prints.	Drains.	Burrow pits.	Dobas.	Ponds.	Tanks.	Wells.	Burrow pits.	Swamps.	Pools under bridges.	Fallow lands.	Paddy fields.		Clear water.	Turbid water.	Irrigated.	Un-irrigated.
A. philippinensis	Number of breeding places ...	...	...	...	...	7	4	12	...	...	1	...	1	1		...	...	...	...
	Incidence of larvæ in numbers ...	...	...	...	...	9	13	13	...	...	1	...	1	1		...	...	...	...
	Percentage breeding	...	...	...	...	17·1	8·2	25·2	...	...	2·6	...	2·6	2·6		54·6	2·3	28·3	17·1
A. culicifacies	Number of breeding places ...	1	2	1	...	...	3	12	...	...	...	...	1	1		...	...	...	...
	Incidence of larvæ in numbers ...	1	8	4	...	...	7	24	...	...	...	2	2	...		...	...	...	...
	Percentage breeding	2·6	4·1	2·6	...	...	6·1	25·2	...	...	...	2·6	2·6	22·8		22·8	23·3	30·9	...

This brings us to a consideration of plant associaton with anopheline larvæ (Tables VI, VII, VIII). In general, plants favourable to anopheline production may be grouped into three categories: (*i*) those actually associated with larvæ and always providing shelter and often food ; (*ii*) those simply providing shelter (or even food) but always in association with other plants and (*iii*) chance associates.

The first category includes partly floating species on or under which the female mosquito deposits her eggs. Later the young larvæ support themselves on the much branched roots of aquatic plants or on leaves of fleshy species. Since it is not uncommon for each plant species to have a number of epiphytic algæ either

on their ventral surface or on their roots; the food problem of larvæ is thus itself solved. Association of such plants as *Eichornia* is intimate because these plants having large airspaces within their floats, provide the necessary oxygen required for the subsistence at or below the water surface. Other plants that might be added to this list are those that form floating mats with formation of numerous roots and floats at nodes of their creeping steams, e.g., *Jussiaea*, *Hygrorhiza* etc.

TABLE VI.

Plant species intimately associated with anopheline larvæ.

Plant species or association	Anopheline species.	Total anopheline species associated.
1. *Chlorella vulgaris*	*hyrcanus, vagus, subpictus*	3
2. *Chara zeylanica*	*hyrcanus*	1
3. *Azolla pinnata*	*hyrcanus*	1
4. *Salvinia natans*	*hyrcanus, annularis, pallidus, ramsayi*	4
5. *Alternanthera sessilis*	*pallidus, hyrcanus*	2
6. *Ceratophyllum demersum*	*hyrcanus*	1
7. *Cardanthera triflora*	*pallidus, philippinensis, hyrcanus*	3
8. *Colocasia antiquorum*	*hyrcanus, barbirostris, vagus*	3
9. *Eichornia speciosa*	*annularis, hyrcanus, barbirostris*	3
10. *Ipomaea aquatica*	*hyrcanus, subpictus, annularis*	3
11. *Jussiaea repens*	*culicifacies, hyrcanus, barbirostris*	3
12. *Limnanthemum indicum*	*annularis, hyrcanus, vagus*	3
13. *Limnophila* sps.	*annularis, pallidus, hyrcanus, vagus*	4
14. *Myriophyllum indicum*	*pallidus, hyrcanus*	2
15. *Nymphaea lotus*	*annularis, pallidus, hyrcanus, vagus*	4
16. *Polygonum* sps.	*annularis*	1
17. *Trapa natans*	*barbirostris*	1
18. *Utricularia* sps.	*annularis, hyrcanus*	2
19. *Vallisneria spiralis*	*hyrcanus, barbirostris, vagus*	3
20. *Pistia stratiotes*	*annularis, pallidus, philippinensis, culicifacies, hyrcanus, barbirostris, ramsayi, vagus*	8
21. *Jussiaea-Scirpus articulatus*	*hyrcanus*	1
22. *Limnanthemum-Scirpus*	*annularis, pallidus, hyrcanus*	3
23. *Polygonum*-grass	*hyrcanus, barbirostris*	2
24. *Hygrorhiza-Hydrilla*	*hyrcanus, subpictus*	2
25. *Grass-Utricularia*	*hyrcanus, subpictus*	2
26. *Alternanthera—Pistia—Eichornia*	*hyrcanus, varuna*	2
27. *Sesbania-Aeschynomene*	*hyrcanus*	1

Whether there exists some sort of correlation between a particular plant and that of anopheline would be a hasty judgement at the moment, as such studies are still in progress, but one thing can be definitely stated with respect to association of some plants with anopheline larvæ in general; e.g., wherever there is *Pistia*, *Jussiaea* or *Limnanthemum* or their association with *Ipomaea aquatica*, *Alternanthera sessilis*, *Limnophila*, *Scirpus articulatus* etc., there must occur *A. hyrcanus*, *A. annularis* or *A. pallidus*; *Nymphaea*, *Limnanthemum* and *Alternanthera* in association with *Spirogyra* (and often *Cladophora*), provide best indicator or breeding place of the probable vector species: *A. philippinensis*, *A. pallidus*, *A. annularis*.

The second category includes *Chara*, *Ceratophyllum*, grasses, naked species with aerial leaves or branches. When occuring singly, these plants might not help in breeding.

Among chance associates are such plants as *Cassia*, *Aponogeton*, *Croton*, etc. These do not always give positive results for larvæ. It was observed on a number of occasions that all dips (at a time 25) gave negative results with respect to, e.g., *Aponogeton*, even though larvæ often fed on leaf epidermis of this plant.

In absence of vegetation, it is noted that roots of palms or bamboos along the banks of ponds compensate for the vegetation and provide the best breeding habitat. The much branched fibrous roots give a good protection against large animalcules and also provide a good substratum for filamentous algæ like *Cladophora*, *Microspora*, *Oedogonium etc.* to grow. These provide food for larvæ.

TABLE VII.

Association of plant communities with anopheline larvæ.

	Plant communities.	Anopheline production.	Total species.
1.	*Marsilea-Cassia-Acanthus-Lemna-Scirpus-grass*	*hyrcanus, barbirostris*	2
2.	*Marsilea-Ipomaea-Jussiaea-Scirpus*	*philippinensis, hyrcanus*	2
3.	*Marsilea-Cassia-Lemna*	*culicifacies, hyrcanus, subpictus*	3

TABLE VIII.

Chance associates of anopheline larvæ.

	Pure association of plants.	Associated anopheline species.
1.	*Cassia tora*	nil.
2.	*Croton sparsiflorus*	nil.
3.	*Eriocaulon sieboldianum*	nil.
4.	*Najas foveolata*	nil/*hyrcanus*.
5.	*Aponogeton crispus* .	nil/*hyrcanus*.

SUMMARY.

Aquatic vegetation is intimately related with anopheline-breeding and it is divided into a few plant types with respect to their relative potential importance in anopheline production with particular reference to *A. pallidus*, *A. annularis*, *A. philippinensis*, *A. culicifacies*, *A. ramsayi*, *A. hyrcanus*, *A. barbirostris*, *A. vagus*, *A. varuna*, *A. aconitus* and *A. subpictus*. The plant types are: floating leaf, flexuous, floating mat, carpet, erect leafy, submerged, pleuston and microscopic respectively.

Changes in the vegetative cover have a bearing on anopheline production: a complete carpet bringing the incidence of larvae to a minimum and a broken mat increasing it to a great extent. In pools, with mixed vegetation, larval incidence is highest in presence of emergent (associated with submerged) vegetation; less so in the submerged vegetation-phase and rare in the absence of both floras.

A. philippinensis prefers clear water breeding pools and *A. culicifacies* has a tendency to breed equally in clear and turbid waters.

Of the plants associated with anophelines, those intimately associated are always providing shelter and food. No particular *anopheline* species is associated with a particular plant species (in *senso stricto*) but some plants like *Salvinia*, *Nymphaea*, *Jussiaea*, *Limnanthemum*, *Alternathera*, *Polygonum*, *Ceratophyllum*, *Eichornia*, *Pistia*, *Hygrorhiza*, *Scirpus* etc. are definitely indicative of anopheline-breeding. Plants like *Cassia*, *Marsilea* and *Aponogeton* are chance associates.

ANNONATED LIST OF PLANTS.

The following list includes the plant species found in some association with anopheline-breeding. The families are arranged alphabetically, as are the genera and species within a family.

1. Acanthaceae.

Alternanthera sessilis L.
Cardanthera triflora Ham.

2. Alismaceae

Butomopsis lanceolata Kunth.
Sagittaria guayanensis H.B.K.

3. Aroideae

Colocasia antiquorum Schott.
Cryptocoryne sps. ?
Pistia stratiotes L.

4. Compositae

Enhydra fluctuans Lour.

5. Ceratophyllaceae.

Ceratophyllum demersum L.

6. Commelinaceae.

Commelina salicifolia Roxb.
C. benghalensis L.

7. Convolvulaceae.

Ipomaea aquatica Forsk.

8. Cyperaceae.

Cyperus haspan L.
C. irio L.
Fimbristylis dichotoma Vahl.
F. monostachya Hassk.
Kyllinga monocephala Rott.
Scirpus articulatus L.
S. grossus L.

9. Euphorbiaceae.

Croton sparsiflorus Morong.

10. Gentianaceae.

Limnanthemum cristatum Griseb.
L. indicum Thwaites.

11. Gramineae.

Andropogon aciculatus Retz.
A. squarrosus L.
Hygrorhiza aristata Nees.
Leersia hexandra Sw.
Panicum colonum L.
Setaria glauca Benth.
Phragmites karka Trin.

12. HALORAGEAE.

Myriophyllum indicum Willd.
M. tuberculatum Roxb.

13. HYDROCHARIDEAE.

Hydrilla verticillata Casp.
Lagarosiphon Roxburghii Benth.
Ottelia alismoides Pers.
Vallisneria spiralis L.

14. HYDROPHYLLACEAE.

Hydrocotea zeylanica Vahl.

15. LEGUMINOSAE

Cassia tora L.
Aeschynomene aspera L.
Neptunia oleraceae Lour.
Sesbania palludosa Prain.

16. LEMNACEAE.

Lemna minor L.

17. LENTIBULARIACEAE.

Utricularia bifida L.
U. flexuosa Vahl.
U. racemosa Wall.
U. stellaris L.

18. NAIADACEAE.

Aponogeton crispum Thumb.
A. monostachyon L.
Najas foveolata A. Br.
Potomogeton crispus L.

19. NYMPHAEACEAE.

Nymphaea cyanea Roxb.
N. esculenta Roxb.
N. lotus L.
N. rubra Roxb.
Nelumbium speciosum Willd.

20. ONAGRACEAE.

Jussiaea repens L.
Trapa bispinosa Roxb.

21. POLYGONACEAE.

Polygonum flaccidum Meism.
P. orientale L.
P. tomentosum Willd.
Rumex meritimus L.

22. PONTEDERIACEAE.

Eichornia speciosa Kunth.
Monochoria hastæfolia Presl
M. vaginalis Presl

23. SCROPHULARIACEAE.

Limnophila conferta Benth. ?
L. gratisioides R. Br.
L. heterophylla Benth.
L. racemosa Benth.

24. MARSILEACEAE.

Marsilea quadrifoliata L.

25. SALVINIACEAE.

Salvinia cucullata Roxb.
S. natans Hoffm.
Azolla pinnata R. Br.

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FILARIASIS IN PATNA (BIHAR).

PART I.

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FILARIASIS is one of the main mosquito-borne diseases which is responsbile for considerable sickness with different types of disease manifestations, among the people of the City of Patna. In order to study the incidence, type and distribution of filarial infection in the corporation area of Patna, a survey was started under the National Filaria Control Programme. A rough survey and study of the associated factors, concerned with the causation and transmission of the filarial infection, have also been undertaken.

The investigation started in the latter half of September, 1955. The findings recorded in the paper relate to the eastern zone of Patna, popularly known as 'Patna City', which forms one of the three zones of the Patna Corporation, the other two being the central or Bankipur Zone, and the western or the new capital area zone.

PHYSIOGRAPHY.

Patna, the capital of Bihar, situated at latitude 25·25 degrees north and longitude 85·5 degrees east, includes the ancient city of Patliputra, the foundations of which were laid as far back as 475 B.C. by King Ajatasatru and later described by Megasthenes and Fahieng. It lies on the right bank of the River Ganga which

forms the northern boundary of the city. The River Poonpoon flows about five miles south of the city and joins the Ganga five miles downstream at a point near Fatwah Town. The city moats and the tracts of water sheets ultimately discharge the excess water into the River Poonpoon during the monsoon season.

Patna is on the main line of the Eastern Railway, and is the terminus of the Patna-Gaya line. It is also on the main route of traffic connecting north Bihar and south Bihar across the Ganga. It is a big centre of river-borne traffic. Several roads radiate from the city to various other districts of the State.

The present Patna Corporation has an area of 24·67 square miles, the length being about 13 miles and the average width being about 1½ miles in the old portions, and about three miles in the new Capital area. At places, the width of the built-up areas, however, is only about half a mile.

According to the 1951 census, the total population of the Patna Corporation was about 3,25,000 with an average density of population of about 13,000 persons per square mile.

For administrative purposes, Patna has been divided into 36 wards. The western or the new capital zone consists of Ward 1 and Wards 33 to 37. The central or Bankipur Zone has Wards 2 to 16, and the eastern or Patna City zone comprises Wards 17 to 32 (Map 1). This paper presents the results of survey of Wards 17 to 32, that is, the eastern zone or 'Patna City' with an area of 3,956 acres, and a population of 1,14,568. The density of population varies between 7·4 per acre in Ward 32 to 116 per acre in Ward 26. The total number of holdings is 21,041.

TOPOGRAPHY.

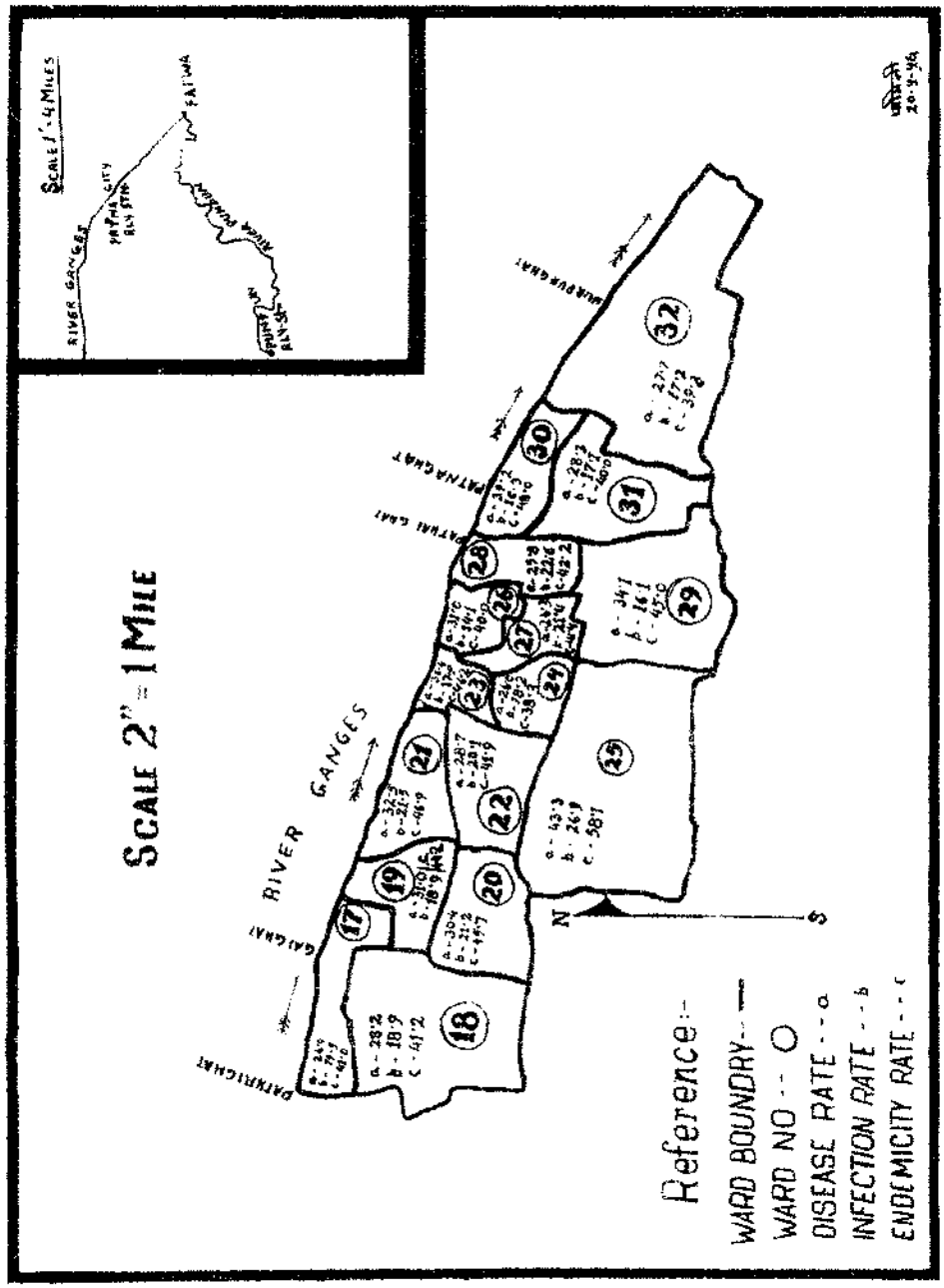
Patna City has an altitude of 150 to 200 ft. above the mean sea-level. The land slopes generally in the south-easterly direction from the bank of the Ganga. The three broad zones may be looked upon as the natural divisions of the town for drainage purposes.

In the central and eastern zones, the Ashoka Rajpath, running parallel to the Ganga, forms the main ridge line and so the surface water flows to both the sides of the road. In the western zone, the Ashoka Rajpath runs along the Ganga.

Prior to the construction of the Chirayantar-Agamkuan dyke along the southern border of the town in 1930, the flood water of the River Poonpoon, used to submerge the whole area south of the Ashoka Rajpath. The dyke now protects the town from the flood water. The storm water accumulated within the city, however, finds no outlet and remains locked up for about two months in the year. The situation is worsened when there is flooding in the Ganga which does not allow the Poonpoon flood to subside quickly, and causes back-flow and over-flow of the drains. Thus, for the greater part of the year, a chain of pools and lagoons are left behind in the southern part of the town, along its entire length, and form potential breeding grounds of mosquitoes.

MAP 1.

Incidence of filariasis in different wards of Patna City.



DRAINAGE.

The city has open surface drains which are mostly *kutcha* and partly *pucca*. These open surface drains are usually choked up by silt, dried leaves, and by the indiscriminate throwing of refuse and garbage in them. Nearly all the main drains, and major portions of the outfall drains flow southwards. Only some portions of the central and western zones are drained northwards into the River Ganga.

There is a very old channel continuing from the outfall of the central zone at its southern end at Agamkuan, which connects with the Poonpoon during the monsoon season. In dry weather, this portion is converted into a chain or water-logged areas with an abundant growth of aquatic vegetations such as water hyacinth (*Eichornia crassipes*), *Pistia lanceolata*, laminæ and other water-weeds.

Major portions of the eastern and western moats of the eastern zone flow southwards. Both these moats drain into the so called southern moat, now only partly in existence in the form of depressions which are converted into pools of water after the rainy season.

The Patna Improvement Trust has taken up schemes for improving the drains of Patna. To cope with the storm water, locked up due to the Poonpoon in flood, the Trust is going to provide high-power pumps at two places in the town.

WATER-SUPPLY.

There is a protected water-supply for a major portion of the town, and for the rest wells are used. Apart from these, there are a large number of disused wells and tanks which have been found to be potential breeding grounds of mosquitoes.

METEOROLOGICAL CONDITIONS.

Temperature and humidity vary in different parts of the year as shown in Graph 1. Rainfall during the different months is shown in Graph 2. Total average annual rainfall, from 1950 to 1955, is 41·5 inches. Except during the height of the monsoon (July-August), favourable conditions appear to exist almost throughout the year for the breeding of mosquitoes.

SOCIOLOGICAL CONDITIONS.

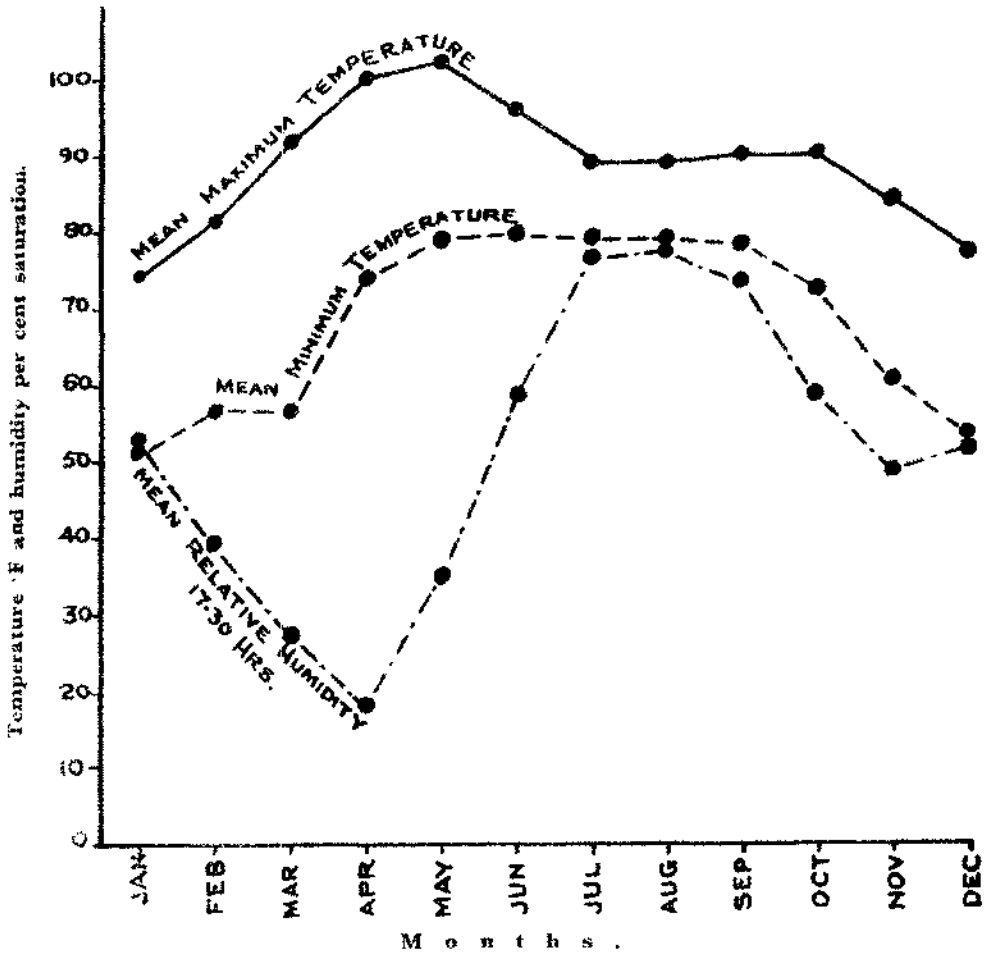
The western zone of Patna accommodates the seat of Government, the houses of the legislature, the High Court, the Rajbhwan, and the residences of ministers and a large number of Government servants.

The central zone, contains the two universities of the state, the general hospital, the civil and cirminal courts, banks, business houses, main bazars, and residences of politicians ; in short, the main intelligentia of the town. The older part of this zone is also congested and extremely insanitary.

The eastern zone of Patna may be looked upon as the centre of grain trade and is occupied by those concerned with the trade. It is most insanitary and

GRAPH 1.

Mean monthly temperature and humidity of Patna (average for 1950 to 1955)



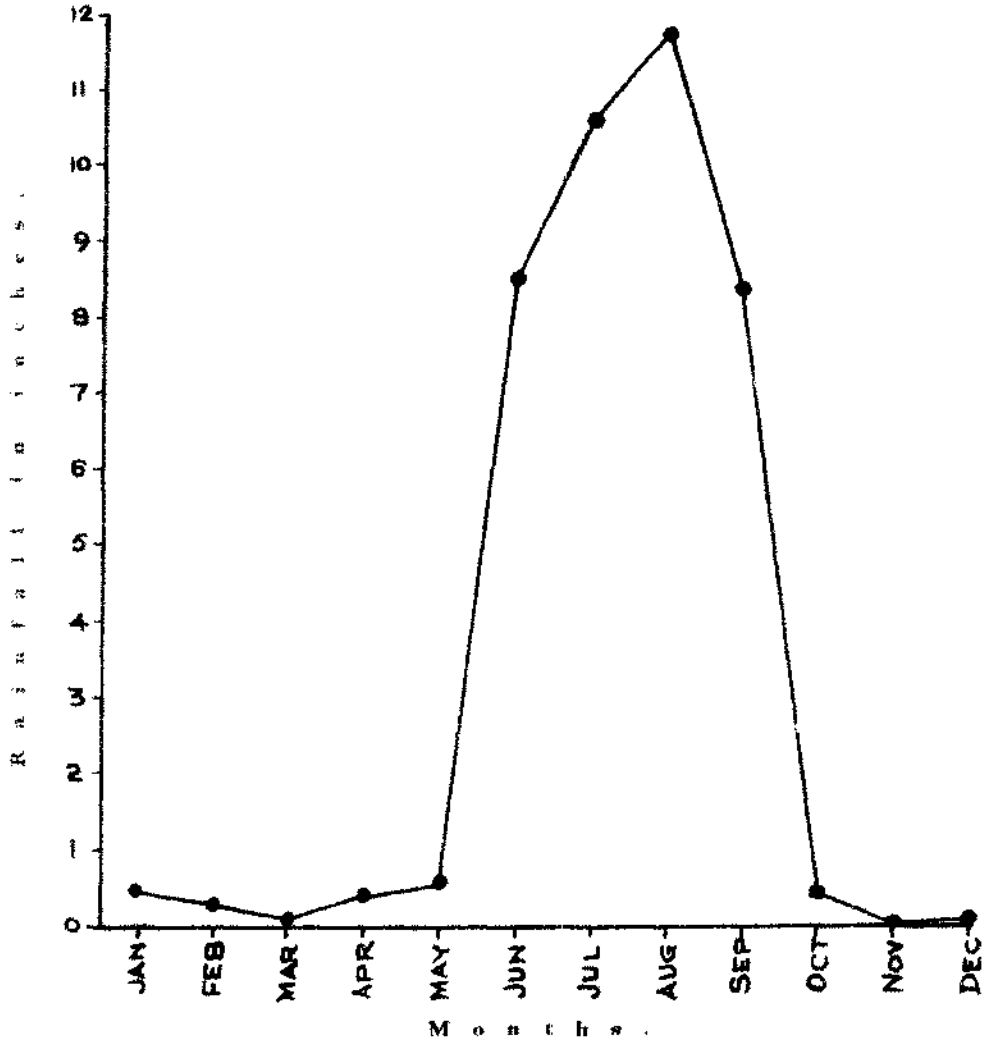
also very highly congested. The houses are mostly back to back and built according to old design. Cowsheds usually are inside the dwelling houses. The people are socio-economically backward. The literacy rate and standard of living is the lowest in this zone.

PREVIOUS WORKS.

Korke (1926:1928:1929) conducted an investigation into the incidence of filariasis in the then Bihar and Orissa. A part of his investigations were carried out in Patna, and in Bihar Town, Paliganj and Maner in Patna District. He

GRAPH 2.

Average monthly rainfall (average of years 1950-1955).



examined eight cases in Patna Town, and sixty cases in Bihar Town. In both these places, the cases were all males, selected on clinical suspicion of filariasis. In Paliganj and Maner, he examined random samples of 113 and 104 persons

respectively. The disease rate calculated from his findings varies between 15·4 and 28·3 per cent and infection rate between 15·0 and 19·2 per cent in the different places. Concomitant infection and disease manifestation was found in 5·7 to 9·7 per cent of the samples of the different places. Infection in all his cases was, invariably, with *W. bancrofti*.

DETAILS OF PRESENT WORK AND FINDINGS.

In order to determine the incidence and type of filarial infection, and the incidence of different filarial disease manifestations in the human community, and to determine the vector species of mosquitoes with the incidence and type of filarial infection in them, a human and entomological survey was conducted.

Human survey.—Random representative samples of the population of all the sixteen wards, covering all age groups in both the sexes, in all the socio-economic strata, were examined. Blood smears were collected in the night between 8-30 p.m. and 12·00 midnight, by house to house visits. The survey parties carried survey cards with columns for name, father's name, age, sex, various columns for history or presence of different signs and symptoms of filariasis, result of blood examination and remarks. Against each person's name, the columns were filled up by suitable enquiry and examination. Approximately 20 c.mm. of blood, representing roughly three big drops of blood, was taken from each case.

On the following day, the blood smears were dehæmoglobinized by gentle shaking with a few drops of tap water, before staining with J.S.B. Solution I for four minutes. The entire smear was examined before declaring a slide to be negative for microfilariæ. In the case of positive smears, the species and the total number of microfilaræ were recorded. The results were entered in the appropriate column on the survey card, analysed, and are presented in Tables I, II and III; Graphs 3, 4, 5, 6, 7 and Map I.

Table I and Graph 6 show the infection rates, disease rates, endemicity rates and average infestation rates of the different wards, and of the entire population examined. Nine thousand four hundred and eighty-five persons were examined in the 16 wards of Patna City during the survey, covering 8·3 per cent of a population of 1,14,568 persons. External manifestations of filarial disease were encountered in 2,860 persons (30·1 per cent) and microfilariæ were detected in the blood of 1,780 persons (18·7 per cent). The species of microfilaria encountered throughout the survey was *W. bancrofti*, without exception. The disease rate varied from ward to ward, the lowest being 25·8 per cent in Ward 28 and the highest being 43·3 per cent in Ward 25. The lowest infection rate was 14·1 per cent in Ward 26 and the highest was 26·9 per cent in Ward 25. The filarial endemicity rates of the different wards varied between 38·3 per cent in Ward 24 and 58·1 in Ward 25. The endemicity rate for the whole of the Patna City comes to 42·7 per cent. Ward 25 in which the infection, disease and endemicity rates are the highest, is a water-logged, semi-rural area where excessive breeding of mosquitoes goes on throughout the year. In Ward 26, the infection rate and the disease rate are significantly low (considering the fact that the proportion of persons of higher age groups was highest in this ward) because of admixture with comparatively recently

settled population from the Punjab, as also of older Punjabi inhabitants who are generally better off socio-economically. Ward 30, in which the disease rate and the endemicity rate are significantly higher, is extremely insanitary and is inhabited by socio-economically lower strata of the people. Except in Wards 25, 26 and 30, the variations of disease rate, infection rate and endemicity rate, from ward to ward, are not statistically of much significance. The variations can be accounted for, largely by the difference in the total number of persons and the difference in the relative numbers of persons of different age groups examined, in the different wards.

TABLE I.

Filaria survey—Patna City Circle—Patna Municipal Corporation (December 1, 1955 to April 27, 1956).

Ward number.	Population.	Number of persons examined.	Percentage of population examined.	With disease		With microfilariae.		Endemicity rate per cent	Average infestation of microfilariae per 20 c.mm.
				Number.	Per cent.	Number.	Per cent.		
17	7,985	674	8·4	178	26·4	132	19·5	41·0	30·0
18	6,004	538	8·9	152	28·2	102	18·9	44·2	18·3
19	5,060	502	9·9	156	31·0	95	18·9	44·2	20·0
20	9,576	738	7·7	225	30·4	157	21·2	15·7	22·9
21	9,931	835	8·4	272	32·5	180	21·5	46·9	24·3
22	8,300	765	9·2	220	28·7	154	20·1	44·0	18·9
23	5,777	385	6·6	141	36·6	69	17·9	46·2	40·5
24	8,164	373	4·5	97	26·0	68	18·2	38·3	41·1
25	7,929	189	2·3	82	43·3	51	26·9	58·1	18·7
26	7,415	1,595	21·5	496	31·0	226	14·1	40·0	39·7
27	5,965	844	14·1	222	26·3	181	21·4	41·4	46·6
28	6,190	336	5·4	87	25·8	76	22·6	42·2	44·0
29	7,995	211	2·7	72	34·1	34	16·1	45·0	54·3
30	7,093	331	4·6	130	39·2	54	16·3	48·0	29·6
31	6,054	874	14·4	248	28·3	150	17·1	40·0	45·8
32	5,130	295	5·7	82	27·7	51	17·2	39·3	44·9
Total	1,14,568	9,485	8·3	2,860	30·1	1,780	18·7	12·7	32·8

TABLE II.

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

Age group (years).	MALES:					FEMALES:					BOTH SEXES:				
	Number examined.	With disease.		With microfilariæ.		Number examined.	With disease.		With microfilariæ.		Number examined.	With disease.		With microfilariæ.	
		Number.	Per cent.	Number.	Per cent.		Number.	Per cent.	Number.	Per cent.		Number.	Per cent.	Number.	Per cent.
2-5	406	16	3·9	35	8·6	371	6	1·6	30	8·0	777	22	2·8	65	8·3
6-10	848	47	5·5	121	14·2	482	23	4·7	75	15·5	1,330	70	5·2	196	14·7
11-20	1,680	471	28·0	326	19·4	664	89	13·4	108	16·2	2,344	560	23·8	434	18·5
21-30	1,686	784	46·5	378	22·4	701	152	21·6	135	19·2	2,387	936	39·2	513	21·4
31-40	809	456	56·3	173	21·3	434	109	25·1	85	19·5	1,243	565	45·4	258	20·7
41-50	631	396	62·7	161	25·5	310	100	32·2	60	19·3	941	496	52·9	221	23·4
Above 50	297	162	54·5	67	22·5	166	49	29·5	26	15·6	463	211	45·5	93	20·0
All ages	6,357	2,332	36·7	1,261	19·8	3,128	528	16·8	519	16·4	9,485	2,860	30·1	1,780	18·7

TABLE III.

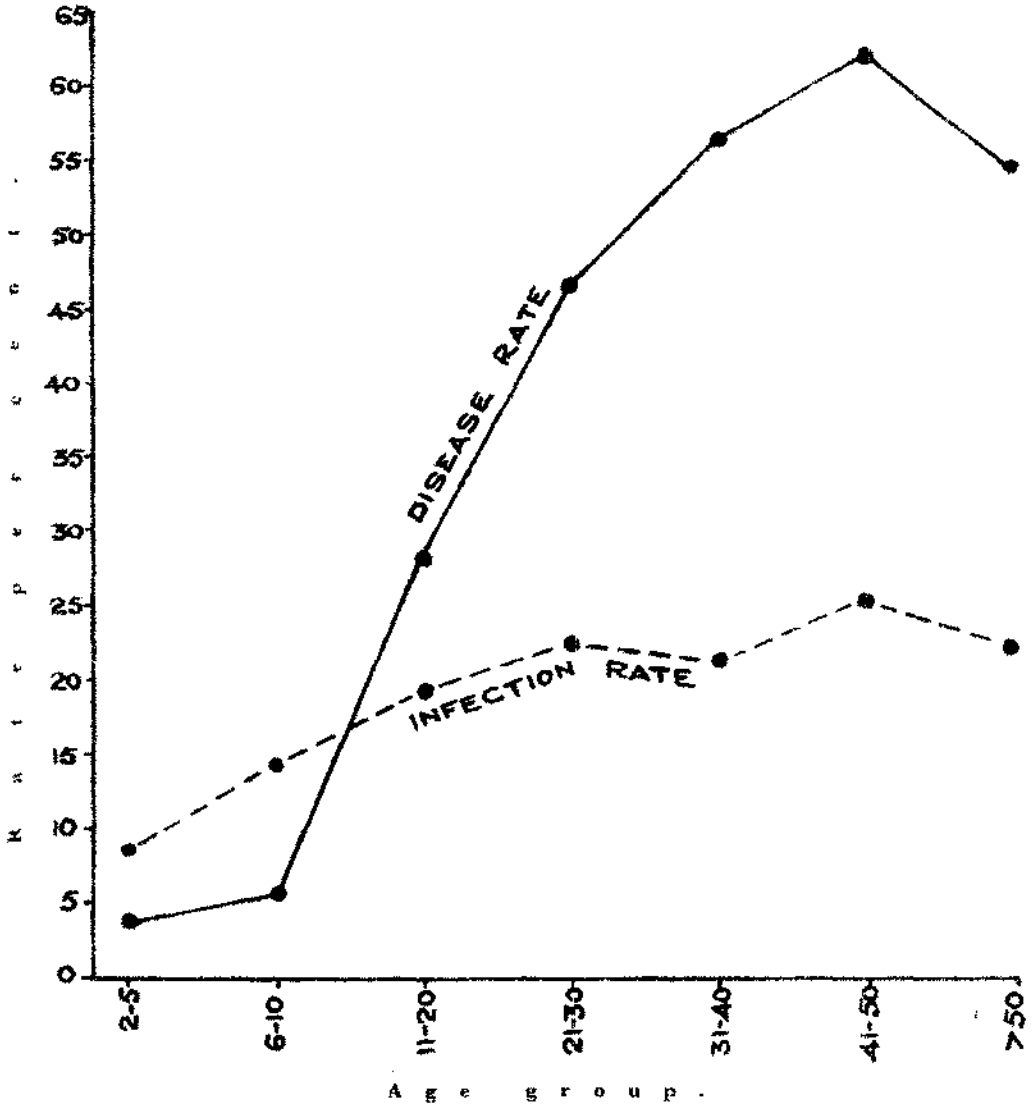
Incidence of filarial diseases manifestations.

Disease manifestations.*	PERCENTAGE INCIDENCE AMONG PERSONS WITH FILARIAL DISEASE.		CASES SHOWING MICROFILARIAE.	
	Males (2,332 cases).	Females (528 cases).	Number	Per cent.
Lower extremities	19·5	50·5	111	15.3
Upper extremities	9·0	30·4	88	23·5
Both extremities	3·0	11·5	23	17·4
Hydrocele	75·9	...	407	22·9
Other genital manifestations ...	1·1	0·3	13	46·4
Filarial fever	7·2	20·8	47	16·8
Chyluria	0·1	...	1	50

*Such individuals as had more than one type of manifestation have been shown in more than one place.

GRAPH 3.

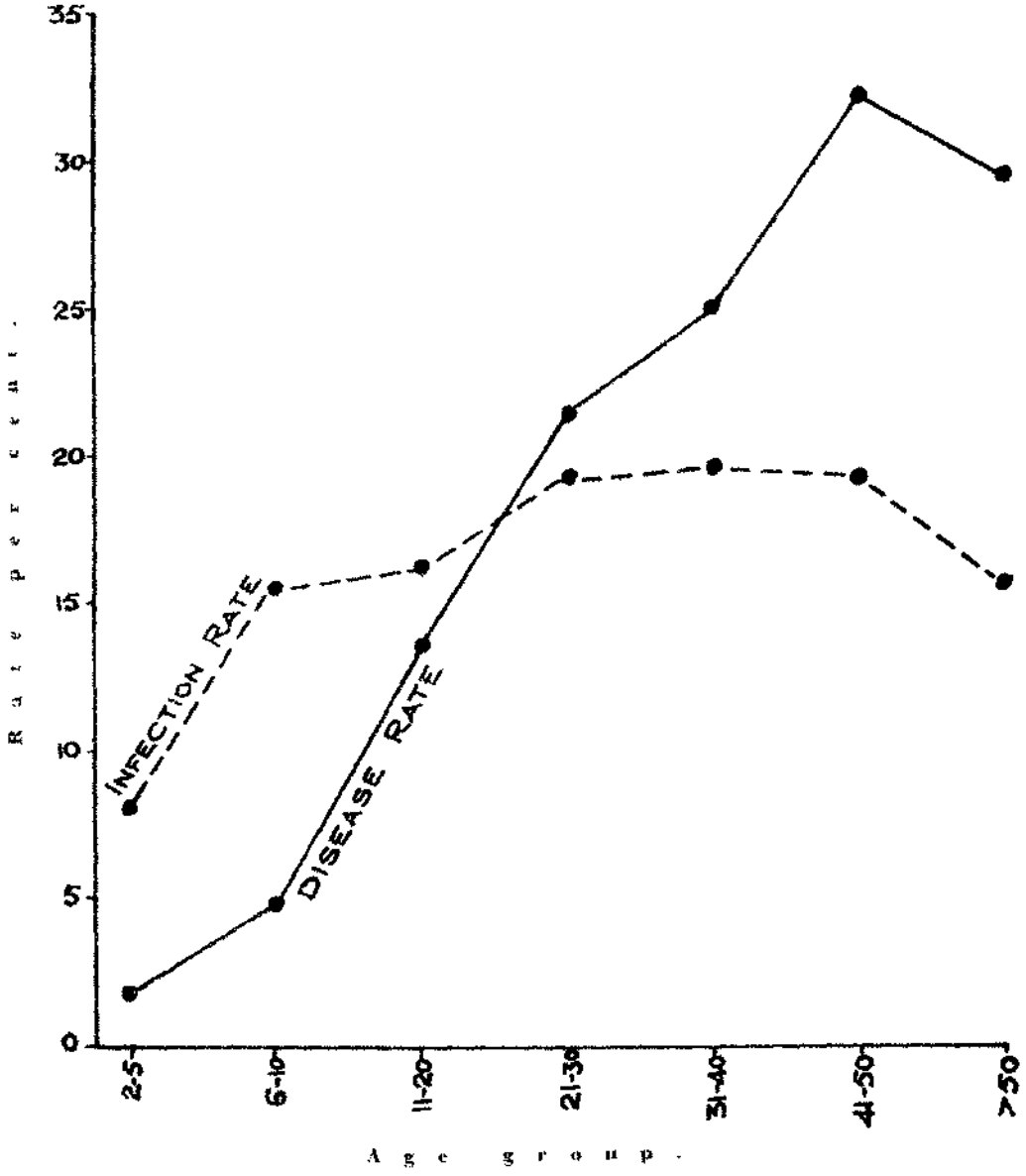
Incidence of filarial disease and infection among males.



The incidence of filarial disease and infection in the different age groups of the two sexes is shown in Table II and in Graphs 3, 4 and 5. The occurrence of external disease manifestation steadily increases up to the age of 50 and shows a slight decline (not statistically significant) above that age. Infection rate increases with age between two and thirty years, and remains more or less steady beyond thirty years.

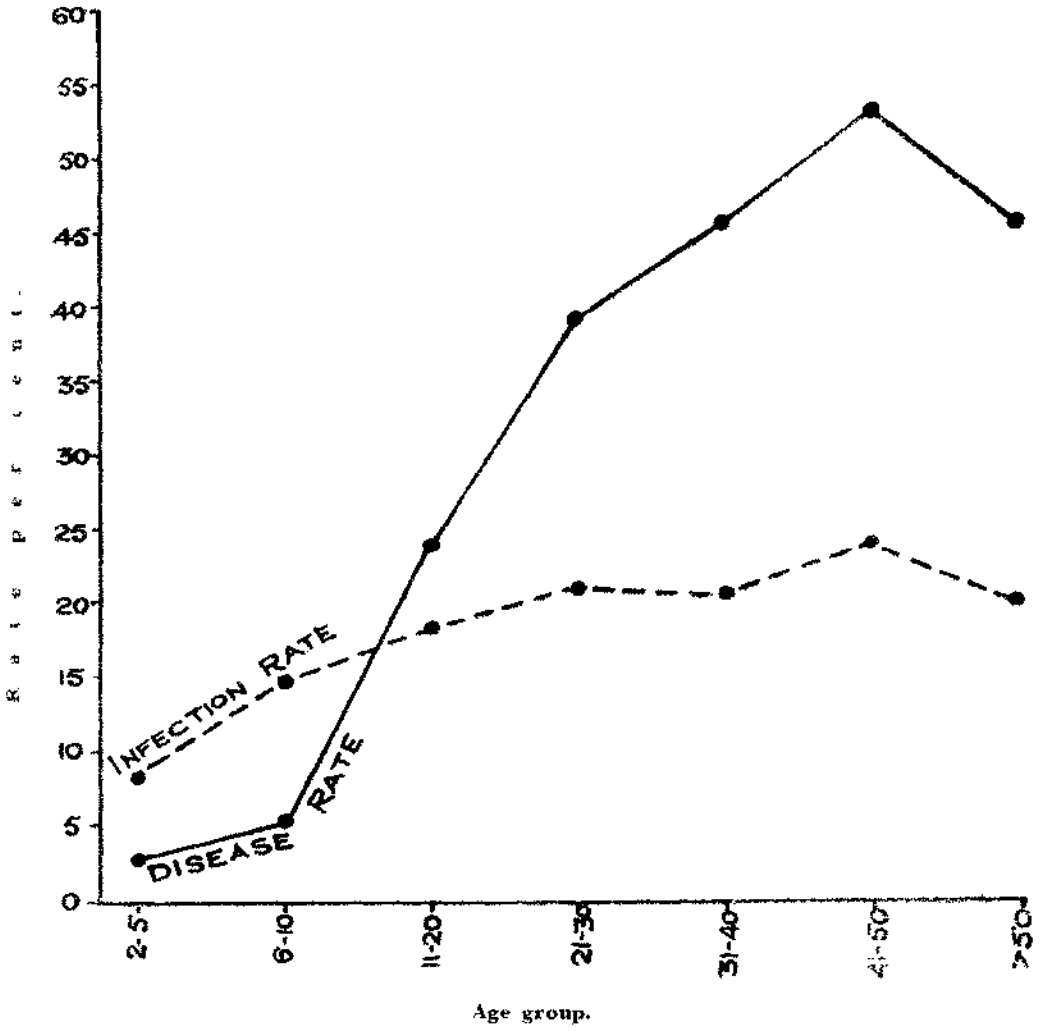
GRAPH 1.

Incidence of plantal disease and infection among females.



GRAPH 5.

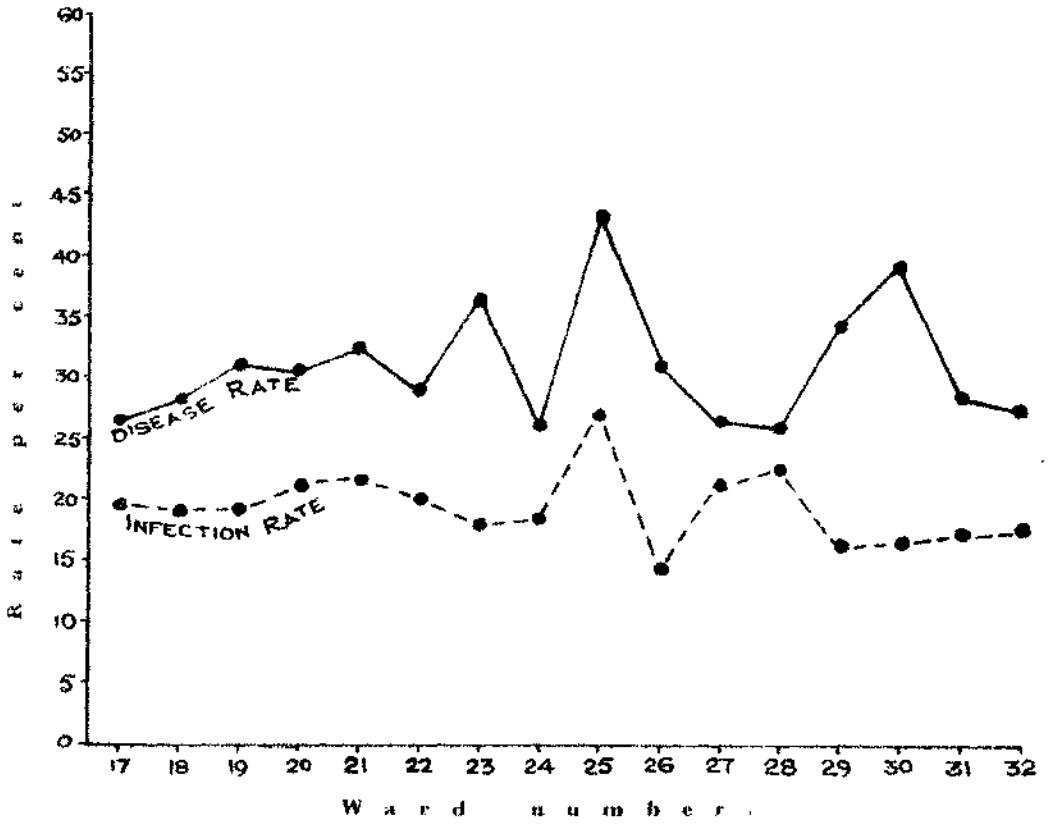
Incidence of filarial disease and infection among both sexes.



The youngest child examined was aged two years. The lowest age at which microfilariæ were detected was two years, and external disease manifestations were not observed below four years. Two male children and one female child of four years of age, showed lower limb swellings of a few months' duration, and one female child of the same age showed upper limb swelling, also of a few months' duration. Hydrocele was observed in a male child aged four years, but definite information, as to whether it was congenital or acquired, could not be elicited.

GRAPH 6.

Filarial disease and infection rates in different wards of Patna City.

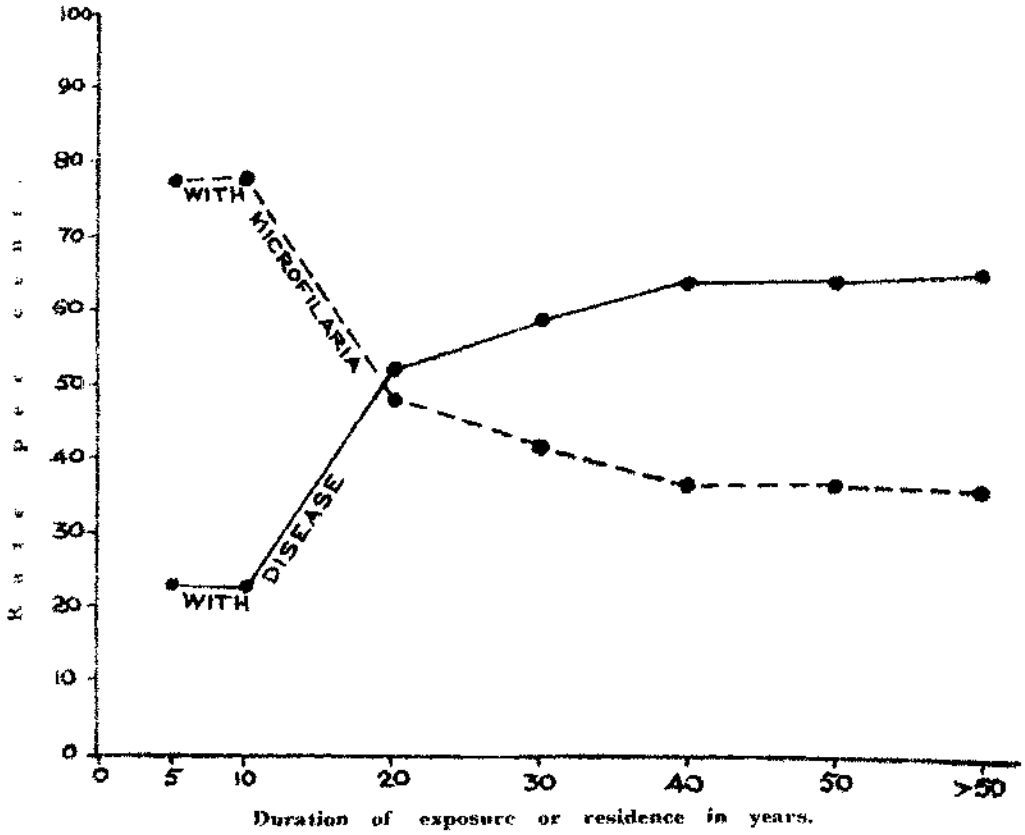


The infection rate in males is somewhat higher (19·8 per cent) than that in females (16·4 per cent). But this is not statistically significant. The difference in disease rate between males (36·7 per cent) and females (16·8 per cent) is statistically significant. But the usual tendency among the local women is to suppress disease manifestations. They are not ready for detailed clinical examination. These may be the reasons of the difference in disease rate between males and females.

The types of disease manifestations recorded during the survey, consisted of swelling of the extremities, upper, lower or both (temporary or permanent), hydrocele, other genital manifestations (including elephantoid scrotum, epidydimio-orchitis, elephantiasis of the vulva etc.), filarial fever, and two cases of chyluria (Table III). In males, the commonest disease manifestation was hydrocele. Next in order of frequency, were lower limb swellings, upper limb swellings, and filarial fever. In females also, this order of frequency prevails, lower limb swellings being the commonest disease manifestation.

GRAPH 7.

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



Occurrence of microfilariæ concomitant with disease manifestation (Table III) was the least among those with swellings of lower extremities (15·3 per cent) and those with filarial fever (16·8 per cent). Infection was present in 23·5 per cent of cases with upper extremity swellings and in 17·4 per cent cases with swellings of both extremities. 22·9 per cent of hydrocele cases, and 46·4 per cent of cases with other genital manifestations, also showed microfilariæ in their blood. Of the two cases of chyluria recorded, only one showed microfilariæ.

The density of microfilarial infestation per 20 c.mm. in the positive persons, was determined by counting all the microfilariæ in the blood smears. The counts ranged from 1 to 777 per smear. The average microfilarial infestation rate, per 20 c.mm. of positive blood, was 32·8 in the community examined (Table I).

Entomological survey.—Adult mosquitoes were collected regularly, at fixed hours in the morning from fixed catching stations, and general collections were made fortnightly, throughout the period of the survey. The catching stations and other shelters from which the collections were made, included human dwellings, mixed dwellings and cattlesheds.

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

C. fatigans,
C. bitæniorhynchus,
C. Vishnui,
Armigeres obturbans,
Aedes (Stegomyia) ægypti,
A. subpictus,
A. vagus,
A. culcifacies,
A. annularis.

The female mosquitoes were dissected soon after collection, on the same day, for determining the vectors of filarial infection in Patna, and developmental stages of microfilariæ were looked for in the abdomen, thorax and head/proboscis. One thousand six hundred and seventyone specimens of different species of anophelines and culicines were dissected and the results are given in Table IV.

TABLE IV.

Mosquito dissection in Patna (November, 1955 to April, 1956).

Mosquito dissection.		Nov. 1955.	Dec. 1955.	Jan. 1956.	Feb. 1956.	Mar. 1956.	Apr. 1956.	Total.
Species.	Other details.							
Culex fatigans	Number dissected	41	258	251	355	380	202	1487
	Number positive	2	32	23	37	37	27	178
	Abdomen	1	22	19	21	10	13	86
	Thorax	1	19	17	35	44	26	142
	Head ...	1	1	1	...	3	2	8
	Infection rate (per cent) ...	4·8	12·4	9·1	10·4	9·7	13·3	11·9
Other culicines	Number dissected	...	...	2	17	2	11	32
	Number positive	...	...	...	...	...	...	...
Anophelines	Number dissected	...	20	93	23	15	1	152
	Number positive	...	...	...	...	...	...	...

Developmental stages of microfilariæ were found only in *C. fatigans.* Out of 1,187 specimens of this species dissected, 178 were found to contain developmental stages of microfilariæ, i.e., an infection rate of 11.9 per cent. Eighty-six showed infection in the abdomen, 142 in thorax and 8 in the head/proboscis.

There were variations from month to month in the infection rate, that for November, 1955 being the lowest (4·8 per cent), and those for the other months deviating from the gross infection rate for all the months (11·9 per cent) by a much lesser extent.

BREEDING PLACES OF INSECT VECTOR.

The following types of water collection were found to be the breeding places of *C. fatigans* :—

Domestic cesspools.—In the majority of houses, there is no planned drainage of waste water from the kitchen, bath, latrine (mostly service latrines), or cowshed, where there is one. Water stagnates in cesspools forming excellent breeding places of *C. fatigans.*

Stagnant pools in drains.—These have already been referred to under the section on drainage. These are perennial breeding places of mosquitoes. Desilting and removal of scum from these innumberable drains, before applying larvicides, is a gigantic problem before the local Filaria Control Unit and the Antimosquito Scheme of Patna. In the southern belt of the city, where all the drainage water collects, there is the added problem of vast stretches of aquatic vegetations like water hyacinth, pistia, lamina, etc.

Tanks, disused wells and ponds.—There is no dearth of these in Patna City and heavy breeding of *C. fatigans* occurs there.

Natural water collections.—Vast sheets of water are formed in the low-lying tracts by storm water with considerable water-logging during the monsoon. These water collections form the most formidable breeding places for months after the monsoon. River pools along the bank of the Ganga also serve as undetected breeding places.

DISCUSSION.

Filariasis is prevalent throughout the Patna City in varying intensities from ward to ward.

Filarial disease rate obtained by Korke (1929) from a sample of 217 persons only, in Patna District was 22 per cent. The disease rate of Patna City, obtained from the present large scale survey, is higher, namely 30·1 per cent. Three thousand six hundred and sixtynine cases of filariasis attended the out-patients and in-patients departments of the two principal hospitals of Patna in 1954. From this it appears that about 1·25 per cent of the population, or about 4 per cent of people with disease manifestations, got themselves treated in the hospitals in 1954. This can be accounted for, to some extent, by the fact that only those persons who had acute manifestations, generally attended the hospitals.

The filarial disease rate is low up to the age of ten years, then rises progressively up to the age of 50 years, and has a slight tendency to fall beyond 50 years, in males as well as in females (Graphs 3, 4 and 5). Krishnaswami (1955) had similar findings in Mangalore.

The common disease manifestations are hydrocele, swellings of the extremities and filarial fever. Out of 108 cases with filarial disease manifestations, examined by Korke (1926:1928:1929), in four different parts of Patna District, 48 had hydroceles (44·4 per cent), 29 showed swellings of lower extremities (26·8 per cent), 9 showed swellings of upper extremities (8·3 per cent) and 46 gave history of filarial fever (42·5 per cent). In the present work, 2,860 persons with filarial disease manifestation were examined, out of which 61·8 per cent had hydroceles, 30 per cent had swellings of lower extremities, 17·6 per cent had swellings of upper extremities and 9·7 per cent gave history of filarial fever. The incidence of different disease manifestations, in the two sexes, is shown in Table III.

The filarial infection rate found by Korke (*loc. cit.*) in Patna District was 18·4 per cent. The infection rate in persons with filarial disease manifestations was 35·4 per cent, and in persons without any disease manifestations, it was 13·6 per cent. In the present work, the infection rate of Patna City is 18.7 per cent of all persons examined, 20 per cent in persons with filarial disease manifestations and 18 per cent in persons without any disease manifestation. The infection rate shows a progressive rise up to the age of 30 years, and beyond that age, it remains more or less constant. In the studies of Krishnaswami (1955) in Mangalore, the progressive rise is only up to 20 years and beyond that it remains more or less constant.

The youngest age at which microfilariæ were found was two years, and the earliest age at which disease manifestations were found was four years. Iyengar (1933) in Trivandrum, found microfilariæ in a child of two years, and external disease manifestations not earlier than eight years. Krishnaswami (1955) also found microfilariæ in a child of two years, but he found external disease manifestations in a child as young as five years.

A high degree of positive correlation between filarial infection rates and disease rates, was observed by Iyengar (1933) in Trivandrum. Similar correlation was observed by Krishnaswami (1955) in Mangalore. The result of the present work agrees with these observations, in most of the wards (Graph 6).

The endemicity rate of Patna City is 42·7 per cent. The variation of endemicity rate from ward to ward does not confirm the observation made by Iyengar (1933), in respect of Trivandrum, that the highest endemicity rates of bancroftian filariasis are to be found in the urbanised centre, and the lowest in the semi-rural or rural periphery of a city. Krishnaswami (1955) also could not confirm Iyengar's observations, in his studies in Mangalore.

An analysis of only those cases having disease and/or microfilariæ in the blood (i.e., excluding such persons as have neither microfilariæ nor disease manifestation), shows that, among these cases, the disease rate increases with age and the microfilaria rate diminishes with age (Graph 7). Iyengar (1933) and Krishnaswami (1955) made similar observations in Trivandrum and Mangalore, respectively, and explained the decline of microfilaria rate as being due to the onset of disease

manifestations. Pandit *et al.* (1929) observed that certain elements, present in the sera of persons with elephantoid swellings, tend to bring about a reduction in the microfilariæ. Krishnaswami (1955) supported this observation, as only 3·4 per cent of his cases with external manifestations of filariasis showed microfilariæ in the blood, whereas 16·2 per cent of those without disease were infected. But in the present series of observations, this view could not be supported, as there was very little difference between the infection rate among persons with disease manifestations (20·0 per cent) and that among persons without disease manifestations (18·0 per cent) (Table V).

TABLE V.

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

Group.	Number examined.	Number showing microfilariæ.	Infection rate per cent.
Persons with filarial disease ...	2,860	587	20·6
Persons without filarial disease ...	6,625	1,193	18·0

SUMMARY.

Observations (from December, 1955 to April, 1956) on the incidence of filariasis in Patna City have been recorded.

The gross disease rate of the city is 30·1 per cent and the microfilaria rate is 18·7 per cent. The filarial endemicity rate is 42·7 per cent.

The average microfilarial infestation per 20 c.mm. of blood is 32·8. The highest density of microfilaria enumerated is 777.

The youngest age at which microfilariæ have been found in the night blood is two years, and the youngest age at which external disease manifestations have been found is four years.

The external disease manifestations observed are swellings of the lower and/or upper extremities, hydrocele, other genital manifestations like elephantoid scrotum, epidydimio-orchitis etc., filarial fever and chyluria.

W. bancrofti is the only species of microfilaria recorded and natural infections are observed only in *C. fatigans*.

The incidence of filariasis has been found to be higher in the more insanitary and socio-economically backward areas.

ACKNOWLEDGEMENTS.

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Meteorological data of 1950-1955 have been obtained from the Director, Regional Meteorological Centre, Calcutta.

The figures of the number of cases of filariasis, recorded in Patna Medical College Hospital and in Patna City Hospital in 1954, have been obtained from the Office of the Civil Surgeon, Patna.

The authors are thankful to the subordinate field staff of the Filaria Control Unit, Patna, for their active and enthusiastic assistance in field work.

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Indian Journal of Malariology, **10**, 3, *September 1956*.

FILARIASIS IN TRAVANCORE-COCHIN STATE.

1. Ernakulam and Mattancherri.*

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[June 5, 1956.]

A FILARIASIS survey of the municipal towns of Ernakulam and Mattancherri was carried out during the period October, 1954 to March, 1956. The observations recorded during the survey are reported here.

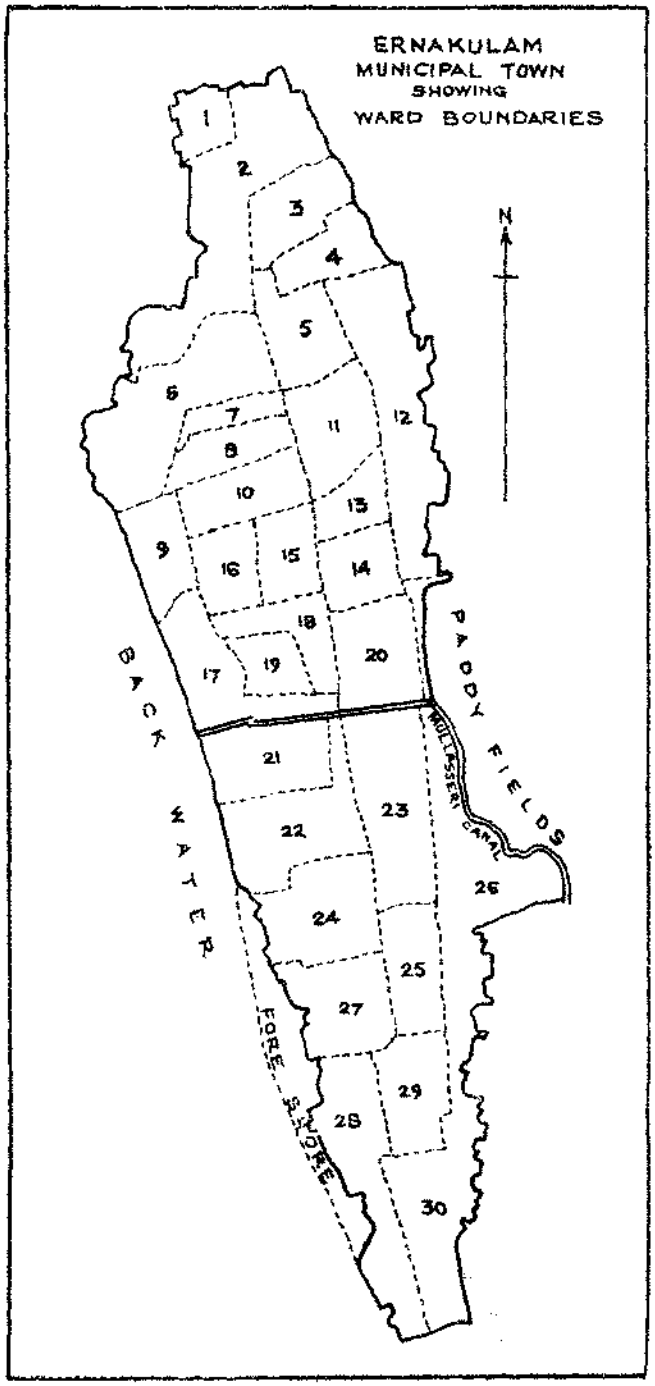
Ernakulam (Map 1), the capital of what was formerly the Cochin State—now merged with Travancore State, is situated on the west coast close to the Arabian Sea. The town is bounded on the west and south by an extensive sheet of backwaters which communicates with the sea by a narrow bar located west of the centre of the town. These backwaters, which extend over about 20 miles northwards up to Cranganore and about 30 miles southwards to form the Vembanad Lake, form one of the important water-ways in the State, both for passenger and goods traffic. There are extensive cultivated fields on the eastern and northern sides of Ernakulam.

The municipal area is about four miles long from north to south ranging from $\frac{1}{4}$ to $1\frac{1}{4}$ miles in width and covers about four sq. miles. The population is about 63,000 according to last census.

Mattancherri (Map 2), also called Cochin by the local people, is another municipal town located to the west of Ernakulam, separated by the backwaters

* The survey was carried out at the request of the Government of Travancore-Cochin State

MAP I.


ERNAKULAM
MUNICIPAL TOWN
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WARD BOUNDARIES
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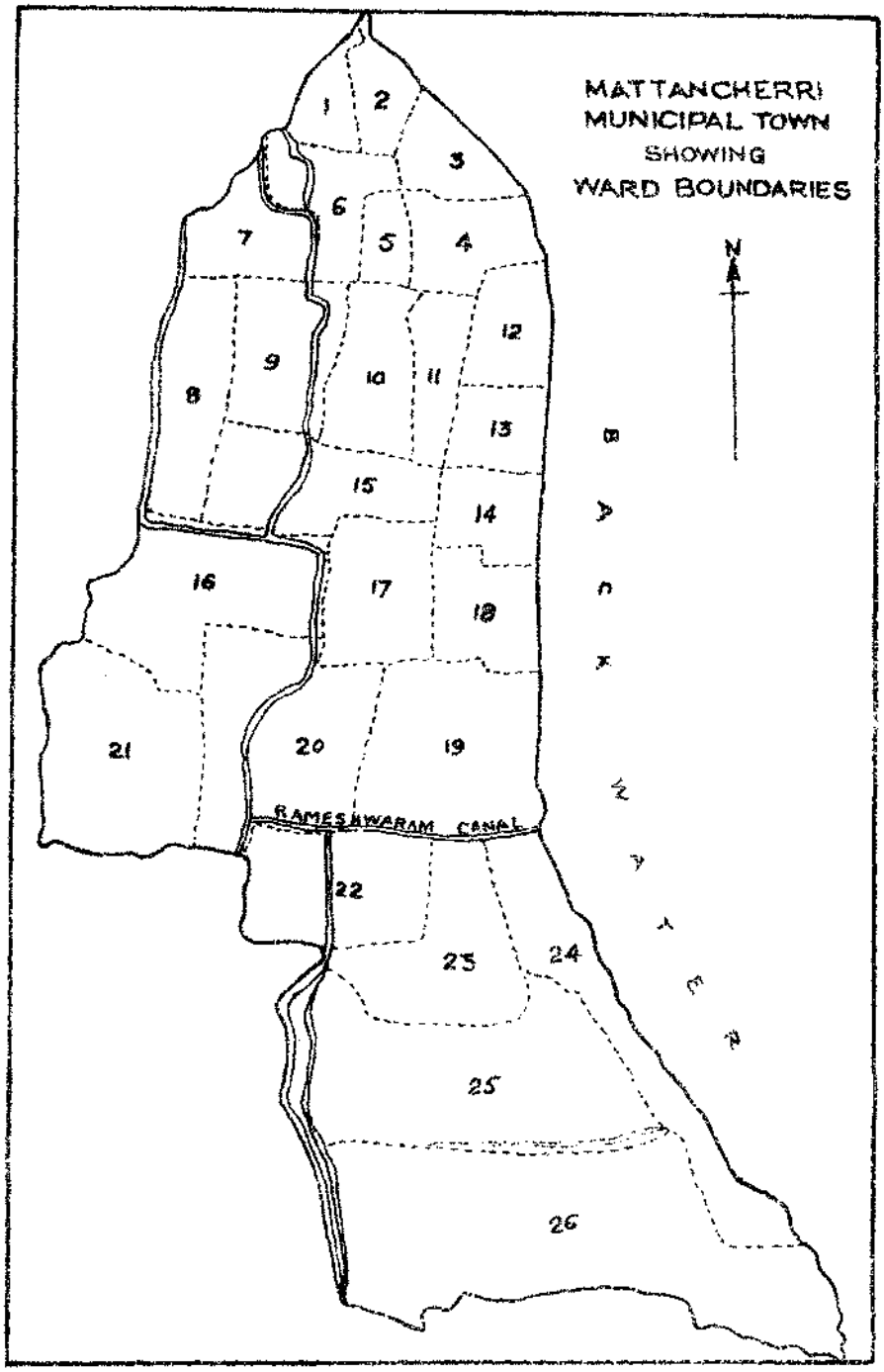
BACK WATER
PADDY FIELDS
MULLASSERY CANAL
FORE SHORE

MAP 2.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RAMESWARAM CANAL
PAMBAN

and the Willingdon Island. This would appear to be the area where Clarke (1709) described the "Cochin leg". This town is separated from the sea by the Fort Cochin Municipality, which belongs to the Madras State. Mattancherri is an important industrial area, and is very thickly populated with an area of just over two sq. miles and a population of 72,000. This town is said to be one of the most crowded areas in the world.

The health conditions in the two towns assume a strategic importance as the defence personnel of the Indian Navy are stationed on the Willingdon Island located on the backwaters. The distance across the backwaters is not more than three to four furlongs.

WATER SUPPLY AND DRAINAGE.

Protected water supply is brought to Ernakulam from the Alwaye River, about ten miles to the north. The supply, as far as this town is concerned, is adequate. The same supply is extended to Mattancherri but is inadequate to meet the local demands there. People of Mattancherri depend mainly on the wells and ponds for their domestic water supply.

There is no underground drainage system in either Mattancherri or Ernakulam. Surface drains exist in some areas, but even these are poorly maintained. Kutcha marginal drains, which are badly clogged and stagnant, are not uncommon even on the main roads of these towns.

HISTORY OF FILARIASIS.

No filariasis survey has been carried out earlier in either of these towns. Mattancherri, however, has been notorious for its filarial endemicity. Cruickshank and Wright (1914) surveyed the area of British Cochin, now called Fort Cochin, a small municipal town belonging to Madras State and adjoining Mattancherri. They recorded a filarial infection rate of 20·9 per cent and an elephantiasis rate of 12·9 per cent among the population.

There is a prevailing impression among the citizens of both Mattancherri and Ernakulam that elephantiasis is a water-borne disease. This is obviously based on their observation that the incidence of the disease is on the decline after introduction of protected water supply in Ernakulam, as compared with Mattancherri, where, as mentioned earlier, the water supply is not as satisfactory.

PRESENT WORK.

A detailed filariasis survey was carried out among the population of the two towns. Night surveys and entomological investigations were carried out with a view to elicit details regarding the epidemiology of filariasis. Random respresentative samples of the population were examined by a house-to-house visit between 8 p.m. and 12 midnight, care being taken to include all age groups of both sexes from different social strata.

Persons were examined from all the different wards of the two towns, and the objective was to cover a minimum of ten per cent of the population.

The procedure in the field regarding the collection of data, obtaining and examination of the blood smears, was as described by Krishnaswami (1955). The smears, after dehæmoglobinisation, were stained the following day with J.S.B. I (Jaswant Singh and Bhattacharji, 1944) for five minutes.

The observations recorded in Ernakulam and Mattancherri are presented separately below:

ERNAKULAM.

Seven thousand three hundred and twenty-eight persons, representing 11·6 per cent of the population, were examined during the period October, 1954 to January, 1955. The details of the findings in the different wards of Ernakulam are recorded in Table I. External manifestations of filarial disease were met with

TABLE I.

Results of filariasis survey of Ernakulam.

(October, 1954 to January, 1955)

Ward number.	Number of persons examined.	Number with disease.	Number with micro-filaria.	Disease rate per cent.	Micro-filaria rate per cent.	Endemicity rate per cent.	Remarks.
1	253	6	6	2·4	2·4	4·3	
2	406	19	36	4·7	8·9	13·3	
3	280	13	21	4·6	7·5	12·1	
4	209	5	10	2·4	4·8	7·2	
5	227	9	16	4·0	7·5	11·5	
6	209	5	9	2·4	4·3	6·7	
7	219	6	16	2·8	7·3	9·6	
8	253	7	20	2·8	7·9	10·7	
9	237	4	18	1·7	7·6	9·3	
10	263	2	24	0·8	9·1	9·9	
11	258	3	21	1·2	8·1	9·3	
12	491	15	17	7·9	8·9	16·8	
13	225	11	17	4·9	7·6	12·5	
14	254	4	23	1·6	9·1	10·2	
15	226	9	22	4·0	9·9	13·9	
16	286	15	19	5·3	6·7	12·0	
17	140	15	16	10·7	11·4	20·0	
18	230	4	29	1·7	12·1	13·4	
19	183	4	19	2·2	10·4	12·6	
20	224	12	21	5·4	9·4	14·3	
21	366	3	23	0·8	6·3	7·1	
22	196	...	20	...	10·2	10·2	
23	140	9	9	6·4	6·4	12·8	
24	95	1	14	1·1	14·7	15·8	
25	202	4	11	2·0	5·4	7·4	
26	131	7	9	5·3	6·9	12·2	
27	220	10	11	4·5	6·4	10·9	
28	469	15	18	3·2	3·9	7·1	
29	286	22	35	7·7	12·2	19·9	
30	141	20	26	4·6	5·9	10·3	
Total ...	7,328	259	558	3·5	7·6	10·6	

among 259 persons while 558 individuals showed overt filarial infection in the peripheral blood; the disease rate in the community was thus 3·5 per cent while the infection rate was 7·6 per cent. The endemicity rate for the town was 10·6 per cent.

TABLE II.

Species of filarial infections at Ernakulam Surveyed during 1954-55.

Age group (years).	Number of persons.				Average infestation rate (Per cent).
	With *W. bancrofti.*	With *W. malayi.*	With mixed infection.	Total.	
1 - 5	18	1	...	19	14·9
6 - 10	45	9	1	55	17·0
11 - 20	168	5	2	175	29·5
Total ...	231	15	3	249	25·6
21 - 30	90	4	3	97	15·8
31 - 40	81	8	2	91	13·4
41 - 50	55	7	...	62	15·9
Above 50 ...	54	4	1	59	10·7
Total ...	280	23	6	309	15·3
Grand Total ...	511	38	9	558	19·9

The incidence of filariasis varied in different wards of the town. The highest disease rate of 10·7 per cent was recorded from Ward 17, while there was not a single person with filarial manifestation among 196 examined in Ward 22. The infection rates in the wards ranged from 14·7 per cent in Ward 24 to 2·4 per cent in Ward 1. The indices of filarial infection and disease in the different parts of the town are shown in Map 3.

Both *W. bancrofti* and *W. malayi* infections were encountered during the survey of Ernakulam. Of the 558 persons who showed microfilariæ in their night-blood smears, 511 (91·6 per cent) were of *W. bancrofti*, while only 9 (1·6 per cent) showed *malayi* infections; the remaining 38 (6·8 per cent) harboured mixed infection of both the Wuchererian infections. All the infections of *W. malayi* were recorded from wards in the peripheral part of the town, where semi-urban or rural conditions prevailed, and factors favourable for the breeding of *Mansonioides sp.* were present.

The filarial disease rate of the town was 3·5 per cent. The commonest clinical manifestations noticed were swellings of the legs and hands and lymphangitis of the groin and axillary glands; a few cases of genital lesions were also encountered. (Table X).

MAP 3.

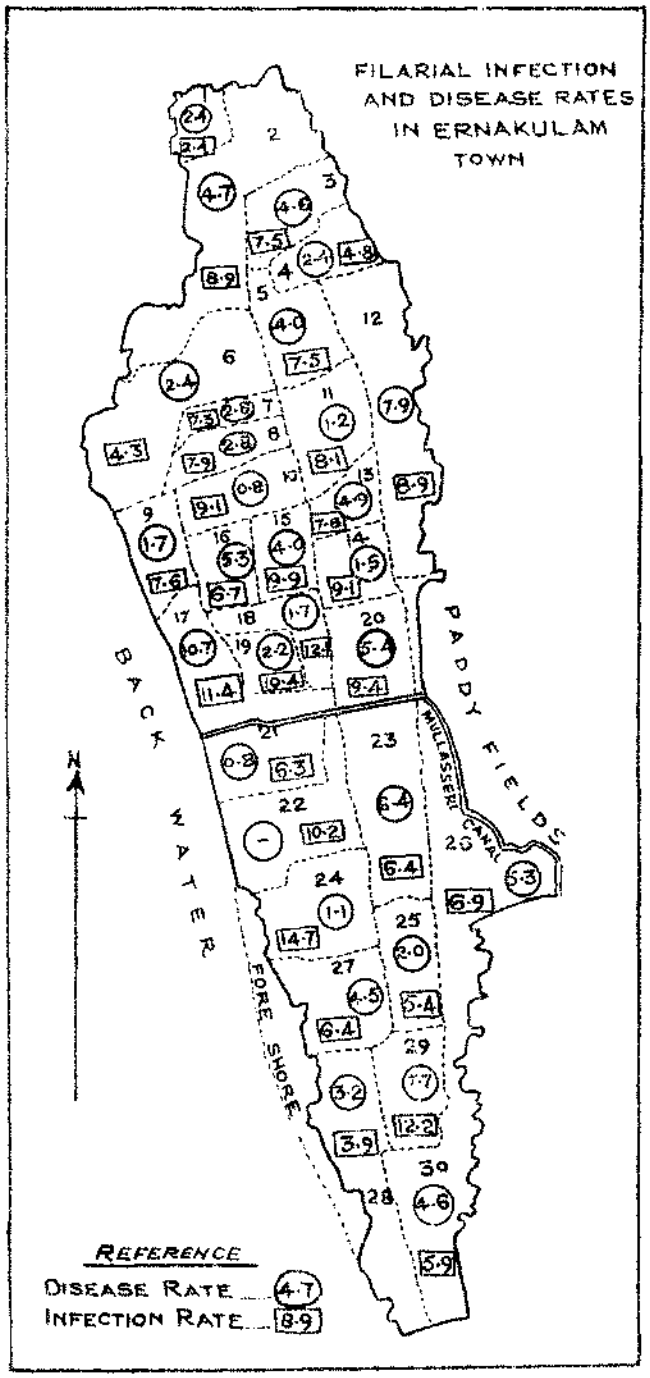

FILARIAL INFECTION AND DISEASE RATES IN ERNAKULAM TOWN
BACK WATER
FORE SHORE
PADDY FIELDS
MULLASSERI CANAL
N
REFERENCE
DISEASE RATE
INFECTION RATE

TABLE III.

Filarial disease and infection rate at Ernakulam.

Age group (Years).	Number examined.	Disease.		Infection.	
		Number.	Rate per cent.	Number.	Rate per cent.
1 - 5	617	1	0·15	19	3·1
6 - 10	689	1	0·14	49	7·1
11 - 20	2,034	30	1·47	172	8·4
21 - 30	1,627	52	3·19	97	5·9
31 - 40	1,068	47	4·4	87	8·1
41 - 50	715	55	7·7	61	8·5
Above 50 ...	557	68	12·2	56	10·1
Total ...	7,307	254	3·47	541	7·4

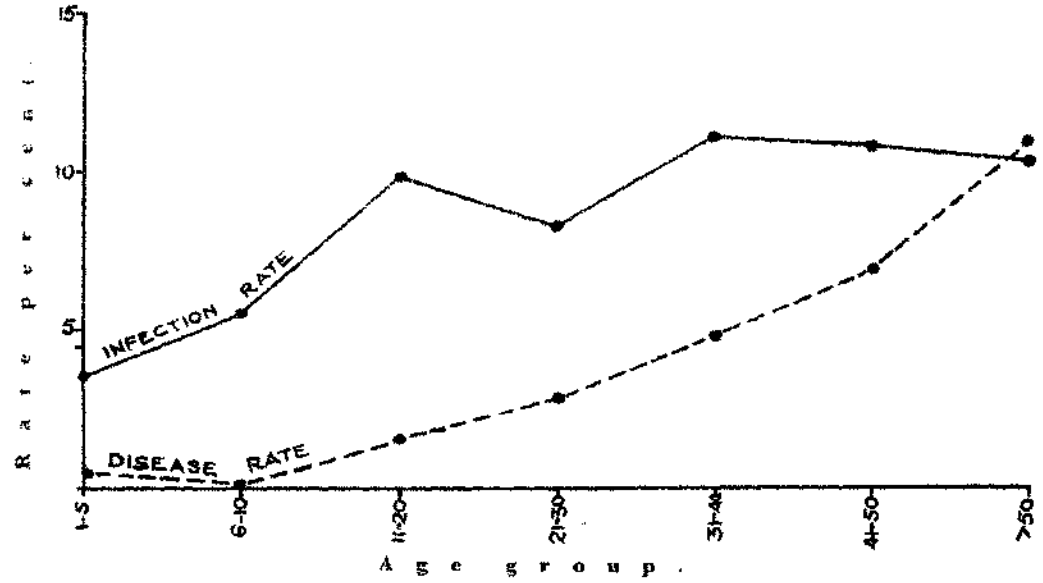
TABLE IV.

Analysis of disease infection rates in Ernakulam surveyed during the year 1954-55.

Age group (Years).	Males.						Females					
	Number examined.	Number with disease.	Number with microfilariæ.	Disease rate per cent.	Microfilariæ rate per cent.	Number with both.	Number examined.	Number with disease.	Number with microfilariæ.	Disease rate per cent.	Microfilariæ rate per cent.	Number with both.
1 - 5	320	1	10	0·3	3·1	*Nil*	291	*Nil*	9	*Nil*	3·1	*Nil*
6 - 10	396	*Nil*	22	...	5·5	*Nil*	293	1	27	3·4	9·2	*Nil*
11 - 20	1,049	16	103	1·5	9·8	1	985	14	69	1·4	7·0	1
Total	1,771	17	135	0·9	7·6	1	1,569	15	105	0·9	7·6	1
21 - 30	874	26	73	2·9	8·3	1	753	26	24	3·5	4·2	*Nil*
31 - 40	564	27	65	4·8	11·5	*Nil*	504	20	22	4·0	4·4	*Nil*
41 - 50	381	26	43	6·8	11·3	*Nil*	344	29	18	8·7	5·4	*Nil*
51 and above	311	44	44	10·9	10·9	4	246	24	12	9·8	4·9	*Nil*
Total	2,130	123	225	5·8	10·6	5	1,837	99	76	5·4	4.1	*Nil*
Grand Total (including all ages)	3,901	140	360	3·6	9·2	6	3,406	114	181	3·3	5·3	1

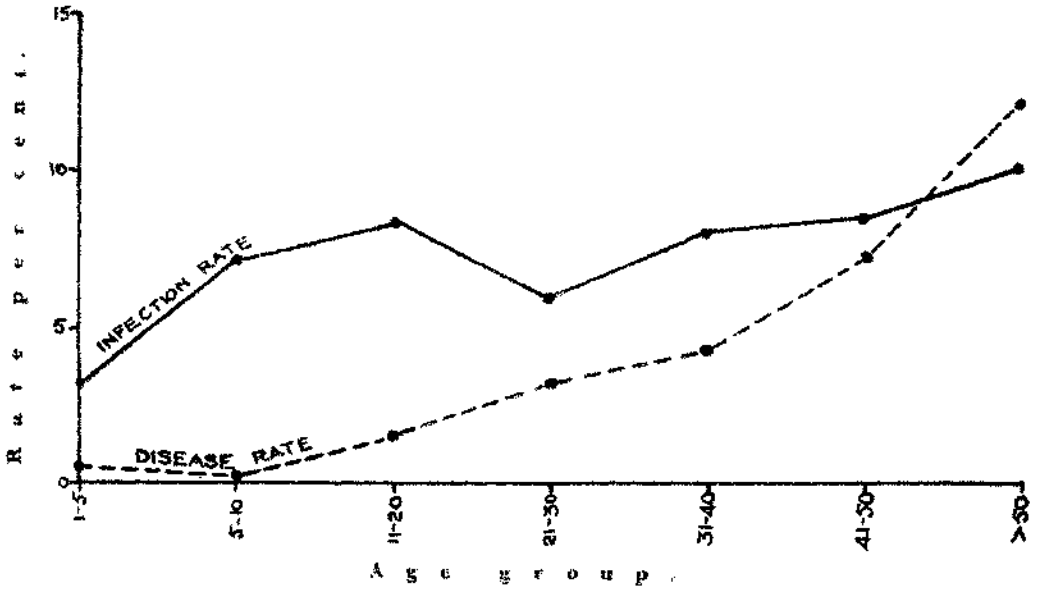
GRAPH 1.

Filarial infection and disease rates among both sexes in Ernakulam.



GRAPH 2.

Filarial infection and disease rates among males in Ernakulam.



GRAPH 3.

Filarial infection and disease rates among females in Ernakulam

GRAPH 4.

Average microfilarial infestation rates in Ernakulam.

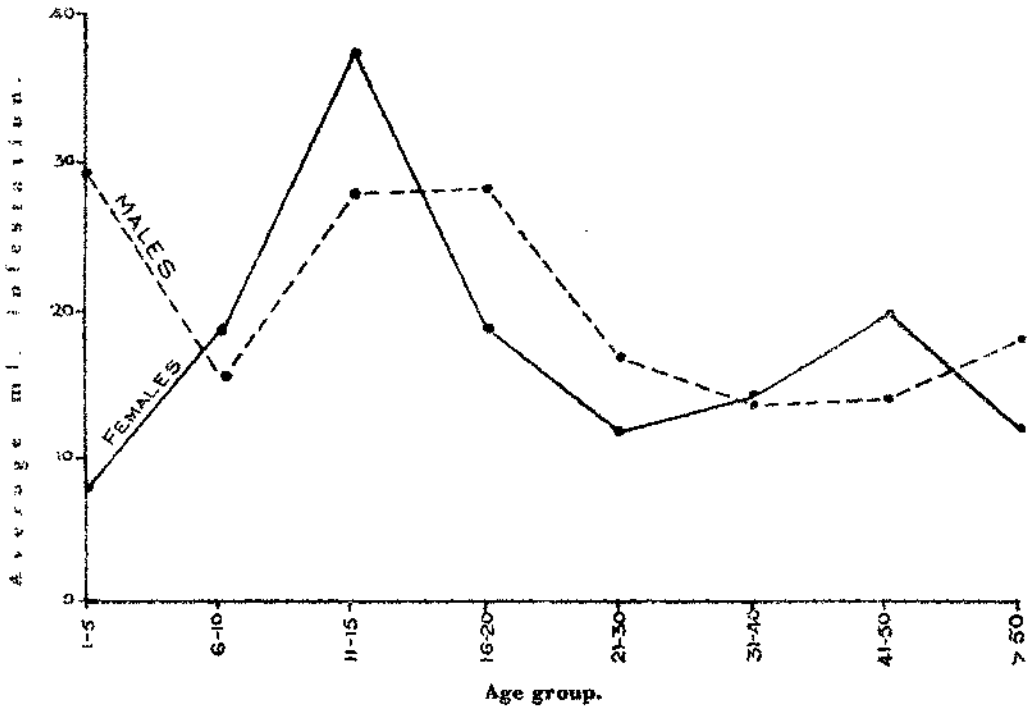


TABLE V.

Average infection rate in the two sexes in different age groups amongst the persons surveyed in Ernakulam.

Age group (years).	MALES.		FEMALES.		BOTH SEXES.	
	Number of positive persons.	Average infestation (Microfilariæ per 20 c.mm.)	Number of positive persons.	Average infestation (Microfilariæ per 20 c.mm.)	Number of positive persons.	Average infestation (Microfilariæ per 20 c.mm.)
1 – 5	6	29·3	14	7·7	20	14·2
6 – 10	27	15·1	29	18·2	56	16·7
11 – 15	45	28·8	39	37·1	84	32·6
16 – 20	63	29·0	32	18·5	95	25·5
21 – 30	77	16·4	24	11·3	101	15·2
31 – 40	68	13·3	23	13·7	91	13·3
41 – 50	44	13·6	20	19·5	64	15·4
Above 50	46	17·9	14	11·6	60	16·4
Total ...	376	19·4	195	19·0	571	19·3

The disease and infection rates in the different age groups are represented in Tables III and IV and Graphs 1, 2 and 3. The infection rate was observed to be low in the early age groups and rose up to 20 years; it remained more or less static in the higher age groups. The incidence of the filarial disease, on the other hand, showed a progressive rise with advancing age.

The microfilarial infestation rate was 19·3 per 20 c.mm. This was analysed according to age groups in the two sexes (Table V and Graph 4). No significant variation was observed in the microfilarial infestation in the two sexes.

MATTANCHERRI.

The survey of Mattancherri was commenced in January and was completed in March, 1955. Public cooperation from the citizens was poor, and only 4,017 persons representing 6·9 per cent of the population could be examined during the survey. Observations recorded in the different areas of the town are presented in Table VI. Both filarial infection and disease were prevalent in all the different areas of the town covered by the sample survey, though their intensity varied. The filarial disease rate ranged from 1·6 to 13·5 (average 7·6) per cent while the infection rate varied from 10·2 to 23·9 (average 14·7) per cent in the different wards. The overall filarial endemicity rate for the town was 21·7 per cent. The distribution of filariasis in the 26 wards of the town is shown in Map 4.

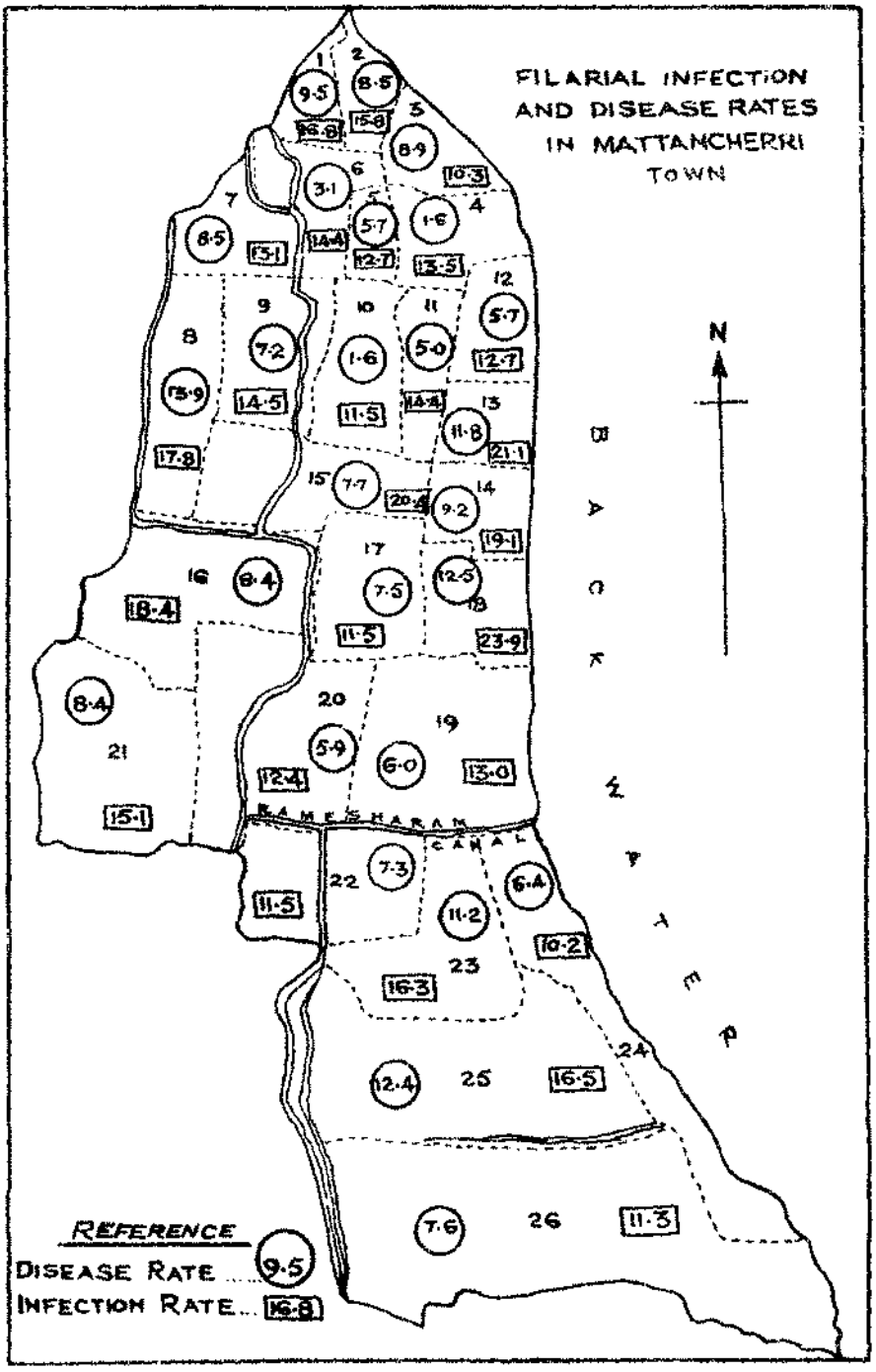
TABLE VI.

Filariasis survey of Mattancheri.

(February and March, 1955).

Ward number.	Number of persons examined.	Number of persons with disease.	Number of persons with microfilariae.	Filarial disease rate per cent.	Filarial infection rate per cent	Endemicity rate per cent.
1.	285	27	48	9·5	16·8	26·3
2.	247	21	39	8·5	15·8	24·3
3.	146	13	15	8·9	10·3	19·2
4.	126	2	17	1·6	13·5	15·1
5.	228	13	29	5·7	12·7	18·4
6.	257	8	37	3·1	14·4	17·5
7.	130	11	17	8·5	13·1	22·3
8.	101	14	18	13·9	17·8	28·2
9.	152	11	22	7·2	14·5	21·7
10.	252	4	29	1·6	11·5	13·1
11.	169	8	23	5	14·4	18·8
12.	228	13	29	5·7	12·7	18·4
13.	76	9	16	11·8	21·1	32·9
14.	173	16	33	9·2	19·1	28·3
15.	207	16	42	7·7	20·4	28·0
16.	131	11	24	8·4	18·4	26·8
17.	174	13	20	7·5	11·5	18·4
18.	176	22	42	12·5	23·9	34·7
19.	215	13	28	6·0	13·0	19·0
20.	202	12	25	5·9	12·4	18·3
21.	179	15	27	8·4	15·1	23·5
22.	192	14	22	7·3	11·5	18·8
23.	276	31	45	11·2	16·3	27·5
24.	187	12	19	6·4	10·2	16·6
25.	242	30	46	12·4	16·5	27·7
26.	275	21	31	7·6	11·3	18·9
Total	5,017	380	737	7·6	14·7	21·7

MAP 4.


FILARIAL INFECTION
AND DISEASE RATES
IN MATTANCHERRI
TOWN
N
BAZAAR STREET
RAMESHARAM CANAL
REFERENCE
DISEASE RATE ..... 9·5
INFECTION RATE.. 16·8

The infection and disease rates in the community, analysed according to the age groups, are presented in Table VII and Graph 5. The former shows a sharp rise up to ten years of age, reaching 15 per cent, and remains almost static at that level thereafter, while the disease rate shows a gradual steady rise with advancing age. Neither of these indices show any significant variation in the two sexes, though both appear to be slightly higher in the male among the sample of population examined (Table VIII, Graphs 6 and 7).

Both *W. bancrofti* and *W. malayi* infections were encountered among the individuals examined from Mattancherri. Of the 737 persons who showed microfilariæ in their night blood, 666 (90·3 per cent) were of *W. bancrofti;* 56 7·6 per cent) were *W. malayi* and 15 (2·1 per cent) were mixed infections. *W. malayi* was encountered in the peripheral wards, especially towards the southern end of the town.

The disease manifestations of filariasis, recorded during the survey of Mattancherri, are presented in Table X. Incidence of lymphadenitis, genital lesions and combined lesion of leg and hand, were comparatively higher than in Ernakulam.

The average microfilarial infestation in the town was 24·9. The microfilarial load at the different age groups and in the two sexes are analysed and presented in Table IX and Graph 8.

ENTOMOLOGICAL OBSERVATIONS.

Routine collections from the different parts of both the towns were made during the survey, and adult mosquitoes of the following species were recorded:—

Culex fatigans,
Culex vishnui,
*Ficalbia chamberlaini,**
Aedes albopictus,
Aedes aegypti,
Armegeres obturbans,
Mansonioides annulifera,
Mansonioides uniformis,
Mansonioides indiana,
Anopheles hyrcanus,
Anopheles jamesi,
Anopheles subpictus,
Anopheles vagus and
*Anopheles jeyporiensis.**

Drains either stagnant or sluggish, cesspools and odd collections of dirty water were the main sources of breeding of *C. fatigans.* A number of ponds, covered with *Pistia stratiotes,* were present especially in the peripheral wards of the town and in some of them breeding of *Mansonioides sp.* was detected.

Dissection of the mosquitoes revealed presence of developing filarial larvæ only in *Culex fatigans, Mansonioides uniformis* and *M. annulifera.* The details are presented in Table XI.

The favourite breeding places of *Culex fatigans,* the vector of the more prevailing infection, *W. bancrofti,* in these areas, are the dirty water collections like domestic cesspools, drains, and ponds. As mentioned earlier, drainage conditions being very poor, culicine breeding is profuse in spite of the antilarval measures instituted by the municipalities.

* Recorded later by the Travancore-Cochin Branch of the Malaria Institute of India.

TABLE VII.

Filarial infection and disease rates among different age groups in Mattancherri.

Age group (years).	Total number of persons examined.	Number with disease.	Number with microfilariæ.	Disease rate per cent.	Microfilaria rate per cent.	Number with both.
1 – 5	239	1	8	0·4	3·3	*Nil*
6 – 10	466	5	68	1·1	14·6	*Nil*
11 – 20	1,382	67	215	4·8	15·5	2
21 – 30	1,302	110	184	8·4	14·13	3
31 – 40	828	116	125	14·0	15·0	3
41 – 50	462	86	72	18·6	15·6	5
Above 50	326	75	51	23·0	15·6	1
Grand total	5,005	460	723	9·2	14·4	14

GRAPH 5.

Filaria infection and disease rates among both sexes in Mattancherri

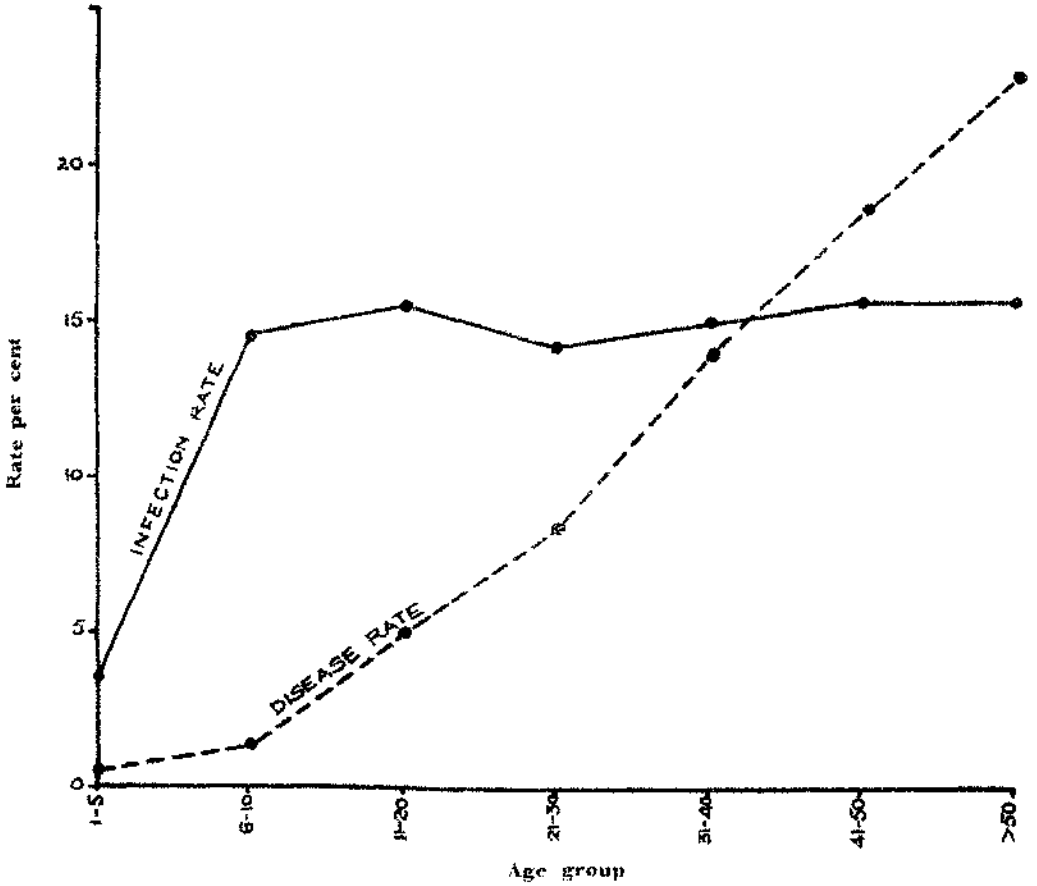


TABLE VIII.

Filarial infection and disease rates among males and females at Mattancherri.

Age group (Years)	Males. Total number of persons examined.	Number examined.	Number with disease.	Number with microfilariæ.	Number with both.	Disease rate per cent.	Microfilaria rate per cent.	Females. Number examined.	Number with disease.	Number with microfilariæ.	Number with both.	Disease rate per cent.	Microfilaria rate per cent.
1 - 5	239	131	1	3	*Nil*	8·0	2.3	108	...	5	...	...	4·6
6 - 10	466	275	4	42	...	1·5	14.3	191	1	27	...	0.52	14·5
11 - 20	1,328	950	42	151	1	4·4	15.9	432	25	64	1	5.8	14·8
21 - 30	1,302	892	84	141	3	9·4	15.8	410	26	43	...	6.3	10·5
31 - 40	828	483	78	81	3	16·1	16.8	345	38	44	...	11	12·7
41 - 50	462	266	55	42	4	20·7	15.8	196	31	30	1	15.8	15·3
Above 50	326	184	43	31	1	23·3	16·8	142	32	20	...	22·5	14·0
Total	2,918	1,825	260	295	11	14·3	16·2	1,093	127	137	1	11·6	12·4

TABLE IX.

Average infestation rate among males and females by different age groups.

Age group (Years).	Male. Total number of positive persons.	Average infestation (Microfilaria per 20 c.mm.)	Female. Total number positive.	Average infestation (Microfilaria per 20 c.mm.)	Both sexes. Number examined.	Average infestation (Microfilaria per 20 c.mm.)
1 – 5	3	6·3	5	2·4	8	3·9
6 – 10	42	15·3	27	22·3	69	18·0
11 – 15	70	19·4	29	27·7	99	21·8
16 – 20	84	30·8	38	24·4	122	28·8
21 – 30	145	27·1	44	22·5	189	26·0
31 – 40	80	26·5	45	30·7	125	28·0
41 – 50	41	26·8	30	24·6	71	25·9
Above 50	32	23·7	21	18·7	53	21·7
Total	497	25·2	239	24·5	736	24·9

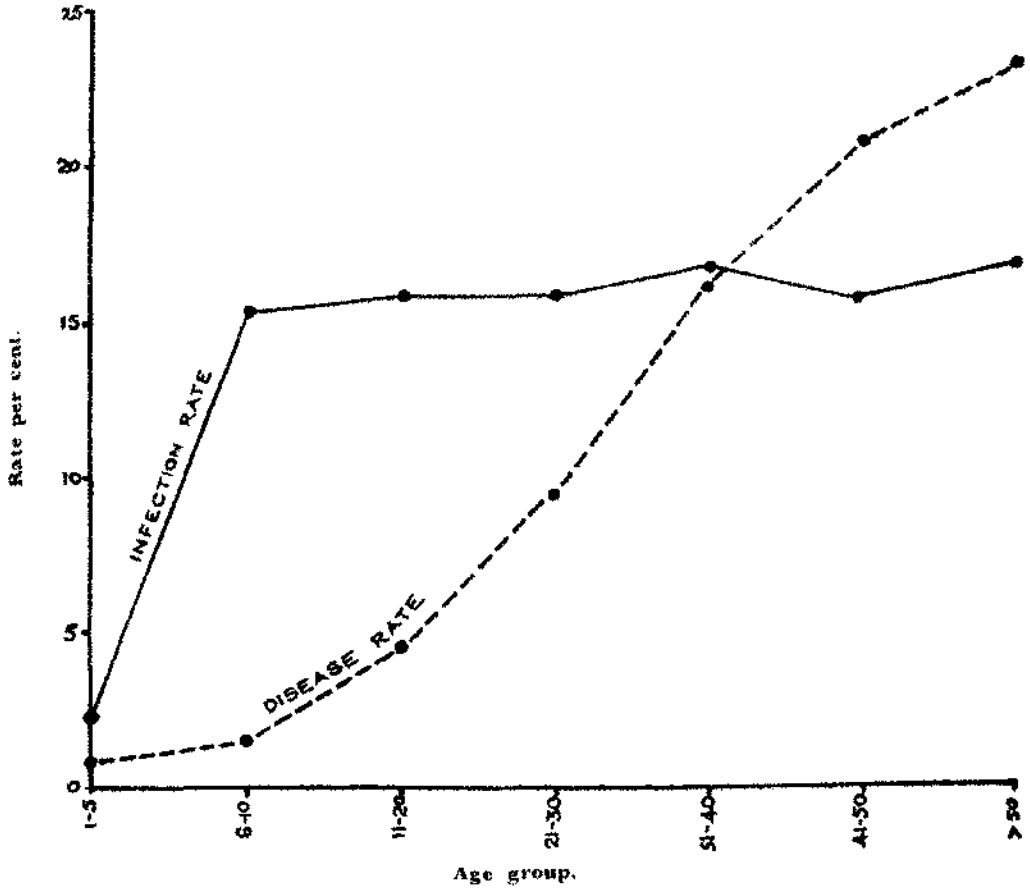
TABLE X.

Analysis of filarial disease manifestations met with at Ernakulam and Mattancherri.

Area.	Number of persons examined.	Number of persons with disease.	Lymphadenitis.	Type of manifestation.				
				Leg only.	Hand only.	Leg and hand.	Genital.	Others.
Ernakulam ...	7,328	245	16	200	24	3	2	...
Mattancherri ...	5,030	586	199	274	18	30	65	...

GRAPH 5.

Filarial infection and disease rates among males in Mattancherri.



GRAPH 7.

Filarial infection and disease rates among females in Mattancherri

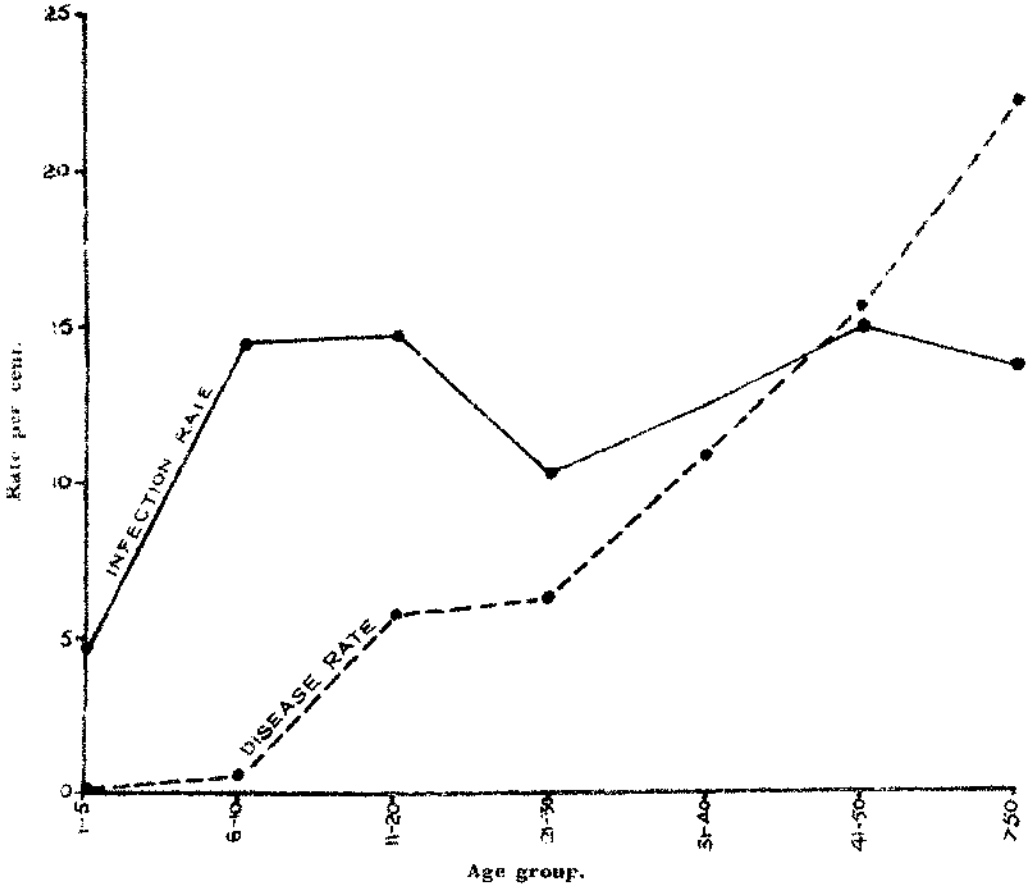


TABLE XI.

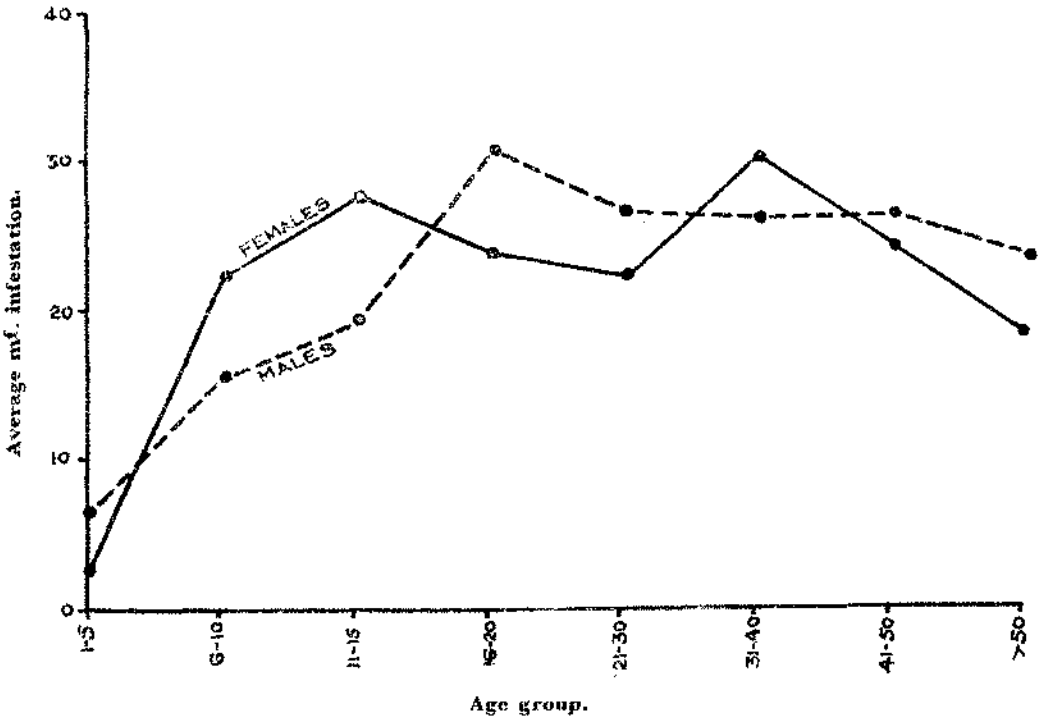
Details of dissections at Ernakulam and Mattancherri.

Area.	Species of mosquito.	Number dissected.	*Number positive.	Infection rate per cent.
Ernakulam ...	*C. fatigans*	1,654	50	3·0
	M. uniformis	350	3	0·9
	M. annulifera	99	1	1·0
Mattancherri ...	*C. fatigans*	1,249	68	5·5
	M. annulifera	56	1	1·8

*All stages of infection.

GRAPH 8.

Average microfilarial infestation rates in Mattancherri.



A large number of ponds in the peripheral parts of these two towns, and especially in Mattancherri, are overgrown with *Pistia stratiotes* which are the sanctuaries of *Mansonioides sp.* breeding. With the advancing urbanised conditions, this problem appears to be gradually on the decline.

SUMMARY.

The findings recorded during a filaria survey of the two towns of Ernakulam and Mattancherri in Travancore-Cochin State, have been recorded.

Filariasis was found to be endemic in both the towns, the endemicity rates for the two towns being 10·6 and 21·7 per cent respectively.

Both the Wuchererian infections were recorded, the incidence of *W. bancrofti* being higher in the areas surveyed.

The developing forms of filarial larvæ were found in *C. fatigans, Mansonioides uniformis* and *M. annulifera.*

ACKNOWLEDGEMENTS.

The authors gratefully acknowledge the help given by Shrimati P. Janaki Amma and Shri M. Kuttykrishna Menon, Chairmen of the Ernakulam and Mattancherri Municipalities, the Commissioners, Health Officers and Councillors of the two towns in the conduct of the surveys. The facilities provided by the Director of Public Health, Travancore-Cochin, and the assistance rendered by Dr. V. Madhavan Pillai, Assistant Surgeon, (Filaria), Travancore-Cochin State, are also gratefully acknowledged. Thanks are also due to Mr. B. S. Krishnamurthy, Malaria Assistant, Malaria Institute of India, for his assistance in the field.

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FIELD STUDIES ON THE COMPARATIVE EFFECTIVENESS OF D.D.T., B.H.C. AND DIELDRIN RESIDUAL SPRAYS AGAINST THE VECTORS OF WUCHERERIAN INFECTIONS.

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RESULTS of field studies on the relative efficacy of residual sprays of D.D.T., B.H.C., and dieldrin against anopheline mosquitoes have been reported by Pal *et al.* (1955) and Viswanathan *et al.* (1955). Both the above reports recorded a high degree of residual toxicity of dieldrin as compared with the other two insecticides. Pal *et al.* (1955) observed the duration of residual toxicity of 12·5 mg. of dieldrin per sq. ft. to last for seven to eight weeks and compared favourably with 50 mg. D.D.T. and 10 mg. gamma B.H.C. Viswanathan *et al.* (1955) found that a single application of dieldrin at 14 mg./sq. ft. established a high degree of successful malaria control as good as an application of D.D.T. at 112 mg./sq. ft. once or twice during the season, while B.H.C. (11 mg. gamma isomer per sq. ft.) did not last beyond six weeks. Davidson (1952) in Africa observed that a deposit of 50 mg./sq. ft. of dieldrin gave lasting effect for six to seven months against culicine mosquitoes.

The present trials were undertaken to study the efficacy of these three insecticides in the control of *Mansonioides sp.*, the vector of *W. malayi;* and of B.H.C. and dieldrin against *C. fatigans*, the vector of *W. bancrofti;* respectively, in India.

VENUE AND PLAN OF OPERATION.

Ernakulam Town and some villages in Shertallai Taluq, both located in the Travancore-Cochin State in South India, were selected for these trials.

Ernakulam (Map 1) is a small municipal town with a population of 63,000 according to the last census and an area of about four square miles. A recent survey of the town (Jaswant Singh *et al.*, 1956) revealed filarial endemicity rate of 11 per cent, majority of the infections being *W. bancrofti* with *C. fatigans* as the vector. Two areas, each about one square mile in area, situated in the northern and southern extremities of the town,were selected for the experimental spraying. The population in each of these areas was about 10 to 12 thousands. All the structures in the area in the north were sprayed with dieldrin while those in the southern area were sprayed with B.H.C. The spraying operations were carried out during the latter half of December, 1954, after making preliminary entomological observations in the areas for two weeks.

A third area in the eastern part of the town, well separated from either of the experimental areas, was taken up as the comparison area for check observations.

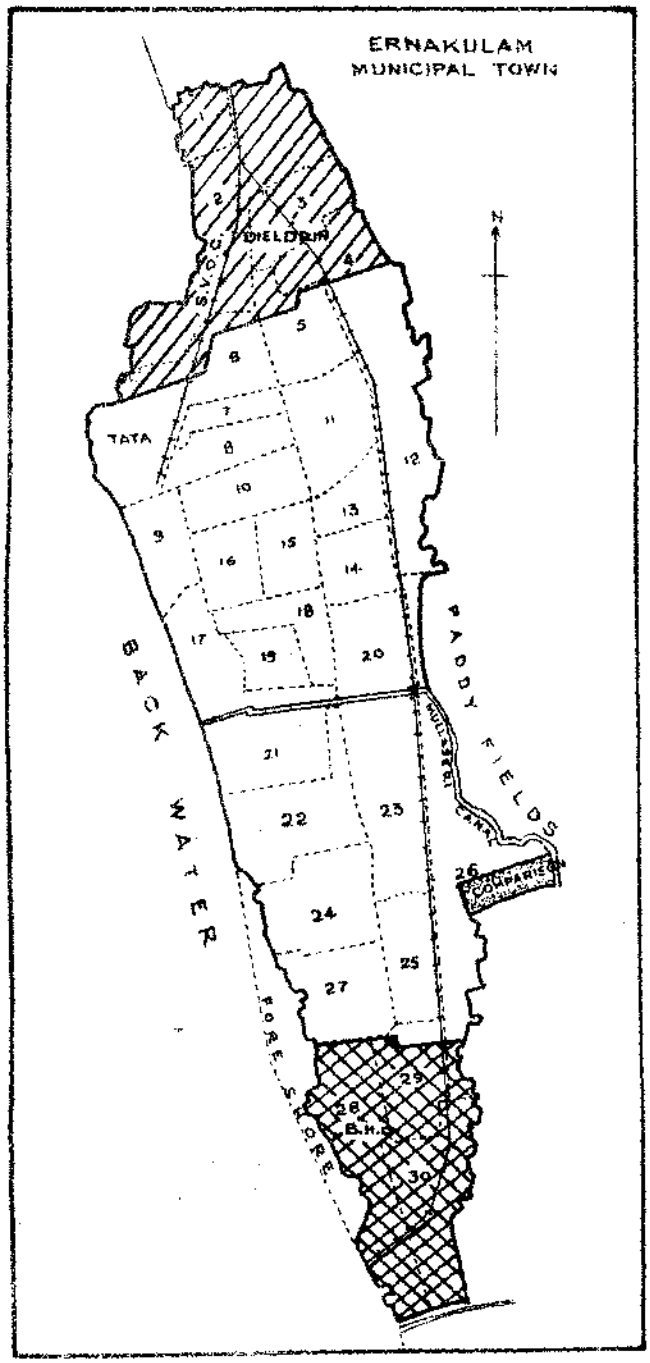
Shertallai (Map 2) is a highly filarious taluk in the coastal area of Travancore-Cochin State. *W. malayi* is the main species of filarial infection prevelent in this area, the filarial endemicity rates ranging in the different parts from 20 to 50 per cent. In these parts, there is no demarcation between one village and another, nor are the houses grouped together to form a village. The houses are scattered, separated from each other by 20 to 50 yards by fields or coconut groves. Administrative divisions called 'Panchayats' and 'Pakuthies' exist. Areas were selected in convenient blocks for the spraying operations (Map 2) . Four areas, each about one square mile, with about 500 houses and two to three thousands population were selected. The details regarding these areas and the insecticidal applications are given in Table I.

TABLE I.

Details of insecticidal applications in Shertallai area.

Area.	Number of houses in the area.	Population.	Insecticide.	Dosage.
Kokodamangalam ...	560	2,690	B.H.C.	44 mg. gamma isomer per sq. ft.
P. V. Bhagam ...	382	1,860	D.D.T.	200 mg./sq. ft.
Kadakarapalli ...	534	2,680	Dieldrin	50 mg./sq. ft.
Vayalar East ...	440	2,240	*Nil*	Comparison area.

MAP I.


ERNAKULAM
MUNICIPAL TOWN
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DIELDRIN
TATA
BACK WATER
PADDY FIELDS
FORE SHORE
B.H.C.
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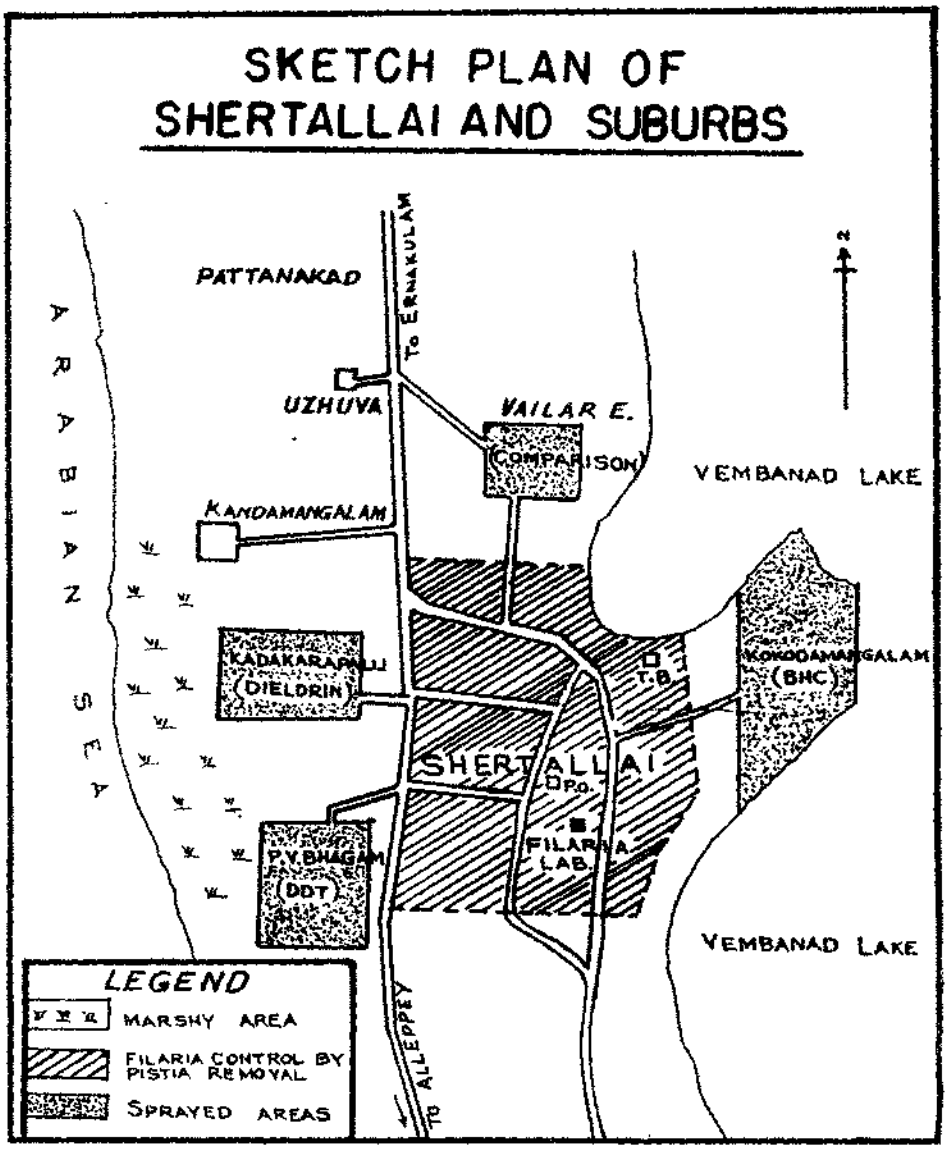
MAP 2.



The interior of all strutures were sprayed reaching as far high up as possible of the walls and roof. About 50 per cent of the structures sprayed at Enrakulam were pucca masonry constructions with plastered walls, while in Shertallai areas such structures formed a negligible proportion. Majority of the structures in the latter area were thatched huts, with plaited coconut leaf roofing, and walls either of the same material or of bamboo matting.

METEOROLOGICAL DATA.

The areas selected for the trials are located on the west coast of peninsular India, noted for its heavy rainfall. The annual rainfall ranges between 120 and 150 inches, the main precipitation occurring between June and September, though frequent showers continue up to mid-November. Temperature remains warm and humidity is high almost all the year round. Iyengar (1938) recorded in Shertallai a low incidence of *Mansoniaides* during the monsoon and a rise in their numbers during the post-monsoon season. The highest prevalence of these mosquitoes was recorded during the months of January to March. A similar observation has been reported for *C. fatigans* in Ernakulam by the local people.

INSECTICIDAL FORMULATIONS AND DOSAGES ADOPTED.

Water dispersible powders of the three insecticides were used during the present trials. All the formulations used gave stable suspensions in spite of the fact that only pond water was available in most of the places. D.D.T. was applied in a dose of 200 mg. sq. ft., B.H.C. in a dose of 44 mg./sq. ft. of gamma isomer and dieldrin in a dose of 50 mg./sq. ft.

PREPARATION OF SPRAYS.

(*a*) *D.D.T.*—One pound of 75 per cent water dispersible powder was suspended in 1·5 gallons of water and sprayed at one gallon per 1,000 sq. ft. Most of the surfaces on which the spray was applied being of non-absorbant material, it was not possible to spray one gallon of spray over 1,000 sq. ft. without considerable running. The required dosage had, therefore, to be carried out by spraying twice over, applying half gallon per 1,000 sq. ft. each time.

(*b*) *B.H.C.*—One and a half pounds of the B.H.C. formulation was suspended in one gallon of water and sprayed over 2,000 sq. ft. Here again a second round of spray, applying a similar dosage, was carried following the first to get the required dosage of 44 mg. gamma isomer per sq. ft.

(*c*) *Dieldrin.*—Dieldrin spray was prepared by suspending eight ounces of the 50 per cent water dispersible powder in one gallon of water and this was sprayed over 2,000 sq. ft.

COLLECTION OF DATA.

Regular weekly collections were made from four catching stations in the comparison areas, two of which were fitted with window traps. Six catching stations were fixed in each of the sprayed areas, three of them sprayed and three unsprayed; one each of these was fitted with a window trap of the type described by Jaswant Singh *et al.* (1951) while the others served for making total catches. Collections of mosquitoes in the window traps as also from the rooms where they were fitted, were made daily in the morning; total collections were made at weekly intervals. The window trap collections were kept in the laboratory under optimum survival conditions for 24 hours after which the mortality among them was recorded.

In addition to the above observations, survival was also studied among mosquitoes exposed in wall cages at weekly intervals in sprayed houses selected at random. *Culex fatigans* and *Mansonioides sp.* mosquitoes, hatched out in the laboratory and kept on glucose feeds for 48 hours, were utilized for these studies. About 10 to 20 mosquitoes were exposed at each time. The above observations, especially on *Mansonioides sp.*, could not be carried out regularly as it was not always possible to hatch out the required number of these mosquitoes in the laboratory. Specimens of *C. gelidus* were used when *Mansonioides* were not available.

RESULTS OF TRIALS AT ERNAKULAM.

A sharp fall in the prevalence of all species of mosquitoes followed the application of spray in both the areas treated with dieldrin or B.H.C.; the residents reported an almost complete freedom from mosquitoes and other insect pests. The follow up in the different areas are presented below.

(1) *B.H.C. area.*—Total collections from the catching stations in this area revealed a complete absence of mosquitoes in the sprayed stations during the week following spraying. From the second week onwards, a low but gradually increasing incidence in the mosquitoes was recorded both in the sprayed and unsprayed catching stations (Table II and Graph 1). The collections from the

TABLE II.

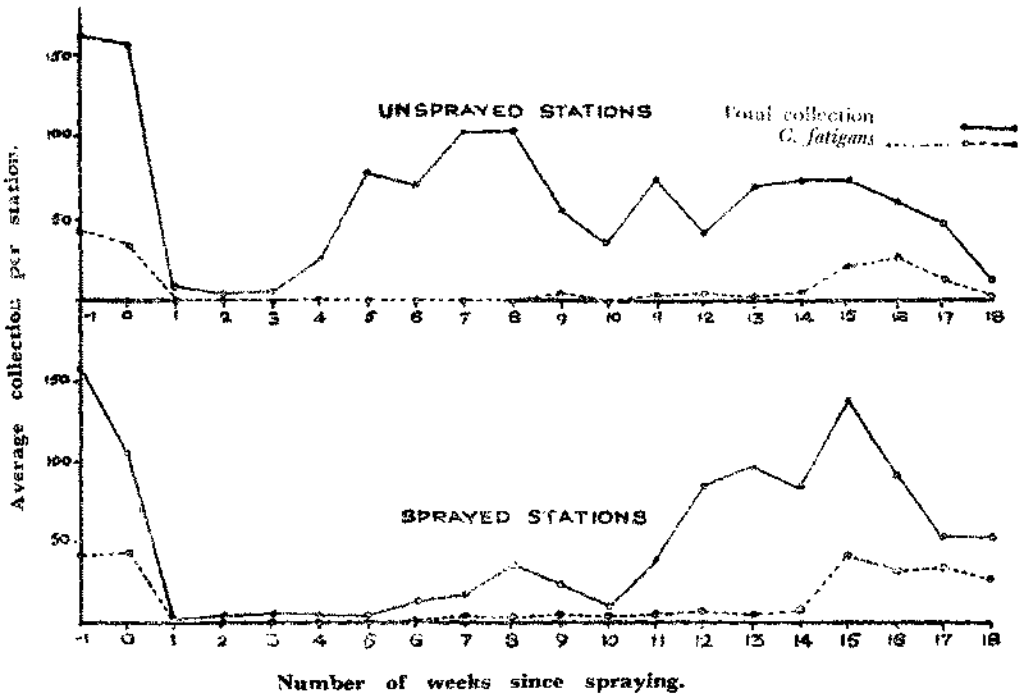
Total number of mosquitoes caught (weekly) (average of two catching stations) and ratio of catches from sprayed and unsprayed catching stations in Ernakulam.

Week.	44 MG. GAMMA ISOMER B.H.C./SQ. FT.			50 MG. DIELDRIN/SQ. FT.			Comparison area.
	Sprayed.	Unsprayed.	Sprayed/ Unsprayed.	Sprayed.	Unsprayed.	Sprayed/ Unsprayed.	
-1	160·5	162·5	0·98	64·8	105·6	0·61	123·7
-2	107·0	156·7	0·67	78·3	128·7	0·60	133·6
1	...	9·0	...	1·0	1·5	0·66	113·0
2	1·5	2·5	0·60	0·5	1·5	0·33	120·5
3	2·0	3·5	0·57	...	0·5	...	150·0
4	3·0	26·0	0·11	...	4·5	...	122·0
5	2·0	80·0	0·02	0·5	4·5	0·11	98·5
6	13·0	72·5	0·17	2·5	3·0	0·83	137·5
7	17·0	103·0	0·16	3·0	6·0	0·5	104·5
8	38·5	102·0	0·37	5·0	23·0	0·21	106·5
9	22·0	57·5	0·38	2·5	11·0	0·22	124·5
10	9·5	37·5	0·25	2·5	9·5	0·24	112·5
11	39·0	77·5	0·50	8·0	8·5	0·94	261·0
12	87·0	43·0	2·00	13·0	114·5	0·11	279·5
13	96·5	73·5	1·31	18·0	99·5	1·31	220·5
14	87·5	77·5	1·13	76·0	76·5	2·39	194·0
15	140·0	78·0	1·80	92·5	72·0	1·28	176·0
16	93·0	62·0	1·50	83·0	88·0	0·94	201·5
17	53·5	50·0	1·70	131·0	134·5	0·98	98·5
18	53·0	13·5	3·92	83·0	98·5	0·84	89·0

stations left unsprayed within the sprayed area (Table II), began rising sharply from the fourth week following spraying and continued to remain at significantly high levels, for the rest of the period. In the sprayed catching stations, however,

GRAPH I.

Mosquito incidence in the area sprayed with B.H.C. at Ernakulam



the collections were low till the tenth week, following which a rise was noticed which was sharp and persistent. An analysis of these collections showed a total absence of *Culex fatigans* up to eighth week—the main bulk of collection being *J. subpictus*—after which there was a gradual increase in their density up to 14 weeks followed by a sharp rise which persisted thereafter (Tables IV and VI-*A*).

Observations on the survival rate among the mosquitoes, found in the window traps and in the rooms fitted with the trap, are interesting. A 100 per cent mortality within 24 hours of collection was noticed among these mosquitoes up to six weeks following spraying. A gradual increase was noticed in the number of mosquitoes surviving the observation period, till it reached a 100 per cent during the fourteenth week when these observations were discontinued (Tables III and III-*A*).

TABLE III.

Catches from window traps and rooms (total for the week) from sprayed catching stations—Ernakulam.

Week	B.H.C.				DIELDRIN.				COMPARISON.			
	W.T.	Per cent survival.	R.C.	Per cent survival.	W.T.	Per cent survival.	R.C.	Per cent survival.	W.T.	Per cent survival.	R.C.	Per cent survival.
-1	No observations during -1 and 0 weeks.											
0												
1	...	...	...	...	12	...	3	...	88	1·1	250	0·8
2	4·0	...	1·0	...	3	...	3	...	43	8·3	272	8·4
3	...	...	2·0	...	3	...	5	...	5	40·0	383	63·7
4	...	...	4·0	...	4	...	5	...	12	50·0	277	79·9
5	6·0	...	16·0	...	3	...	6	...	6	83·3	220	79·0
6	3·0	...	42·0	...	2	...	3	...	...	...	255	60·0
7	2·0	...	48·0	4·16	8	...	7	...	...	...	201	72·1
8	9·0	11·1	94·0	4·2	3	...	4	...	...	...	264	85·2
9	15·0	6·6	127·0	7·8	3	33·3	12	...	...	...	205	82·9
10	6·0	16·6	117·0	16·2	...	...	3	...	...	...	293	94·1
11	14·0	14·2	286·0	26·8	8	81·5	18	44·4	...	...	343	96·2
12	13·0	30·7	340·0	45·0	3	33·3	30	50·0	...	...	369	92·1
13	5·0	40·0	258·0	8·9	...	...	79	76·9	...	...	289	44·7
14	3·0	100·0	188·0	90·9	1	100·0	63	84·1	...	...	229	90·8
15	...	...	66·0	78·7	1	100·0	62	95·1	...	...	140	98·5

W.T. = Window-trap. R.C. = Collections from rooms where window traps were fixed.

TABLE III-A.

Survival rate among mosquitoes collected from window-traps and the rooms where these were fitted.

Week	B.H.C.				DIELDRIN.				COMPARISON.			
	W.T.	Per cent survival.	R.C.	Per cent survival.	W.T.	Per cent survival.	R.C.	Per cent survival.	W.T.	Per cent survival.	R.C.	Per cent survival.
-1	No observations during -1 and 0 week.											
0												
1	5	...	6	...	14	...	5	...	88	1·1	250	0·8
2	8	...	3	...	9	...	7	...	43	8·3	272	8·4
3	10	...	14	28·5	16	...	16	...	5	40·0	383	63·7
4	11	...	8	37·5	5	...	12	...	12	50·0	277	79·9
5	14	...	14	21·3	12	...	11	...	6	83·3	220	79·0
6	3	...	13	15·3	11	...	20	...	...	...	255	60·3
7	2	...	25	20·0	11	...	31	...	...	...	201	72·1
8	...	...	17	11·7	7	...	33	...	...	...	264	85·2
9	...	...	16	31·2	14	...	21	...	...	...	205	82·9
10	...	...	22	63·6	6	...	11	...	...	...	293	94·1
11	3	...	78	61·0	11	...	87	9·8	...	...	343	96·2
12	...	...	119	58·1	13	7·6	94	15·8	...	...	369	92·1
13	1	...	152	38·1	9	33·3	73	23·2	...	...	289	44·7
14	...	...	71	84·6	10	50·0	94	74·4	...	...	229	90·8
15	...	...	61	98·3	29	96·5	91	98·9	...	...	140	98·5

W.T. = Window-trap. R.C. = Collections from rooms where window traps were fixed.

TABLE IV.

Weekly total mosquitoes and total C. fatigans in sprayed catching stations, Ernakulam.

Week.	B.H.C.		Dieldrin.		Comparison.	
	Total mosquitoes.	*Culex fatigans.*	Total mosquitoes.	*Culex fatigans.*	Total mosquitoes.	*Culex fatigans.*
−1	160·5	42·5	64·8	22·6	123·7	55·7
0	107·0	14·7	78·3	26·8	133·6	68·5
1	...	...	1·0	...	113·0	77·5
2	1·5	...	0·5	...	120·5	75·0
3	2·0	...	...	...	150·0	101·5
4	3·0	...	...	...	122·0	62·0
5	2·0	...	0·5	...	98·5	47·0
6	13·0	...	2·5	...	137·5	60·5
7	17·0	...	3·0	...	104·5	35·5
8	38·5	1·0	5·0	...	106·5	29·5
9	22·0	3·0	2·5	...	121·5	38·5
10	9·5	0·5	2·5	0·5	112·5	33·5
11	39·0	1·0	8·0	1·5	261·5	99·5
12	87·5	5·5	13·0	3·0	297·5	70·5
13	96·5	1·0	118·0	87·5	220·5	76·5
14	87·5	6·0	176·0	111·0	194·0	102·0
15	140·0	42·0	92·5	75·5	176·0	116·0
16	93·0	31·5	83·0	64·0	201·5	89·0
17	53·5	36·0	131·0	109·5	98·5	52·0
18	53·0	17·0	83·0	64·0	89·0	45·5

(2) *Dieldrin area.*—Almost a complete mosquito vacuum was noticed in the sprayed catching stations for four weeks following application of insecticide while an insignificant low incidence was recorded during this period from the unsprayed observation stations within this area. Total collections from both these types of catching stations showed a low incidence of mosquitoes up to eleven weeks. The twelfth week's collection revealed a sharp rise of mosquito density in the unsprayed houses, while a similar rise occurred in the sprayed stations during the following week. The first specimen of *Culex fatigans* was recorded from the area

during the eighth week from one of the unsprayed catching stations, and two weeks later from a sprayed station. A significant persistent rise in the vector species occurred from the thirteenth week following the spraying (Table I and Graph 2).

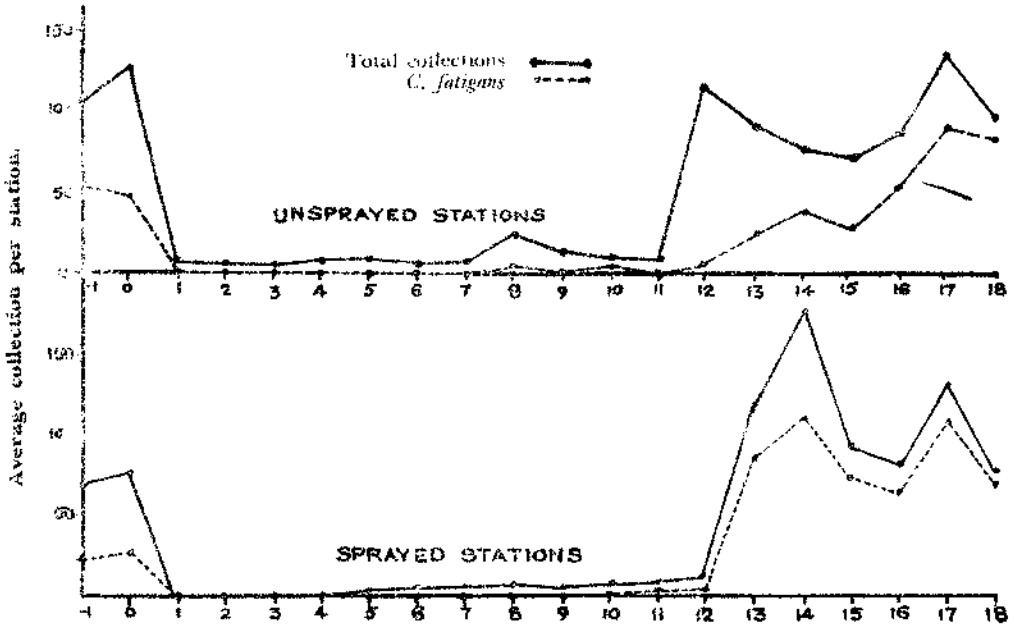
All mosquitoes collected from the window traps died within 24 hours of collection during eight weeks following spraying. A gradual increase in the number of survivors was noticed thereafter but did not reach 100 per cent up to the fifteenth week when the window trap observations were discontinued (Table III and Graph 4).

TABLE IV-A.

Total mosquitoes and total C. fatigans *in unsprayed catching station, Ernakulam.*

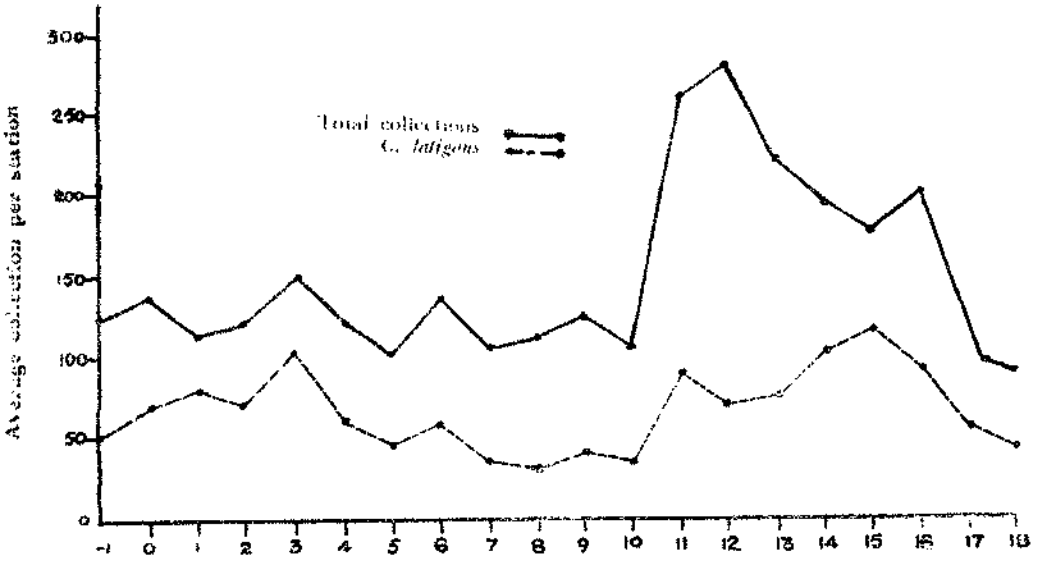
Week.	B.H.C.		Dieldrin.		Comparison.	
	Total mosquitoes.	*Culex fatigans.*	Total mosquitoes.	*Culex fatigans.*	Total mosquitoes.	*Culex fatigans.*
1	462·2	43·7	405·6	51·4	123·7	55·7
0	156·7	35·5	127·7	48·1	133·6	68·5
1	9·0	...	1·5	...	113·0	77·5
2	2·5	...	1·5	...	120·5	75·0
3	3·5	...	0·5	...	150·0	101·5
4	26·0	...	4·5	...	122·0	62·0
5	80·0	...	4·5	...	98·5	47·0
6	72·5	...	3·0	...	137·5	60·5
7	103·0	...	6·0	...	104·5	35·5
8	102·0	...	23·0	0·5	106·5	29·5
9	57·5	1·0	41·0	...	124·5	38·5
10	37·5	...	9·5	0·5	112·5	33·5
11	77·5	1·5	8·5	...	161·5	90·5
12	43·0	1·0	114·1	5·5	297·5	70·5
13	73·5	3·5	9·5	23·5	220·5	76·5
14	77·5	5·0	76·5	39·5	194·0	102·0
15	78·0	20·5	72·0	28·5	176·0	116·5
16	62·0	27·5	88·0	51·4	201·5	89·0
17	59·0	15·5	134·5	88·5	98·5	52·0
18	13·5	3·5	98·5	82·0	19·0	45·5

GRAPH 2.



Number of weeks since spraying.

GRAPH 3.

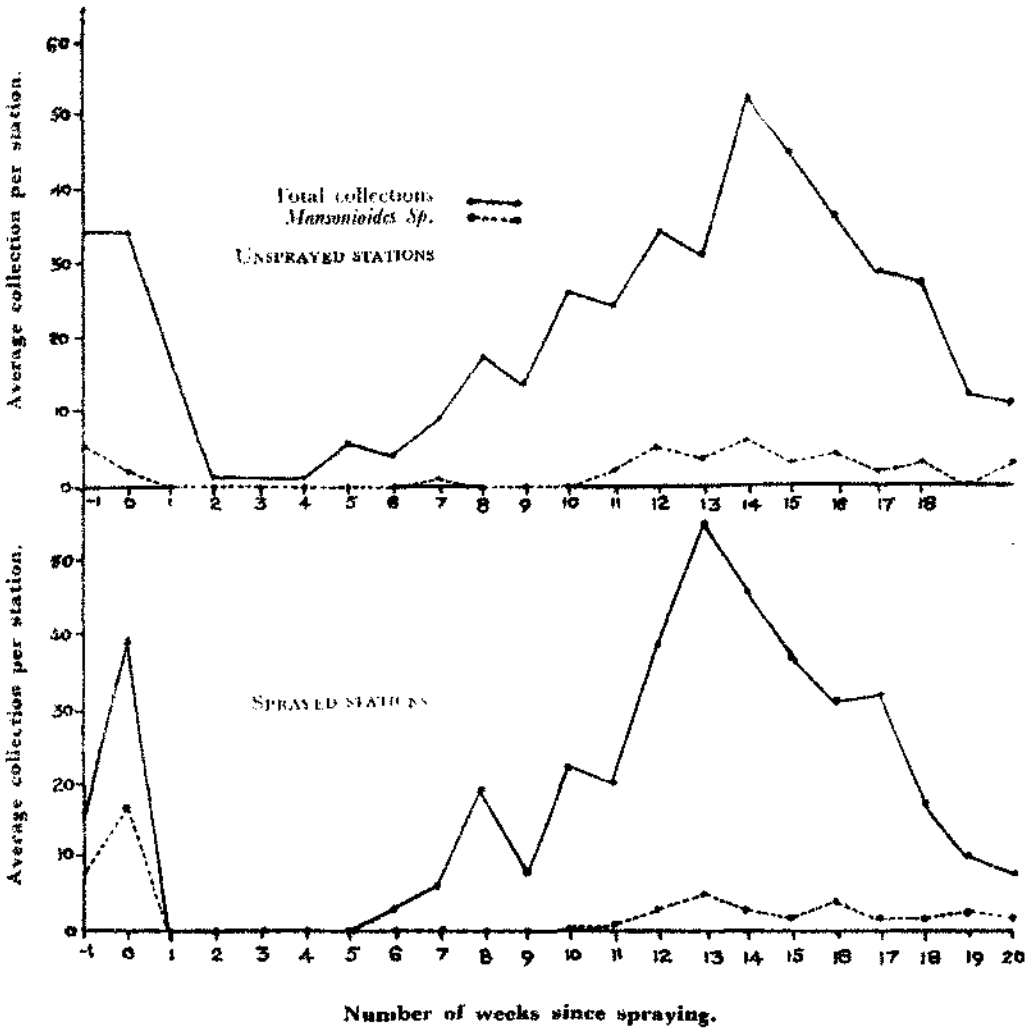


Number of weeks since spraying.

(3) *Comparison area.*—Parallel observations recorded in the comparison area are presented in Table II and Graph 3. The total incidence of mosquitoes as well as of *Culex fatigans* (Table IV) remained high throughout the period of observation. A natural decline in the mosquito incidence was noticed from the seventeenth week following the commencement of the obesrvations. All observations were discontinued at this stage.

Observations on the survival among window trap collections, were discouraging in the early stages, as a high mortality was noticed even among the

GRAPH 4.



collections from the comparison area (Table III). This was obviously due to contamination with some insecticide. A complete replacement of the collecting equipment and cages was followed by a high survival among these collections.

TABLE V.

Total number of mosquitoes caught from the sprayed and the unsprayed catching stations—Average of two catching stations—Shertallai.

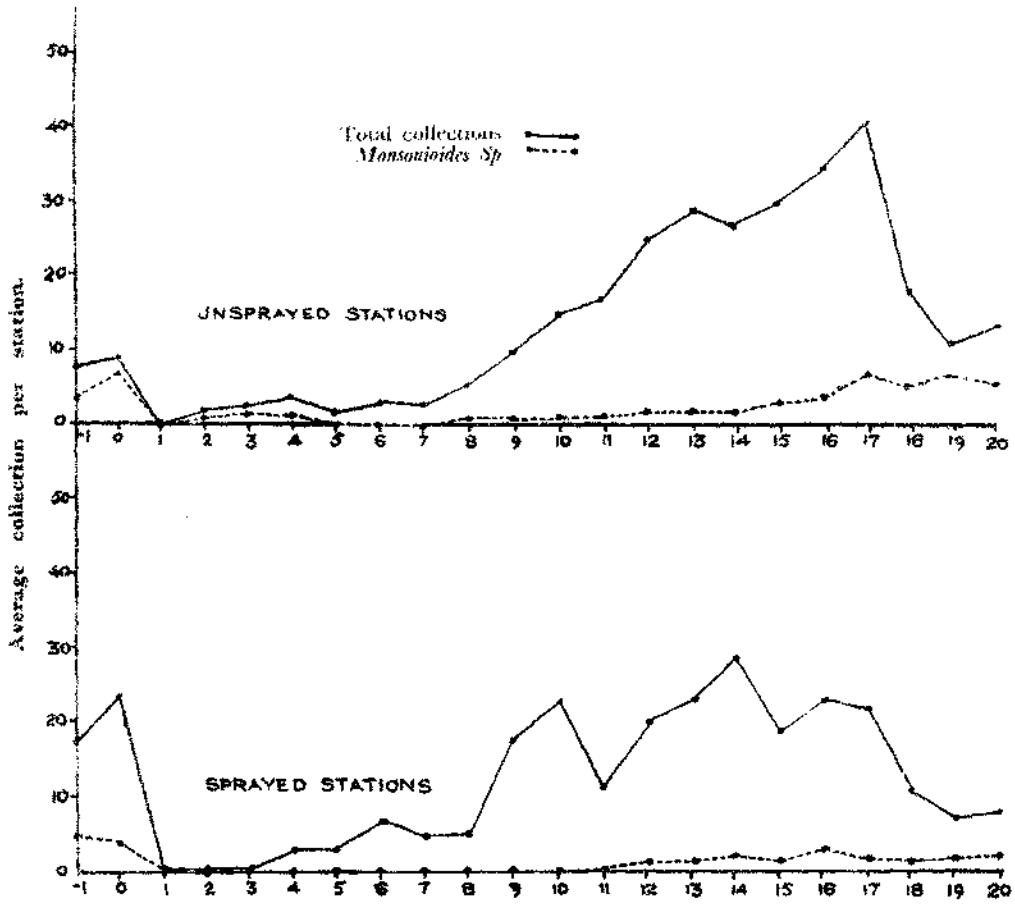
Week.	AVERAGE NUMBER OF MOSQUITOES PER CATCHING STATION.						
	44 mg. gamma B.H.C. per sq. ft.		200 mg. D.D.T. per sq. ft.		50 mg. dieldrin per sq. ft.		Comprison area.
	Sprayed.	Unsprayed.	Sprayed.	Unsprayed.	Sprayed.	Unsprayed.	Unsprayed.
-1	16·5	34·0	17·5	8·5	14·0	14·0	19·0
0	39·5	34·5	24·0	9·5	23·0	32·5	26·0
1	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	23·0
2	...	0·5	...	1·0	...	...	39·0
3	...	...	...	3·0	1·5	1·0	67·0
4	...	0·5	2·5	3·5	1·5	3·0	94·0
5	...	5·5	3·0	2·0	3·0	6·5	114·5
6	2·5	4·0	7·0	3·5	0·5	2·5	103·5
7	5·5	9·0	4·5	2·5	2·5	7·0	100·0
8	19·0	16·5	4·5	5·5	2·0	17·0	75·5
9	8·5	14·0	18·0	10·5	6·0	12·5	97·5
10	22·0	26·0	23·0	15·0	7·5	15·5	115·0
11	20·0	24·0	12·0	17·0	4·0	20·5	99·5
12	39·0	34·0	20·0	25·0	9·0	34·6	142·5
13	55·0	31·5	23·0	29·0	21·0	36·0	162·0
14	16·0	52·0	29·0	27·0	27·0	31·5	53·0
15	37·0	40·8	19·5	30·0	15·0	27·5	60·0
16	30·5	30·0	23·0	34·5	19·0	31·5	93·0
17	31·5	28·0	22·5	41·5	8·5	16·0	48·5
18	17·0	26·5	11·0	18·0	9·0	11·5	26·0
19	10·5	12·0	8·5	11·5	7·0	9·5	27·5
20	8·0	11·5	9·0	13·0	5·0	10·0	19·0

RESULTS OF TRIALS AT SHERTALLAI.

The observations recorded in the sprayed and comparison areas are presented below:

(1) *B.H.C. area.*—(Kokodamangalam Village): Mosquitoes started appearing in the sprayed catching stations from the sixth week following spraying and from the fourth week in the unsprayed station. The density increased gradually up to the eleventh week following which there was a sudden rise in the density, which continued up to the seventeenth week, after which there was a natural recession (Table V, Graph 4), as shown by collections from the comparison area (Table V, Graph 7).

GRAPH 5.



Total freedom from mosquitoes as reflected in the window trap collections was observed for a period of two weeks following spraying. A steady increase in the incidence of mosquitoes was recorded from the seventh week onwards, though survival was negligibly low among them (Table VI).

(2) *D.D.T. area.*—(P. V. Bhagam Village): Following application of a dose of 200 mg. of D.D.T. per square foot, absence of mosquitoes as determined by total catches from fixed catching stations, was noticed for a period of one and three weeks respectively in the unsprayed and sprayed catching stations. The number of mosquitoes collected, however, remained low up to eight weeks, following which there was a sharp persistent increase in the mosquito density (Table V, Graph 5).

Efforts at hand-collections from the rooms, and daily examination of window traps for any mosquito trapped therein, showed a complete absence of mosquitoes up to eight weeks, after which mosquitoes were found coming in regularly.

(3) *Dieldrin.*— (Kadakarapalli Village): Complete absence of mosquitoes, as revealed by total collections, was recorded for two weeks following application of dieldrin at 50 mg. per sq. ft. The density, however, remained low for a period of 12 weeks, following which an abrupt rise was noticed which persisted during the rest of the observation period (Table V and Graph 6) up to twenty weeks following spraying.

Records of mosquitoes coming into window traps, and survival rate among the mosquitoes collected showed that though varying numbers of mosquitoes continued to appear in the house, survival rate among the mosquitoes entering the sprayed houses was negligibly low up to at least 14 weeks following application of spray. Observations had to be discontinued at this stage due to onset of monsoon.

Analysis of mosquitoes collected in the three areas showed that the main bulk (70 per cent) of the house-frequenting mosquitoes consisted of *Culex gelidus* and *Culex vishnui* while a good proportion (25 per cent) consisted of *A. subpictus*. The other species met with in the collections were *A. vagus*, *A. jamesi*, *A. barbirostris*, *C. (Lutzia) fuscanus* and *Armegeres obturbans*.

Total collections and window trap collection from the comparison area (Vayalar East) were satisfactory (Table V). Survival rate among mosquitoes collected from houses and from window traps was comparatively high, and ranged from 11·3 to 77·2 per cent (Table VI).

DISCUSSION.

Observations on the efficacy of the modern synthetic insecticides, in controlling vectors of filarial infections, have not been carried out on any large scale.

Indoor residual spraying with D.D.T. as a water dispersible powder, sprayed at a dose of 167 mg. per sq. ft., was followed up for three weeks by Iyengar (1951) in Thailand. The mosquito density fell from 55·4 to 5·4 per man-hour, while the density of *Mansonioides sp.*, the local vector, fell from 44·8 to 1·3 per man-hour. Prior to spraying, the infection rate among the vectors was 7·6 per cent, while

TABLE VI.

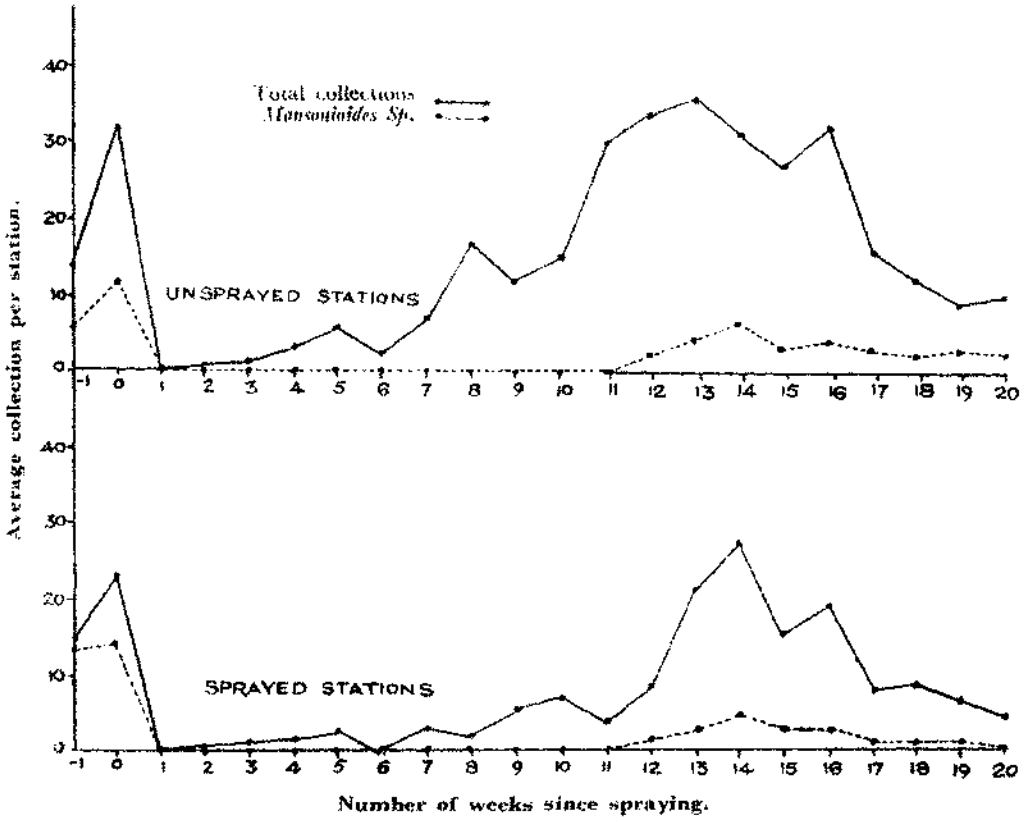
Weekly collections from window-traps and rooms in Sheriallai.

Week.	B.H.C.						D.D.T.						Dieldrin.						Comparison					
	W.T.			R.C.			W.T.			R.C.			W.T.			R.C.			W.T.			R.C.		
	Collection.	Survival.	Per cent survival.	Collection.	Survival.	Per cent survival.	Collection.	Survival.	Per cent survival.	Collection.	Survival.	Per cent survival.	Collection.	Survival.	Per cent survival.	Collection.	Survival.	Per cent survival.	Collection.	Survival.	Per cent survival.	Collection.	Survival.	Per cent survival.
−1	21	6	28·5	11	...	...	7	...	...	8	...	...	12	...	...	9	...	...	5	...	...	47	...	...
1	...	...	...	...	...	...	...	...	...	...	...	...	3	...	...	...	...	...	3	...	...	38	...	...
2	...	...	...	...	...	...	...	...	...	...	...	...	3	...	...	1	...	...	2	...	...	53	6	11·3
3	2	...	...	...	...	...	...	...	...	...	...	...	12	...	...	6	...	...	7	2	28·5	78	7	8·9
4	...	...	...	...	...	...	...	...	...	...	...	...	14	...	...	...	...	...	14	4	28·5	129	58	44·9
5	9	1	11·1	...	...	...	...	...	...	...	...	...	18	...	...	7	...	...	12	8	66·6	104	38	36·5
6	3	1	33·3	...	...	...	...	...	...	...	...	...	18	2	11·1	14	...	...	5	4	80·0	104	38	36·5
7	17	2	11·7	3	1	33·3	...	...	...	...	...	...	17	1	5·8	14	...	...	22	12	54·5	97	45	46·3
8	10	1	10·0	20	3	15·0	9	...	...	...	...	...	16	...	...	12	1	8·3	12	3	25·0	142	36	32·1
9	21	...	...	16	2	12·5	16	2	12·5	2	...	...	20	...	...	12	...	...	15	7	46·6	102	47	46·07
10	13	1	7·6	10	6	60·0	6	...	...	2	...	...	22	1	4·5	21	...	...	11	9	81·8	155	81	52·2
11	14	...	...	25	9	36·6	9	1	11·1	7	...	...	25	...	...	10	...	...	8	6	75·0	149	101	67·7
12	12	3	25·0	23	7	30·4	10	2	20·0	2	...	...	19	...	...	8	...	...	13	7	46·6	131	84	64·1
13	18	2	11·1	41	20	48·7	9	2	22·2	6	2	33·3	15	...	...	27	5	18·5	11	4	36·3	180	139	77·2
14	9	1	11·1	40	14	35·0	1	...	...	9	3	33·3	11	...	...	32	6	18·7	...	...	...	161	97	60·37

W.T.=Window-traps. R.C.=Collections from rooms where window-traps were fixed.

three weeks after the spraying, the infection rate in the same area was zero. Studies in Orissa (*Indian Council of Medical Research, Report*, 1953) showed that D.D.T. was suitable for the control of *C. fatigans* when sprayed at a dose of 200 mg./per sq. ft.

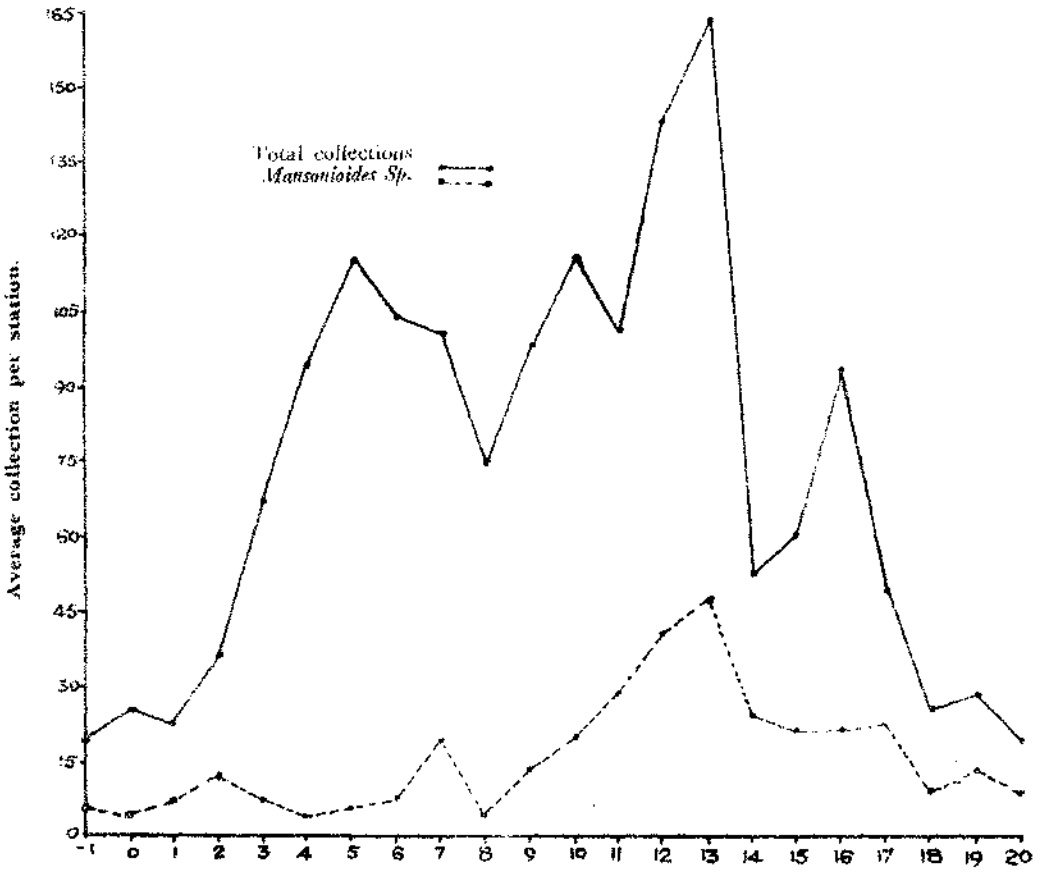
GRAPH 6.



Davidson (1952) reported highly favourable results with dieldrin against *C. fatigans;* he recorded a mortality of 60 to 95 per cent lasting over six to seven months with this insecticide sprayed at 50 mg. per sq. ft.

At the dosages applied in these trials, B.H.C. and dieldrin appear to be equally effective against the vector of *W. bancrofti* infection. The absolute as well as relative density of *Mansonioides* mosquitoes continued to remain significantly low, as compared with those in the comparison area of Vayalar, throughout the observation period of twenty weeks following spraying.

GRAPH 7.



Dissections of mosquitoes collected from the sprayed and comparison areas also bear out the above observations. While 60 (6·6 per cent) of 905 specimens of *Mansonioides annulifera* and *M. uniformis* from the unsprayed area were positive for filarial infections, not a single specimen was positive out of 634 that were dissected from the sprayed areas during the same period (Table VII). These observations would appear to indicate that the residual toxicity of an undisturbed deposit on thatch and bamboo matting might last as long as or longer than 20 weeks under experimental conditions. But under rural conditions in India, some kind of interference is bound to take place either by renewal of the thatch or replastering of the wall with mud or cowdung. Actually in the area where the sprays were applied, the residents appreciated the freedom from insect pests so much that they delayed replacement of the thatch which was an annual feature with the approach of the monsoon.

TABLE VII.

Results of mosquito dissections.

Area.	Species mosquito.	Before spraying.		After spraying.	
		Number dissected.	Number positive.*	Number dissected.	Number positive.*
Ernakulam					
Dieldrin ...	*C. fatigans*	798	5	248	1†
B.H.C.	..	341	4	100	1†
Comparison	..	1,645	50	627	6
Shertallai					
Kokodamangalam (B.H.C.)	*Mansonioides*	122	1	232	*Nil*
P. V. Bhagam (D.D.T.) ...	..	93	2	199	*Nil*
Kadakarapalli (Dieldrin) ...	..	120	2	203	*Nil*
Vayalar East (Comparison)	..	23	2	905	60

* All stages at development.
† 14th week following spraying.

Residual toxicity of dieldrin (50 mg. per sq. ft.) and B.H.C. (44 mg. gamma isomer per sq. ft.) lasted from 13 to 14 weeks against *C. fatigans*, the vector of bancroftian filariasis. *Mansonioides sp.* would thus appear to be more susceptible than *C. fatigans* to the different insecticides.

The duration, for which mosquitoes other than the vector were controlled, varied with the insecticide, being lowest (eight weeks) with D.D.T., 10 to 11 weeks with B.H.C., and 12 to 13 weeks with dieldrin.

With the formulations of the different insecticides made available for these trials, the procedure had necessarily to be modified for each preparation in order to apply the required dosages on the surfaces sprayed. It has been mentioned earlier that the structures treated were mostly of non-absorbent material, and the quantity of spray applied had to be restricted to an optimum of one gallon per 2,000 sq. ft. of wall surface—with a view to avoid running as also to maintain a convenient speed by the operator. In the case of B.H.C., the spray suspension contained 1·5 lb. per gallon—higher concentration became too thick to go through the nozzle—and in order to deposit 44 mg. gamma isomer it was essential to distribute two gallons of the spray over 2,000 sq. ft. This was done by first applying one gallon over the area, and again repeating the same dosage after a few minutes. This technique enabled the application of the required dosage *without* any wastage in running. A similar procedure was adopted to apply 200 mg. D.D.T. per sq. ft. using a five per cent spray and applying two gallons per 2,000 sq. ft. This double application of the spray in the case of D.D.T. and B.H.C., necessarily resulted in considerable increase in the time of spraying, and almost doubled the cost of operation and supervision.

In the case of dieldrin, however, it was possible to apply the required dosage of 50 mg. per sq. ft. with a single application of one gallon of a 2·5 per cent spray over 2,000 sq. ft.

TOXICITY TO HIGHER ANIMALS.

A variety of domestic pests and vermins were found succumbing to the insecticides, especially during the spraying operations; lizards, scorpions, cockroaches and crickets were killed by hundreds, especially in the rural areas of Shertallai. The inmates of the sprayed houses reported relief from these pests for considerable periods following the application of sprays. The residents were warned to sweep off and bury the vermins, and not to feed their poultry on the dead insects. In spite of this, one or two accidents happened and it was not possible to ascertain whether the reported deaths among poultry were due to the direct toxicity of the insecticide or due to their having fed on the dead insects. In one area, large scale death of fish was reported in one pond but this was due to the workers having washed the buckets and pumps in that pond after finishing their work the previous evening. Except for this, there was no other such accident reported.

SUMMARY.

1. Results of field trials with B.H.C. and dieldrin against *C. fatigans*, the vector of *W. bancrofti*, and of D.D.T., B.H.C. and dieldrin against *Mansonioides sp.*, the vector of *W. malayi*, are reported.

2. Water dispersible powders of all these insecticides were used, applying a dosage of 200 mg., 50 mg. and 44 mg. gamma isomer of D.D.T., dieldrin and B.H.C., respectively, per sq. ft.

3. Applied at the above dosages, B.H.C. and dieldrin were equally effective against *C. fatigans*, the residual effect lasting for 13 to 14 weeks.

4. In three areas sprayed with B.H.C., dieldrin and D.D.T. in the rural areas of Shertallai, the density of *Mansonioides sp.* remained significantly low throughout an observation period of 20 weeks following spraying. The effect of B.H.C., dieldrin and D.D.T. appeared to last for 11, 12 and 8 weeks respectively, against the other culicines in the same area. The vectors of *W. malayi*, appeared comparatively more susceptible to these insecticides than *C. fatigans*.

5. The practical difficulties in the application of the required dosages of D.D.T. and B.H.C., using the formulations made available, have been discussed.

ACKNOWLEDGEMENTS.

The help given by the Director of Public Health, Travancore-Cochin State and his staff, particularly Dr. V. M. Pillai and Dr. A. J. Thommen, is gratefully acknowledged. Dieldrin and B.H.C. formulations used in the above trials were made available by the courtesy of Messrs. Burmah Shell (India) Ltd., and Messrs. Imperial Chemical Industries (India) Ltd.

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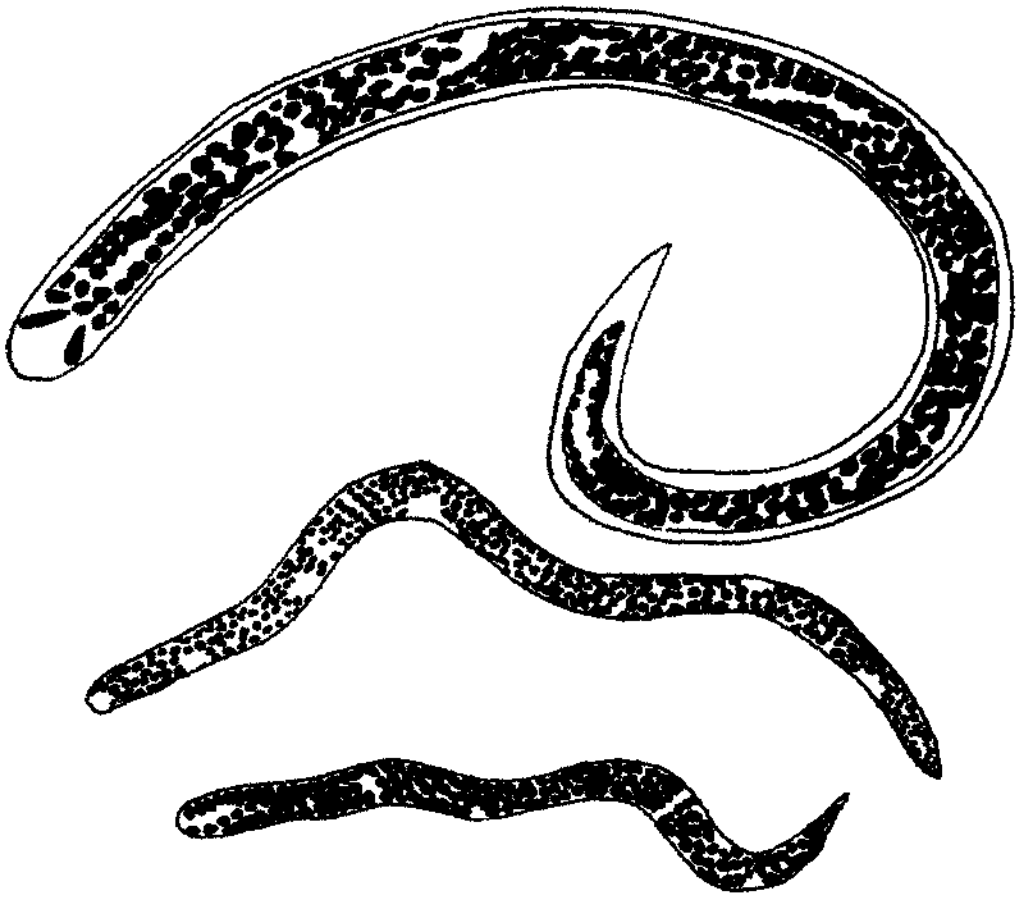
PLATE IV.



Mylonchulus brachyurus from domestic fowls. (Camera Lucida drawings.)

Indian Journal of Malariology, **10**, 3, *September 1956.*

MICROFILARIÆ IN DOMESTIC FOWLS.

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[June 8, 1956.]

FILARIAL infection in domestic fowls has been described both from India and abroad. Ramanujachari and Alwar (1953) described a single microfilaria found on postmortem of a Desi fowl in Madras, India. Kuppuswamy (1936) reported the presence of microfilariæ in 17 out of 237 fowls examined in Malaya. In the Philippines, Dy (1955) and Rozeboom (1956) also noted microfilariæ in some of the local fowls.

This note deals with a description of three types of microfilariæ encountered in the peripheral blood of domestic fowls in Ernakulam and Shertallai of Travancore-Cochin State (Pattanayak, 1955).

Blood smears from fifteen country fowls of both sexes from Ernakulam and Shertallai were examined. Nine of them showed microfilariæ in their peripheral circulation, each containing one or more of three types of sheathed microfilariæ, both in fresh drop and on staining by J.S.B. I (Jaswant Singh and Bhattacharji, 1944). The details of the above microfilariæ and of those described by Ramanujachari and Alwar (1953) as also by Kuppuswamy (1936) are set out in Table I.

The measurements of the forms described now are the average of ten microfilariæ of each type. Apart from the differences in measurements, the following morphological variations were noted in the three types of microfilariæ. Camera Lucida drawings of the three forms are appended (Plate IV).

The cephalic end of the nuclear column in the largest (Type 1) of the microfilariæ, terminated in two elongated oval nuclear masses which enclosed a

V-shaped space with the wider end pointing anteriorly. This character as well as coarse nuclei of the nuclear column with a tapering posterior end with a clear caudal space, was a distinct feature of these large microfilariæ.

TABLE I.

The measurements of microfilariæ found in fowls. (All measurements are in microns.)

Characteristics of microfilariæ.	Type I*.	Type II*.	Type III*.	Microfilariæ described by Ramanujachari and Alwar (1953).	Microfilariæ described by Kuppuswamy (1936).	
Length	176·04	97·80	65·20	71·0	80·0	80·0
Breadth, head-end ...	6·52	4·89	4·89	5·0	4·8	6·4
Distance from the anterior end to the nerve ring ...	32·60	22·82	16·30	18·0	...	...
Distance from the anterior end of the excretory pore ...	57·05	34·23	27·71	27·0	17·2	24·0
Distance from the anterior end to the anal pore ...	...	...	62·0	60·0	...	...
Length of the cephalic space	3·26	2·89	1·63	...	...	...

* Type I - III as described in this paper.

The medium sized microfilariæ (Type II) also had coarse nuclear bits, with a small cephalic space, and a blunt tail end which was packed with nuclear material. The sheath could be easily made out in a majority (nearly 80 per cent) of this type of microfilariæ. This microfilaria compared well with that described by Kuppuswamy (1936) from Malaya.

The smallest sized microfilariæ (Type III) had very little cephalic space, and the nuclear column bifurcated a little behind the proximal end and ran in two parallel lines along the edges on either side. The caudal end was uniformly tapering and packed with nuclei. This type of microfilariæ appeared to be similar to that described by Ramanujachari and Alwar (1953).

Two fowls, showing all the three types of microfilariæ, were sacrificed but no adult worms were recovered in spite of careful examination. In the absence of the adult worms, it has not been possible to identify the species of microfilariæ encountered in these fowls.

Kuppuswamy (1936) surmised that mosquitoes would be the vectors. Dy (1955) and Rozeboom (1956) reported unsuccessful attempts to infect mosquitoes with fowl filariæ in the Philippines.

The following species of laboratory-bred mosquitoes have so far been utilized without success to study their ability to transmit the filarial infections reported in this paper.

Species.	Number dissected.
C. inuzia	25
C. fatigans	1,909
C. sitiens	25
Aedes aegypti	500
M. annulifera	9
A. subpictus	40

The possible role of ectoparasites of the fowl, e.g., ticks, lice and mites as vectors, is being investigated.

SUMMARY.

A description of three types of sheathed microfilariæ found in domestic fowls in Ernakulam and Shertallai (Travancore-Cochin State) is given.

Attempts to infect experimentally different local species of mosquitoes have proved unsuccessful. The possibility of ectoparasites of the fowls being the vectors, is being investigated.

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STUDIES ON REPRODUCTIVE SYSTEM OF *CONISPICULUM GUINDIENSIS*.

1. Production of microfilariæ 'in vitro' by *C. guindiensis*.

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[June 8, 1956.]

PANDIT *et al.* (1929) described in the garden lizard, *Calotes versicolor*, around Guindy, Madras, a naturally occurring filarial infection with a non-periodic sheathed microfilariæ. Webber (1954*a*; 1954*b*) and Hawking (1954) reported their studies on the reproductive system of *Litosomoides carinii* from cotton rats. Hawking (1954) reported the number of microfilariæ produced 'in vitro' by female adult worms *L. carinii* and results of similar studies on adult females of *C. guindiensis* are recorded in this note.

MATERIAL AND METHODS.

Adult female worms of *C. guindiensis* were collected from infected *Calotes versicolor*, obtained through the courtesy of the Director, King Institute of Preventive Medicine, Guindy, Madras. The adult females were identified from the description by Menon *et al.* (1944), and for their parturition by examination in normal saline under the binocular dissecting microscope. Individual worms were kept in small receptacles with 25 per cent animal serum and 75 per cent Ringer solution containing 0·2 per cent glucose and incubated at 75°F. After 2, 3, 4, 19, 22 and 48 hours, 30 c.mm. of the fluid was withdrawn and the number of microfilariæ were counted. After withdrawal of fluid each time a similar amount of fluid only, from a separate container, was replenished. All possible aseptic precautions were observed.

Five sets of observations, using eleven worms, were carried out. Of these, two sets (Serial 1 to 4, and 8 and 10 of Table I) using four and two worms, respectively, were normal; i.e., incubated in serum and Ringer's solution alone, while in another experiment three worms (Serial 5, 6 and 7 of Table I) were incubated for three hours in the normal manner, whereafter 60,000 units of penicillin were added to study the effect of the antibiotic on the production of microfilariæ. In the fourth experiment, to a receptacle containing one worm (Serial 9 of Table I), a similar amount of penicillin was added after only two hours of incubation in serum alone. The last experiment was to study the action of diethylcarbamazine after two hours of incubation in normal serum (Serial 11 of Table I). Results are set in Table I below.

TABLE I.

Numbers of microfilariæ produced by adult worms of C. guindiensis *in the different experiments.*

Serial number of receptacle containing adult worms.	Quantity of fluid used (c.c.).	Number of microfilariæ present after: 2 hours.	3 hours.	4 hours.	19 hours.	22 hours.	48 hours.	Remarks.
1.	2	...	...	2,520	...	8,100	8,440	Snake serum in Ringer's solution.
2.	6	...	...	1,680	...	7,200	7,200	,, ,,
3.	3	450	...	...	1,350 8,010 (ova)	...	...	Buffalo serum in Ringer's solution.
4.	3	90	...	...	11,880	...	...	,, ,,
5.	3	.	360	...	..	...	...	,, plus 60,000 units of penicillin after three hours incubation.
6.	3	..	1,710	.	.	...	...	,, ,,
7.	3	...	450	.	...	...	...	,, ,,
8.	3	1,530		..	1,530	11,340	87,660	Buffalo serum in Ringer's solution.
9.	3	2,160		..	...	...	...	,, plus 60,000 units penicillin after two hours incubation in normal serum.
10.	3	2,970	.	.	2,970	6,570	12,090	Buffalo serum in Ringer's solution.
11.	3	2,340	..	.	12,690	44,730	28,710	,, plus 2·5 mg. diethylcarbamazine after two hours incubation to normal serum.

RESULTS.

From a study of the worms incubated in 25 per cent serum as above, it is noted that an adult female worm of *C. guindiensis* maintained at 75° F. 'in vitro' produces 1,350 to 87,660 (average 19,517) microfilariæ in 48 hours. Most of the microfilariæ are extruded between the second and twenty-second hours, though the worms remain alive and motile for a longer period. The above observations correspond generally to those recorded by Hawking (1946) for the adult female worm of *L. carinii*.

In the series where pencillin was added, though the worms were actively parturating just prior to the addition of the antibiotic, further extrusion of microfilariæ ceased immediately after the addition of the antibiotic. In all these cases, the worms remained alive and motile for over 48 hours. Further studies are in progress on the effect of other antibiotics and production of microfilariæ by *C. guindiensis* 'in vitro'.

As regards Serial 11, diethylcarbamazine was found to have no effect on the extrusion of microfilariæ. This result is in conformity with those reported by Malaria Institute of India (1953) on the lack of effect of diethylcarbamazine 'in vitro' against the adults or microfilariæ of *C. guindiensis*. Studies on the effect of antifilarial compounds against the reproductive capacity of *C. guindiensis* 'in vitro' have been planned. These studies, it is expected, will throw some light on the utility of using the reproductive capacity of the whole worm 'in vitro' for the screening of 'filaricides' as against screening against *Mf. C. guindiensis* only as suggested by Hawking (1946).

ENUMERATION OF MICROFILARIÆ AND EGGS IN AN ADULT FEMALE WORM OF *C. GUINDIENSIS*.

Attempts to count microfilariæ and eggs in adult female worms in histological sections gave unreliable results, as the microfilariæ get cut into small pieces. Accordingly fresh preparations were used. The female worms were cut into 1 mm. sections, each of which was transferred to a separate slide in a drop of saline, minced, spread, dried, stained and examined under the microscope. The total number of microfilariæ and ova was counted in each slide. One worm 12 cm. long showed 3,35,040 microfilariæ and ova according to this technique. Another worm 8·7 cm. long showed 1,04,175 microfilariæ and ova.

It appears that the total count of ova (all stages) in an adult female *C. guindiensis* is of the order of 2,28,000.

SUMMARY.

1. An adult female *Conispiculum guindiensis* maintained 'in vitro' at 75°F. produced 1,350 to 87,660 (average 19,517) microfilariæ in about 48 hours. The parturition started in two hours and reached a maximum in about twenty-two hours.

2. The possible inhibitory effect of penicillin on the parturition of *C. guindiensis* is set out.

3. The possible role of study of effect on parturition of female *C. guindiensis* as an 'in vitro' technique for screening of filaricides and related compounds, is brought out.

4. A mature female *C. guindiensis* contained about 1,04,175 to 3,35,040 ova (average 2,28,856) in all stages of development up to the mature microfilariæ.

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DIVIDING FORMS OF *P. FALCIPARUM* IN THE PERIPHERAL BLOOD OF ADULTS.

BY

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AND

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[June 8, 1956.]

INTRODUCTION.

The occurrence of schizonts and tenue forms of *P. falciparum* in the peripheral blood may be seen in some cases of malaria. The influence, under which these forms are detectable in the peripheral blood, remains undetermined. However, the presence of the dividing forms in the blood smears is usually associated with very low tolerance of the patient and consequently denotes grave implications regarding the progonosis of the case (Jolly, 1936; Schwetz, 1938; Raper, Wilson and Wilson, 1945).

This communication describes the appearance of the developing forms and schizonts in two cases of *P. falciparum* infection not in any way seriously ill. Both made uneventful recoveries with the standard treatment.

CASE HISTORY.*

Case number 1. Adult male, aged 23 years, presumably semi-immune, visited his village home in Aligarh District, U.P., during the transmission period and obviously contracted the infection there. On the third day of fever, the patient was admitted into the hospital, and his temperature was recorded as 104·2°F. The following day, the patient was afebrile, but on the fifth day the temperature rose up to 105°F. The blood was examined at this stage and *P. falciparum* rings,

*Both patients were from amongst the Police Force of the Delhi State and were treated at the local Police Hospital.

trophozoites and schizonts were seen. Nothing abnormal was detected in the lungs and heart. The liver was not enlarged nor was the spleen palpable. The general condition of the patient was good.

The patient responded well to oral dose of chloroquine.

Case number 2.—Adult male, aged 23 years, was admitted into the hospital with a history of fever for three days associated with cough and cold. The temperature recorded was 104·2 F. Throat was congested. The spleen was not palpable. The blood was examined and *P. falciparum* rings and schizonts were detected. The general condition of the patient was good. The patient responded satisfactorily to intravenous chloroquine.

DENSITY OF PARASITES.

The maximum parasitæmia, observed in case number 1, did not exceed eight parasitized cells per 10,000 erythrocytes while in case number 2, the highest parasitæmia recorded was 46 parasitized cells per 10,000 erythrocytes.

MORPHOLOGY.

The infected erythrocytes were uniformly normal in shape and size, some were stippled with Maurer's dots. Tenue forms of parasites were common in case number 1, while in the other the typical *falciparum* rings were present in large numbers. Double chromatin dots and accole forms were encountered frequently. Immature schizonts containing 4 to 10 merozoites were encountered in fair number in both the cases. The morphological character has been presented in Plate V (camera lucida drawings). Gametocytes were not seen.

DISCUSSION.

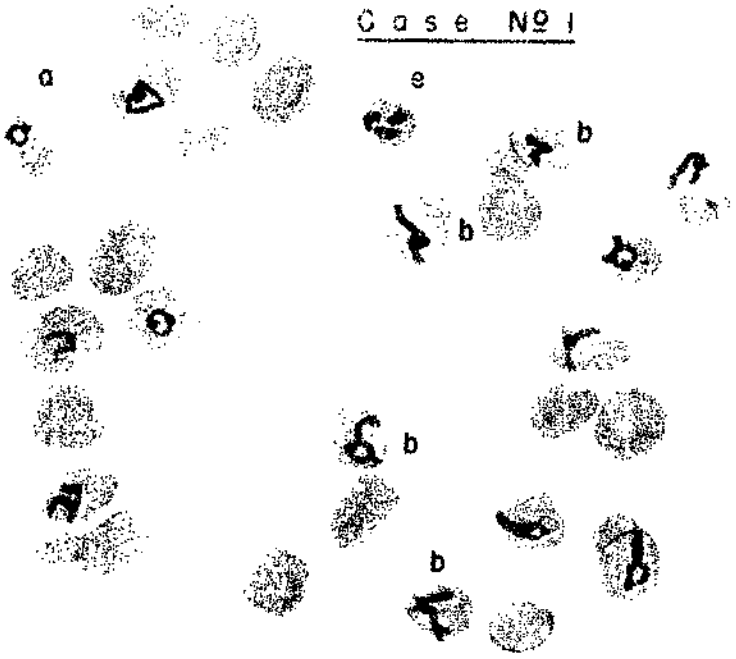
Dividing forms of *P. falciparum* in the peripheral blood have been observed in young children with marked splenic enlargements and are associated with high density of parasites (Jolly, 1936). Such unusual cases are considered as indicating either low tolerance and grave prognostic consequences, or of indifferent significance (Schwetz, 1938).

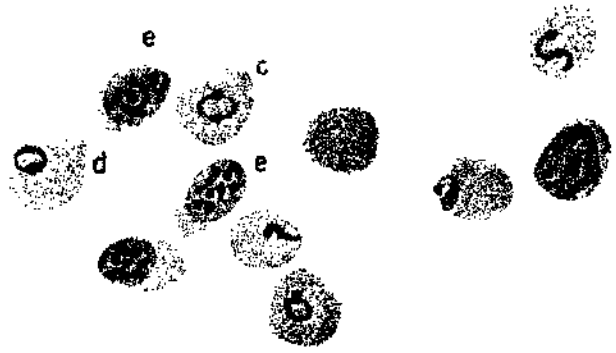
In the cases under review, the patients were adults and presumably semi-immune from endemic areas. Further, the low density of parasites (less than 0·5 per cent cell infection) indicates a fair amount of tolerance. So, the association of the dividing forms with low tolerance (as in children), splenic enlargement and high parasite density, is not applicable in these cases.

Raper *et al.* (1945) reported similar occurrence of the dividing forms of *P. falciparum* in African adults who recovered with minimal treatment or no treatment at all. They suggested that there may be a race or strain of *P. falciparum* in which peripheral schizongony may occur. Probably a strain difference is also applicable in the present infection.

Both the cases were easily amenable to antimalarial treatment. The parenteral administration of chloroquine was more in nature of a suitable therapeutic trial of the drug and bore no relation to the gravity of the case.

PLATE V.





a. Ring forms.
b. Tenue forms.
c. Double chromatin dots.
d. Accole forms.
e. Schizonts.

SUMMARY.

Two cases of *P. falciparum* infection in adults, with peripheral schizogony, are described. The cases were without the grave implications. The morphological features of the parasites are presented.

The infections were amenable to oral and parenteral chloroquine treatment.

The suggestion that peripheral schizogony may be a strain character, is further stressed.

ACKNOWLEDGEMENT.

The authors are thankful to Dr. K. C. Chopra of the Police Hospital, Delhi, for extending facilities to undertake these studies.

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MALARIA IN BURMA

A Review.

BY

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INTRODUCTION.

Burma being generally a highly malarious country, a number of attempts were made in various localities by different authors to study the problem but very little of it is known outside the local official circles as the bulk of the information has not been published. No attempt has been made to collate available information from various sources and present a picture of the malaria situation of the country as a whole. Following the assistance afforded by the World Health Organization and other allied international agencies, in the demonstration of modern methods of malaria control and in the training of malaria workers of different categories, the Government of the Union of Burma have launched a five-year country-wide programme of malaria control which is soon expected to cover all the malarious areas of the country. There is also the distinct probability of this programme being developed into a malaria eradication project. It is thus desirable, and perhaps even necessary, to take stock of the position as it existed prior to the inauguration of the malaria control plan and sum up the available information within the compass of a single paper in order that a proper comparison might be possible with conditions prevailing at a later date when malaria is brought under control, or has been eradicated, as the case may be.†

* The authors wish to express their thanks to the authorities of the World Health Organization, South-East Asia Region, and the Deputy Director of Health Services (Public Health), Burma, for their permission to publish this paper and to Dr. E. J. Pampana and Dr. E. Burford Weeks of the Malaria Section, World Health Organization, for helpful criticism and suggestions.

†A malaria control demonstration project, assisted by the World Health Organization, was in operation in the Lashio area of the Northern Shan States form October 1951 to March 1954, during which period detailed studies were made on the epidemiology of malaria in the area and on the bionomics of the local vector, *A. minimus*. These studies are not included in this paper, as a complete report on the activties of the project is likely to be published elsewhere. A brief summary of the results of these operations has, however, been furnished by Dy (1954).

Burma is situated in the Oriental Region and is a rather narrow strip of country, its greatest length form north to south being about 1,300 miles and the greatest width east to west 600 miles. The area of Burma is about 2,62,000 square miles. Its population, according to the census of 1941, is about 17 millions but detailed records are not available. The density of population over the whole area is 65 per square mile but it varies from about 30 in the hill tracts to over 500 in certain parts of Southern Burma.

The country is largely made up of a series of mountains and valleys running from north to south, some of the mountain peaks rising to about 10,000 feet above the sea level while the valleys hardly reach the 1,000 feet level. Each of the valleys is traversed by a major river, which is fed by numerous tributaries. Only one part of Burma is really flat and that is the main valley of the River Irrawaddy constituting a large part of Central Burma. Many of the mountains consist of hard crystalline rock but there are also a few newer sedimentary rocks, specially in Central Burma, where oil occurs. A large part of the Shan Plateau, however, consists of lime stone, where grass lands, rather than cultivable soils, are formed. Several valuable minerals are found in areas covered by hard rocks, such as tin at Tavoy, lead and silver at Namtu and rubies and other precious stones at Mogok (French and Dudley Stamp, 1941).

According to rainfall, the country has been divided into three zones, *viz.*, the coastal zone having an average annual rainfall of about 200 inches, the northern zone, mainly consisting of hill tracts, having an average annual rainfall of about 80 inches and the dry zone in Central Burma, where the annual rainfall ranges between 20 and 40 inches.

The Tropic of Cancer passes through Burma just north of Mandalay; therefore, a major part of the country lies in the torrid zone. However, the weather is generally very pleasant in the hill tracts (which constitute about 60 per cent of the whole country) but the dry zone is very hot in the summer months, the temperature in shade sometimes rising beyond 113°F.

The autochthonous races inhabiting Burma are the Burmans, Mons, Karens, Shans, Kachins and Chins, the last-named three peoples being confined mostly to the northern hill region and the Shan Plateau. A large part of the area, known as Tenasserim, is claimed to be the territory of the Mons, while the delta region is populated by a large number of Karens. At the southern tip of the Shan Plateau, there are Kayahs, who are not more than a million in strength. In addition, there are, specially in the hill tracts, several tribes who appear to be different from the other racial groups mentioned above. The largest tribe among them are the Palaungs inhabiting mainly the Northern Shan States. The Wa Tribes inhabit the extreme north-eastern area called the Wa States. This area is covered with mountains and forests and is very inaccessible. This is a poorly developed area and the people are extremely backward. The Burmans constitute the majority of the population, outnumbering all others put together.

None of these, however, are the original inhabitants of Burma (Harvey, 1947) with the possible exception of the Wa tribe. The earliest inhabitants were Indonesians but they are nowhere in the country now and have left not even a trace of their existence here. They were displaced by various Mongolian tribes, who

are the forerunners of the present inhabitants and who migrated into Burma in successive waves, beginning only a few centuries before Christ. The latest immigration was that of the Shans, who are of the same stock as the people of Thailand, in the twelfth and thirteenth centuries A.D.

There are also appreciable numbers of foreigners at present in Burma, mostly Indians, Chinese, Nepalese and Pakistanis, their total number probably not exceeding one million. Many of them have been in Burma for over five or six decades and are likely to be gradually absorbed into the local population.

For the purpose of the present study, the country is divided into four regions instead of the seven "natural" regions described by French and Dudley Stamp (*loc. cit.*). The four regions are:—

(*i*) The Hill Tracts, comprising the western and northern hill ranges and the Shan Plateau:

(*ii*) The Plains, comprising the Dry Zone forming Central Burma;

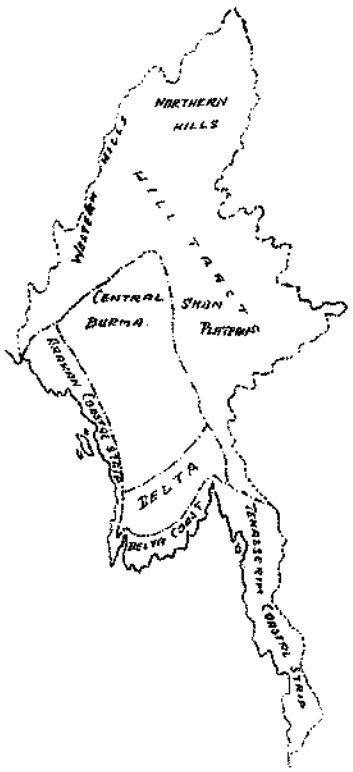
(*iii*) The Delta consisting of south central Burma; and

(*iv*) The Coastal Tracts consisting of Arakan, the coastal areas of the Delta and the Tenasserim Coastal Strip.

Map 1 shows the boundaries of the regions.

MAP 1.

The four malaria regions of Burma



SOURCES OF STUDY.

There are, for the whole pre-war period, only a few published records of malaria in Burma. One of them deals with the malaria in the mining area of Mawchi by James (1929), another relates to the study of the anopheline fauna of the Arakan Region by Grewal (1937) and the last deals with the clinical aspects of malaria among troops by Stott (1916). However, there are many unpublished records of investigations in several localities of Burma which are available in official records, all of which are referred to in the present study.

The most important among recent reports is the thesis by Fox (1949), in which he not only summarised the existing information on the anophelines of Burma but also recorded his own observations on them. A few other records of war-time work in Burma by other authors have since been published.

THE HILL TRACTS.

A number of investigations were carried out in the hill tracts by various workers at different times. They were mostly undertaken in isolated localities when specific reasons necessitated the studies, such as the posting of army or police personnel in any hill station or the occurrence of an unusual amount of sickness among government officials. The studies were, therefore, restricted to towns and covered only limited periods, the longest not exceeding five months. A few villages, situated close to towns, were studied on a few occasions. Only in a few cases, these studies were followed by control measures as at Lashio in the Shan States. In spite of these limitations, the reports contain interesting observations on spleen and parasite rates and, to a lesser extent, on malarial morbidity and mortality as well, from which it is possible to gain some idea of the malaria situation as a whole. In certain cases, dissections of the local anophelines were made and vector species indicated, which enabled the workers to make a brief study of the bionomics of the vectors.

Spleen rates.—Some of the investigations were made during the rainy season, while others were made either before or after the rains. If, as will be shown later, the bulk of transmission occurs during the monsoon period (May-October), the figures obtained before the commencement of rains, and even during the rainy season, may not afford a true picture of the situation and may tend to minimise the actual hazard. The ages of the children examined are not always furnished. Only Lalor (1912) and Feegrade (1926) specifically mentioned that the children examined by them were under ten years of age, while Maung Gale (1927) stated that he examined children who were not more than twelve years old. The method of palpation has not been mentioned except by Maung Gale (*loc. cit.*). He measured the enlargement by the finger-breadth, a practice common at the time and the same method was most probably adopted by the other workers too. Senior White (1930) alone recorded the different sizes of enlargements.

Table I shows the spleen rates recorded by various authors in this region.

TABLE I.

Spleen rates among children under 12 years of age recorded in the Hill Tracts of Burma.

Author.	Year.	Locality.*	Number of children examined.	Spleen rate per cent.
Lalor	(1912)	Wuntho Town	?	71
,,	(1912)	Villages near Wuntho	?	86–95
Fcegrade	(1925)	Bhamo	?	11
,,	(1926)	Lashio Town	?	34–56
,,	(1927)	Hsipaw Town	858	23–54
,,	(1931)	Mong-Yai	117	52
Maung Gale	(1926)	Papun	?	52
Jolly	(1928)	Mawlaik	?	44
U Tin	(1937)	Wuntho	?	79
,,	(1953)	Lauksawk	?	50
Senior White	(1930)	Villages near Namtu	26	88

Except at Bhamo, spleen rates above 50 per cent are recorded from all other localities of this region. The varying spleen rates in certain towns are explained by the authors as being due to the proximity or otherwise of the breeding places, those living on the outskirts of the town or close to running streams showing more splenic enlargements than those living in the heart of the town.

That the spleen rates in the villages were generally higher than in towns, was specifically mentioned by Lalor (1912) who found higher rates in villages near the town of Wuntho than in the town itself.

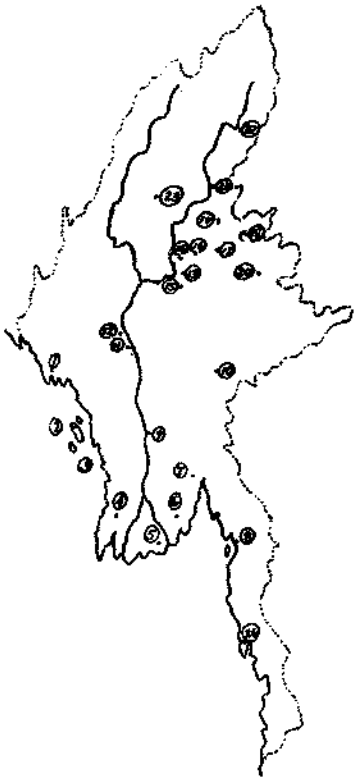
There is only one record of adult spleen rate by Fcegrade (1925) from Bhamo. The very low rate (2 per cent) recorded here cannot be of any special significance as the spleen rate among children was also very low, being just above the hypo-endemic level.

Considering the extent of the region, which is very large, the splenic examinations made may appear to be very meagre. Also, the fact that most of the examinations were restricted to towns, limits the usefulness to a certain extent. It may, however, be justifiable to postulate on the basis of available information that the hill tracts, as a whole, are hyperendemic for malaria, an observation which is shared by Fox (*loc. cit.*) and other authors.

*Reference is invited to Map 2 for the location of the towns referred to in this paper.

MAP 2.

Towns mentioned in the text.



1 Akyab
2 Kyauk Pyu
3 Sun Do Way
4 Bassein
5 Pya Pon
6 Rangoon
7 Pegu
8 Moulmein
9 Prome
10 Pyin Ma Na
11 Min Bu
12 Pwin Byu
13 Mandalay
14 Madaya
15 Amarapura
16 Maymyo
17 Hsi-Paw
18 Lashio
19 Namtu
20 Mong Yai
21 Bha Mo
22 Myit-Kyi-Na
23 Wun Tho
24 Tavoy

Parasite rates.—Most of the above-mentioned authors recorded results of blood examinations made by them. Lalor (1912) and U Tin (1937) found a parasite rate of over 50 per cent in Wuntho, U Tin (1953) recorded a 60 per cent rate in Lauksawk, Maung Gale (1927) found the parasite rate ranging between 21 and 33 per cent in Papun, and Feegrade (1926 *a*) found a 30 per cent rate in Lashio and between 20 and 30 per cent in Hsipaw. U Tin (1937) recorded a 74 per cent gametocyte rate among 101 children examined in Wuntho. These figures, which were recorded in widely distributed localities at different times, are more conformable to endemic than to epidemic conditions.

The three common species of malaria parasites, *viz.*, *P. falciparum*, *P. vivax*, and *P. malariæ* were encountered everywhere during these investigations. The first two species were much more prevalent than the last but there appears to be some conflicting information about the relative prevalence of the first two species. *P. vivax* was observed to be the dominant species in Wuntho (U Tin, 1937), in Papun (Maung Gale, 1926) and in Hsipaw (Feegrade, 1927). However, Feegrade (1925:1926*a*) found that *P. falciparum* dominated the picture in Bhamo and Lashio throughout the transmission season. These disparities might be due to the small

number of subjects examined in certain instances. The difficulties experienced in collecting children for taking blood smears have been fully described by Feegrade (1930); he cites an instance where among 469 children examined for splenic enlargement, only 82 blood films could be collected. The best evidence, as to the relative prevalence of these two species, has probably been adduced by Senior White (1930) from Namtu. He reviewed the results of blood examination of 34,525 malaria cases admitted into the Burma Corporation Hospital during the year 1929 and concluded that the dominant species during June-December was *P. falciparum* and that, during the rest of the year, *P. vivax* predominated. This indicates that a large majority of fresh infections occur during June-December and that the bulk of the incidence in January-May is due to relapses.

Feegrade (1925) casually mentioned in his report on malaria in Bhamo that "cases of *P. ovale* and *P. tenue* were included under *P. falciparum*". This is indeed the first time that *P. ovale* is recorded in Burma and it would be interesting to ascertain if there are any further records about this species. However, why *P. ovale* came to be included, along with *tenue*, in *falciparum* has not been explained.

Meteorological records.—Observations recorded in Lashio from 1922 to 1926 show that the average annual rainfall there is 62·8 inches and that most of it is precipitated between June and September. The mean daily maximum temperature is 85°F. and the mean minimum 65°F., the highest being 101°F. in May and the lowest 43°F. in January. The average relative humidity is 89 per cent, the maximum being 96 per cent and the minimum 77 per cent.

Conditions in other parts of this region are not dissimilar, though in places like Kutkai and Taunggyi, which are situated at an elevation of about 4,500 feet above the sea level, the rainfall may be heavier and the temperatures lower.

Though the relative humidity remains favourable for malaria transmission practically throughout the year, the temperature appears to be definitely unfavourable during the cold months of December and January. The distribution of rainfall renders the streams and other running waters unsuitable for anopheline breeding in June-August, whereas optimum breeding conditions exist in the post-monsoon months and in the early monsoon period when the flushing effect of rains is not very effective. However, as Feegrade (1926) has observed, a certain amount of vector breeding goes on unchecked during June-August in the terraced ricefields of the valleys, which thus become the temporary breeding places for the vector.

Transmission season.—Though no specific mention of the transmission season was made anywhere, most workers have agreed that the people suffer from malaria more during the rainy season—May to October—than during the rest of the year. U Tin (1953), however, suggested that transmission is perennial in this region. Feegrade (1926*a*) observed that, though the bulk of transmission is restricted to the rainy season, there are two peaks, the first in May-June and the second in Spetember-October, and that the morbidity of later months is made up largely of relapses. Except Senior White (*loc. cit.*), other authors based their arguments on morbidity figures from hospitals and dispensaries where malaria is diagnosed on clinical grounds alone. However, in highly endemic areas such as

the one under study, clinical diagnosis may be accepted as fairly correct and authentic.

Since dissections of vector Anopheles were not continued to cover one year or 'season' in any locality, the conclusions about the length of transmission season based on morbidity cannot be verified. It was, of course, not feasible to obtain blood films from infants to determine the length of the period of transmission; it has already been shown that collection of blood films even from grown-up children was very difficult.

It may be stated on the basis of available information that transmission probably lasts throughout the year, with a very low intensity during the cold months and with two peaks, one immediately after the onset of the south-west monsoon and the other towards the end of the monsoon period, the latter being invariably the higher. In spite of enviromental conditions being favourable, there is a reduced incidence during the heavy monsoon rains; this is obviously due to the flushing effect of the rains on streams and other lotic waters where the local vector, *A. minimus*, breeds. The very low incidence observed in the dry months of March and April may be due to the reduction in vector output as a result of their breeding places becoming dry.

Morbidity and mortality.—There are clear indications in the reports of various authors that a large proportion of the adult population actually suffers from malaria every year. Feegrade (1926) has shown from the hospital figures of Hsipaw that about 80 per cent of the total population, served by that hospital, suffered from the disease during 1926. Referring to the hospital records of Namtu, Senior White (*loc. cit.*) stated that one-third of the total admissions in 1929 were malaria cases, diagnosed miroscopically, among whom there were many adults.

The spleen and parasite rates among children, already furnished, show that many of them are exposed to the risk of malaria rather early in their lives and continue to suffer from it afterwards. Malaria is probably their main health hazard.

Data on vital statistics are available in only a few reports. U Tin (1937*a*) says that, during 1931 and 1932, deaths from "fevers" in Kalemyo accounted for more than 75 per cent of the total mortality. Maung Gale (1926) has shown that about 60 per cent of the mortality at Papun, in 1926, was due to malaria. Both these localities are endemic for malaria.

Blackwater fever.—Lalor (1912) alone makes specific mention of blackwater fever at Wuntho. The fact that other authors made no mention of it, may not mean that it had not been a problem. Several medical men, with whom the present authors had discussions, are agreed that blackwater fever, though not very common, was prevalent in parts of Mytikyina, Namtu, Lashio and Loilem. It, however, appears that, since the newer synthetic drugs have practically eliminated the use of quinine, blakwater fever has tended to disappear.

Economic effects of malaria.—The effects of endemic malaria on the people's economy has been studied only by Senior White (*loc. cit.*), who made a detailed calculation of the amount of money lost by the Burma Corporation at Namtu in 1929 on account of malaria. In this study, he took into consideration (*a*) the loss of working days among the Asiatic and non-Asiatic staff employed; (*b*) the cost

of hospitalization of malaria cases and (*c*) the cost of medication of out-patients and came to the conclusion that, for a population of about 12,000, the Corporation had lost Rs. 88,780 during that year. Thus, the loss *per capita* was Rs. 6.56. He, however, added that the indirect cost, due to practical inefficiency of employees having quinine treatment (which was the only treatment then available) and recovering from attacks and of absence of key personnel from time to time, was not taken into account in the above calculation and it was almost " unestimable '.

Namtu is an industrial settlement, where medical aid is always available and the wages of staff members are easily ascertainable, thus simplifying the process of estimating the financial loss due to malaria. Its economic effects in rural areas cannot be computed with the same degree of accuracy, mainly because of the difficulty in assessing the actual number of man-days lost. It has been stated by some workers that, in such areas, the agricultural operations are carried out in the absence of the sick adult members by other members of the family who otherwise have no work to do and there is, therefore, no actual loss in terms of money. It is, however, doubtful if this somewhat complacent view is applicable to thinly populated areas like the one under study. The density of the rural population in this region is only about 32 per square mile and there is very little likelihood of the loss of man-days being easily made up by surplus population. The sickness among the people must, therefore, operate adversely on their economy. Here too, the indirect effects of a large part of the working population being frequently sick and physically poor remains " unestimable ".

Endemicity and immunity.—There is not much information in the available records to show whether the hill region is hyperendemic or holo-endemic for malaria. The only adult spleen rate taken by Feegrade (1925) was in Bhamo Town, where the child spleen rate was itself very low, being very near the hypo-endemic level and a low spleen rate among adults does not, therefore, signify anything. Vital statistics are virtually absent. The following recorded facts may, however, be considered in this respect:—

(*a*) The spleen rates among children are not constantly above 75 per cent; in fact, in a number of cases, they are not far above 50 per cent;

(*b*) The information on morbidity furnished by almost all the authors shows that there is an appreciable amount of sickness among adults;

(*c*) The available data on mortality show that malaria is an imporant cause of death in all age-groups; and

(*d*) There is evidence to indiate that transmission is not continuous; even during the monsoon period, which is described as the active transmission season, there is reduced incidence between the two peaks, whereas the winter months are markedly healthier.

The hill tracts of Burma, therefore, appear to be hyper-endemic for malaria. Probably, there is no area in this country where holo-endemic conditions are known to prevail.

Anopheline vectors.—Table II shows the results of dissections carried out by different authors in this region.

TABLE II.

Results of dissections.

Species.	Number dissected.	G+	Gl+	Infection rate per cent.	Sporozoite rate per cent.
*A. minimus**	3,283	50	29	1·5	0·9
A. leucosphyrus	1,296	?	12	?	1·0
*A. culicifacies**	303	2	...	0·07	...
A. jeyporiensis†	161	...	...	...	...
A. maculatus	287	1	...	0·4	...
A. philippinensis	686	2	1	0·3	0·1

*Dissections of these two species carried out in the Southern Shan States by U Tin (1928) and Singh (1940) are not included in this Table as gut and gland infections were not shown separately.

†Infections were, however, found in the Arakan region and Burma-Yunnan border.

All the authors are agreed that, of the above mentioned species, *A. minimus* is the most important vector of malaria in the whole region. In summing up the position in the hill tracts, Fox (*loc. cit.*) said that *A. minimus* is chiefly responsible for the intense post-monsoon transmission that occurs in all highly endemic foothill areas and in the equally important mountain valleys of the western hill range and the Shan Plateau. In areas where breeding extends through the pre-monsoon months, he suggested, it is probably responsible for pre-monsoon transmission as well.

The adult bionomics of vector Anopheles are of great importance in malaria control through the use of residual insecticides and it may, therefore, be profitable to discuss the subject here in some detail in respect of the above mentioned species.

Most of the work referred to above was done before the advent of D.D.T. and at a time when anti-larval operations were the only method available for malaria control. It is not, therefore, surprising that some studies were made on the ecology of anopheline larvae but none on adult bionomics which, therefore, merits some discussion here.

(*i*) *A. minimus.*—Macan (1948) has stated that *minimus* enters houses at about 9 p.m. and the peak is reached at midnight, but most of the feeding takes place after midnight. He also made the interesting observation that, in September, the blood-fed percentage collected at night was only 10 but, in October, the percentage increased to 39 under identical conditions, which is an indication of the increasing activity of the mosquito in the latter month.

It may, however, be emphasized that these collections were made in army camps where the use of mosquito nets was universal and, therefore, the number of unfed specimens must be high.

Fox (*loc. cit.*) found likewise that the invasion of houses by *minimus* started between 9 and 10 p.m. and the peak was reached at about midnight, after which it tailed off. But, feeding continued throughout the night, as catches made before midnight always contained 60 to 70 per cent of unfed specimens.

The same author has also observed that, when *minimus* enters the house for feeding, it rests on the inside wall for a long period before feeding.

The day-time collections in houses made in Burma by various authors are shown in Table III.

TABLE III.

Day-time collections of anophelines in houses in Burma.

Author.	Year.	Total anophelines collected.	Number of *minimus* collected.	Percentage of *minimus* to total.
Feegrade ...	(1926)	925	525	78·0
U Tin ...	(1928)	3,077	2,861	92·0
Idem ...	(1937)	2,749	2,723	99·3
Fox ...	(1949)	422	394	93·3

A. minimus is thus shown to rest in houses for a few hours before feeding and for a much longer period after feeding. Muirhead-Thomson (1940) stated that, in Assam *minimus* feeds and rests entirely in houses and is thus a thoroughly domestic mosquito but later he modified this view and remarked that, if window traps and such other methods were available in his Assam work, he would have been able to show conclusively whether the mosquito is as highly domestic as he had imagined (Muirhead Thomson, 1951). Macan (1948) in Burma said that "a proportion, possibly a large proportion, of *minimus* leaves the house after feeding and seeks resting places outside", though in villages situated in open country with no jungle in the immediate neighbourhood, it is common indoors. By examining the digestion stages of individual specimens, Fox (*loc. cit.*) concluded that, in the Mandalay-Maymyo foot-hills, a large majority of *minimus* that had fed on one night left the house the following night to rest out-doors, thus corroborating the findings of Senior White and Venkat Rao (1944) on the same group of anophelines in India.

It is thus shown that, during every gonotrophic cycle, *minimus* spends at least 24 hours resting in houses.

A. minimus is generally recognized to be a highly anthropophilous insect; however, there are no precipitin test records in Burma to prove this. Instead of precipitin tests, facilities for which are not easily available, Fox (*loc. cit.*) carried out extensive night collections in houses and cowsheds as the mosquitoes came to feed and, from the information so gathered, estimated the anthropophilism of various anopheline species. He considers that, provided the numbers are large, this method is just as reliable as the other and has the added advantage of no

special equipment being necessary. In this manner, he collected 846 *minimus* in houses against 51 in cowsheds and concluded that this species feeds predominantly on man and that, even when cattle are present, only a very small proportion is deviated from human beings.

The main breeding places are shallow sluggish streams, seepages and other running waters but Feegrade (1926*a*) has shown that during the rainy season when these waters are frequently flooded and flushed, larvae are found in low-lying ricefields and, to a limited extent, in small ponds and burrow-pits containing clear water.

(*ii*) *A. leucosphyrus.*—This Anopheles is usually associated with jungle conditions and had till recently been supposed to be somewhat a rarity. Christophers (1933), therefore, stated that it is not thought to play any part in malaria transmission in the Indian region. Clark and Chaudhury (1941) were the first to demonstrate its importance in Assam. They dissected 859 specimens and found 8 gut and 19 gland infections, giving an infection rate of 3.4 per cent and a sporozoite rate of 2.2 per cent. Subsequent observations made by workers during the war have shown that *leucosphyrus* is definitely important in the northern hill region of Burma (Macan, 1948; Kuitert and Hitchcock, 1948), though its status in the eastern hill region and the Shan Plateau is still uncertain.

Of the various races or varieties constituting the *leucosphyrus* complex, only the type form and *balabacensis* have so far been recorded in Burma. McArthur (1950) quoting Reid has stated that the form found in this country is the type form, which is by far the most widely distributed. Colless (1950) has, however, stated that he had seen specimens from Burma collected by Macan and Kuitert and that they were all *balabacensis*. Both forms are probably present in Burma, or Reid may have identified the specimens as the type form because he considered *balabacensis* as a synonym of the type form and not as a distinct variety. The identity of the form or forms present in Burma is thus left in some uncertainty but both the forms are known to be vectors in the areas of their distribution and may be considered together in the present context.

The day-time resting habits of *leucosphyrus* have not been studied in detail and the meagre information so far obtained is somewhat conflicting. Macan (1948) observed that it is mainly a jungle rester, though some specimens could be collected from tents occupied by troops. Clark and Chaudhury (*loc. cit.*) on the other hand found many specimens in houses but none in cowsheds. There is, however, a good deal of unanimity on the anthropophilism of this mosquito, all authors having agreed that it feeds predominantly on man but it appears to be a lazy insect, flying for not more than a hundred yards or so in search of a blood meal. The feeding time, according to Macan, is between 9 p.m. and midnight but other authors (Colless, 1956) have shown that more feeding occurs after midnight.

The behaviour of the mosquito in the house, when it comes to feed, has also been studied in detail by Colless (1956). He has stated that, though the mosquito does not rest in houses during the day, it rests for a little while on the walls before feeding and for a longer time, possibly till about day-break, after feeding.

The breeding places are stagnant collections of water such as burrow-pits, pools and swamps under thick cover or overhanging shade. Clear water pools in

the beds of jungle streams, also serve as good breeding places. When, however, the shade is removed, the breeding largely disappears.

(*iii*) *A. culicifacies.*—Feegrade (1926) found only one gut infection out of 113 dissected at Hsipaw. But U Tin (1928) and Singh (1940) dissected altogether 29 specimens in the Southern Shan States and reported an infection rate of 7 per cent. Whether these are gut or gland infections, has not been stated.

A. culicifacies is pre-eminently a vector of plains areas, specially irrigated tracts, and has not so far been found to be a vector of practical importance in hill regions. Fox (*loc. cit.*) considers that its importance in Burma is restricted to Central Burma and that elsewhere, it is virtually harmless. Discussion of this species will, therefore, be resumed in the next section when Central Burma will be considered.

(*iv*) *A. jeyporiensis.*—Both the forms of this complex are recorded in Burma. Christophers (1933) stated that he examined some specimens from Mandalay District and confirmed them as the variety, *candidiensis.* However, when dissections were made by various authors in this country, they did not maintain the distinction between the two forms and designated all specimens as *jeyporiensis* on the plea that the morphological differences between the two forms are too ill-defined for precise identification.

The evidence on the day-time resting places of this species complex appears to be somewhat conflicting. Macan and Watson (quoted by Fox (*loc. cit.*) failed to find adults in houses and cowsheds in Arakan whereas Feegrade (1925) was able to find them in some numbers in the Bhamo area.

In India, *jeyporiensis* can be found in houses and cowsheds but some specimens are also found in outdoor shelters (Senior White, 1947).

The *jeyporiensis* complex in Burma appears to be more anthropophilous than zoophilous, unlike in India where it is mainly zoophilous. More feeding takes place in the second part of the night than in the first. Its behaviour in houses during the short period of its stay there, has not been studied owing to paucity of numbers.

Larvæ of *jeyporiensis* have been found in shallow waters in valleys and other stagnant waters, and at the edges of slow running streams under shade.

(*v*) *A. maculatus.*—The only gut infection found in this species was at Lashio by Feegrade (1926*a*), who considered this as a secondary vector in that region. However, after scrutiny of all available information, Fox (*loc. cit.*) concluded that *maculatus* cannot be regarded as an important vector anywhere in Burma.*

There is very little information on the adult bionomics of this mosquito. It is rarely seen feeding on human beings and is absent from both houses and cowsheds during the day. Very few specimens are seen actually feeding on cattle too and Macan (*loc. cit.*) says that he was absolutely unable to find where *maculatus* was feeding.

(*vi*) *A. philippinensis.*—This species has a very wide distribution in Burma and is present in all regions, from Bhamo in the north to Moulmein in the south,

*Under the heading "The Delta Region", some evidence on the vectorial status of this species is furnished.

and from Kengtung in the east to Akyab in the west, but the only infection found was in the hill region at Bhamo.

Generally speaking, *philippinensis* is associated with the plains where weed-choked tanks are plentiful and is properly not an important mosquito of the hill tracts.

Observers in Burma are agreed that, in spite of prolific breeding, it is somewhat difficult to find *philippinensis* adults in good numbers in houses and cowsheds during the day. Its out-door shelters have not yet been ascertained.

There appears to be some diversity of opinion regarding the feeding habits of this mosquito. Macan (1948) observed that, in the Kabaw Valley, large numbers were seen to attack people sleeping on the verandahs of houses in the immediate vicinity of cattle. Macan and Watson (quoted by Fox, *loc. cit.*) stated that this species was the most abundant biter of man out of doors in certain areas before the rains and during the rainy season, it began to exceed *minimus* biting under cover. On the other hand, Fox (*loc. cit.*), in his night collections, was able to find only 84 specimens in houses as against 11,938 near cattle, denoting almost exclusive zoophilism. He, therefore, concluded that *philippinensis* cannot be regarded as a major vector in Burma. Probably, on account of certain peculiar conditions obtaining in Bhamo, *philippinensis* is a vector of purely local importance there.

The breeding places of *philippinensis* in Burma, as in India, are tanks and ponds with a fairly thick cover of vegetation like *Ceratophyllum demersum* and *Hydrilla verticilata*. In India, Iyengar (1944) found that *Eichhornia speciosa*, on the other hand, inhibits the breeding of *philippinensis*.

THE PLAINS REGION (DRY ZONE).

It has been agreed by various authors that a large part of this region is practically malaria-free and that the disease assumes importance in (*a*) the neighbourhood of foot-hill areas both in the east and the west and (*b*) in areas where extensive irrigation systems exist, malaria being hyperendemic or nearly so in both cases. In dry un-irrigatd areas, interest centres around periodical localised epidemics of a more or less severe nature. Malaria in the foot-hills is similar to that in the hill region with the same vector problems, which have already been described in the preceding section. The problem in the other two types of localities, is discussed below.

Spleen rates.—Feegrade (1930) carried out a detailed survey in nine villages constituting the " village tract " of Mezali in Minbu District. These villages are situated in latitude 20° 19 N and longitude 94° 30 E on the banks of Mon River and have a total population of approximately 4,000. He examined 469 children (age group 0-10 years) and found an over-all spleen rate of 41.6 per cent. He attributed this rather high spleen rate to " poverty, nearness to the river and heavy foliage " characteristic of the area.

Other data on spleen rates in this region are found in the old records maintained in the Harcourt Butler Institute of Public Health, Rangoon. The spleen examinations were made in most cases by sub-assistant surgeons on epidemic duty,

but in a few instances senior public health inspectors, without any special training in malariology, were also entrusted with this work.

According to these data, the small town of Madaya, just north of Mandalay, was shown to have a child spleen rate of 25·3 per cent among 87 children examined. In the Madaya Township, 45 villages were visited, in 30 of which the spleen rate was well below 50 per cent and, in the remaining villages, above that level. In Amarapura Township adjoining Mandalay, 36 villages were visited, of which 30 showed spleen rates below 50 per cent and the remaining villages above that figure. It is not known whether these spleen examinations were made in post-epidemic periods or during inter-epidemic years. The area as a whole is probably meso-endemic but this view cannot but be of a very general nature.

Parasite rates.—Feegrade (1930) found a parasite rate of 15.8 per cent among the children of Mezali Village tract. He encountered the three common species of malaria parasites and *P. ovale* " in almost equal proportions ". U Tin (1933), however, says that, at least in the irrigated tracts, M.T. is much more predominant than B.T.

Meteorological records.—The main meteorological feature of the dry zone is its low rainfall, which ranges between 20 and 40 inches annually. The Mezali area, however, seems to be better favoured in this respect. Feegrade collected rainfall records of this area for the years 1911 to 1928, which show an average of 47.8 inches per year. This is perhaps due to its being situated near the Arakan mountain range (on the western side of the same mountain range, the rainfall is 200 inches per year). Around Mandalay, the average annual rainfall is only about 30 inches. Hot summers prevail in the whole region, the maximum temperature during summer being over 113° F. The winters are often mild and pleasant, the minimum seldom falling below 42° F.

Transmission season.—Only a brief mention of the transmission season is made in the available reports. Fox (*loc. cit.*) has stated that the transmission season extends from July to March, with a peak in December or January. Other authors are in general agreement with this view.

Morbidity and mortality.—Records of morbidity are not available in this region chiefly because of lack of hospital and dispensary facilities. Even where there is a dispensary within a reasonable distance from a village, cases of malaria are not always reported there. Only cases of serious injuries are brought to hospitals and, in the words of Feegrade (1930), " all other ailments and minor injuries are left untreated or treated by the local " sesayas " (practitioners of indigenous system of medicine) who do not maintain any records.

As regards clinical forms of malaria, U Tin (1933) says that cerebral malaria is fairly common in certain parts of the region but blackwater fever is rare.

Records of mortality in the Mezali area during " non epidemic " years of 1918-1928 furnished by Feegrade (1930) show that, with a few exceptions, the mortality from " fevers " exceeds that from all other causes put together. Commenting on these figures, he says that, even allowing for grave errors in the classification of the causes of death by village headmen, the fact cannot be denied that fevers, chiefly of malarial origin, account for the largest proportion of the total mortality.

There is no information from other areas of the dry zone on this subject.

Epidemics of malaria.—The factors responsible for epidmics in this region are somewhat obscure; in some years they appear to have been associated with failure of the monsoon while in others extensive flooding has been held responsible. Occasionally, an extension of the irrigation system has been a precipitating factor (Fox, *loc. cit.*).

It has been emphasized that epidemics are likely to be superimposed on hyperendemic conditions in some of the irrigated tracts (U Tin, 1933).

Irrigation and malaria.—Malaria in irrigated tracts of Burma appears to have followed the same pattern as it did in India, as is shown in the area covered by the Mon River irrigation system. In this particular instance, the factors responsible are stated to be a radical interference with the natural drainage of an endemic area together with the introduction of new sources of infection in imported labour (Jolly, quoted by Fox, *loc. cit.*). In India, it has been shown that, as a result of unrestricted supply of irrigation water, the sub-soil water rises practically to ground level. The houses become damp, unhealthy and almost uninhabitable. Lack of proper drainage channels results in tanks and ponds remaining always full, and the seepages, arising out as a consequence thereof, create conditions favourable for the breeding of dangerous anophelines (Rao, 1945). The effects of irrigation on agriculture are at first favourable, bumper crops being harvested in the first few years but subsequently progressive deterioration will be observed, as the effects of water-logging, preventing soil aeration, begin to be felt (Covell, 1946). These remarks apply to this part of Burma in almost every detail.

Anopheline vectors.—Table IV shows the dissections made in this region by various authors:

TABLE IV.

Dissection of anophelines.

Species.	Number dissected.	G I	G I+	Infection rate per cent.	Sporozoite rate per cent.
A. aconitus ...	119	...	...	...	...
A. culicifacies ...	351	4	1	1·4	0·3
*A. minimus** ...	520	...	...	...	...

*All specimens are from foot-hill areas of this region.

In spite of negative dissections, *A. minimus* may be regarded as the principal vector of foot-hill areas. So far as the irrigated and unirrigated areas are concerned, the only gland infection found was in *A. culicifacies* which should, therefore, be strongly suspected and control measures applied accordingly unless, of course, further work shows up another and more powerful vector species. It may be remembered that, in India, *culicifacies* has shown to be the vector in many irrigated tracts, specially those which have been newly brought under irrigation.

A. culicifacies appears to be a highly domesticated Anopheles. In Pwinbyu, Feegrade (1930) recorded that this species made up 97 per cent of the day-time catches in houses and cowsheds, and adults were seen resting even in food store baskets in the smaller bamboo huts. Fox (*loc. cit.*) found more adults during day-time catches at Shwebo than in his night collections. He was always able to collect adults " corresponding in numbers to the larvae in breeding places ". No specimens were collected from outdoor shelters. These observations are in complete agreement with Senior White *et al.* (1945) who observed in India that *culicifacies* spends all but a very short part of its gonotrophic cycle in houses and cowsheds and that, in this respect, it differs entirely from the *minimus* group of mosquitoes.

Many workers have observed that *culicifacies* is mainly a zoophilous Anopheles. Fox refers to precipitin tests made on 14 blood smears of *culicifacies* by an army unit, which were all positive for cattle. Macan (1948) observed that, in the Kabaw Valley, *culicifacies* fed on men only when cattle were absent. The personal observations of Fox were very few and did not admit of generalization but he stated that the specimens, found resting in houses by day, had probably fed on cattle the previous night.

The precipitin tests obtained in India are somewhat disappointing. In South India, where *culicifacies* is the only vector, its anthropophilic index is only 2·5 per cent. In the Delhi area where it is a vector, the anthropophilic index ranged between 0·9 and 3·2 per cent according to the absence or presence of a sufficient number of cattle. On the other hand, in the Jeypore Hills where it does not play any part in transmission, the anthropophilic index is four times higher (Senior White, 1947).

In spite of a low anthropophilic index, this mosquito is capable of assuming the role of a major vector in certain areas owing mainly to its numbers. (Russell and Ramachandra Rao, 1942).

Its breeding places are shallow pools, burrow-pits and slow running streams containing clear water but no vegetation. Whether it is also breeding in the ricefields of irrigated tracts and, if so, whether the breeding is restricted to the first two months or so of the plant growth, has not been shown anywhere.

THE DELTA REGION.

Very little information about this region is furnished in the available records. Only some general observations are found in the reports of U Tin (1947) and Fox (*loc. cit.*).

Malaria incidence.—The distribution of malaria in this region is very patchy and the degree of endemicity varies from one locality to another, though the region as a whole is either hypo-endemic or only mildly mesoendemic. Towards the Pegu hills, however, there is a higher degree of endemicity but the area is rather sparsely populated. The seaward margin is mainly mangrove swamp but this area will be discussed in the next section dealing with coastal tracts.

A peculiar feature of this region is the occurrence of sharp localized epidemics at somewhat irregular intervals, often associated with failure of the monsoon.

As an example, U Tin (personal communication) has stated that a severe epidemic occurred at Insein near Rangoon in 1929. The spleen rate recorded, when the epidemic had subsided, was 100 per cent. Regarding the transmission season, Fox said that the seasonal incidence is not clearly defined but is " probably mainly post-monsoon, from September to December ".

The rainfall in this region is about 100 inches per year. The climate is mildly temperate, extremes of temperatures being pratically unknown.

Anopheline vectors.—The meagre information available indicates the presence of three vector species in different localities, *viz.*, *A. minimus*, *A. maculatus* and *A. culicifacies*. Probably, as elsewhere, foot-hill malaria is transmitted by *minimus*. No dissections of *culicifacies* are made and thus its complicity in epidemic outbreaks has not been proved. According to a personal communication from U Tin, three hundred *maculatus* were dissected in Insein during the 1929 epidemic, and two specimens were found with gland infections, giving a sporozoite rate of 0.66 per cent. This is, in fact, the first record of gland infections in *maculatus* in Burma. Whether *maculatus* is responsible also for outbreaks in other parts of this region, is not known.

THE COASTAL REGION.

For the sake of convenience in discussion, this region may be divided into three areas, *viz.*, the Arakan area, the coastal areas of the Delta and the Tensaserim coastal strip.

The Arakan area may be further sub-divided as follows: -

(*a*) A series of small mountain ranges, running parallel to the coast and covered by bamboo jungle and dense forest. This locality is sparsely populated.

(*b*) A hillock area formed by clay and shales, where perennial streams are abundant and the population larger in numbers; and

(*c*) The coastal strip, which narrows down from north to south and is cut into by numerous tidal creeks. The Ramree and Cheduba islands are also included in this locality. The largest and the most densely populated villages are found in this locality, where rice is grown wherever salt water can be excluded by drainage of tidal creeks.

MALARIA INCIDENCE: (i) *Arakan Region.*—There is not much information about the area covered by low mountain ranges. Malaria is probably hyper-endemic there, as in similar areas elsewhere in the country. The hillock area, where there are many perennial streams beyond the tidal influence, is also probably hyperendemic but the incidence is stated to be higher in the rural areas than in towns. Th coastal area and the islands off the coast, are known to be highly malarious.

In certain localities like the Ramree Island, malaria appears to have been introduced in recent times. Tyssul Jones (1950) observed that, in this island, malaria did not probably exist before the second half of the last century. Arakan was ceded to the British in 1826 and they established a military station at Kyaukpyu

(the chief town of Ramree Island) but later abandoned it for purely military reasons. There was no malaria among the troops. During the second half of the century, systematic working of oil wells was started, a road was built and the embankment already existing was repaired and raised in height. For these works, labour was imported in great strengh from Bengal, Orissa and Madras, several parts of which were notorious for malaria. The malaria history of the island begins at this time. Whether gametocyte carriers were introduced or vector Anopheles was transported in the process, is not known. In any case, the situation was so completely altered that, towards the end of 1895 the town of Kyaukpyu became highly malarious and the military police, who were brought in, were all stricken with the disease and had to be repatriated.

As the malaria situation deteriorated, investigations were carried out first by Lalor (1913) and subsequently by Williams and Jolly (quoted by Fox, 1949) and Feegrade (1924). All these authors found hyperendemic conditions and suggested, as remedial measures, reclamation of swampy areas around towns and villages and substitution of rice cultivation by vegetables and fruits. A limited drainage scheme was also recommended.

The last survey made by Tyssul Jones (*loc. cit.*) has shown that the situation has remained pratically unchanged, at least in the Ramree Island, for the last thirty years.

There is virtually no information about malaria in the coastal areas of the Delta.

The Tenasserim coastal area is a very narrow strip of land, projecting southward and lying between the coast and numerous jungle-covered hills running from north to south. It also contains the small delta of the Salween River. Being nearer to the equator than the rest of Burma, the climate is a little hotter but the rainfall is very heavy, usually exceeding 200 inches per year. There is a limited amount of rice cultivation, but the chief importance of the area lies in its rich mineral resources which are associated with the granite intrusions of the hills. Rubber is also grown extensively.

The only report about malaria in this area is made in a general way by U Tin (1947). He says that malaria is wide-spread in the whole area.

Spleen rates.—Lalor (1913) who carried out the first investigation of Arakan area, found spleen rates ranging between 60 and 87 per cent in the Ramree Island. About thirty years later, Tyssul Jones (*loc. cit.*) visited the same villages and examined 541 children. He found an over-all spleen rate of 46·8 per cent, the range being between 24 and 70 per cent. Out of eight villages examined, spleen rate exceeding 60 per cent was found in four villages and lower than 50 per cent in the others.

U Tin (1936) visited Sandoway town and examined a total of 804 children. The over-all spleen rate was as low as 13·9 per cent but he pointed out that, in certain parts of the town which are close to the breeding places, the spleen rate was 64 per cent. He extended the investigation to villages in the immediate vicinity of the town and found an over-all spleen rate of 33·1 per cent among 278 children examined. Of the six villages visited, the spleen rates were above 50 per cent in three (two of them showed 100 per cent) and below that figure in the other villages.

Parasite rates.—Data on parasite rates are available only for Sandoway. Altogether 187 blood smears were examined from the town, of which 7.5 per cent were positive. Of the 131 smears taken from the surrounding villages, 24.4 per cent were positive. There is no information about infant parasite rates in any of the available reports.

The species incidence shows a preponderence of *P. vivax*, followed in order of prevalence by *P. falciparum* and *P. malariæ*. Mixed infections were also observed on a few occasions.

There are no spleen and parasite rate records for the coastal areas of the Delta and the Tenesserim coastal strip.

Meteorological records.—The Arakan and Tenasserim areas are the wettest in the country, receiving an annual rainfall of over 200 inches. On the Delta Coast, however, the rainfall is less, being about 120 inches. The distribution follows the usual pattern of areas affected by the south-west monsoon. The first four months of the year are practically dry and the next five months receive over 150 inches, the rain then petering out gradually to the dry month of December.

The maximum temperature at Sandoway is 99° F. and the minimum 44° F. whereas the relative humidity ranges between 74 and 93 per cent. Similar conditions probably prevail in the other parts of the Arakan area*; thus both the temperature and relative humidity are favourable for transmission throughout the year. There are no records for the other two areas of this region, but there is no reason to suppose that conditions there are far different.

Transmission season.—Referring to hospital records in Sandoway, U Tin (1936) said that, besides the dry months preceding the monsoon, October, November and December are the most malarious months in the year. Fox (*loc. cit.*) has said that transmission is probably perennial, though it is very much reduced during the monsoon period. Summing up the records, Macan and Watson (quoted by Fox, (*loc. cit.*) have postulated as follows:—

(*a*) *Mountain area.*—A definite pre-monsoon season (April-May) with possibly some transmission during the monsoon;

(*b*) *Hillock area.*—A long pre-monsoon season (February-May) with a lower peak in the post-monsoon period till the cold weather sets in; and

(*c*) *Coastal area.*—Sporadic transmission at any period but there appears to be a definite increase associated with the spring and autumn equinoctal tides. The intensity of malaria varies from year to year and isolated epidemics are liable to occur.

Morbidity and mortality.—Accurate information is available only in the report of U Tin (1936) for Sandoway. The figures furnished by him relate to the years 1933-36. According to these figures, the number of cases, hospitalized for malaria, was never less than 20 per cent of the total indoor patients. During 1936, altogether 429 persons were admitted as in-patients, of whom 141 or about 33 per cent were for malaria.

*The minimum temperature in Ramree Island is definitely higher, being about 70° F. (Tyssul Jones, 1950).

The mortality figures are also significant. The percentage of deaths due to malaria, during the years 1927-36, exceeded ten. During 1936, which was most probably an epidemic year, the mortality was about 70 per cent.

Anopheline vectors.—Table V shows the results of dissections of some anophelines carried out in this region.

TABLE V.

Dissection of anophelines.

Species.	Author.	Year.	Place.	Number dissected	G+	Cl+	Infection rate (per cent)	Sporozoite rate (per cent)
A. minimus ...	U Tin	(1936)	Sandoway	36	...	...	...	...
A. minimus ...	Macan and Watson	(1944)	Akyab area	71	...	1	1·4	1·4
A. annularis ...	Lalor	(1913)	Kyaukpyu	742	...	5	...	0·7
A. annularis ...	Feegrade	(1944)	Akyab	573	3	2	0·9	0·3
*A. sundaicus** ...	Lalor	(1913)	Kyaukpyu	480	...	...	...	...
A. sundaicus	Feegrade	(1924)	Akyab	30	...	...	...	...
A. sundaicus ...	Macan and Watson	(1944)	Arakan	378	...	...	...	...
A. jeyporiensis ...	Macan	(1944)	Bengal-Burma Border	124	...	2	1·6	1·6

It will be noted that no dissections were made either in the coastal area of the Delta Region or in the Tenasserim coastal strip.

(*i*) *A. minimus* is probably the main vector in the montane and submontane areas of the region as a whole though *A. leucosphyrus* may also be suspected as a carrier of at least secondary importance in certain limited localities. The bionomics of these two species have been discussed in previous pages.

(*ii*) *A. annularis* exists all along the coastal areas of Arakan along with *A. sundaicus* and, as shown above, infections have so far been noted in the former but not in the latter species.

A. annularis has a very wide distribution in Burma, having been found in large numbers in all regions but it appears to be a vector only on the Arakan Coast and not elsewhere.† This Anopheles is highly zoophilous and feeds mainly on cattle. In his night collections, Fox (*loc. cit.*) obtained 49 specimens in houses and 319 in cowsheds, the proportion being 1 : 6·5 . Elsewhere the proportions

*According to unpublished reports, a few gut infections were recorded in this species at Akyab by an American team of workers during 1951-53.

†Outside Burma, infections are reported from (*a*) certain localities of Assam and Orissa in India and (*b*) Yunnan in China near the Burma border.

are different, varying from 1 : 6 at Toungoo to 1 : 50 in the Mandalay-Maymyo foot-hills. The precipitin tests made by Senior White (1947) in India have shown that its anthropophilic index there, is only about 1·5 per cent. There is, however, evidence to show that, when cattle are scarce or under certain other conditions described below, *annularis* is easily diverted to man.

The resting habits of *annularis* deserve some mention. Unlike in India where it is a domestic insect, *annularis* in Burma has been observed to be largely an outdoor rester. Feegrade (1930) noted adults feeding on cattle at night in Pwinbyu but did not find them in houses or cowsheds by day. In the Mandalay-Maymyo foot-hills, Fox (*loc. cit.*) obtained 298 adults in night collections, but not a single specimen was found during the day. Only small numbers were found by him in houses at Tamu. Tha Gyaw (1927) has recorded that adults could be seen during the day, resting in scrub jungle around their breeding places but not in houses.

The breeding places of *annularis* are tanks, ponds and burrow-pits overgrown with certain types of aquatic vegetation. During the late rainy season, when its maximum density is reached, breeding overflows into ricefields.

How such a markedly zoophilous insect could become responsible for highly endemic conditions, has not been properly understood. Commenting on his work in India, Senior White (1947) observed that, in areas where it is a vector as well as in areas where it is not, the anthropophilic index is about the same and is so low that it is inconceivable that the mosquito should play any part in malaria transmission at all. Probably the factors mentioned below will serve partly to explain the position:—

(*a*) In India, Senior White *et al.* (1943) postulated, on the basis of relative prevalence of larvae and adults, that the longevity of this mosquito increases during Septembr, October and the first half of November, during which period the surplus population overflows into houses rather than cowsheds. At this time, there seems to be an increased urge to feed on man.

(*b*) During the later part of the rainy season and in the autumn, a certain proportion of the *annularis* population, usually about 20 per cent, enter into a condition described as "gonotrophic discordance", whereby the mosquitoes are fixed in houses or cowsheds as the case may be for prolonged periods, as the ovulation period is greatly extended. Those specimens which are fixed in houses establish greater contact with man and are thus enabled to feed during the short periods when the parasites in human carriers rise in numbers (Venkat Rao, 1947).

There is some evidence of increased longevity in *annularis* of Burma during certain periods. Fox (*loc. cit.*) quoted Stott as stating that, in Mandalay, adults were more prevalent in houses during December-February although breeding took place throughout the year. Whether *annularis* in the Arakan is also dependent on the phenomenon of gonotrophic discordance for its ability to transmit malaria, requires investigation.

(*iii*) Besides the Arakan coastal area, *A. sundaicus* has been recorded also from Mingalun (Feegrade, 1933) and Pyapon (Singh, 1939), both in the southern delta region of Lower Burma. Dr. Ohn Pe, Senior Malariologist for Burma,

has informed the authors that he collected a few specimens in Asain village of Ye Township, just south of Moulmein on the Tenasserim Coast. Apparently *sundaicus* exists all along the sea coast of Burma.

The status of *A. sundaicus* in the Arakan area has recently been discussed in some detail by the present authors (Postiglione and Venkat Rao, 1956) and, therefore, the question will be dealt with only briefly here. Discussing the position of *sundaicus*, Fox (*loc. cit.*) stated that, though not a vector of regular annual importance, *sundaicus* is responsible for "sharp local outbreaks in different years at different places". In the course of an examination of the malaria situation in Ramree Island, Tyssul Jones (*loc. cit.*) remarked *on the basis of indirect epidemiological evidence* that *sundaicus* is the primary vector there, and not *annularis* as previously observed.

As no sporozoite infections have so far been found in *sundaicus* in this area, the observations of Fox (*loc. cit.*) and Tyssul Jones (*loc. cit.*) are obviously based on the notoriety of *sundaicus* in other regions, and cannot be regarded as having been proved facts for this country. Though *sundaicus* is generally a dangerous carrier in many regions, evidence has recently been adduced to show that, at least in certain localities, there are races or forms of the species which, if at all, are very poor vectors of purely secondary importance. Taylor (1943) has said that, in certain areas of Malaya, there was not always a direct correlation between *sundaicus* prevalence and malaria incidence and suggested that there might be two races, one being a vector and the other not. Some evidence was collected which showed that this hypothesis might prove correct but, owing to the world war, the work could not be proceeded with further. Senior White *et al.* (1947) have shown that, in the Chilka Lake area of India, there are two forms of *sundaicus*, one breeding in fresh and the other in saline waters and that the former was the most important vector there whereas the latter was practically harmless. [It has been subsequently shown that the fresh and salt water forms of both India and Sumatra can be distinguished from each other by certain morphological differences (Venkat Rao and Ramakrishna, 1950; Bonne-Wepster and Swellengrebel, 1953)].

There is thus nothing improbable in the presence of an innocuous form of *sundaicus* in Burma. If, on the other hand, *sundaicus* is really a vector here, negative dissections must have been due to pure chance. It may be noticed that dissections here were carried out at long intervals of time and sometimes in small numbers, not enough to show infections if the infection rate in the mosquito is low. The position is still obscure and requires further, may be prolonged, investigation.

(*ii*) *A. jeyporiensis* has been found with gland infections in only the Burma-Bengal border of this region and appears to be a vector of some importance in that area. As the type form has not been distinguished from the variety, it is not possible to determine which of the two forms is responsible for the transmission. Only *candidiensis* was established as a vector in the southern tip of India (Iyengar, 1934); elsewhere in that country, it is either absent or is prevalent but in small numbers. The type form constitutes the vast majority of the complex and has been repeatedly shown to be a non-vector. In Indo-China too, *candidiensis* but not *jeyporiensis* is the vector form (Toumanoff, 1936). It may, therefore, be necessary to establish the identity of the form in this region.

SUMMARY.

The malaria situation, as it was known prior to about 1951, is reviewed in the light of the information available, mostly in the form of unpublished records.

In the Hill Tracts, comprising about 60 per cent of the extent of Burma, malaria is hyperendemic and transmission lasts almost throughout the year but with two peaks, the first in April-May and the second in the post-monsoon period, the latter always being the higher. The winter months are markedly healthier.

The main malaria vector is *A. minimus* but, in the thickly wooded parts of the northern hill range, *leucosphyrus* is also an important vector.

The Plains Region is divided into three areas for an assessment of the malaria situation. Malaria is hyperendemic in foot-hill areas and is nearly so in irrigated areas. The unirrigated areas are free from endemic malaria. Periodical epidemics are, however, liable to occur in the last mentioned area as well as in irrigated areas. These epidemics are associated with both heavy and short monsoon rains. The transmission season extends from July to March, with a peak in post-monsoon months.

Whereas *A. minimus* is the undoubted vector in foot-hill areas, the vector in the other areas has not been definitely established. Only one gland infection has so far been observed in *A. culicifacies*, which is, therefore suspected as the culprit.

A large part of the Delta Region is generally malaria free. Hyperendemic malaria is restricted to the Pegu Hill range which, however, is sparsely populated. Localized epidemics have occurred in this region at somewhat irregular intervals and they are associated with failure of monsoons. The mosquito responsible for the epidemic outbreaks is not definitely located; *A. maculatus* was incriminated once in Insein.

The coastal region, extending from Arakan to the tip of Tenasserim, is generally hyperendemic. Malaria transmission is probably perennial but the bulk of it occurs in the dry months, the peak being observed in October-December.

The vector of mountain and hillock areas is *A. minimus* but, towards the Arakan border in the north, *A. jeyporiensis* (type form or variety, not known) is also concerned. In Moulmein, the presence of *A. leucosphyrus* has recently been located and this may also be taking part in the transmission, at least in that area.

The main vector of the coast appears to be *A. annularis*. Though *A. sundaicus* is present in fair strength in the Arakan, dissections have so far failed to prove its vectorial status. It now appears that this species extends into the coastal areas of the Delta and Tenesserim also. Further studies are indicated in order to prove whether *sundaicus* in this region is a vector or not.

The bionomics of the various vectors have been briefly discussed.

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SCREENING OF ANTIMALARIALS AGAINST *P. GALLINACEUM* IN CHICKS.*

Part VI. Evidence of two-fold† acquired resistance to chloroquine diphosphate in a strain of *P. gallinaceum* in chicks.

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DEVELOPMENT of high degree of acquired resistance to antimalarial drugs like proguanil and pyrimethamine in several species of avian, rodent, simian and human plasmodia has been demonstrated during experimental studies by a number of workers (Bishop and Birkett, 1947; Williamson *et al.*, 1947; Hawking and Perry, 1948; Schmidt *et al.*, 1949; Adams and Seaton, 1949; Cooper *et al.*, 1950; Hawking and Thurston, 1951; Jaswant Singh, Ray, Basu and Nair, 1952 and Jaswant Singh *et al.*, 1953:1954; Schmidt and Genther, 1953). Such high degree of resistance to other antimalarials has not been reported so far. For example, Knoppers (1947) was able to demonstrate only a two-fold resistance to quinine in *P. gallinaceum*, whereas Bishop and Birkett (1947) and Williamson and Lourie (1947) were unable to show any such acquired resistance to mepacrine. Similar unsuccessful findings were reported by Bishop and McConnachie (1952*a*) in respect of chloroquine of the 4-aminoquinoline series. With regard to pamaquine of the 8-aminoquinoline series, only a slight degree of acquired resistance in plasmodia was reported by Fulton and Yorke (1941) and Bishop and Birkett (1948).

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†Since submission of this paper, the strain has now (November, 1956) developed a three-fold resistance. Further details will be reported in due course.

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The present paper records the findings of the development of a two-fold acquired resistance to chloroquine diphosphate in a strain of *P. gallinaceum* after continuous exposure to the drug for nine months during which period 57 serial passages were made.

PROCEDURE.

The studies were undertaken in laboratory-hatched 7-day old, white Leghorn chicks infected with a strain of *P. gallinaceum*. The dose of inoculum (0.5×10^6 parasitized cells per gm. of body weight), technique of inoculation (through the right jugular vein), method of drug administration etc. were similar to those described earlier by Jaswant Singh, Basu and Ray (1952).

The chloroquine preparation used was avloclor (chloroquine diphosphate) received through the courtesy of Messrs. Imperial Chemical Industries (India) Ltd., and each dosage schedule consisted of a total of seven doses. The first dose was administered on the zero day (day of inoculation) followed by two doses daily for the subsequent three days.

On Day 4, parasites were counted against 10,000 erythrocytes (with the help of an Ehrlich's eyepiece) in thin smears stained with J.S.B. (Jawant Singh and Bhattacharji, 1944).

The procedure adopted was first to determine the smallest dose of chloroquine diphosphate (the M.E.D.) which would reduce parasitæmia in infected chicks to approximately 25 per cent as compared to that in the untreated batch at or near peak of infection (Class I effect of Shannon). Subsequently, treatment was commenced in a fresh batch of infected chicks, commencing with a dose lower than the M.E.D. and later passaging the infection when the peak infection was attained (Day 4).

As a rule, the same dosage schedule was repeated several times in succeeding batches, sometimes up to 12 times, before any further increase was made. This depended on the degree of parasitæmia. If any particular schedule was effective in reducing parasitæmia to a very low level, it was repeated many times in succeeding batches till there was evidence of significant rise in infection. Along with each treated batch, consisting usually of five to seven chicks, a similar batch of infected chicks was kept untreated for comparison.

OBSERVATIONS.

For determining the M.E.D. of chloroquine diphosphate, the dosage schedules adopted were 0·05, 0·0571, 0·0615, 0·066, 0·08, 0·088, 0·1, 0·114 and 0·123 mg. per 50 gm. of the body weight, which are equivalents to 1/32, 1/28, 1/26, 1/24, 1/20, 1/18, 1/16, 1/14, and 1/13 of the M.E.D. of quinine, (1·6 mg. per 50 gm. chick) ,respectively. It was observed that with doses of 0·05 and 0·0571, the parasitæmia reached beyond 60 per cent (average) of the comparison group. With 0·0615 (1/26 M.E.D. of quinine), it was between 19 and 33 per cent; with 27 per cent as the average. This was repeated several times. In all other dosage schedules, the infection varied from 2·5 to less than one per cent, proportional to the dosage schedules.

In some of the higher dosage schedules, there was complete clearance of parasites in some of the birds. On the basis of these findings, it was established that the M.E.D. of chloroquine diphosphate was 0·0615 mg.

The actual investigation was commenced with a dose of 0·05 mg. per 50 gm. chick, and it was repeated three times in succeeding passages in fresh batches of chicks. In all cases, the parasitæmia was fairly high ranging from 70 to 80 per cent of the comparison group. Similar findings were noted with a dose of 0.057 under which parasitæmia ranged between 62 and 70 per cent. After six passages, the dose was increased to 0·0615 mg. (M.E.D.) and the individual variation in parasitæmia was observed to be between 8 and 60 per cent. However, with a dose of 0.08 mg., significant reduction in parasites was detectable. This dosage was repeated 12 times and during the last two to three passages, the parasitæmia was found to rise up to about 30 per cent against a maximum of about 5 per cent in the early stages.

The same procedure was followed throughout till a dose of 0·123 mg. (1/13 of M.E.D. of quinine) showed increased parasitæmia during the last few passages. Up to two subsequent passages, with the treatment schedule increased to 0·133 mg. (1/12 M.E.D. of quinine), the parasitæmia was found to be of a much lower order (average six per cent).

At this stage, a ' check test ' was undertaken in which both the parent strain as well as the one exposed continuously to chloroquine, were used. The same dosage schedules were administered in both the series. Under each series, an untreated batch was kept for comparison, so that the degree of parasitæmia could be calculated as percentage of the comparison group. The results are tabulated in Table I.

TABLE I.

Data on ' Check test '.

Schedule number.	Dosage in mg. per 50 gm. chick.	Dosage equivalent of quinine.	PARENT STRAIN.	RESISTANT STRAIN.
			Percentage (average) of infection as compared to comparison group.	Percentage (average) of infection as compared to comparison group.
I	0.0571	1/28	66.4	75.0
II	0.0615	1/26	28.2	62.5
III	0.066	1/24	2.5	Not undertaken.
IV	0.114	1/14	0.018	33.3
V	0.123	1/13	0.069	27.0
VI	0.133	1/12	Not undertaken	6.0

From Table I, it may be noted that under Schedule I, the degree of parasitæmia in both the series was somewhat similar. However, significant

difference was noted in the next schedule under which the parasite count was less than half (28·2 per cent) in respect of the parent as compared to the resistant strain (62·5 per cent). Under schedules IV and V, the parasitæmia was observed to be extremely low (less than one per cent) in respect of the parent strain, whereas it was found to be 33·3 and 27·0 per cent, respectively, in the resistant strain.

In view of this, it was not considered necessary to find the degree of parasitæmia under Schedule VI so far as the parent strain was concerned. But in the resistant strain, the degree of parasitæmia was found to be significantly low (six per cent) at least during the initial stage (up to two passages). However, further studies are being continued under this schedule to observe if, after several more passages, the infection became high.

However, from the data now available, it is noted that the intensity of infection (28·2 per cent) under Schedule II (0·615 mg.) in the parent strain is about the same (27·0 per cent) in respect of the sub-strain under dosage Schedule V (0·123 mg.). It is, therefore, most likely that the sub-strain of *P. gallinaceum* has developed at least a two-fold resistance to chloroquine.

DISCUSSION.

Attempts to develop induced resistance to drugs of the 4-aminoquinolines in simian and human (*P. vivax*, Chesson strain) plasmodia were found to be unsuccessful (Schmidt *et al.*, 1949 and Cooper *et al.*, 1950).

More or less similar observations were reported by earlier workers in respect of avian malaria. Thomson (1948) was unable to develop acquired resistance to amodiaquine or chloroquine in *P. lophuræ* in chicks up to sixtieth passage. The initial dose of amodiaquine used by the above worker was 0·001 and 0·0013 per cent of the diet which, according to the data supplied, appears to be 0·093 and 0·12 mg. per 50 gm. chick, respectively. The same in respect of chloroquine was 0·002 per cent of the diet or 0·172 mg. per 50 gm. chick. In the final analysis, it was observed that no acquired resistance could be built up. However, the initial dosage schedules would appear to be rather too high, so much so that the doses suppressed the infection so strongly that even the maintenance of the strain was difficult and there was always the danger of losing the strain (Thomson, 1948).

Similar attempts made by Bishop and McConnachie (1952*b*) to develop resistance to chloroquine in *P. gallinaceum* in chicks, proved unsuccesful. The initial dose used by them was 0·04 mg. per 20 gm. of the chick administered daily (equivalent to 0·1 mg. per 50 gm.) . But subsequently the doses were increased mostly to two-fold each time (0·04, 0·08, 0·16, 0·2 and 0·4). Although, up to a dose of 0·08 mg., parasitæmia was high, in still higher dosage the density was found to be extremely low, so much so that the above workers considered it necessary to pass the strain alternately through birds receiving 0·16 mg., and birds receiving no drug.

From the data obtained from the present investigation, it would be evident that a two-fold resistance could be built up during the course of nine months after 57 serial passages.

On the face of previous experiences by earlier workers, the present findings would naturally appear to be rather surprising. Perhaps the explanation may be sought in the method of approach to the problem.

The usual procedure adopted for studies on the development of acquired resistance to antimalarials in rodent, simian or human, plasmodia is to commence treatment with sub-effective doses of the drug at the initial stages. This may either effect deceleration or temporary clearance of parasitæmia. After cessation of treatment and when sufficient amount of the drug has been excreted, there is usually rise in parasitæmia when the strain is sub-passaged and treatment commenced. Unfortunately the same procedure cannot be adopted in avian infection like *P. gallinaceum* as after the acute phase the chicks usually die on account of development of exo-erythrocytic forms. As such, the initial and subsequent dosage schedules should be so regulated that parasites are not completely knocked down and that sufficient number should be available for growth after sub-passages. This is perhaps very essential when dealing with drugs of the 4-aminoquinolines which are excreted very slowly.

During the present investigations, this principle was adhered to strictly in that the initial dose administered was quite small (0·05 mg. per 50 gm. chick) so as to allow parasitic development reasonably well, the increases in the schedules were made very slowly, and whenever there was any evidence of strong action on the parasites under any particular schedule it was repeated many times during subsequent passages and thus the parasites were kept exposed to drug continuously.

It is, therefore, likely that these factors helped essentially in the development of acquired resistance to chloroquine diphosphate even though it is at the moment only two-fold. However, the studies are continuing for evidence of further increase in resistance.

SUMMARY.

A dose of 0·0615 mg. per 50 gm. chick (equivalent to 1/26 the M.E.D. of quinine) of chloroquine diphosphate was established as the minimum effective dose against a strain of *P. gallinaceum*.

This strain was exposed to chloroquine diphosphate for a period of nine months during which period successive blood passages were made. The initial dosage schedule adopted was 0·05 mg. (equivalent to 1/32 M.E.D. of quinine). Subsequently, the schedules were increased gradually during sub-passages but not until the same dose was repeated several times (three to twelve times) in fresh batches of chicks.

A ' check test ' at the end of 57 passages showed that, in the parent strain the degree of parasitæmia, with a dosage schedule of 0·0615 mg., was almost the same in the sub-strain as with double the dosage (0·123 mg.), whereas under the latter dosage schedule parasitic density was extremely low (0·009 per cent) when the parent strain was exposed to it.

This indicated that a two-fold resistance to chloroquine diphosphate has been built up in the sub-strain. Further investigations are in progress.

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ESTIMATION OF B.H.C. IN SCRAPINGS FROM SPRAYED SURFACES.

BY

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Technical B.H.C. is a mixture of several isomers of hexachloro-cyclohexane, amongst which, gamma isomer is largely responsible for insecticidal activity (Slade, 1945). This insecticide has been extensively used both in agriculture and public health fields, and in order to determine its purity, adequate application, residual deposition, metabolism etc., several methods for quantitative estimation of B.H.C. have been successfully worked out. Important amongst these are, mass isotope dilution (Trenner *et al.*, 1949), ultraviolet spectroscopy (Davidon and Woodward, 1949), infra-red spectroscopy (Baasch, 1947; Baasch and Smith, 1947), partition chromatograhy (Aepli *et al.*, 1948; Ramsey and Patterson, 1946), polarographic analysis (Dragt 1948; Nakasima and Oiwa, 1950), biological assay (Furman and Hoskins, 1948), colorimeteric (Armstrong, *et al.*, 1951; Schechter and Hornstein, 1952), turbidimetric (Howard, 1947) and methods based upon total and hydrolysable chlorine (Goldenson and Sass, 1947; World Health Organization specifications for B.H.C., 1955; Bami, 1957). Extensive use of B.H.C. as a residual insecticide in several public health programmes in India, emphasized the necessity of having a simple and direct method for the estimation of B.H.C. on sprayed surfaces, preferably on the line of Alessendrini test for D.D.T. (Alessendrini, 1948: 1950; Bami, 1955) which has been successfully adopted for routine work. Amongst the analytical methods indicated above, estimation of B.H.C. through hydrolysable chlorine method, could serve as a simple and direct analytical procedure. This method has now been investigated and successfully adopted for estimating B.H.C. in scrapings, obtained from surfaces, sprayed with technical B.H.C. formulations.

Technical B.H.C. is stable when dry and is not affected by nitric acid or high temperature. However, it is very sensitive to alkalies and, in their presence, is rapidly dehydro-dehalogenated to give a mixture of isomeric trichlorobenzenes even at ordinary temperature.

$$\underset{\text{(B.H.C.)}}{C_6H_6Cl_6} \xrightarrow{\text{alkali}} \underset{\text{(trichlorobenzene)}}{C_6H_3Cl_3} + 3\,HCl \quad \text{(hydrochloric acid)}.$$

Amongst four major isomeric constituents of technical B.H.C., beta isomer is most resistant to alkali dehydrodehalogenation (Slade, 1945). On the other

hand, excessive alkali treatment may further dechlorinate trichlorobenzenes first formed (Linden, 1938). Goldenson and Sass (1947), while investigating dehydrodehalogenation of B.H.C. with alkali in acetone solution, recommended a period of two hours of refluxing in order to achieve quantitative conversion of B.H.C. into trichlorobenzenes. Similarly, the World Health Organization Expert Committee on Insecticides (1955) recommended at least an hour's refluxing to complete the above reaction. Considering that B.H.C. is as easily dehydro-dehalogenated as D.D.T. (for which only 15 minutes of reaction time at room temperature is sufficient), this aspect was reinvestigated. Lindane (pure gamma B.H.C.) and standard quality B.H.C. were dehydro-dehalogenated with one normal alcoholic potash in acetone medium with varying time and temperature of reaction (Bami, 1957). The results, calculated in terms of purity of the product, are given in Table I.

TABLE I.

Dehalogenation studies.

Serial Number.	Properties tested.	Lindane (Gamma B.H.C.) Per cent.	B.H.C. Technical (Standard product) Per cent.
1.	Gamma B.H.C. content	99·5	13·14
2.	Technical B.H.C. content (by total chlorine method)	99·99	98·9
3.	B.H.C. content by hydrolysable chlorine method		
	(*a*) 15 minutes at room temperature ...	96·7	91·2
	(*b*) 15 minutes at boiling water bath ...	99·99	98·9
	(*c*) 30 minutes refluxing	99·99	98·9
	(*d*) 60 minutes refluxing ...	99·99	98·9

It was evident that acetone helps in dispersion of B.H.C. in alkaline medium and the reaction is completed in 15 minutes of heating on a water-bath. Longer time for reaction and higher strength of alkali were found to be unnecessary. Acetone solutions of standard technical grade B.H.C. (World Health Organization specifications, 1955) containing 1·0 to 10·0 mg. of insecticide, were dehydro-dehalogenated for 15 minutes with 1 N alcoholic potash. The reaction mixture was acidified and treated with excess of 0·04 N silver nitrate solution. Its chlorine content was suitably determined by Volhards method (1874; 1878) as detailed later. The results were directly expressed in terms of amount of B.H.C. (Table II) using the formula given below:—

$$\text{B.H.C. in gm.} = (B-A) \times N \times \cdot 03546 \times \frac{100}{36 \cdot 58}$$

$$= (B-A) \times N \times \cdot 09694$$

where A = ml. of KCNS used for titrating the sample.
B = ml. of KCNS used for blank determination.
N = normality of KCNS.

In other series, varying amounts of B.H.C. in acetone solution were added to mud samples (0·5 gm. each) and the solvent evaporated. These mud samples, having known amounts of B.H.C. sorbed in them, were extracted by shaking with acetone and decanting. This acetone extract (nearly 20 ml.) was dehydro-dehalogenated with alcoholic potash in the manner described. The results of recovery of B.H.C. by this technique are also given in Table II, and it is evident that almost quantitative recovery of B.H.C. can be affected, although experimental variations are more significant for the lower dosage as indicated by coefficient of variation percentage. It was also observed that normal inorganic chloride impurities in mud had almost negligible effect on the final results, but in the case of muds known to have a high inorganic chloride content, it would be advisable to run the blank with an untreated sample of such mud. A suitable portion of the sprayed surface could therefore be scraped; the scraped material extracted with acetone and its B.H.C. content estimated by hydrolysable chlorine method as described later.

TABLE II.

Analysis of technical B.H.C. in wall-scraping by hydrolysable chlorine method.

B.H.C. taken (or sorbed in mud).	B.H.C. estimated directly.	B.H.C. estimated* in mud samples having given quantity of B.H.C. sorbed in them.				Coefficient of variation (Per cent).
		I	II	III	IV	
1·0 mg.	1·0 mg.	1·1 mg.	1·0 mg.	1·0 mg.	1·0 mg.	8·5
2·0 ,,	2·0 ,,	2·0 ,,	2·0 ,,	2·1 ,,	1·9 ,,	8·3
3·0 ,,	3·0 ,,	3·0 ,,	2·9 ,,	3·0 ,,	3·1 ,,	4·7
4·0 ,,	4·0 ,,	4·0 ,,	3·9 ,,	4·0 ,,	4·0 ,,	2·2
5·0 ,,	4·9 ,,	5·0 ,,	4·9 ,,	5·1 ,,	5·0 ,,	2·8
6·0 ,,	6·0 ,,	6·0 ,,	5·9 ,,	6·0 ,,	6·0 ,,	1·5
7·0 ,,	7·0 ,,	7·0 ,,	6·8 ,,	6·9 ,,	7·1 ,,	3·2
8·0 ,,	8·1 ,,	7·0 ,,	7·9 ,,	7·9 ,,	8·0 ,,	1·1
9·0 ,,	9·1 ,,	8·9 ,,	8·9 ,,	8·9 ,,	9·0 ,,	1·0
10·0 ,,	10·0 ,,	10·0 ,,	9·8 ,,	9·9 ,,	10·0 ,,	1·7

*As described in the text.

The area to be scraped for estimation of B.H.C. content was fixed at 5 × 5 cm. due to ease of handling, familiarity of the staff with this technique in connection with D.D.T. scraping (Alessendrini, 1948:1950 ; Bami, 1955) and variations in the current B.H.C. dosage schedules which would be effectively covered by this technique.

As in the case of D.D.T., about 0·5 gm. of the scraped material was most convenient for extraction. The extraction itself should be done with acetone C.P. (at least the acetone should be dry and colourless as far as possible), and after

each treatment with acetone, reasonable care should be exercised to avoid the mud being transferred along with the extract. Alcoholic potash solution should not be coloured. For this purpose, it could be made freshly every time or if need be, the alcohol (methanol or ethanol) be distilled over alkali (Bami, 1957) prior to its use. During reaction with alcoholic potash, there will be some reduction in volume due to evaporation of solvents, which is considered desirable, because it would aid in getting a sharper end point during the final titration. However, care should be taken that the solvent did not dry up completely. Treatment with excess of silver nitrate and digestion of the silver chloride precipitate on water-bath for 15 minutes, was also quite sufficient (Bami, 1957). The digested solution can be directly titrated with standard potassium thiocyanate (nearly 0·01N), using one ml. of 10 per cent ferric alum as indicator. Appearance of pale orange colour, which can be best seen against white background, would indicate the end point of the titration. The sensitivity of the end point, is improved by keeping the volume of the final titration solution as small as practicable. The blank determination can be done with pure acetone or the extract of an untreated sample of mud but normally there is practically little difference in these two values, and a direct blank is quite adequate, within the accuracy of the method.

The amount of B.H.C. estimated in 25 sq. cm. of the sample, may be multiplied by 36 in order to get mg. of B.H.C. per sq. ft. while its multiplication with 5 (assuming a gamma isomer content of 13 – 14 per cent) would directly offer gamma B.H.C. concentration on sprayed surfaces. In actual practice, in order to avoid individual calculations for each sample, the normality of KCNS can be so adjusted that value of $(B\text{-}A)$ in ml. would directly correspond to mg. of B.H.C. in the sample. With the present formula, potassium thiocyanate solution with a normality of 0·0103 would permit value of $(B\text{-}A)$ being directly read as mg. of B.H.C. in the sample. This has been found to be very convenient and a standard KCNS solution of a higher concentration can be directly diluted to this strength. The normality of KCNS can be standardized by Volhard's method, using sodium chloride A.R. (Bami, 1957) or by any other convenient technique. The laboratories, undertaking testing of chlorinated hydrocarbon type of insecticides, can conveniently dilute the standard reagents like silver nitrate and KCNS for the present determination.

LIMITATIONS.

The accuracy of this method is primarily dependent upon the accepted variable limits of hydrolysable chlorine for technical B.H.C. (World Health Ogranization specifications, 1955) which cover the range of 97.4 to 103.8 per cent purity. Coupled with this, are the factors like slower rate of dehydro-dehalogenation of beta isommer of B.H.C., chloride contamination of the acetone extract and accuracy of the end point determination with weak solutions. From the results in Table II, and the considerations listed above, it is evident that a variation of up to ±10 per cent in the final results, can be reasonably expected. However, for the intended purpose, this variation has no appreciable effect on the conclusions to be drawn. Some of the other aspects like number of samples to be analysed and interpretation of the results, have already been discussed in connection with D.D.T. scraping analysis (Bami, 1955) and are applicable to the present method also.

This method is only applicable to technical B.H.C. formulations, when used alone. B.H.C. formulations having gamma B.H.C. (Lindane) as the active ingredient, cannot be estimated by this technique as it is not sensitive enough for the purpose. The present technique could effectively estimate 18 mg. (2·5 mg. gamma B.H.C.) to nearly 684 mg. (95 mg. gamma B.H.C.) of technical B.H.C. per square foot of the sprayed area, which would effectively cover the variations in the dosage sprayed and also serve to detect any chemical deterioration of B.H.C. with time.

REAGENTS AND APPARATUS.

1. *Alcoholic potassium hydroxide I normal.*—Potassium hydroxide (56 gm.) was dissolved in minimum of water (about 30 ml.) and diluted with ethanol to a volume of one litre. (The reagent should be colourless or only lightly coloured. Ethanol can be replaced by pure methanol without any loss of accuracy).

2. *Silver nitrate* 0·04 *normal.*—Dissolve silver nitrate (6·8 gm.) in distilled water and make up the volume to a litre in a dark container.

3. *Nitric acid I normal.*—Nitric acid (specific gravity 1·42; 65 ml.) was diluted with distilled water to a volume of one litre.

4. *Potassium thiocyanate* 0·0103 *normal.*—Dissolve potassium thiocyanate A.R. (9·4 gm.) in distilled water and make it up to a volume of one litre. This reagent is approximately 0·1 N and should be standardised by any of the conventional method such as described by Bami (1957). Having known the exact normality of the concentrated solution, it is diluted to the required strength with distilled water. Volume of strong KCNS solution in ml. which should be diluted to 1,000 ml. in order to get the final normality of 0·0103.

$$= \frac{10 \cdot 3}{\text{normality of KCNS}}$$

(Owing to deliquescent nature of KCNS, exact weighing is not possible).

5. *Ferric ammonium sulphate.*—Ten per cent aqueous solution.

6. *Sodium chloride A.R.*

7. *Acetone C.P.* (colourless solvent grade would also be suitable).

8. *Volumetric flasks* (100 ml. and 1000 ml.).

9. *Pipettes graduated* (5 ml. and 10 ml.).

10. *Conical flasks* (100 ml. capacity).

11. *Test-tubes* 6 *inch* × ¾ *inch in wooden racks.*

12. *Burette* 50 *ml.* (with stand).

13. *Water-bath* (at least 12 × 12 inches).

14. *Balance analytical with weight box.*

15. *Laboratory glassware and hardware like, beakers, watch-glass, funnels, etc.*

PROCEDURE FOR TESTING OF B.H.C. IN SCRAPINGS.

1. Scrap thoroughly only 25 sq. cm. (5×5 cm.) of the sprayed surface with a penknife and collect about 0·5 gm. of the scraping.

2. Transfer the material to a test-tube and add acetone (5 ml.) to it. Shake well and allow the material to settle for 1 - 2 minutes. Decant the clear extract to a 100 ml. conical flask.

3. Acetone extraction, as above, is repeated three times more, and about 20 ml. of the extract is thus obtained.

4. Add alcoholic potash (2·5 ml.) and keep the flask on a hot water-bath for 15 minutes.

5. Cool the contents and add dilute nitric acid (5 ml.) followed by silver nitrate solution (4 ml.).

6. Digest the precipitate on a boiling water-bath for 15 minutes with occasional shaking.

7. Add ferric ammonium sulphate solution (1 ml.) after cooling and titrate the solution against 0·0103 normal potassium thiocyanate. The end point is appearance of pale orange colour which does not fade away. (This gives value of *A* in ml. of KCNS used).

8. Take blank mud sample (0·5 mg.) and proceed as above or take acetone (20 ml.) in a 100 ml. conical flask and proceed from step 4 to 7 as above. (This gives value of *B* in ml. of KCNS).

9. (*B-A*) gives mg. of B.H.C. present in the sample which can be multiplied by 36 to get mg. of B.H.C. per sq. foot of the sprayed surface (when technical B.H.C. with 13 - 14 per cent gamma isomer content has been used, the result may be multiplied by 5 to give mg. of gamma B.H.C. per sq. ft. of the sprayed surface).

Samples in batches of about ten, can be conveniently and efficiently handled simultaneously by this method, and for a given set of samples from the same locality a single blank is sufficient. The relevant data regarding each sample should indicate locality of sampling, type of wall surface, formulation and dosage applied, date of application and date of collection of the sample.

SUMMARY.

Dehydro-dehalogenation of technical B.H.C. has been used as a basis for evolving a simplified technique of B.H.C. estimation in scrapings collected from sprayed surfaces. According to this method, a sample representing 25 sq. cm. area, is extracted with acetone and the solvent extract employed for dehydro-dehalogenation with alcoholic potash. B.H.C. content was directly calculated from the amount of chlorine liberated which was estimated by Volhard's method. This method has a normal minimum accuracy of ±10 per cent and would cover a range of 18 mg. (2·5 mg. gamma B.H.C.) to nearly 684 mg. (95 mg. of gamma B.H.C.) of technical B.H.C. The method is not sensitive enough for pure gamma B.H.C. formulations.

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

Part III. Morphology of the developmental forms in *C. fatigans*.

BY

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JASWANT SINGH, Ramakrishnan and David (1950) and David and Nair (1955) recorded the finding of trypanosomes in the salivary glands of laboratory bred *C. fatigans* fed on sparrows naturally infected with *P. relictum*. A striking peculiarity of this species of trypanosome is that it is not patent in the blood of the avian hosts but the developmental forms could be easily traced in *C. fatigans* when fed on birds known to be previously infected naturally or artificially. Rats, monkeys and various species of birds were found susceptible to this parasite. Sites of infection due to this trypanosome, both in birds and mosquitoes (*C. fatigans*), were experimentally studied by Nair and David (1956). In this paper, the morphology of the developmental forms, as found in *C. fatigans*, is briefly described.

MATERIALS AND METHODS.

In attempts at experimental transmission and determination of the sites of infection in birds and mosquitoes (Nair and David, 1956), a large number of laboratory bred *C. fatigans* were fed on heavily infected birds, chiefly sparrows and nightingales. These mosquitoes were dissected in batches at periodical intervals, the first batch four hours and the last one 72 hours after their blood feed. The sites in the mosquitoes where the developmental forms of the flagellate were found, were recorded. At the same time, the different stages of developmental forms

seen, interval taken for these forms to appear after the blood feed of the mosquitoes and their morphology, were studied. J.S.B. stain (Jaswant Singh Bhattacharji, 1944) was used throughout for staining the parasites.

MORPHOLOGY OF THE DEVELOPMENTAL FORMS OBSERVED IN MOSQUITOES.

Smears made from the stomach contents 4 to 12 hours after the infective feed, showed on one occasion only a structure resembling a crithidial form of the parasite with very coarse granules scattered in the cytoplasm (Plate VI, Fig. 20).

The forms found in the stained preparations of the stomach contents (partially digested blood), 28 to 36 hours after the blood feed, are shown in Plate VI, Fig. 21 and Plate X, Fig. 48 to 51. These were the longest of the different forms found in the mosquitoes and appeared to represent the adult forms. They were thin and elongated in shape. The nucleus and the kinetoplast in majority of the forms, were located in the middle third of the parasite. The free flagellum varied from short to medium length. Coarse granules, scattered over the cytoplasm, were present.

In the smears made between 48 and 60 hours, leishmania (Plate VII, Fig. 23*a* to 23*e*) and crithidial (Plate VII, Fig. 33*a*, 33*b*) forms were made out.

In some dissections, made 10 days after the infective feed, midgut showed long adult forms (Plate VII*a*, Fig. 24 and 25*b*), elongated crithidial forms resembling almost the adult ones (Plate VII*a*, Fig. 25*a* and 26), dividing forms (Plate VII, Fig. 29*a* and 29*b*) and leishmania forms (Plate VII, Fig. 27*a*, 27*b*, 28*a*, 28*b* and 28*c*).

Hindgut in some mosquitoes, when dissected between fourth and nineteenth day of blood feed, appeared almost packed with the developmental forms of the parasite. Camera lucida drawing of its general appearance is shown in Plate IX, Fig. 47. A gentle pressure on the coverslip, over the packed gut, would force out the individual forms and scatter them widely. From these, developing leptomonads (Plate VI, Fig. 11; Plate VII, Fig. 31*a* and 31*b*; Plate XI, Fig. 53 to 56), and crithidial (Plate VII, Fig. 31*c*, 32*a* and 32*b*; Plate XI, Fig. 52) forms could mostly be made out.

In the rectal papillæ, four days after the feed, structures, similar to the developing leptomonad forms, were observed (Plate VII, Fig. 30*a* to 30*d*).

Infection in foregut was always in the form of culsters (Plate VI, Fig. 18). These were tightly packed and appeared as though the whole lumen was blocked. On pressure, they got scattered and with high dry lens, leptomonad (Plate VI, Fig. 17 and Plate VIII, Fig. 45), crithidial (Plate VI, Fig. 8, 10*b* and 13), dividing (Plate VIII, Fig. 43, 44 and 46) and adult stages could be made out. Very great plemorphism was observed among the adult ones, and consisted mainly of small slender (Plate VI, Fig. 4, 9*a* to 9*f*, 10*a*, 10*c*, 13; Plate VIII, Fig. 40 and 41), medium (Plate VIII, Fig. 39 and 42) and long (Plate VIII, Fig. 38) forms. Dissections made 5 to 32 days after the infective feed, revealed all these stages in the foregut and no correlation whatsoever could be noted between the appearance of any particular stage of the parasite and the day of dissection of the mosquito.

PLATE VI.

DEVELOPMENTAL FORMS OF THE TRYPANOSOME.

Adult (Slender forms) Metacyclic forms).—
From salivary glands: Fig. 1, 3, 7, 15, 16.
From foregut: Fig. 4, 9*a* to 9*f*, 10*a*, 10*c*, 13.

Cluster forms in foregut.— Fig. 18.

Adult (Medium sized).—
From salivary glands: Fig. 14.

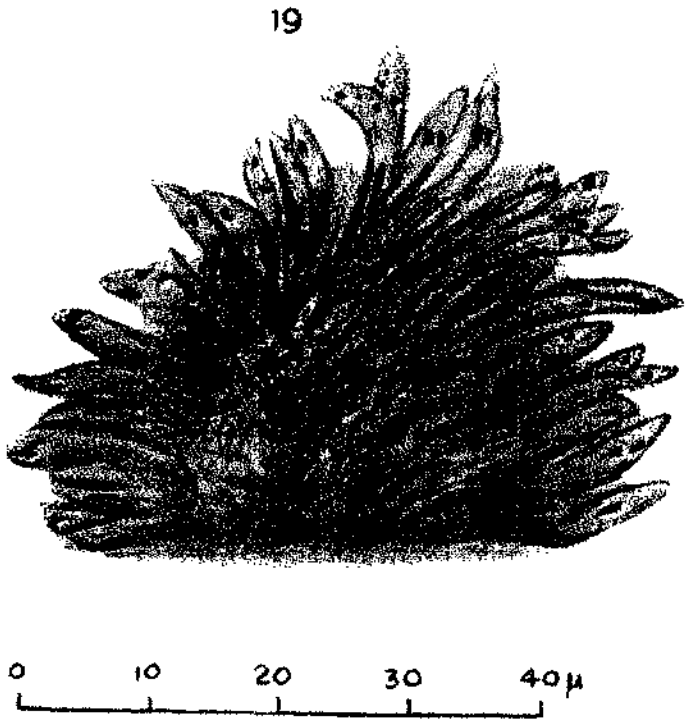
Adult (Long forms).—
From midgut: Fig. 21, 22.

Leptomonad from.—
From hindgut: Fig. 11.
From foregut: Fig. 17.

Crithidial forms.—
From salivary glands: Fig 2, 6*a*.
From foregut: Fig 8, 10*b*, 12.
From midgut: Fig 20.

Dividing forms.—
From salivary glands: Fig. 5, 6*b*.

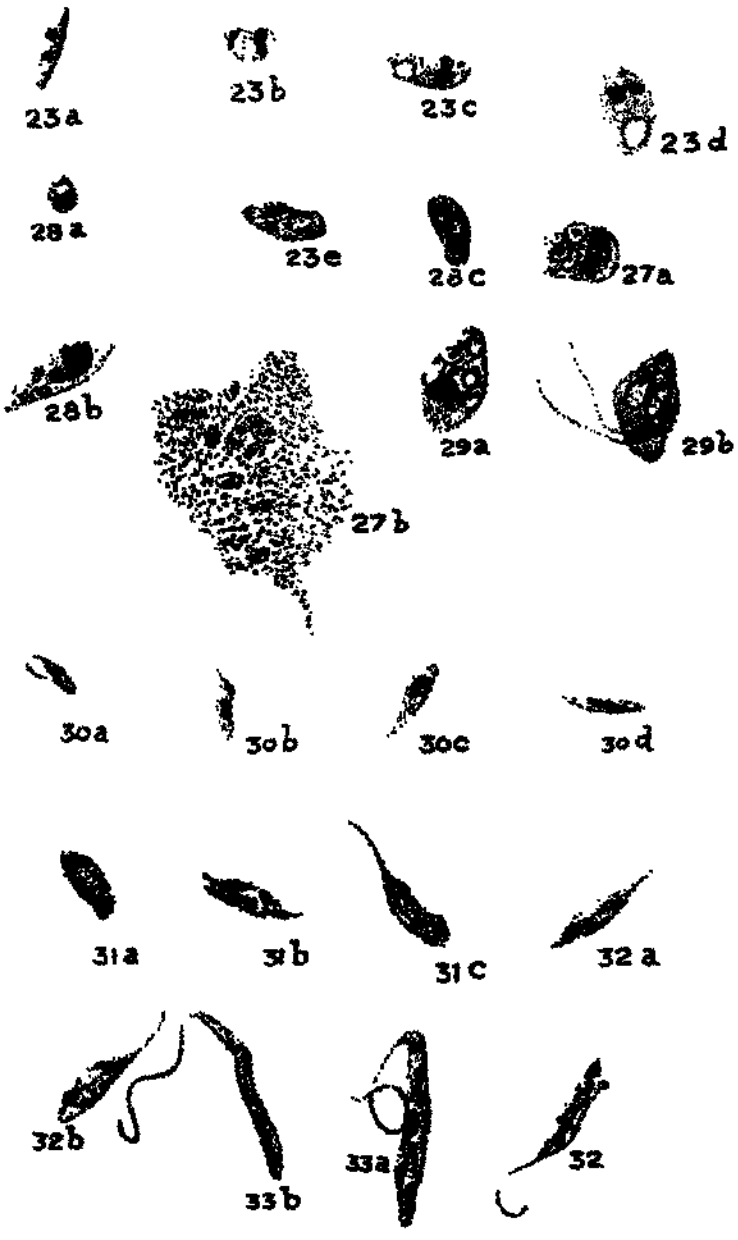
PLATE VIa.



Cluster arrangement of sporozoites. — Fig. 19.

PLATE VII.

DEVELOPMENTAL FORMS OF THE TRYPANOSOMES.



Leishmania forms.
From midgut: Fig. 23a to 23c.
27a, 27b, 28a to 28c.

Herpetomonad forms.—
From rectal papillae. Fig. 30a. to 30d.
From hindgut: Fig. 31a, 31b.

Davaine forms.
From midgut: Fig. 29a, 29b.

Crithidial form.—
From midgut: Fig. 33a, 33b.
From hindgut: Fig. 31c, 32a. 32b.

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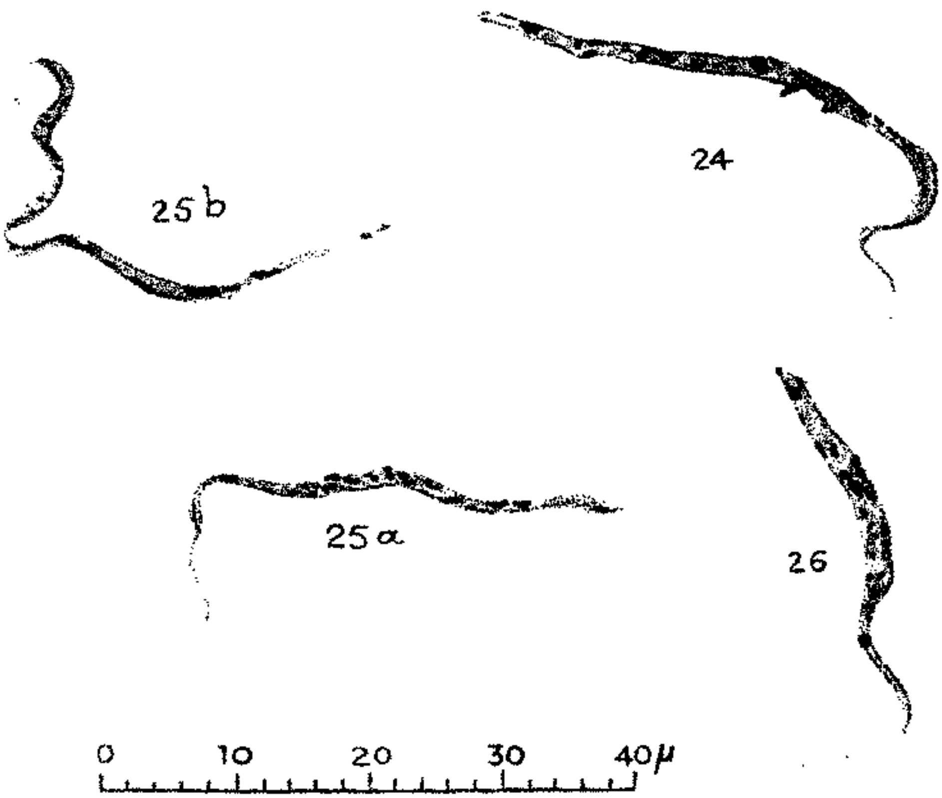
PLATE VIIa.

DEVELOPMENTAL FORMS OF THE TRYPANOSOME.

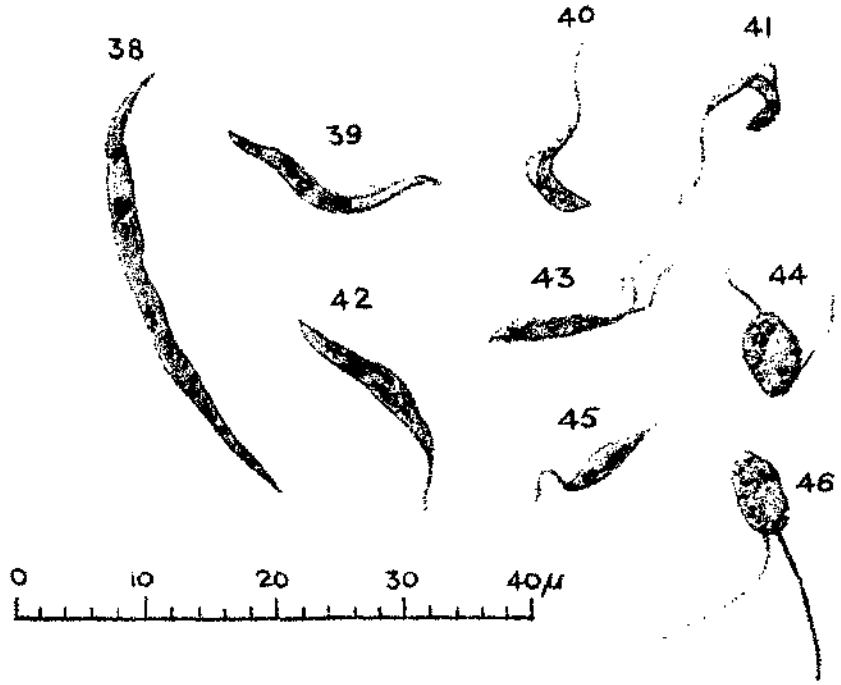


[illegible] forms.
From midgut: Fig. 25a, 26.

[illegible] Long forms.—
From midgut: Fig. 24, 25b.

PLATE VIII.

DEVELOPMENTAL FORMS OF THE TRYPANOSOME.



Adult Long forms -
From foregut: Fig. 38.

Adult Middle sized -
From foregut: Fig. 39, 42.

Adult Slelender forms ? Metacyclic forms -
From foregut: Fig. 40, 41.

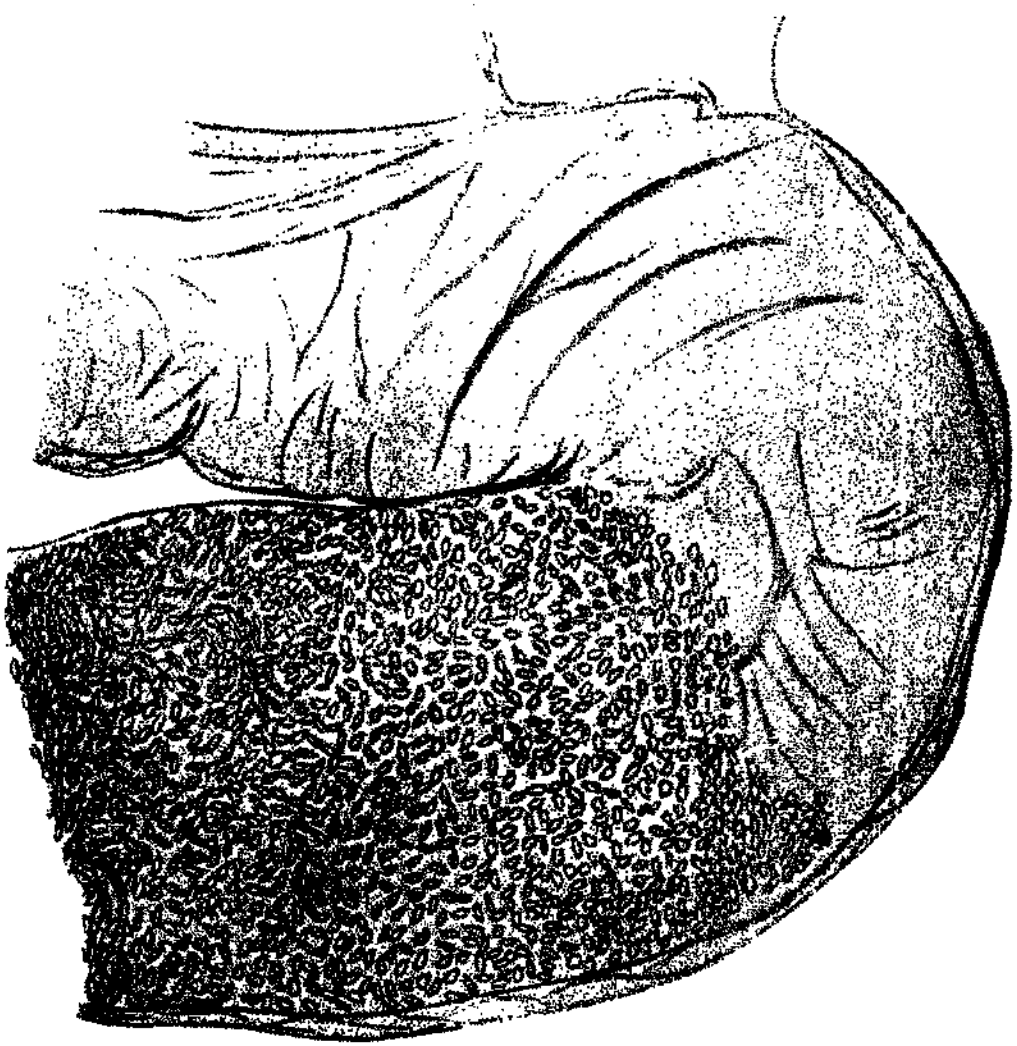
Dividing forms.—
From foregut: Fig. 43, 44, 46.

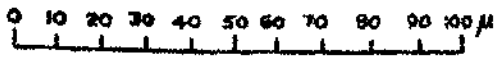
Leptomonad forms. -
From foregut: Fig. 45.

PLATE IX.

DEVELOPMENTAL FORMS OF THE TRYPANOSOME.

47

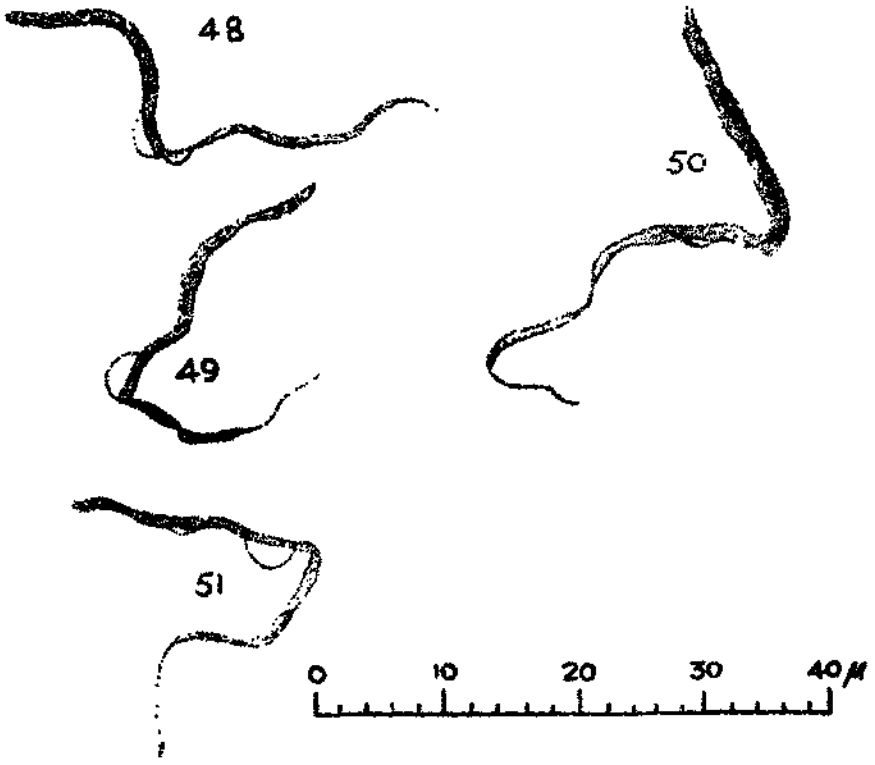




Hmalgui infection. General appearance. Fig. 47.

PLATE X.

DEVELOPMENTAL FORMS OF THE TRYPANOSOME.

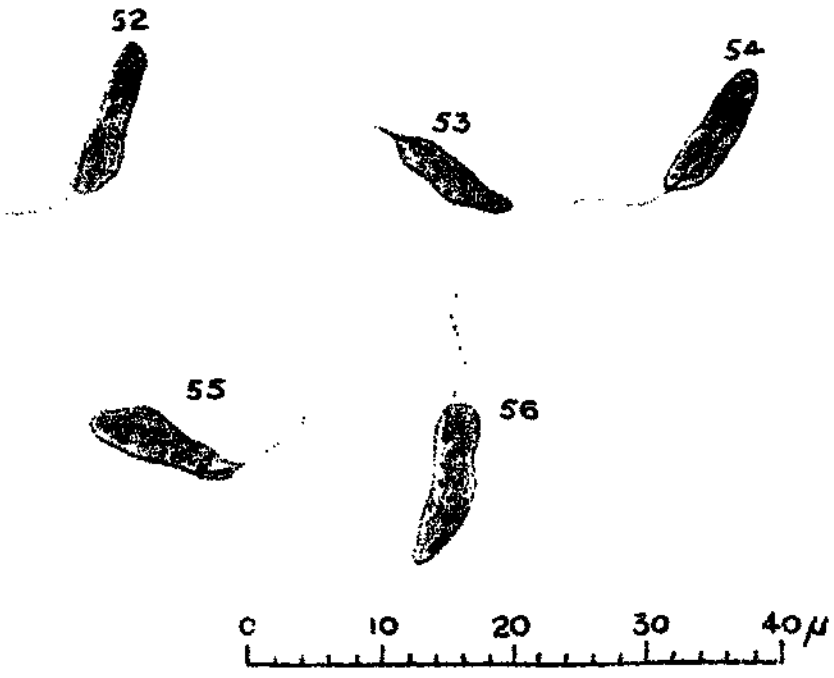


Adult (*Long forms*).
From midgut

Fig. 48 to 51

PLATE XI.

DEVELOPMENTAL FORMS OF THE TRYPANOSOME.



Trinidad farm.—
From hindgut: Fig. 52.

Leptomonad form.—
From hindgut: Fig. 53 to 56.

Infection in the salivary glands was also in the form of culsters (Plate VI*a*, Fig. 19). Breaking up of the culsters, with gentle pressure on the coverslip, revealed crithidial (Plate VI, Fig. 2 and 6*a*), dividing (Plate VI, Fig. 5 and 6*b*) and adult forms. The latter constituted mostly of the slender shaped variety (Plate VI, Fig. 1, 3, 7, 15 and 16), as previously described by Jaswant Singh, Ramakrishnan and David (1950) but a few were also of the medium sized type (Plate VI, Fig. 14). In some cases, crithidial and dividing forms were present even after 30 days, but in a few instances, adult forms appeared as early as eight to ten days.

The nucleus in the slender and medium sized adult, irrespective of the site of infection, appeared towards the posterior third, close to kinetoplast and was invariably thick and somewhat elongated. In all adults, one to two folds of undulating membranes could be made out. In the slender forms, the flagellum was comparatively long, but was short in the medium and long varieties. Coarse granules appeared in large numbers in the long type, whereas they were practically absent in the slender forms and only a few in the medium sized ones.

DISCUSSION.

From the general studies made regarding the morphology of the different forms found in different sites in the digestive tract of the mosquitoes and also in the salivary glands, the probable cycle of the parasite in the mosquito appears to be as follows. Presumably adult forms are taken up by the mosquito, and would seem to remain in the midgut without appreciable change for a period of 24 to 48 hours. Thereafter they undergo multiplication, during which period they go through the stages of leishmania, leptomonad, crithidial and dividing forms. From the midgut, these developmental forms pass on to the hindgut on occasions. From these sites, the developmental forms migrate to foregut. In this site, the final stage of development occurs and metacyclic forms are seen. As mosquitoes are probably not the suitable intermediate hosts, only a few parasites develop into these final stages. These as well as other stages, due to the same cause, adhere to each other by the flagellar end of the body, and thus agglutinated clusters are formed. Some of these forms, on occasions, succeed in reaching the salivary gland and there also a phenomenon of agglutination occurs. The metacyclic forms in the clusters, resemble the small slender adult forms.

SUMMARY.

Morphology of the developmental forms of the trypanosomes (that exist as a natural cryptic infection in sparrows) seen in *C. fatigans*, is given and the probable life cycle of the parasite in this mosquito discussed.

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Jaswant Singh, Ramakrishnan, S. P. and David, A. (1950) ... ... *Ind. J. Mal.*, **4**, p. 189.
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Indian Journal of Malariology, **10**, 4, *December 1956*.

FILARIASIS IN TRAVANCORE-COCHIN STATE.*

II. Shertallai Taluk.

BY

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[October 10, 1956.]

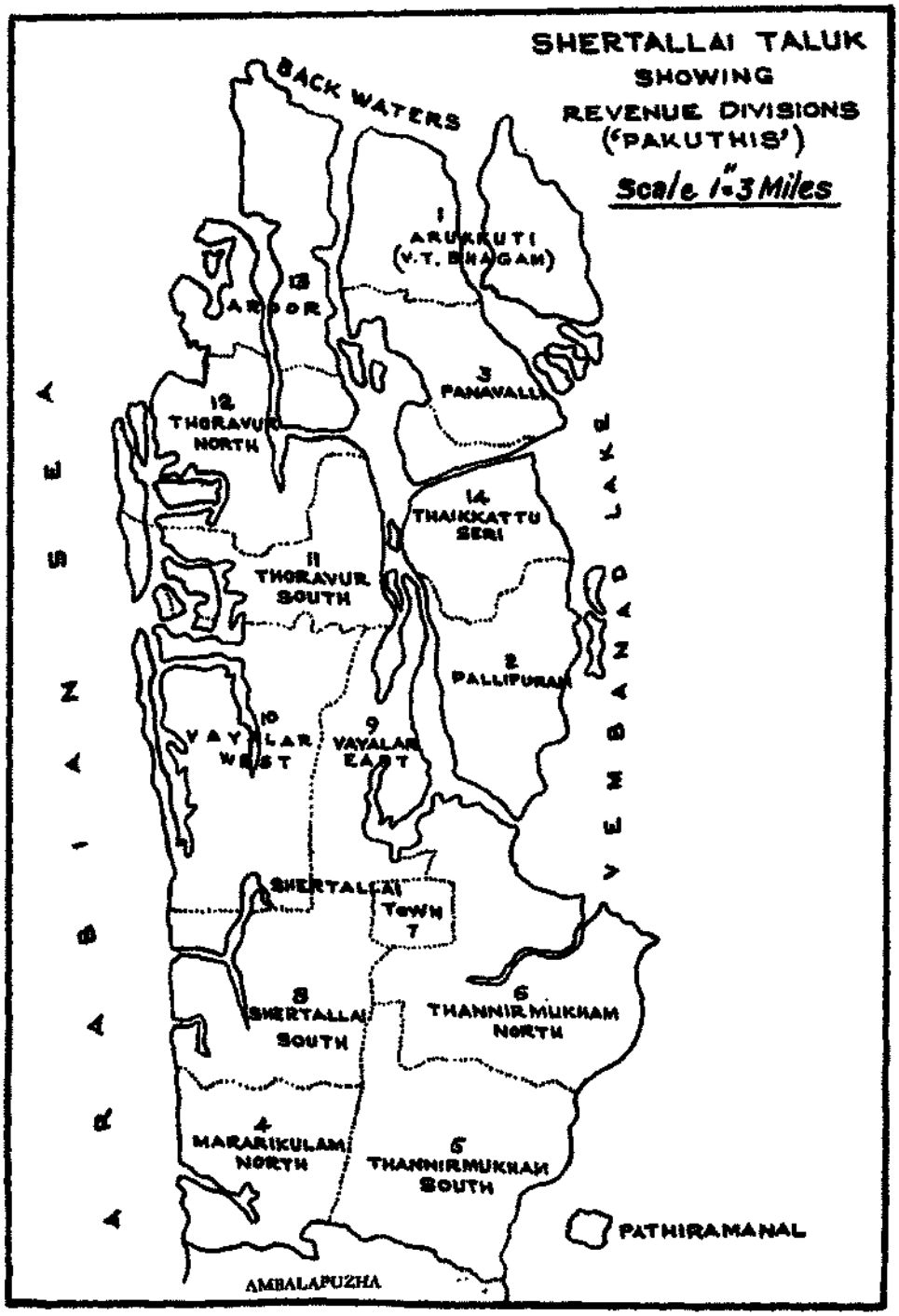
SHERTALLAI TALUK in Travancore-Cochin State, is located on the west coast of the southern end of Peninsular India. This taluk consists of two areas: (*i*) a coastal strip located between the Arabian Sea and the Vembanad Lake, continuous with the mainland on the southern side, and (*ii*) Pallipauram Island in the Vembanad Lake, to the north-east of the coastal strip. The entire taluk is notorious for its high filarial endemicity. The area of the taluk is about 124 sq. miles, and the population, according to the last census, is 2,54,774. The taluk has been divided for administrative and revenue purposes into a number of divisions called "*Pakuthies*", with Shertallai Town as its headquarters (Map I).

The entire taluk consists mainly of rural areas and there is no clear cut demarcation between one village and another. The houses are scattered, each having its own compound wall made of plaited coconut leaves. The dwellings in most places remain separated from one another by 50 to 100 yards, except in some bazaar areas on the roadside and in Shertallai Town where semi-urban conditions prevail.

The whole taluk is sandy and abounds in extensive, dense coconut plantations, which form the chief source of income to the residents. No part of the coconut tree is wasted—the different parts being utilized as timber, thatching, fencing, fuel, matting etc., oil and edible fats are extracted from the kernel of the nut, while the fibres are utilized for making coir. Coir manufacture is one of the chief cottage industries in the taluk.

* This survey was carried out at the request of the Government of Travancore-Cochin State.

MAP 1.


SHERTALLAI TALUK
SHOWING
REVENUE DIVISIONS
('PAKUTHIS')
Scale 1"=3 Miles
BACK WATERS
1 ARUVAKUTI (V.T. BHAGAM)
13 ARTHOR
3 PANAVALLI
12 THORAVUR NORTH
14 THAIKKATTU SERI
11 THORAVUR SOUTH
2 PALLIPURAM
10 VAYALAR WEST
9 VAYALAR EAST
SHERTALLAI Town 7
8 SHERTALLAI SOUTH
6 THANNIRMUKHAM NORTH
4 MARARIKULAM NORTH
5 THANNIRMUKHAM SOUTH
PATHIRAMANAL
AMBALAPUZHA

There are a number of ponds—at least one, often more in each compound—utilized mainly for watering the coconut plants and for retting the fibres. The coconut husk is kept soaked in the ponds for 4-6 months, when the soft parts rot and soften. The fibres are then separated by beating up the rotten husks with wooden clubs. During the process of such soakage, the organic content of the water goes up very high. This factor, together with the presence of pistia plants, makes conditions ideal for the profuse breeding of *Mansonoides sp.*, the vectors of *W. malayi* infection.

WATER SUPPLY AND DRAINAGE.

Sub-soil water level is high and is within a few feet below ground level. People in Shertallai depend on the numerous ponds for their water supply, both for watering the plantations and for domestic use. A majority of the ponds are overgrown with vegetation. There are few wells in the larger villages and in the semi-urbanised areas of Shertallai. There is no drainage, the waste water being led off either to the plantations or allowed to soak in the sandy soil.

METEOROLOGICAL CONDITIONS.

Like other parts of the West Coast, Shertallai has a damp, humid climate. The temperature remains between 70° and 90° F. and the humidity rarely falls below 60 per cent. The rainfall is heavy, the average being more than 100 inches. The monsoon sets in sometime towards the later part of May and continues till August-September. Intermittent showers, however, continue to occur till mid-November.

PREVIOUS SURVEYS.

Iyengar (1938) surveyed Shertallai and recorded a high incidence of filariasis, with endemicity rates ranging from 31·2 to 65·2 per cent in the different *pakuthies*. The average infection rate was 29 per cent and exclusively due to *W. malayi* infection (Table I). *W. bancrofti* was conspicuous by its absence in the taluk.

PRESENT WORK.

A detailed filariasis survey of Shertallai Taluk was carried out during the period March-May, 1955. Night surveys and entomological investigations were carried out with a view to elicit all details regarding the epidemiology of filariasis locally prevalent. The survey covered all the *pakuthies* of the taluk, examining random, representative samples, covering all age groups and both sexes by a house-to-house visit in selected parts of different villages between 8 p.m. and midnight.

Eight thousand four hundred and sixty-three persons, covering 3·3 per cent of the population, were examined during the survey for filarial infection and disease. External manifestations of filarial disease were met with among 2,011 persons,

TABLE I.

Filariasis survey of Shertallai Taluk (Travancore State); Iyengar (1938).

Index number in Map I.	Locality (*Pakuthi*).	Number examined.	Number positive for microfilaria (*W. malayi*).	Filarial infection rate per cent.	Number with filarial disease.	Filarial disease rate per cent.	Filarial endemicity rate per cent.
1	Arukutti ...	302	69	19·1	45	12·4	31·2
2	Pallipuram ...	420	115	27·4	53	12·6	38·8
3	Panavalli ...	300	72	24·0	58	19·3	41·0
4	Murarikulam North	289	78	27·0	94	32·5	52·2
5	Thannirmukkam South ...	470	123	26·2	93	19·8	41·9
6	Thannirmukkam North ...	680	199	29.3	280	41·2	60·9
7	Kakothamangalam	380	147	38·7	85	22·4	55·8
8	Shertallai south ...	510	191	37·5	151	29·6	59·0
9	Shertallai north ...	748	246	32·9	177	15·6	47·3
10	Vayalar East ...	92	25	27·2	39	42·4	65·2
11	Vayalar West ...	431	139	32·3	171	39·7	64·0
12	Turavoor south ...	302	95	31·5	73	24·2	53·3
13	Turavoor north ...	540	154	28·5	85	15·7	43·5
14	Aroor	510	103	20·2	88	17·3	35·9
15	Thykattusseri ...	370	100	27·0	41	11·1	37·0
	Total for Shertallai Taluk ...	6,404	1,856	29·0	1,473	23·0	48·1

while 1,766 persons showed microfilariæ in their night blood. The disease rate recorded is likely to be slightly higher than the actual figure as the sample was, to some extent, biassed by a number of over-anxious people with elephantoid swellings coming forward for being examined. These people had to be included in the routine survey in the interest of maintaining good public relations.

The parasite rates in the different parts varied from 9·4 to 33·2 per cent. No part of the taluk was free from filariasis. The average microfilaria rate for the taluk was 20·8 per cent. The disease and infection rates in the different *pakuthies* of Shertallai are presented in Table II.

An analysis of the disease and infection rates in the different age groups are given in Table III and Graph 1.

TABLE II.

Filariasis survey of Shertallai Taluk.

Index number in Map 1.	Area surveyed.	Number of persons examined.	Number with disease.	Number with microfilaria.	Disease rate.	Infection rate.	Endemicity rate.	Average infection rate.
7	Shertallai Town ...	1,059	111	190	10·5	17·9	28·3	23·0
8	Shertallai South ...	688	170	228	24·7	33·1	56·0	40·3
6	Thannirmukam North	864	97	84	11·2	9·7	20·9	32·2
5	Thannirmukam South	598	217	125	36·3	22·8	53·9	32·0
10	Vayalar West ...	839	258	189	31·1	22·5	53·1	34·8
9	Vayalar East ...	315	99	88	31·4	27·9	57·1	34·3
1	Arukutti	275	16	25	5·9	9·0	14·9	21·8
14	Thykattusseri ...	314	48	64	15·3	20·4	34·7	30·9
2	Pallipuram ...	563	113	112	20·1	19·9	37·9	22·3
3	Panavalli ...	514	84	110	16·3	21·4	35·4	27·0
4	Murarikulam North	638	247	185	37·1	29·0	62·4	40·5
11	Thuravoor South ...	340	114	84	33·5	24·7	57·1	27·9
12	Thuravoor North ...	705	248	115	35·2	16·3	50·5	30·1
13	Aroor	751	189	167	25·2	22·2	45·0	20·2
	Total for Shertallai Taluk	8,463	2,011	1,766	23·8	20·9	42·9	33·7

From Table III and Graph 1, it will be noted that no age group is exempt from filarial disease. The disease rate in the lowest age group (1 - 5 years) is 2·7 per cent., and shows a gradual steady rise with advancing years, reaching 45·4 per cent in the age group above 50 years. The microfilarial infection rate, from 13·5 per cent in the youngest age group, reaches 23·5 per cent in the adults (21 - 30 years) and then shows a slight decline, remaining steady at about 20 per cent in the adults.

The youngest age, at which microfilaria was detected, was in a girl one year old in Vayalar West *pakuthi*. Elephantoid swelling of both legs was recorded in a boy three years old in Thuravoor North.

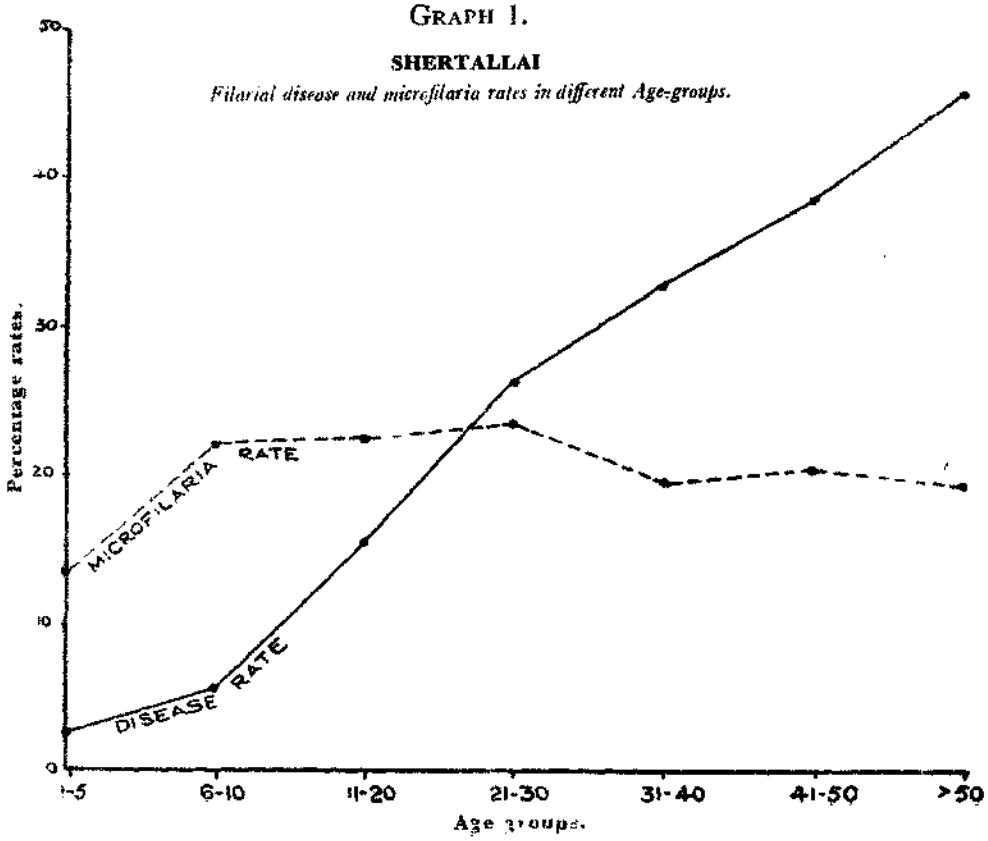
The microfilaria rate is low among the persons with manifestations of filarial disease, in comparison with that among the apparently normal persons. During

TABLE III.

Disease and microfilaria rates in the different age groups in Shertallai.

Age group.	Number of persons examined.	Number with disease.	Number with microfilaria.	Number with both.	Disease rate per cent.	Infection rate per cent.
1–5 years ...	819	22	111	2	2·7	13·5
6–10 ,, ...	915	59	202	8	6·5	22·0
11–20 ,, ...	1,739	270	390	29	15·5	22·4
21–30 ,, ...	1,977	519	465	46	26·2	23·5
31–40 ,, ...	1,265	417	250	28	32·9	19·7
41–50 ,, ...	894	344	182	16	38·4	20·3
Above 50 years ...	854	389	166	28	45·5	19·4
Total	8,463	2,011	1,766	157	23·8	20·9

GRAPH 1.



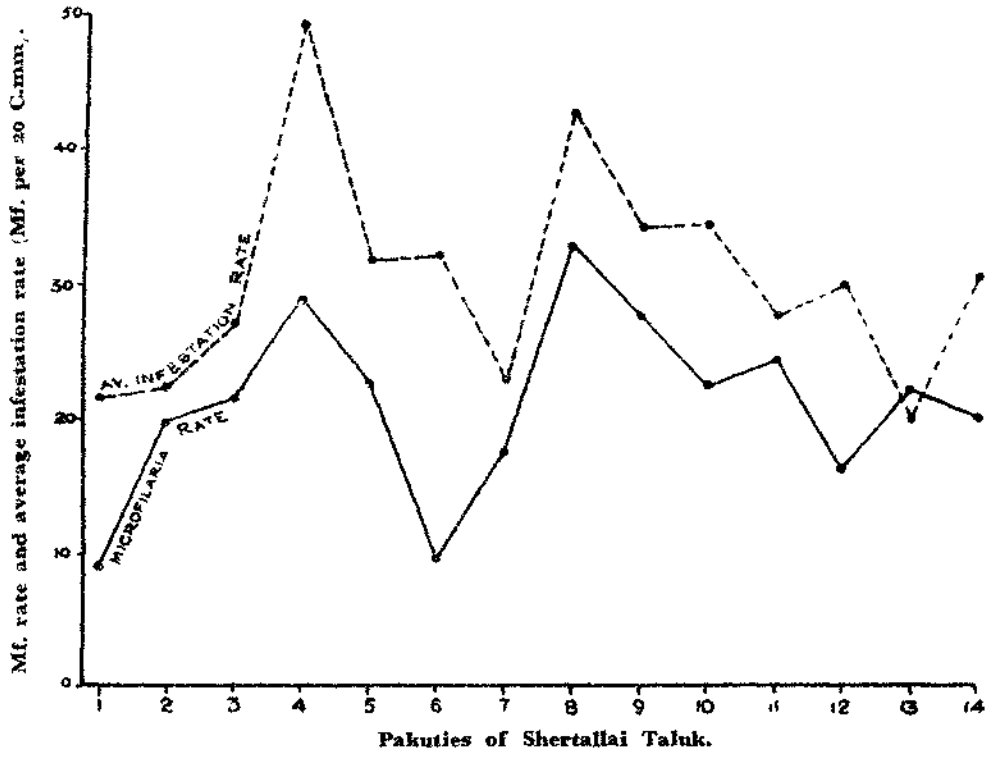
the present survey of 6,452 apparently normal persons, 24·9 per cent or 1,609 persons had microfilaria in their night blood; while only 7·8 per cent or 157 of 2,011 persons with disease, were positive among those with disease manifestations. The question whether the lowering of the microfilaria rate in persons, with disease, is a result of any possible immunity mechanism, has been discussed in general by Iyengar (1938).

The average infestation ranged from 20·2 to 49·5 microfilariæ per 20 c.mm. in the different *pakuthies*, the average for the taluk being 33·7 microfilariæ per 20 c.mm. of blood. There appears to be a positive correlation between the microfilaria rates and microfilarial infestation rates in the different *pakuthies* of Shertallai (Graph 2).

GRAPH 2.

SHERTALLAI.

Microfilaria rates and average infestation rates in different areas.



Iyengar (1938) recorded a prevalence of exclusively *W. malayi* infection throughout the taluk. In the present survey, however, sixteen cases of *W. bancrofti* were also recorded from Shertallai Town, seven of them in association with *W. malayi* infection. It is interesting to note that with the advancing urbanisation, *W. bancrofti* has started establishing itself in this town. It would be worthwhile

instituting periodic surveys in this town to watch the progress of *W. bancrofti* infection with the advance of urban conditions.

Of the 8,463 persons examined in Shertallai, 2,011 showed visible external signs of filarial disease and/or gave a history of acute attacks of filarial fever. An analysis of the types of filarial disease processes, is as follows:—

Elephantiasis of one or both legs	1,298	persons
,, ,, ,, ,, hands	44	persons
,, ,, ,, ,, legs and hands ...	142	,,
Filarial scrotum	11	,,
Fever and lymphangitis	516	,,

The rarity of genital lesions in *W. malayi* cases was in accordance with the observations recorded by Iyengar (1938) and Raghavan and Krishnan (1949). These authors, however, had recorded that persons with genital lesions in *W. malayi* areas were imported cases from *W. bancrofti* areas. In the present observations, however, two of the five persons, with genital lesions, confirmed having always lived in Shertallai Taluk all along.

ENTOMOLOGICAL OBSERVATIONS.

The following species of mosquitoes were collected from Shertallai Taluk:—

C. fatigans, C. vishnui, C. gelidus, C. sitiens, Lutzia fuscanus, M. annulifera, M. uniformis, M. indiana, Armegeres obturbans, A. subpictus, A. jamesi, A. vagus and *A. barbirostris.*

Specimens of all the above species were dissected but developing filarial forms were found only in *M. annulifera* and *M. uniformis.* The details of dissection are given below:—

Species.	Number dissected.	Number positive.	Percentage positive.
M. annulifera ...	1,830	92	5·03
M. uniformis ...	62	1	1·64

The important vector of *W. malayi* in Shertalai Taluk was *M. annulifera; M. uniformis* playing a secondary role. The favourite breeding places of the vectors were the ponds with *Pistia stratiotes* and especially those where the coconut husks were allowed to rot.

Manual removal of *Pistia* has been in force, as a method of control of *W. malayi,* in some parts of this taluk for nearly 20 years. A comparison of the filarial endemicity in the various parts of the taluk, as determined by Iyengar (1938) and the present survey, is set out in Table IV. Except in the *Pakuthies* of Arukutti and Thannirmukham North, this index would be noted to have remained unchanged.

TABLE IV.

Indices of filarial endemicity of different pakuthies in Shertallai Taluk in 1938 and 1955.

Locality.	INFECTION RATE, PER CENT.		DISEASE RATE, PER CENT.		ENDEMICITY RATE, PER CENT.	
	1938	1955	1938	1955	1938	1955
Arukutti ...	19·1	9·0	12·4	5·9	31·2	14·9
Pallijuma ...	27·4	19·9	12·6	20·1	38·8	37·9
Panavalli ...	24·0	21·4	19·3	16.3	41·0	35·4
Mararikulam North	27·0	29·0	32·5	37·1	52·2	62·4
Thannirmukkam South	26·2	22·8	19·8	36·3	41·9	53·9
Thannirmukkam North	29·3	9·7	41·2	11·2	60·9	20·9
Shertallai South ...	37·5	33·1	29·6	24·7	59·0	56·0
Vayalar East ...	27·2	27·9	42·4	31·4	65·2	57·1
Vayalar West ...	32·3	22·5	39·7	31·1	64·0	53·2
Thuravoor South ...	31·5	24·7	24·2	33·5	53·3	57·1
Thuravoor North ...	28·5	16·3	15·7	35·2	43·5	50·5
Aroor	20·2	22·2	17·3	25·2	35·9	45·0
Thykathusseri ...	27·0	20·4	11·1	15·3	37·0	34·7
Total for Shertallai Taluk ...	29·0	20·9	23·0	23·8	48·1	42·9

ACKNOWLEDGEMENTS.

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INCREASED CITRIC ACID CONTENT IN RESISTANT *CULEX FATIGANS*.

BY

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INTRODUCTION.

MANY workers have attempted to explain the complex mechanism of resistance to D.D.T. in insects. It has been found that resistant houseflies are able to convert D.D.T. to D.D.E.—a relatively non-toxic derivative (Perry and Hoskins, 1950:1951; Lindquist *et al.*, 1951; Fletcher, 1952). The detoxifying enzyme has also been isolated from resistant flies (Sternburg, Vinson and Kearns, 1953). This theory, however, fails to explain how and why after partial detoxification of D.D.T. *in vivo*, the remaining unchanged D.D.T. which will kill susceptible flies, does not exert its lethal action on resistant flies (Perry and Hoskins, 1950:1951; Winteringham, Loveday and Harrison, 1951; Fletcher, 1952). The unchanged D.D.T. is found scattered in various tissues of the resistant flies (Hoskins, 1952).

Fullmer and Hoskins (1951) have found that exposure to D.D.T. causes much greater increase in the rate of respiration in susceptible flies than in resistant individuals. There is also some evidence to show that D.D.T. inhibits the respiratory enzyme cytochrome oxidase. The resistant flies contain more of this enzyme and thus are able to withstand a partial blocking of this vital metabolic pathway. This observation clearly suggests the important rôle played by oxidation-reduction reactions in D.D.T. resistance, possibly as envisaged by Sacktor (1950) through its link with the D.D.T. converting enzymatic mechanism.

The observations recorded above and most of the knowledge on resistance is based on studies on houseflies (*Musca domestica L.*). The present communication attempts to point out certain biochemical changes that occur in D.D.T.-resistant strain of *Culex fatigans*.

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MATERIALS AND METHODS.

A brief résumé of some of the salient points about D.D.T.-resistant strains of *C. fatigans* has already been reported by one of the authors (Mohan, 1955). The high level resistance, exhibited by the adults, was also shown by the larvæ which were at no time subjected to the action of D.D.T.; the adults only were exposed to D.D.T. at each successive generation. The concentration of D.D.T. which caused 100 per cent mortality among larvæ of the normal strain, was found to have little or no effect on larvæ of the resistant strain of the same age, under identical conditions.

In these experiments, resistant insects were of the fiftieth and fifty-second generation. Usually 30 specimens each of adults, pupæ and larvæ of both the strains, were used for the determination of citric acid. The glucose-fed adults were killed with chloroform and pupæ and larvæ in warm water at about 50° C. The samples were then dried in air oven at 70°C. for two to three hours. It has been shown by Dikshit, Joshi and Patwardhan (1956) that such heating did not affect the citric acid content but on the other hand facilitated its complete extraction in the subsequent treatment. Citric acid content in the dried samples of adults, pupæ and larvæ, was determined by the method of Ettinger, Goldbaum and Smith (1952) employing N-Heptane as the solvent. The results are expressed on individual basis.

RESULTS.

The results of citric acid content of individual samples of adults, pupæ and larvæ of resistant and non-resistant strains of *C. fatigans*, are set out in Table I. It would be seen that the resistant strains contained appreciably greater amounts of

TABLE I.

Citric acid content in different stages of resistant and non-resistant strains of Culex fatigans.

Stage.	RESISTANT.				S.E.	NON-RESISTANT.				S.E.	t	p
	Number of batches analyzed	Total Number of insects.	Citric acid content per insect in mg. Range.	Mean.		Number of batches analyzed.	Total Number of insects.	Citric acid content per insect in mg. Range	Mean			
Adults	10	300	0·606 to 2·075	1·343	0·116	10	290	0·292 to 1·710	0·929	0·132	2·36	< 0·05
Pupae	6	180	2·478 to 3·950	3·398	0·216	6	165	2·130 to 3·330	2·693	0·199	2·40	< 0·05
Larvae	11	330	0·937 to 2·310	1·527	0.158	11	330	0·480 to 1·939	1·005	0·137	2·50	< 0·05

citric acid than their susceptible counterparts. This is evident not only in adults but also in pupæ and larvæ. In the adults, pupæ and larvæ of the resistant strain, the average content of citric acid per specimen was respectively 45, 26 and 52 per cent more than at the corresponding stages in the non-resistant strain. Even the eggs of resistant strains were found to possess a slightly higher amount of citric acid than those of the susceptible strain. These results on eggs are not included in the Table because of the small number of observations.

Thus at every stage of mosquito development, one notices that the citric acid content was appreciably higher in the resistant strain. It should, however, be mentioned that in the larvæ and adults there was considerable variation both in the resistant and susceptible strains. It is interesting to note that in spite of this variation, the samples of the resistant strain contained consistently more citric acid than the corresponding samples of the susceptible strain. In pupæ, the variation from sample to sample was much less. Even here one finds that all the batches of pupæ from resistant strain contained relatively greater amounts of citric acid than those from non-resistant strain. It is inconceivable that coincidence could play any rôle in the higher trend observed on the different batches of larvæ, pupæ and adults.

DISCUSSION.

A few attempts to investigate this problem of resistance from a biochemical aspect, have been on record. The present communication has given some additional evidence in favour of considering the problem from this standpoint.

The phenomenon of resistance could be closely associated with the acceleration of certain metabolic reactions. The finding that resistant flies contain greater amounts of cytochrome oxidase, lend support to this hypothesis. The authors felt that if the respiratory enzymes could show an increase, the possibility of energy furnishing reactions being also increased in the resistant groups, would have to be considered.

The rôle of tricarboxylic acid cycle, as the prime energy furnishing process, has been well known for over a decade. The acceleration of the energy furnishing reactions would naturally result in the increased formation and probably accumulation of certain intermediate metabolites. One such compound is citric acid, which forms from the condensation of pyruvate and oxaloacetate under the influence of specific enzymes. Citrate estimations were, therefore, carried out with a view to determining, if any changes occurred in mosquitoes resistant to D.D.T.

As the results clearly show, there was an increase in citrate in the various stages of mosquito development in only the D.D.T.-resistant strain. Any explanation advanced at this juncture to connect the observed increase in citrate with the phenomenon of acquired resistance, would be in the realm of speculation. The question whether increase in citric acid *per se* had anything to do with the phenomenon of resistance, is worth studying.

SUMMARY.

An investigation concerning biochemial changes in resistant *Culex fatigans* in different stages was undertaken. There was an increase in the citrate content of resistant strain.

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MALARIA IN NORTH-EAST FRONTIER AGENCY (INDIA).

BY

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THE North-East Frontier Agency, as the name implies, is on the north-eastern part of India, bordering Burma, Tibet and Bhutan. Naturally it has its own strategic and political importance. The Government of India have taken all possible measures to ensure not only the security and integrity of this part of the country, but also the social and economic development of the tribal people.

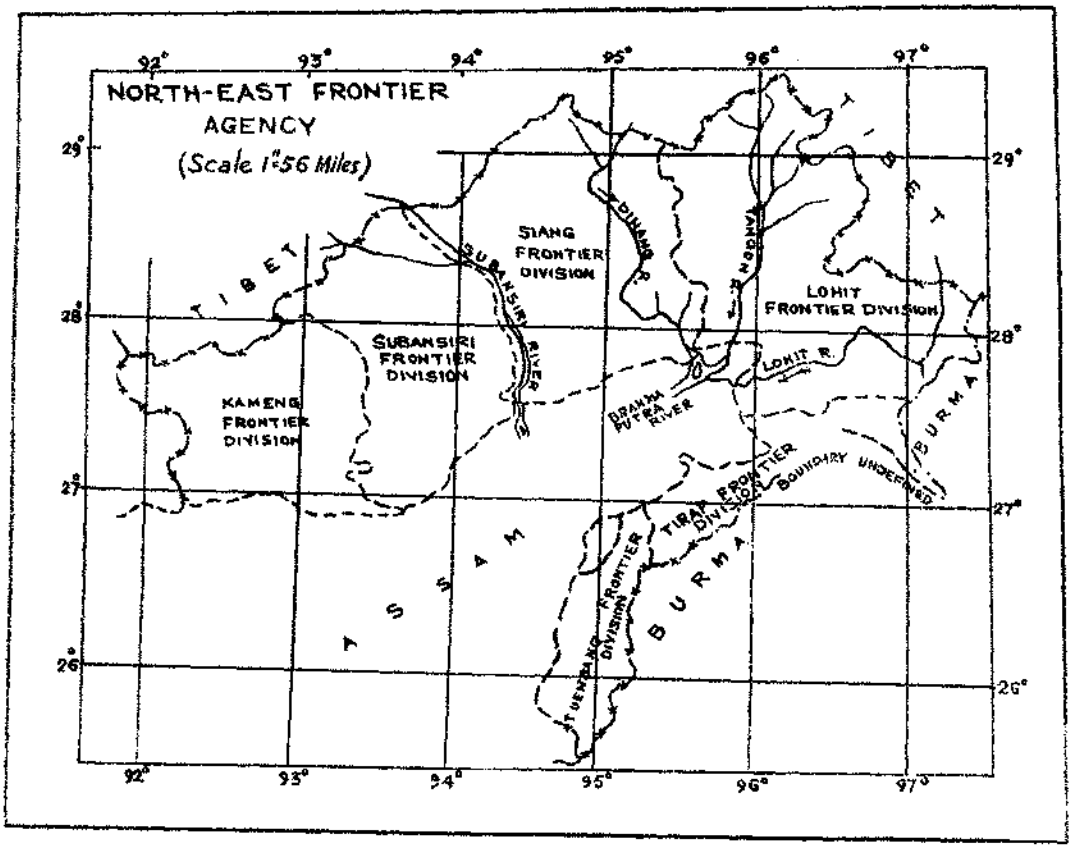
Malaria control in the North-East Frontier Agency was started on a small scale in 1952 but no detailed epidemiological or entomological surveys were ever undertaken. Since its recent participation in the National Malaria Control Programme, inaugurated in April, 1953 (Jawsant Singh, 1953), it was considered necessary to carry out a thorough malaria survey in the agencies before instituting proper control measures under the programme. The survey aimed at elucidating the extent and distribution of malaria and at incriminating the vector or vectors responsible for the transmission of the disease. This paper records the observations made during the survey which lasted for about six weeks (November 28, 1955 to January 14, 1956).

AREA AND TOPOGRAPHY.

The North-East Frontier Agency is a hilly tract and covers an area of 34,000 square miles. It is situated between 25°30′-29°30′ north and 91°38′-97°30′ east. The highest altitude is over 17,000 feet above sea level which, in the submontane region, is somewhat less than 1,000 feet. The general slope of the land is from north to south.

Tibet lies to the north and Assam to the south of the Agency, while Bhutan and Burma form its western and eastern boundaries respectively. The area is densely wooded and is traversed by a number of small rivers, hill-streams and streamlets all of which are the tributaries of Brahmaputra, the great river that flows through Assam into the Bay of Bengal. When in spate, this devastating river brings in its wake the waves of miseries to the people and ruin to a number of beautiful towns in Assam. These streams are flooded during summer months and some places in the Agency remain completely cut off and inaccessible for about eight months in the year.

MAP 1.



For administrative purposes, the Agency is divided into six divisionst(Map 1), namely, Tuensang Frontier Division, Tirap Frontier Division, Lohi Frontier Division, Siang Frontier Division, Subansiri Frontier Division and Kameng Frontier Division. All but Tuensang Frontier Division have been named after the rivers of similar names flowing in the area. Tuensang Division, however, has derived its name from Tuensang, a village situated in the area. Each of these divisions has got more or less a centrally situated headquarter, and a number of outposts distributed throughout its length and breadth. Road communications in the entire agency area are extremely poor. Barring only two fair-weather roads to divisional headquarters, one to Teju in Lohit Frontier Division and the other to Tuensang in Tuensang Frontier Division, and a few more to some outposts in the hilly regions, approach by road to the rest of the places is not possible. The rest of the divisional headquarters can only be approached either by foot through the dense forest in hilly tract highly infested with leeches, or by ponies where tracks are present. Ziro, the divisional headquarter of Subansiri Frontier Division, is approachable only by air. All attempts are being made to construct a number

of fair-weather roads and airstrips in the agency and this will undoubtedly ease the communication problem to a considerable extent in the near future.

POPULATION.

The agency is sparsely populated. The inhabitants comprise mainly about 8 lakhs tribal people. Of these, only one lakh inhabit the foot-hill areas. In the course of this survey, it was gathered that there are in all about 30 tribes, and again each tribe has got a number of sub-tribes. These tribal people have very little intercourse with the people of the plains. Tangsu, Singhpo, Wanchow, Noktey, Khamti, Misimi, Daphla, Aka, Abores, Nagas, etc., were the only few tribes met with during the present survey. Each tribe, and in some cases each sub-tribe, has a language of its own. It was stated that about 60 dialects are spoken in the agency. The tribals in the hills are, as a rule, healthy but those residing in the foot-hill regions, have poor health. They are, in general, ill-clad and spend most of their time outdoors, irrespective of whether their habitation is on the high hills or on the submontane regions.

HOUSING CONDITION.

The huts are characteristically built. The framework of the huts stands on an elevated platform at a height of two to five feet from the ground level. The platform consists of a wooden frame with bamboo matting, and forms the floor of the hut. Ladders, made of wood, are provided for climbing up to the huts. Bamboo mattings, all round, form the walls and the roof is of thatch. Each house has invariably a covered varandah in front where guests are usually accommodated. Houses are in general quite big and, there being no window, are pitch-dark inside. Some of the houses of comparatively well-to-do persons, have provision for bedroom, bath-room, laterine, etc., made by partition walls with bamboo-matting. The space under the platform, is fenced all round and thus utilized as pig-sties.

AGRICULTURE.

The main crop of the land is rainfed paddy and the people there, are mainly rice-eaters. They practise terraced cultivation of rice locally known as '*jhum*' cultivation. Since the administration is introducing wet rice cultivation, they are gradually getting used to it, and it appears that this practice is becoming popular amongst them. Besides rice, cotton and tapioca are also grown but to much less extent. Forests yield timber and firewood.

CLIMATE.

Sufficient data for rainfall, temperature and humidity were not available. However, rainfall data for the last four years, as recorded at Pasighat Agricultural Farm, is presented in Table I. Rains set in as early as March, and continue till October. Highest precipitation is in the month of July. Rains are mostly from the south-west monsoon. Average annual rainfall for the last four years is 236·95 inches. The climate is hot and humid throughout the year, excepting a short winter during December and January.

TABLE I.

Rainfall record of Pasighat recorded at Pasighat Agriculture Farm.

Month.	1952 (Inches).	1953 (Inches).	1954 (Inches).	1955 (Inches).
January	0·79	2·98	2·46	0·81
February	3·48	5·24	7·52	4·37
March	6·66	11·79	1·76	7·83
April	3·35	10·49	11·58	4·96
May	13·18	27·73	24·59	15·69
June	28·42	34·84	64·76	31·38
July	45·21	45·49	84·15	98·10
August	49·49	13·84	58·63	43·12
September	33·93	42·79	33·38	16·95
October	15·72	18·11	10·415	18·92
November	1·34	0·49	0·025	0·42
December	0·39	0·03	5·59	0·37
Total rainfall during the year	192·74	213·43	299·07	242·58

MORBIDITY RATE.

Malaria morbidity, and total morbidity figures, for all the divisions, as available from the office of the Chief Medical Officer, are presented in Table II. Perusal of the data for the last three years, would show that malaria cases are encountered throughout the year. Maximum number of cases were recorded in the months of August, September and October. Malaria figures in relation to total morbidity figures each month, would definitely indicate that incidence of malaria is least during the winter months.

EPIDEMIOLOGICAL DATA.

Spleen survey.—Spleen survey was carried out in all the divisions from November 28, 1955 to January 14, 1956. During the survey, 35 villages were covered, and 877 children between 2 - 10 years of age were examined. The results are presented in Table III. The spleen rate varied from 26·6 to 100 per cent in the foot-hill regions, and the average enlarged spleen, worked out according to Hackett's method (Hackett, 1944),was found to be between 1 and 4·6.

The foot-hill region showed the highest spleen rates, whereas in Mon, a village at a height of about 3,000 feet above sea level, the spleen rate was only 2·6 per cent.

TABLE II.

Morbidity figures, month by month, during the years 1953, 1954 and 1955, in North-East Frontier Agency (India).

Name of division.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1953												
Tuen Sang Fr. Division												
Malaria	685	948	315	293	881	932	1,106	1,370	1,275	1,479	1,387	1,290
Total all diseases ...	2,185	3,323	1,315	1,030	3,347	4,543	4,727	5,224	5,233	4,959	4,723	4,397
Tirap Fr. Division												
Malaria	...	538	931	1,872	1,270	1,283	1,640	1,855	1,776	1,419	1,408	1,260
Total all diseases ...	...	2,026	2,578	3,042	4,060	3,437	3,477	3,591	3,519	2,991	2,700	2,900
Lohit Fr. Division												
Malaria	971	612	388	679	786	1,469	1,439	1,268	1,349	1,196	1,046	698
Total all diseases ...	1,288	1,224	1,550	1,651	1,806	1,919	1,649	1,686	1,715	1,572	1,505	1,154
Siang Fr. Division												
Malaria	1,019	1,416	1,169	1,239	1,107	1,382	1,801	1,872	881	2,039	1,239	823
Total all diseases ...	2,051	2,245	2,749	3,076	2,715	3,141	3,117	1,776	3,009	3,858	2,646	2,099
Subansiri Fr. Division												
Malaria	153	194	270	167	220	411	516	606	575	674	498	518
Total all diseases ...	531	709	694	586	609	1,046	1,017	1,065	1,040	864	1,210	1,204
Kamang Fr. Division												
Malaria	311	376	640	634	765	790	621	718	494	469	481	314
Total all diseases	1,133	1,305	1,836	2,220	1,825	1,885	1,829	2,110	1,564	1,843	2,088	2,170
Total for the year 1953												
Malaria	3,139	4,084	3,913	4,934	5,031	6,287	7,213	6,698	7,341	7,276	6,057	4,918
Total all diseases ...	7,188	10,832	10,722	12,505	14,362	15,971	15,816	15,452	16,071	16,027	14,872	13,027

TABLE II (*Contd.*).

1954

Name of division.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Tuen Sang Fr. Division												
Malaria	1,216	1,205	1,010	1,110	1,023	1,161	616	1,460	1,232	1,349	Figures not available.	...
Total all diseases ...	4,899	5,731	5,255	6,086	5,617	5,505	2,873	5,915	4,999	5,561		...
Tirap Fr. Division												
Malaria	864	876	1,176	1,172	1,190	1,697	1,873	1,426	1,309	1,028	,,	...
Total all diseases ...	2,367	2,577	3,082	3,287	3,519	3,216	3,305	3,034	2,894	3,628	,,	...
Lohit Fr. Division												
Malaria	573	620	593	553	585	871	907	962	1,064	1,014	,,	...
Total all diseases ...	1,286	1,842	2,006	2,046	1,842	1,933	2,035	2,220	2,238	1,967	,,	...
Siang Fr. Division												
Malaria	1,096	561	1,094	1,116	666	1,690	1,557	1,683	1,752	1,254	,,	...
Total all diseases ...	2,633	2,193	3,782	4,085	2,072	3,642	3,231	3,832	3,850	3,155	,,	...
Subansari Fr. Division												
Malaria	441	881	398	577	697	882	875	1,180	885	914	,,	...
Total all diseases ...	1,767	2,234	1,647	1,753	2,168	2,603	2,632	2,546	2,267	2,312	,,	...
Kamang Fr. Division												
Malaria	327	350	392	614	622	634	767	659	637	515	,,	...
Total all diseases ...	1,790	1,950	2,284	2,303	2,088	2,189	3,002	3,009	3,102	2,725	,,	...
Total for the year 1954												
Malaria	4,517	4,493	4,663	5,142	4,783	6,935	6,595	7,370	6,879	6,074	,,	...
Total all diseases ...	14,742	16,527	17,956	19,650	17,306	19,088	17,078	20,556	19,360	19,348	,,	...

TABLE II. (*Concld.*)

1955

Name of division.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Tuen Sang Fr. Division												
Malaria	801	707	712	697	707	772	724	678	585	815	1,006	...
Total all diseases ...	5,303	5,678	4,811	4,980	5,391	6,443	5,743	6,396	4,909	6,700	7,346	...
Tirap Fr. Division												
Malaria	...	...	...	...	...	714	1,122	1,676	1,495	973	997	...
Total all diseases ...	...	...	...	...	...	2,652	3,972	4,734	4,005	3,515	2,343	...
Lohit Fr. Division												
Malaria	...	...	...	...	...	766	589	639	739	959	686	...
Total all diseases ...	...	...	...	...	...	1,799	1,703	1,833	1,714	1,555	1,341	...
Subansari Fr. Division												
Malaria	428	388	521	732	791	853	1,003	949	741	756	560	...
Total all diseases ...	2,302	2,148	2,654	2,847	2,809	2,857	2,989	3,029	2,783	2,307	2,275	...
Kamang Fr. Division												
Malaria	185	245	275	445	326							
Total all diseases ...	2,093	2,162	2,495	2,797	1,906			Figures not available				
Total for the year 1955												
Malaria	1,414	1,340	1,508	1,874	1,824	3,105	3,438	3,942	3,560	3,503	3,249	...
Total all diseases ...	9,788	9,988	9,960	10,624	10,100	13,751	14,407	15,991	13,411	14,077	13,305	...

TABLE III.

Spleen rate amongst children.

Name of division.	Serial number.	Name of the village.	Number of children examined.	Number with enlarged spleen.	Spleen rate per cent.	Average enlarged spleen.	Remarks
Tuen-Sang Fr. Division	1	Mon village and school	38	1	2·6	1·0	At a height of about 3,000 ft. above sea level.
Tirap Fr. Division	2	Namgoy	20	14	70·0	3·3	
	3	Khachang	12	11	91·7	3·4	
	4	Namphai					
	5	New Champoo	16	16	100·0	3·10	
	6	and Sampoo Old					
	7	Hardumsa					
	8	Imbo	21	21	100·0	2·7	
	9	Gujo					
	10	Muhang Mura	29	27	93·1	2·1	
	11	Kusum Pathare					
	12	Kherum Mura	22	22	100·0	2·3	
	13	Kherum Kacheri	19	16	84·2	2·35	
	14	Bardumsa School	12	7	58·3	3·6	
	15	Bardumsa Camp and village					
	16	Namsang village and school	34	27	79·4	2·26	
Lohit Fr. Division	17	Timai	13	12	92·3	2·6	
	18	Teju High School	70	29	41·4	2·45	
	19	Mumang village and school	29	11	38·0	2·0	
	20	Chowkhum School	28	13	36·4	1·8	
	21	Ruing L. P. School and village	23	14	60·9	2·5	
	22	Samak (MekaVillage)	19	17	89·5	3·0	
Siang Fr. Division	23	Balak group of villages	89	48	53·9	1·8	
	24	Mirum Camp and L. P. School.	38	37	97·4	2·84	

TABLE III.—*(Contd.)*

Name of division.	Serial number.	Name of the village.	Number of children examined.	Number with enlarged spleen.	Spleen rate per cent.	Average enlarged spleen.	Remarks.
	25	Pasighat L. P. School	95	37	39·0	2·27	
	26	Rani Village	14	14	100·0	4·6	
	27	Oyan	66	60	90·9	2·6	
	28	Sciile	15	15	100·0	3·7	
	29	Tarang and Langko	74	61	82·4	2·34	
Subansiri Fr. Division	30	Kimin Camp	15	4	26·6	2·2	
	31	Gumto	14	13	93·0	2·8	
	32	Ranor	11	10	90·9	3·1	
	33	Doimukh	16	13	82·2	3·6	
Kamang Fr. Division	34	Foot-hill	11	6	54·5	1·8	
	35	Dafang Basti	14	14	100·0	3·7	
		Total ...	877	590	67·2	2·65	

One hundred and eleven adults, from ten villages, were also subjected to spleen examination and the spleen rate amongst them worked out to be 67·6 per cent.

PARASITE SURVEY.

Children examined for spleen survey, were subjected to blood examination at the same time. Parasite rate recorded (Table IV) was as high as 81·8 per cent in one area. Children from Teju High School were regularly kept on suppressive treatment with antimalarials, and hence parasites could not be detected in their blood. Children from Mon, a village with negligible child spleen rate, showed no parasitæmia. The predominating species in the agency, during the survey, was *P. falciparum* which constituted 65·8 per cent; while *P. vivax*, *P. malariæ* and mixed infections accounted for 15·5 per cent, 16·1 per cent and 2·6 per cent respectively.

INFANT BLOOD EXAMINATION.

All efforts were made to examine each and every infant in the village, visited by door to door visits. In spite of this, the number of infants examined was low, indicating a comparatively small infant population. One hundred and twenty-four infants between the age of one month to one year, from 28 villages, were examined for parasites. The results are recorded in Table IV. Infant parasite

TABLE IV.

Parasite rate amongst children.

Name of division.	Serial number.	Name of village.	Number of children examined.	Number positive for parasites.	Parasite rate per cent.	Species.			Mixed infection.	Remark.
						P. falciparum.	*P. vivax.*	*P. malariæ.*		
Tuen-Sang Fr. Division	1	Mon village and school	38	...	...	...	...	...	...	At a height of about 3,000 feet above sea level.
Tirap Fr. Division	2	Namgoy	20	2	10·0	1	1	...	...	
	3	Khuchang	12	3	25·0	2	1	...	...	
	4	Namphai								
	5	New Champoo	16	2	12·5	2	...	...	...	
	6	Sampoo Old								
	7	Hardumsa								
	8	Imbo	21	10	47·6	7	1	2	...	
	9	Gujo								
	10	Mohang Mura	29	20	68·9	14	3	3	...	
	11	Kusum Pathare	22	18	81·8	10	4	1	3	
	12	Kherum Mura								
	13	Karam Kacheri	19	13	68·4	10	2	1	...	
	14	Bardumsa School								
	15	Bardumsa Camp and village	12	2	16·6	1	...	1	...	
	16	Namsang village and school	34	7	20·6	4	2	...	1	
Lohit Fr. Division	17	Timai	13	1	7·7	...	1	...	...	
	18	Teju High School	70	...	...	...	...	...	...	
	19	Momang village and school	29	3	10·3	3	...	...	...	
	20	Ruing L. P. School and village	23	5	21·7	3	1	1	...	
	21	M. E. School, Chowkhum	28	1	3·6	...	1	...	...	
	22	Samak (Meka)	19	7	37·0	3	2	2	...	

TABLE IV.—(*Contd.*)

Name of division.	Serial number.	Name of village.	Number of children examined.	Number positive for parasites.	Parasite rate per cent.	Species			Mixed infection.	Remark.
						P. falciparum.	*P. vivax.*	*P. malariæ.*		
Siang Fr. Division	23	Balak group of villages	89	8	8·9	6	...	2	...	
	24	Mirum Camp and L. P. School	38	8	21·0	5	2	1	...	
	25	Pasighat L. P. School	95	3	3·2	1	...	2	...	
	26	Rani Village	14	3	21·4	2	...	1	...	
	27	Oyan	66	13	19·7	10	...	3	...	
	28	Scille	15	3	20·0	2	1	...	...	
	29	Tarang and Lungko	74	5	6·9	3	...	2	...	
Subansiri Fr. Division	30	Gumto	14	3	21·4	1	1	1	...	
	31	Ranor	11	3	27·3	3	...	...	...	
	32	Doimukh	16	4	25·0	3	1	...	...	
Kamang Fr. Division	33	Kimin Camp	15	1	6·6	1	...	...	...	
	34	Foot-hill	11	1	9·1	1	...	...	...	
	35	Dalang Basti	14	6	43·0	4	1	1	...	
Total per centage			877	155	17·7	102	24	25	4	
						(65·8 per cent)	(15·5 per cent)	(16·1 per cent)	(2·6 per cent)	

rate ranged from 23 to 83 per cent. Parasite rate, amongst infants, was invariably high in the villages away from outposts; and the extremely low rate in the outposts themselves, accounted for presumably by the effective control of malaria in the outposts. *P. falciparum* was the predominant infection constituting 63 per cent of the total infection; *P. vivax, P. malariæ* and mixed infections accounted for 22·2 per cent, 11·1 per cent, 3·7 per cent respectively (Table V).

ENTOMOLOGICAL DATA.

Adult mosquito collections were made from almost all the villages visited for spleen and parasite surveys. Night collections were also made in certain villages. The density of mosquitoes, during this part of the year, was found to be extremely low. The species of mosquitoes collected were *A. maculatus, A. aconitus, A. vagus,*

A. minimus, *A. annularis* and *A. pallidus* in order of prevalence. *A. minimus* formed 9·7 per cent of the total collections.

Mosquito collections were made from human dwellings only. Cattlesheds are practically non-existant there. Searches for adult mosquitoes were of course made in some of the pig-sties too but mosquitoes were not found in them, probably because they were mostly open on all sides.

TABLE V.

Infant parasite rate.

Name of division.	Number of villages visited.	Number of infants examined.	Number of positive.	Parasite rate per cent.	Species of parasites			Mixed infection.	Remarks.
					P. falciparum.	*P. vivax.*	*P. malariæ.*		
Tirap Fr. Division	13	35	29	83	20	7	...	2	
Lohit Fr. Division	4	16	4	25	2	...	2	...	
Siang Fr. Division	6	54	14	26	18	3	3	...	
Subansiri Fr. Division ...	4	13	3	23	3	...	...	...	
Kamang Fr. Division ...	2	6	4	66·6	1	2	1	...	
Total ...	28	124	54	43·5	34	12	6	2	
					(63 per cent)	(22·2 per cent)	(11·1 per cent)	(3·7 per cent)	

Breeding places.—Perinneal streams were the only source where the mosquitoes could breed. No systematic search for detection of mosquito breeding was made. On casual inspection, some of these streams showed anopheline larvæ. They were first and second instars and hence no attempt for identification of species was made.

Dissection of mosquitoes.—All the adult mosquitoes that were collected during the survey, were dissected and one *A. minimus* was found positve (Table VI). The infective mosquito was caught on December 9, 1955, from a human dwelling in Kheram Mura, a village some five miles away from Bordumsa outpost. The gut of the mosquito showed 25 oöcysts, some of them nearing maturity and the glands showed very scanty infection. Thus *A. minimus* is a vector in the area (Misra, 1956).

Even though *A. leucosphyrus* could not be collected during the present survey, yet from the topographical and other considerations the possibility of this species, playing some rôle in transmission of malaria, cannot be ruled out. Further detailed investigations, during the malaria season, are indicated for more definite knowledge on the transmission of malaria in the area.

TABLE VI.

Results of dissection.

Species.	Number dissected.	Number positive.		Remarks.
		Gut.	Gland.	
A. maculatus ...	21	...	...	...
A. aconitus	16	...	...	...
A. minimus	7	1	1	The same mosquitoe was found positive for gut and gland.
A. vagus	6	...	...	...
A. annularis ...	1	...	...	...
A. pallidus	1	...	...	...

DISCUSSION.

Malaria problem confronting the Agency may now be conveniently classified as (1) malaria endemicity in the foot-hill region; (2) imported malaria to high hills due to migratory habits of the tribal people, and (3) possible malaria hazards consequent upon the developmental work. Each of these problems will be considered separately for the purpose of discussion.

From the epidemiological and malaria morbidity data, it is quite evident that the foot-hill areas are highly malarious. Observations made in Assam, a contiguous State, by various workers (Chalam and Young, 1923; Watson, 1924; Strickland, 1925; Ramsay, 1930; Manson, 1931; Rice and Savage, 1932) have shown that relative proximity of perennial streams to habitations, is a factor in determining the extent and distribution of malaria. The villages in the foot-hill areas are invariably situated in the proximity of perennial streams.

A. minimus has been incriminated as the significant vector species in Assam by Chalam and Young (1923), Strickland (1925; 1929) and Ramsay (1930). Manson (1931), Gupta *et al.* (1932), Rice and Savage (1932) and Manson and Ramsay (1932) further confirmed these findings in the plains of Brahmaputra Valley of Assam and Cachar. *A. minimus* has been incriminated as vector species in the neighbouring Manipur and Tripura states (Misra, 1954), the terrain of which is similar to the one under report.

A. minimus is perhaps the most important vector in this area. It is well known that the principal habitat of *A. minimus* larvæ is moving water, be it perennial river, stream, drain or any other water source of this description. Hence such a distribution of malaria in the foot-hill areas is to be expected.

The density of anopheline population being very low during the period of the survey, the collections made may not represent the true picture of anopheline fauna of the area. *A. minimus* formed only a very small percentage of the total

collection. These findings are similar to what have been observed during cold months in Assam by various workers (Manson and Ramsay, 1932); Rice and Savage, 1932; and Vishwanathan *et al.*, 1941). In so far as the infectivity of *A. minimus* is concerned, there is ample evidence to show that the same species could be positive for oöcyst and sporozoites throughout the year in Assam. During the cold months of January and February, the infection rate may, however, be very low due to the low density of adult mosquito population, on account of lack of perennial breeding grounds (Rice and Mohan, 1936; Vishwanatham *et al.*, 1941). During the present survey, finding of one *A. minimus* positive for oöcysts and sporozoites on December 9, 1955, may indicate the possibility of this species behaving in the same way as in Assam.

Malaria cases are encountered in the high hills where there is remote chance of indigenous transmission for want of breeding places. This only happens because in certain areas the hill people, for purposes of cultivation and collection of necessary commodities, come down to the plains where they stay overnight and contract the infection. Further, for any movement in the Agency tract, porters are indispensable. These porters are mostly from the higher regions and are exposed to infection when they work in the foot-hills.

The possible malaria hazards which may emerge out of the developmental work are (*a*) creation of breeding places and interference with the natural drainage of the terrain in the construction of roads, and (*b*) collection of administrative personnel and labour from different parts of the country (tropical aggregation of labour). These may lead to a severe outbreak of malaria chiefly due to the introduction of fresh strains of malaria parasites under conditions favourable for the local transmission.

CONTROL MEASURES.

Malaria control in the North-East Frontier Agency is indeed a difficult problem, but given money and time malaria can, no doubt, be effectively controlled. Concerted effort should be directed towards the fight against this scourge. Hence the organization should be a well-knit one. If malria is effectively controlled, more than half of the public health problems would be straight away solved. Decline of the population in certain areas may directly or indirectly be attributed to the ravages of malaria. Malaria control, if successfully carried out, may go a long way to arrest this catastrophe.

Taking into consideration, the terrain, the sparse population, lack of communications and the malaria problems as already discussed, the following measures are suggested.

(1) Recurrent antimalaria measures in the form of residual spraying of houses with insecticides.

(2) Suppressive treatment with 4-aminoquinolines under proper supervision.

(3) Avoidance or treatment of freshly created malariogenic conditions to render them harmless.

RECURRENT ANTI-ADULT MEASURES.

From the epidemiological data, it is evident that the foot-hill areas are hyperendemic for malaria. Morbidity figures indicate that malaria cases start cropping up in March and April, and reach its peak during August, September and October. Active malaria transmission thus starts from mid-April and continues till first week of December, height of transmission being probably in September-October. Protection from malaria has, therefore, to be provided during this period.

All houses in the villages, labour camps and outposts have to be treated with residual insecticides. The roofs and walls of the platforms forming the floor of the houses should not be left without spray. Arrangement may also be made with the States, bordering North-East Frontier Agency, for the protection of villages on their side of the border. Because of the enormous malaria risk involved, due to the developmental works in the shape of new contracts taken up in the Agency, it is considered necessary that the area should receive three rounds of spray at the initial stage for the next two years. First round should commence from mid-March and the spacing of the subsequent rounds should be worked out according to what local studies would reveal about the duration of effectiveness of the residual insecticide employed. The total dosage of D.D.T. would be 200 mg. per square foot of sprayed surface, as recommended under the National Malaria Control Programme in the country. As the possibility of transmission in the villages situated on the plateau of high hills exists, a single round of spray with 200 mg./sq. ft. may be sufficient to cover the risk. Detailed epidemiological and entomological studies will be necessary to decide this issue.

SUPPRESSIVE TREATMENT.

All migratory people and tribal porters should be put under suppressive treatment with camoquin or resochin in the weekly dosage of 400 mg. and 300 mg. respectively. They should start taking the drug the day they come down to the plains and continue taking the same at weekly intervals for the period they would be required to stay in the foot-hill regions. This measure would be necessary only to start with, and may be withdrawn when once the effective control is achieved through residual spraying.

AVOIDANCE OF CREATION OF MALARIOGENIC CONDITIONS.

In the construction of roads, natural drainage of the terrain should, as far as possible, be maintained by sufficient number of bridges and culverts. Such a provision should be insisted upon in every engineering project contemplated in the area.

To obviate the risk involved due to " tropical aggregation of labour ", the labour camps, outposts, etc., should be properly sited as far away as possible from the local villages. All new entrants in the area should be examined, and those found positive for malaria parasites on examination of their blood, should at once be put under a course of treatment with camoquin or resochin.

Since evidence is there to show that *A. minimus* in India could be effectively controlled with residual insecticide (Puri and Krishnaswami, 1947; Krishnaswamy, 1952), it is to be expected that residual spraying of houses will undoubtedly bring malaria in the agency well under control. Further, the migratory habits of the tribals and aggregation of people from outside, warrant the use of suppressive treatment which, in its turn, will reduce the active incidence of the disease and thereby enhance the effectiveness of malaria control to a great extent.

SUMMARY.

North-East Frontier Agency is a hilly tract with an area of about 34,000 square miles. The population is only eight lakh, of which one lakh inhabit the foot-hill areas. A large number of streams and streamlets criss-cross the country.

There are 30 tribes in the agency and again each tribe has a number of sub-tribes.

Foot-hill areas are hyperendemic for malaria. The spleen rate in the villages, covered during the survey, varied from 26·6 to 100 per cent. The parasite rate amongst children was high, in one case it was 81·8 per ent. School children examined showed lowest parasite index.

The infant parasite rate ranged from 23 to 83 per cent. On all occasions *P. falciparum* was the predominating species prevalent. *P. vivax* , *P. malariæ* and mixed infections were also encountered.

Morbidity figures would indicate the presence of malaria cases throughout the year. Maximum malaria morbidity is encountered during September and October and minimum during December and January.

Mosquito density was very low during the period of the present survey. From the collection made, six species of anopheline mosquitos were identified.

A. minimus has been established as a vector species in the foot-hill regions of the Agency.

Recommendations for malaria control measures have been made.

ACKNOWLEDGEMENTS.

The author wishes to express his gratitude to Col. P. N. Luthra, Development Commissioner, North-East Frontier Agency and to Dr. S. R. K. Iyengar, Assistant Surgeon, for the facilities offered throughout the period this survey was undertaken. Thanks are due to Drs. D. Phukan, K. H. Hazarika, D. N. Bora, K. Chowdhury, Mr. S. Singh and to all the Medical Officers of the outposts visited, for their co-operation and help rendered. Thanks are also due to Shri Puran Singh, Technician, Malaria Institute of India, and Shri N. R. Dutta, Malaria Inspector, N.E.F.A., for their valuable assistance.

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EFFECT OF INSECTICIDES ON VECTOR SPECIES AND DEVELOPMENT OF RESISTANCE, IF ANY, IN DIFFERENT VECTOR SPECIES IN HIRIYUR AREA, MYSORE STATE*.

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[October 2, 1956.]

INTRODUCTION.

At the Annual Meeting of the Indian Council of Medical Research held in 1954, it was proposed to carry out certain investigations in different parts of India to determine whether malaria vectors are developing any resistance towards residual insecticides. The areas recommended for these trials were those which were under continuous treatment with residual insecticides for over four years. In Mysore State, Hiriyur area in Chitaldrug District, was recommended for these investigations. The vector species in this area are *culicifacies* and *fluviatilis*, although *stephensi* may play a minor rôle (Nursing, Rao and Sweet, 1934).

AREA OF STUDIES.

Hiriyur area is in the south-eastern part of Chitaldrug District on the main Bangalore-Dharwar Road, 99 miles from Bangalore. The average annual rainfall is below 20 inches and it is a semi-irrigated area. Hiriyur Town lies on the right bank of the Vedavati River. A large dam was built in 1898 across the Vedavati River, 12 miles above Hiriyur Town, at Vanivilasapura. There are two large irrigation channels taking off on either side of the river. Water from the channel on the north bank is used to irrigate sugarcane, plantain, and to a small extent paddy fields. The south bank channel irrigates paddy fields to the west of

*The studies reported in this paper were conducted by the Bureau of Malariology, Department of Public Health, Government of Mysore.

Hiriyur Town and also a few acres of sugarcane and plantain. Water in the canals is let in for one week and turned off during the next, the on and off periods alternating with each other.

There are five primary health units working at Hiriyur, Vanivilasapura, Ranganathapura, Dharmapura and Maradihalli, the first three being served by the Vanivilasa Sagar Canal.

The area has been under residual insecticidal treatment since 1951 and at the commencement of these studies, June 1955, had received eleven rounds of spray. The insecticides and dosages employed were:—

D.D.T.—50 per cent wettable powder ...	One round of 100 mg./sq. ft.
Gammexane—P.520 wettable powder ...	Two rounds of 22 mg./sq. ft.
D.D.T.—Emulsion	Four rounds of 100 mg./sq. ft.
D.D.T.—75 per cent wettable powder ...	Four rounds of 100 mg./sq. ft.

PLAN OF EXPERIMENT.

Reconnaissance surveys were carried out in 41 villages of Hiriyur, Ranganathapura and Vanivilasapura Health Units, with the object of selecting certain villages where sufficient numbers of *A. culicifacies*, *A. fluviatilis* and *A. stephensi* could be collected for experimental purposes. When these surveys were taken up in June, 1955, the area had been sprayed in April 1955 under the National Malaria Control Programme, and consequently the mosquito densities were at a very low level. As densities of vector species would build up only after October (Sweet, 1933), the October round of spray in 1955 was with-held in four villages (Nandihalli, Bharmagiri, Krishnambudhi, and Pitlali) which were selected for studies. The anopheline species recorded were *aconitus*, *annularis*, *barbirostris*, *culicifacies*, *fluviatilis*, *hyrcanus*, *jamesi*, *jeyporiensis*, *pallidus*, *splendidus*, *stephensi*, *subpictus*, *tessellatus*, *turkhudi*, *vagus* and *varuna*.

Mosquitoes were collected between 7.00 and 11.00 a.m. in groups of two or three in test tubes, and vector species isolated and released into clean cages. Treatment of filter papers with insecticides, and construction of exposure chambers were carried out as laid down in Technical Report No. 80 of the Expert Committee on Malaria, World Health Organization. Young and freshly-fed mosquitoes were exposed for one hour to the treated filter papers and after exposure, released into clean lantern globes covered with moist lint and provided with soaked raisins. Temperature and relative humidity were recorded before and after exposures. Controls for all tests were run, exposing mosquitoes to filter papers treated only with the solvent. Mortality counts were recorded after 24 hours at room temperature. Tests were carried out during January, March, April, May and June 1956. Temperature and relative humidity ranges at the time of exposure are recorded in Table I.

RESULTS.

Young freshly-fed females of *A. culicifacies*, *A. fluviatilis* and *A. stephensi* were exposed in batches to filter papers treated with 2·0, 1·0 and 0·5 per cent solutions

of technical D.D.T. in risella oil, diluted with ethylene dichloride. Controls were exposed to filter papers treated only with risella oil and ethylene dichloride. Results of exposures are summarized in Table II.

TABLE I.

Temperature and relative humidity.

Month.	Dry bulb Temp. °F.	Wet bulb Temp. °F.	Relative humidity (per cent).
1956			
January ...	83—85	64—69	41—48
March ...	82—92	68—74	26—63
April ...	82—93	68—74	26—64
May ...	81—90	72—76	50—68
June ...	76—84	70—75	61—74

TABLE II.

Results of exposure.

Test insect.	Concentration of D.D.T. (Per cent)	Number exposed.	Number dead after 24 hours.	Per cent mortality.	Number exposed as controls.	Number dead after 24 hours.	Per cent mortality.
	2·0	36	36	100·0	12	12	100·0
A. culicifacies	1·0	36	35	97·2	12	11	91·7
	0·5	36	36	100·0	12	12	100·0
Total ...		108	107	99·0	36	35	97·2
	2·0	40	40	100·0	13	13	100·0
A. fluviatilis	1·0	36	36	100·0	12	11	91·7
	0·5	36	36	100·0	10	10	100·0
Total ...		112	112	100·0	35	34	97·1
	2·0	36	26	72·2	15	5	33·3
A. stephensi	1·0	36	26	72·2	10	6	60.0
	0·5	36	25	69·4	12	7	58·3
Total ...		108	77	71·3	37	18	47·7

It can be seen from the above figures that, in the case of *A. culicifacies* and *A. fluviatilis*, in all the three concentrations of D.D.T. tested, there has been almost 100·0 per cent mortality. Even in the controls, there has been a similar mortality rate. This has to be ascribed to the fact that these controls are not "true controls" as they have also been collected from sprayed villages. In the case of *A. stephensi*, the difference in mortality between the controls and mosquitoes exposed to D.D.T., appears to be significant.

TABLE III.

Results of exposure of female anophelines to D.D.T.

Species.	D.D.T. Concentration.			Per cent mortality for all three concentrations.	
	2·0 per cent.	1·0 per cent.	0·5 per cent.		
	Per cent mortality				
A. culicifacies	100·0	97·2	100·0	99·0	
A. fluviatilis	100·0	100·0	100·0	100·0	
A. stephensi	72·2	72·2	69·4	71·3	
Per cent mortality of all species in each concentration ...	91·07	89·8	89·8	90·2	Overall per cent mortality.

TABLE IV.

Results of exposure of female anophelines to solvent alone as parallel controls for the anophelines exposed to the dosages of D.D.T. mentioned in Table III above.

Species.	Per cent mortality.			
A. culicifacies ...	100·0	91·7	100·0	97·2
A. fluviatilis ...	100·0	91·7	100·0	97·1
A. stephensi	33·3	60·0	58·3	47·7
	75·0	82·3	85·3	80·5

From Table III, it can be made out that *A. fluviatilis* is still highly susceptible; next come *A. culicifacies* and *A. stephensi*. There is not any perceptible difference in mortality of all species in each concentration, as compared to each other, *viz.*, 91·0, 89·8 and 89·8 and also to that of overall per cent mortality of all species in all concentrations, *viz.*, 90·2.

The overall mortality of 80·5 among controls and 90·2 among treated, indicates that the above species are still susceptible to D.D.T.

Experiments carried out in an untreated village, Bhimanhalli of Nagamangala Taluk, with *A. culicifaces* showed that in concentrations of 0·5 per cent and 1·0 per cent D.D.T., there was 100·0 per cent mortality. As similar results are obtained in Hiriyur area, it can be stated that *A. culicifacies* has not built up any resistance to D.D.T. The same may hold good in the case of *A. fluviatilis.*

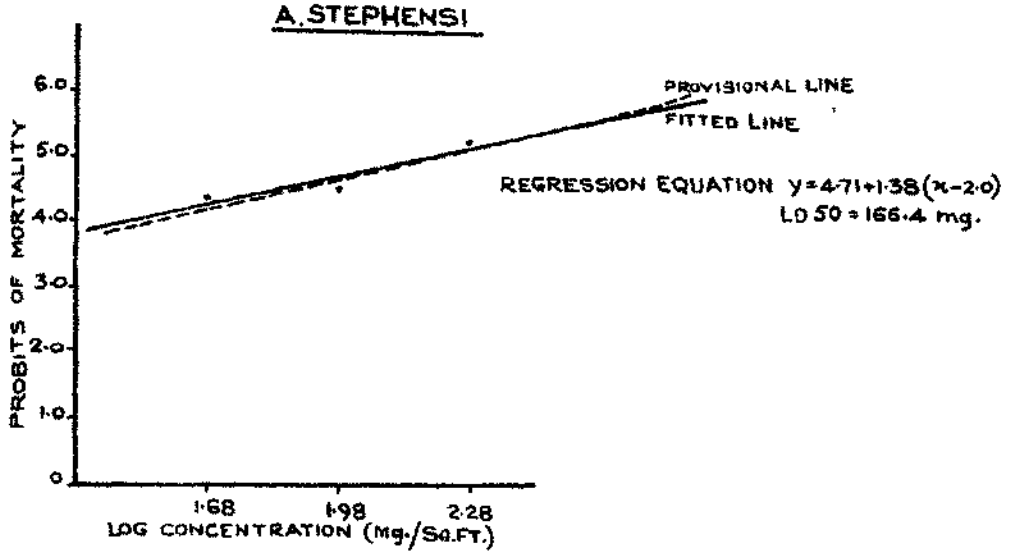
The following formula was used to arrive at the corrected mortality rate for the three concentrations of D.D.T. tested:—

$$\text{Corrected mortality} = \frac{\left\{\text{Observed mortality percentage} - \text{Per cent mortality in controls}\right\}}{100 - \text{Per cent mortality in controls.}} \times 100$$

From the corrected mortality rates for each concentration, log. concentration/probit regression line has been drawn (Graph 1). The calculations involved in this are described in Appendix 1, and the medium lethal concentration is 166·4 mg. for *A. stephensi.*

GRAPH I.

Log. concentration/Probit regression line in respect of different concentrations of D.D.T.



CONCLUSIONS.

In Hiriyur area of Mysore State, which is under residual insecticidal treatment for over five years, investigations to determine whether malaria vectors are developing any resistance towards residual insecticides were carried out as per plans contained in the Technical Report No. 80 of the Expert Committee on Malaria, World Health Organization.

The results indicate that the three vector species, *viz., A. culicifacies, A. fluviatilis* and *A. stephensi* are still susceptible to D.D.T.

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Appendix 1.

Calculations involved in plotting log. concentration/probit regression line.

Test insects—*A. stephensi.*

Log. concentration (x)	Number of insects tested (n)	Number of insects killed (r)	Percentage kill (corrected from controls).	Empirical probit (Y')	Expected probit (Y)	Weighting coefficient (w)	Working probit (y)	nw	nwx	nwy	nwx^2	$nwxy$
1·68	36	26	26·6	4·37	4·20	0·503	4·38	18·108	30·421	79·313	51·107	133·244
1·98	36	26	30·5	4·48	4·65	0·600	4·47	21·600	42·768	96·552	84·672	191·173
2·28	36	25	58·3	5·20	5·10	0·634	5·20	22·824	52·039	118·685	118·685	270·603
								62·532	125·228	294·550	254·464	595·020

$\bar{x} = \frac{Snwx}{Snw} = \frac{125·228}{62·532} = 2·00.$

Equation 1. $Snwx^2 = \frac{(Snwx)^2}{Snw} = 254·464 - \frac{(125·228)^2}{62·532} = 3·7$

$\bar{y} = \frac{Snwy}{Snw} = \frac{294·550}{62·532} = 4·71.$

Equation 2. $Snwxy = \frac{(Snwx)\ (Snwy)}{Snw} = 595·020 - \frac{125·228 \times 294·550}{62·532} = 5·1$

$$b = \frac{\text{Equation 2}}{\text{Equation 1}} = \frac{5·1}{3·7} = 1·38$$

Substituting values for x, $Y = \bar{y} + b\ (x - \bar{x}) = 4·71 - 1·38\ (x - 2·00)$.
$Y = 4·27$, 4·68 and 5·1.
L.D. 50 = 166·4 mg.

$$\text{Corrected mortality} = \frac{(\text{Observed mortality percentage} - \text{Per cent mortality in controls}) \times 100}{100 - \text{Per cent mortality in controls.}}$$

2 per cent D.D.T. $\frac{(72·2 - 33·3)\ 100}{100 - 33·3} = \frac{38·9 \times 100}{66·7} = 58·3$ per cent.

1 per cent D.D.T. $\frac{(72·2 - 60·0)\ 100}{100 - 60·0} = \frac{12·2 \times 100}{40·0} = 30·5$ per cent.

0·5 per cent D.D.T. $\frac{(69·4 - 58·3)\ 100}{100 - 58·3} = \frac{11·1 \times 100}{41·7} = 26·6$ per cent.

(*Contd.*)

APPENDIX 1.—(*Contd.*)

Calculation of mg. per sq. ft.

Area of filter paper used for tests $= \pi r^2 = \frac{22}{7} \times 5{\cdot}5 \times 5{\cdot}5$ sq. cm.

100 c.c. of the solution contains 2 gm. or 2,000 mg. of Technical D.D.T. (for 2 per cent concentration)

Therefore 1 c.c. of solution contains 20 mg. of D.D.T.

As 1 c.c. of the solution is applied to each filter paper, 20 mg. of D.D.T. are deposited on an area of $\frac{22}{7} \times 5{\cdot}5 \times 5{\cdot}5$ sq. cm.

Therefore 194·6 mg. are despoited per sq. ft. in the case of 2 per cent concentration.
97·3 mg. ,, ,, ,, ,, 1 per cent concentration.
48·6 mg. ,, ,, ,, ,, 0·5 per cent concentration.

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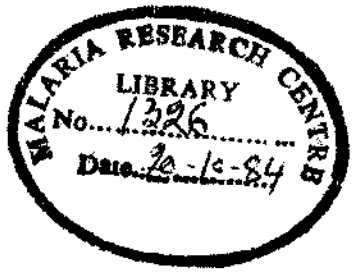

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