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NOTICE

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1964

Applications are invited for award of four **'Shakuntala Amir Chand Prizes'** of the value of Rs. 300 each for best published research work in any subject in the field of medical science including clinical research. The term 'clinical research' covers research into the mechanism and causation of diseases and its prevention and cure, and includes work on patients in hospitals, field studies in epidemiology and social medicine and observations in general practice.

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NOTICE.

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MSS. should be sent in **duplicate** only after careful revision and correction, otherwise they are liable to be refused. Authors are asked especially (*a*) to verify the proper logical arrangement and sequence of their headings and sub-headings, and (*b*) to maintain uniformity in the use of contractions, capitals, italics, etc. A short summary of the salient points of the paper should be placed at the end.

Tables, charts, etc., should be numbered and alluded to in the text as 'Table I', etc., and not as 'the following table', etc.

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

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The following are some of the abbreviations in use :

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

NOTICE.

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Indian Journal of Malariology, **17**, *1*, *March 1963*.

NEED FOR RESEARCH IN FILARIASIS

BY

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AND

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[August 15, 1962]

INTRODUCTION

FILARIASIS has been under investigation for a number of years under the auspices of the Indian Research Fund Association and subsequently the Indian Council of Medical Research. Initially, attempts were made for determining the incidence of the disease in some parts of the country, and also for studying its clinical aspects. In 1950, the Indian Council of Medical Research established a pilot project in Orissa—a highly endemic region for filariasis—in order to evolve methods for its control and ultimate eradication. Time was opportune then to undertake these studies because of the development of a drug, diethylcarbamazine (hetrazan), for its treatment and because of the knowledge available in dealing effectively with the mosquito species. In that pilot project, attempts were made to evaluate the relative merits of mass treatment of the population with hetrazan, and measures against the larval and adult stages of mosquitoes in the eventual control of the disease. Based on these studies, the Government of India initiated the National Filariasis Control Programme in certain endemic areas of the disease in the Second Five-Year Plan period.

In order to facilitate the development of this programme in the Third Five-Year Plan period, the Government of India requested the Indian Council of Medical Research to appoint a Committee to evaluate the work done under the aegis of the National Control Programme and to suggest measures for future development. Report of that Committee has been sent to the Ministry of Health, Government of India. During the course of the evaluation referred to above, however, it became evident that, to carry the control programme to its logical conclusion, it was essential to have information on many aspects of filariasis which may be pertinent to the successful implementation of that programme. Attempt is, therefore, made in this note to highlight some of the problems which require

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intensive study, on the basis of experience gained so far, in the control of the disease.

THE PROBLEM

It would be recalled that in 1953, with the then existing data, it was estimated that 25 million people were living in areas exposed to the risk of filariasis. As a result of surveys carried out by the National Filaria Control Programme since then, it is now known that about 64 million people are living in areas of varying degrees of filarial endemicity. However, the actual size of the problem cannot yet be defined as there are many areas yet to be surveyed.

Surveys carried out recently, mostly of the reconnaissance type, by the Malaria Institute of India (now the Central Institute for Communicable Diseases) have shown that filariasis transmission is occurring in areas where it did not exist before. For instance, in Sonepat, 30 miles north of Delhi, infected and infective mosquitoes have been detected. In Brindaban, 90 miles south of Delhi, a rapid survey, during which 40 persons were examined, showed 4 persons with microfilariae. 90 mosquitoes were dissected of which 3 showed infection and one mosquito had infective larvae. In the Nagpur zone, the infection has probably been introduced within the last 10 years and has resulted in a microfilariae rate in the population of the order of 10 to 15 per cent. In the three areas, however, the disease has not yet been established and it is foreseeable that if the existing transmission progresses unhampered, a proportion of the people will manifest the varying stages of the disease, including elephantiasis, in the next few years. It is likely that other foci of infection will come to light if attempts are made to find them. It would appear that migration of people from endemic zones, with microfilariae in the blood, to these areas has been responsible for this state of affairs.

The filariasis problem in India can, therefore, be classified under the following headings :—

1. Areas where the disease rate is very high, indicating that the transmission is of a very long duration, perhaps a century or more (*e. g.*, Mattancherri in Kerala State).

2. Areas like Porbandar in Gujarat State where the disease rate is 10·1 per cent and the infection rate is 6·8 per cent (Raghavan, 1951) and Mangalore, where the disease rate is 9·5 per cent and the infection rate is 15 per cent (Krishnaswami, 1955). Local information available in these areas shows that the transmission has been occurring for the last 25 to 40 years approximately.

3. Areas where the infection rate is about 10 per cent and the disease rate is negligible, indicating that the transmission is only since about a decade or so (local history).

4. Areas where the infection rate is between 5 and 10 per cent, but disease rate is nil (as in Rajkot in Gujarat), indicating that the transmission is less than 10 years old (local history).

5. Areas where the microfilaria rate is less than 5 per cent., with a low infection and infectivity rates in local mosquitoes, indicating that the transmission has probably commenced within the last 2 or 3 years, as for example in Sonepat (Punjab) and Mathura (Uttar Pradesh).

6. Areas where the *Culex fatigans* density is comparatively high, with no infection or infectivity in them, can be classified as areas with a potential risk to filariasis transmission.

7. Areas where the *Culex fatigans* density is low, and infection and infectivity rates are nil, can be classified as those without risk of filariasis.

All that has been said above, is in respect of bancroftian filariasis and is applicable to *B. malayi* filariasis also. The latter, however, as far as is known, is restricted to limited areas of Kerala, Andhra Pradesh, Orissa and Assam.

RESEARCH PROGRAMME

1. RESEARCH IN EPIDEMIOLOGY OF THE DISEASE.

A broad outline of the problem, as it exists today, has been described. It is essential to carry out field surveys in order to delimit the areas of filariasis in the country and prepare a filariasis map of India. These studies will indicate where attention has to be concentrated in the first instance in the adoption of the control measures against the disease and where extension of work on the lines of the present programme will have to be undertaken. The point for consideration is whether areas, showing low microfilaria rates in the population but an appreciable amount of infection in mosquitoes, should not receive high priority in any programme for the control of this disease. Present experience has shown that the control programme is likely to be of long duration. While attempts are made, therefore, to deal with the existing areas of high endemicity, it would be necessary to see that extension of the infection to other areas, which will ultimately be an additional burden on our resources, is avoided. Control measures, in areas where infection is not yet fully developed, might prove more economical in the long run than in areas of high endemicity. It is from these aspects of the field surveys that to define areas of filariasis seems to be important.

In the study of epidemiology of this disease, two basic factors have to be kept in mind, namely, the probable long incubation period of the disease and the need for repeated infection before the disease gets finally a foothold. It was shown during the Second World War that soldiers infected in one region lost the infection when transported to areas free from filariasis. A problem of some importance in this connection is to determine whether filariasis is a household or familial infection. Work done earlier has suggested that this is a possibility. The insect vector, *Culex fatigans*, which breeds in cesspools around houses, does not seem to travel too far but remains confined, so far as its feeding habits are concerned, to houses in very close proximity to the breeding areas. If these observations are

finally confirmed, they would provide some indications on where to adopt antilarval measures for dealing with them with ease and economy.

2. FACTORS RELATED TO TRANSMISSION ENTOMOLOGICAL RESEARCH.

From what has been said above, it would be clear that there are many other aspects which need investigation, *e.g.*, those which favour or retard the transmission of infection from mosquitoes to human population. It is well known that bancroftian filariasis is endemic in many parts of the country with varying climatic conditions like rainfall, temperature, humidity, etc. However, there is reason to believe that the spread of infection in any area occurs slowly. It is possible that, in some areas the transmission is perennial while in others it is seasonal. If such differences exist, there must be differences in the quantum of transmission which will eventually influence the incidence of infection as well as that of the disease in a population.

In view of these considerations, the subjects of study could be defined as follows :—

(*a*) the areas of the reservoir of infection ;

(*b*) vector density ;

(*c*) vector longevity ;

(*d*) gonotrophic cycle and the biting rate ; and

(*e*) the influence, if any, of the immune status of the host on the developmental cycle of the parasite in the mosquitoes.

Researches, carried out on the above mentioned aspects, will provide basis for working out a 'transmission index' which is still lacking and which is essential in order to elucidate the evolution of endemicity as well as for the precise evaluation of results of control.

Entomological research has other facets also. The possibility or otherwise of other species of mosquitoes, besides *Culex fatigans*, or any other arthropod playing a role in the transmission of infection, has to be borne in mind.

3. IMMUNOLOGY OF FILARIASIS.

Many factors concerning the specific immunity to infection and disease, either inherent or acquired, have yet to be studied. It has been observed that once the disease has established itself, there is a progressive decrease in the number of microfilariae per cubic millimeter of blood. In a majority of cases microfilariae totally disappear. Whether this phenomenon is attributable to immunity or to the physical death of the adult worm, is debatable. It is also known that in cases of established elephantiasis further exposures to infection are of no avail. Pandit *et al.* (1929) demonstrated an immunity phenomenon, called the 'adhesion phenomenon', in filariasis. In the presence of serum from a case of elephantiasis, leucocytes adhere to microfilariae, bringing about their death. The presence of an

antibody in the serum of a case of elephantiasis could thus be demonstrated. It seems worthwhile to pursue this work on a quantitative basis, using refined procedures which are now available for the purpose.

Again in the early stages of infection and disease, microfilariae may not be demonstrable by routine microscopy. In such cases the role of antigens prepared from filarial worms, and the elucidation of cutaneous reaction with their use, should be explored. It may be pointed out that so far only antigens, prepared from adult worms of animal filariasis, have been used. It might be worthwhile investigating the action of antigens prepared from infective larvae of specific infections harvested from infected mosquitoes. The specificity of the reaction can then be demonstrated more readily by this technique than would be possible otherwise. In dealing with the problem of transmission, reference was made to the immune status of the host in determining the infectivity of the vector. This is also an associated phenomenon in immunity which needs study.

4. CLINICAL STUDIES.

Filariasis, like syphilis and malaria, appears to be protean in its manifestations. Apart from lymphadenitis and lymphangitis, other symptoms of the disease do not receive much attention in the differential diagnosis of many other conditions. Experienced clinicians and surgeons have often noted a large number of clinical conditions which may be simulated by filariasis. Instances of acute abdomen, amenable to specific filarial therapy, are known. However, such cases have not been properly documented or studied. One of the methods to build up specific and classified knowledge on this subject would be to submit every case of ill-health in endemic areas, to the examination of night-blood smear. When microfilariae are thus demonstrated in any case, that finding should receive consideration in the differential diagnosis of the clinical conditions under investigation. Such a procedure may also yield valuable results in the study of allergic conditions which are manifestly of diverse etiology. In discussing the immunology of the disease, reference has been made to the studies of filarial antigens. Apart from their utility in the early diagnosis of filariasis, they would also bring to light the filarial etiology of such conditions as tropical eosinophilia. Apparently, there is some evidence that at least a proportion of cases of tropical eosinophilia is caused by filarial infections. It would be worthwhile to compare the antigens, prepared from infective larvae of different animal filarial infections, in the elucidation of some aspects of tropical eosinophilia. Preliminary work, carried out at the Malaria Institute of India (now the Central Institute for Communicable Diseases), indicates the possibility of the use of a haemaglutination test for such diagnostic purposes.

5. THERAPEUTIC TRIALS.

In the mass therapy programme instituted with diethylcarbamazine (hetrazan), certain interesting results have come to light. The occurrence of reactions, either mild or severe, have been noted. Severity of the reactions apparently depends on

the degree of infection in an individual, as well as the extent to which disease process has been established. The occurrence of such reactions has been a great hindrance in the successful execution of mass therapy for the control of the disease. The varying factors, which precipitate such reactions, have to be studied from all aspects, *i. e.*, biochemical, as well as immunological. Again, it is necessary to determine an adequate or optimum single dose therapy in such cases, which might be a more feasible proposition for adoption in mass therapy than the current five-day treatment in use in the programme.

The possibility of inducing immunity in individuals with the use of specific antigens, prepared from infective larvae from mosquitoes, should also be investigated. If it can be shown that antibodies against infective larvae do develop, it would be a contribution of immense value from the standpoint of prophylaxis.

6. ANIMAL FILARIASIS.

Research in the problem of filariasis in general cannot be considered comprehensive unless attention is also paid to the problem of animal filariasis. Many animals show filarial infections with different types of filarial worms. In so far as is known, no evidence exists of a zoonotic relationship of *W. malayi* to any animal infection in India, as has been demonstrated in Malaya. *W. bancrofti* does not appear to have any zoonotic background. Many of these animal filarial infections, as has been stated above, may play a role in the etiology of tropical eosinophilia the incidence of which in this country is said to be the largest in the world. Again, the morphological and developmental forms of different filariae in the mosquito, as has been shown by King, Pandit, *et al.* (1929), may provide the basis for a proper evaluation of epidemiological results and the role of mosquitoes in transferring the infection.

As has been stated earlier, these problems have come up because of the experiences gained in the evaluation of the National Control Programme now under way. It is possible that as research progresses, many other facets would come to light which would need careful study.

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THE COURSE OF MITE-INDUCED INFECTION OF *LITOMOSOIDES CARINII* IN ALBINO RATS TREATED WITH DIETHYLCARBAMAZINE*—ABSENCE OF ANY EVIDENCE OF EFFECT ON ADULT WORMS.

BY

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[August 20, 1962.]

HEWITT *et al.* (1947) originally claimed that prolonged treatment with hetrazan destroyed the adult worms of *L. carinii*. Kenney and Hewitt (1949) discussed the results of the trials with hetrazan against bancroftian filariasis in Guianese patients. In their opinion, the failure of microfilariae to recur in the peripheral blood in most cases, 4 months after treatment, indicated that the adult worms were dead or had been sterilized of their reproductive functions.

Hawking *et al.* (1950) were not able to confirm that adult worms were destroyed by diethylcarbamazine. The highest mortality rate of adult worms in the treated animals was not more than 10 per cent. The persistence of a proportion of microfilariae in some treated cases was considered by Hawking (1950) to be due to their location in the sites inaccessible to the drug. McGregor *et al.* (1952) considered that since the effects of hetrazan on the microfilariae persisted for more than 10 months, it was probable that hetrazan produced a permanent effect (death or sterilization) upon the adult worms of *W. bancrofti*. They also said that it was difficult to demonstrate such an effect in experimental *Litomosoides* infection in cotton-rats.

The studies reported here were carried out to determine the effect of diethylcarbamazine, if any, on the adult female *L. carinii* in albino rats.

*The four different preparations containing diethylcarbamazine used were : (*1*) Hetrazan (1-diethylcarbamyl-4-methylpiperazine dihydrogen citrate) of Lederle Laboratories, American Cyanamid Company ; (*2*) Banocide (1-diethylcarbamyl-4-methyl piperazine citrate) of Burroughs Wellcome & Co. (India), Ltd. ; (*3*) Supatonin (diethylcarbamazine citrate) of Tanabe Seiyaku Co. Ltd., Japan ; (*4*) Unicarbazan (diethylcarbamazine citrate) of Unichem Laboratories (Bombay), India.

MATERIAL AND METHODS.

Albino rats* with mite-induced infection* (Ramakrishnan *et al.*, 1961) were treated with diethylcarbamazine at different dosage schedules at different stages of the infection (Table I).

TABLE I.

Dosage schedules of diethylcarbamazine for the treatment of L. carinii *infection in albino rats*†.

Rat number.	Duration, in days, of patency before treatment.	Preparation of diethylcarbamazine used.	Route of administration.	Daily regime.	Dose mg./kg.	Number of days of treatment.
71	106	Hetrazan	Intraperitoneal	Once	250	2
72	61	—do—	—do—	—do—	250	2
91	131	Banocide	Oral	Twice	25	9·5
93	47	—do—	—do—	—do—	25	9·5
98	35	—do—	—do—	—do—	25	9·5
99	48	(i) Hetrazan	Intraperitoneal	Once	250	2
		(ii) Hetrazan	Oral	—do—	250	2
		(iii) Unicarbazan	Intramuscular	—do—	100	2
		(iv) Banocide	Oral	Twice	250	9·5
192	28	Supatonin	Intraperitoneal	—do—	0·8	8
203	57			—do—	3·0	8
205	58			—do—	3·0	8
219	34			—do—	1·2	8
226	39			—do—	2·0	8
228	92			—do—	2·5	8

Blood smears (2 c.mm.) from the tails of the animals were obtained before, during and after treatment. The slides were dehaemoglobinised, fixed, stained and examined for microfilariae and enumerated when found, as described by Dalip Singh and Raghavan (1962). When the 2 c.mm. samples of peripheral blood of these animals had become negative for microfilariae and remained negative for varying periods of 1 to 6 weeks, the animals were sacrificed and autopsied. The adult worms were recovered from the thoracic cavity of each animal and transplanted into the abdominal cavity of a normal adult albino rat. The blood smears from the recipient animals were examined from the 3rd day after transplantation of adult worms up to a maximum period of 15 weeks (Table III). The recipient animals were allotted the same number as of the donors (from whom adult worms were removed at autopsy) with the letter 'T' affixed, indicating transplantation of worms. This numbering facilitated the follow-up of results of treatment, and transplantation (From the animals mentioned in Table II to the animals mentioned in Table III).

* The rats and mites were obtained from the colony maintained at the Institute.

† The age of rats, at the time of treatment, varied from 3 months 22 days to 9 months 18 days and weight from 130 gm. to 210 gm. The age, at the time of exposure to infection, was 6 months for rats numbers 98 and 99 but for the other animals, it varied from 19 to 30 days.

RESULTS.

1. RESULTS OF TREATMENT.

Particulars in regard to the formulation of diethylcarbamazine used, the route of administration, the dose and the number of days of treatment are mentioned in detail in Table I. The results of treatment are shown in Table II.

It will be observed from Table I that rats numbers 91, 98, 99, 205 and 228 had been given a prolonged course of treatment before the microfilariae cleared from the peripheral blood. Rats numbers 91 and 98 were treated for 9·5 days. But they became negative only after 25 and 10 weeks respectively after the completion of treatment. Rat number 99 was treated for a total of 15·5 days and its peripheral blood became negative to microfilariae 5 weeks after the treatment. Rat number 205 had a total of 48 mg./kg. in 8 days and the peripheral blood was not cleared up to 6 weeks except during intervals in a total period of 10 weeks. Rat number 228, which had a total of 40 mg./kg. in 8 days, continued to have microfilariae in the peripheral blood for a period of 6 weeks. It was clear, therefore, that the individual animals reacted differently to similar dosages of the same drug.

The duration of patency, before the treatment, did not appear to be related to the time taken for the disappearance of microfilariae. This was evident from the response to treatment of rats numbers 71, 91, 99 and 219 (Tables I and II). The average infestation, prior to treatment, did not also appear to be related to the time taken for the clearance of microfilariae at the end of the treatment.

2. RESULTS OF TRANSPLANTATION OF ADULT WORMS AFTER TREATMENT.

Table III depicts the results of transplantation into normal albino rats of adult worms taken out of the thoracic cavities of albino rats treated with diethylcarbamazine.

It may be seen from above that, out of the 12 normal albino rats into which living adult worms from treated albino rats (Tables II and III) were transplanted, all, except two animals (rats numbers 91-T and 93-T), showed microfilariae in their peripheral blood on the 3rd to 7th day of transplantation. The animals, whose peripheral blood became positive for microfilariae after the transplant, remained patent for periods of 5 to 15 weeks of observation. The peak infestation (400 microfilariae in 2 c.mm. blood) was highest in rat number 219-T and lowest (9 microfilariae) in rat number 205-T. Though the numbers of adult worms transplanted were not always counted to avoid damage, rats numbers 91-T and 93-T, which never showed microfilariae in their peripheral blood, received only about 4 female worms each.

From the results depicted in Table III, it was quite clear that the adult female worms were not sterilized by diethylcarbamazine as they still produced microfilariae when transplanted into normal albino rats.

TABLE II.

Results of treatment of L. carinii *infection in albino rats with diethylcarbamazine.*

Rat number.	Number of microfilariae per 2 c.mm. tail-blood														
	Before treatment.	After treatment as shown in Table I.													
		24 hours.	48 hours.	3 days.	5 days.	7 days.	2 weeks.	3 weeks.	4 weeks.	5 weeks.	6 weeks.	10 weeks.	13 weeks.	25 weeks.	26 weeks.
71	1,139	7	N	5	N	N	N	N	N	N	N	*			
72	135	14	1	1	N	N	N	N	N	N	*				
91	493	4	5	31	135	137	294	104	60	97	101	98	166	12	N*
93	52	1	1	N	N	N	N	6	3	7	6	N	*		
98	264	20	1	2	1	1	9	40	159	126	74	N	N	*	
99	468	27	7	..	19	N	3	128	52	N	N	N	*		
192	93	9	8	6	..	1	7	N	N	N	N	*			
203	85	77	5	1	4	N	N	*							
205	66	53	2	N	1	N	6	12	10	9	N	N	*		
219	78	26	3	2	N	N	N	N	N	N	*				
226	68	32	N	N	N	N	N	N	N	N	*				
228	109	34	14	3	N	1	5	47	48	58	125	N	N	*	

N = Negative for microfilariae in tail-blood. * The animals were autopsied during the week.

TABLE III.

Results of transplantation, into normal white rats, of adult L. carinii *worms taken out of albino rats after treatment with diethylcarbamazine.*

Number of microfilariae in heartblood of donor rat at autopsy.	Rat into which worms were transplanted.	Microfilariae per 2 c.mm. blood, weeks after worm transplant:															
		Days.	Weeks.														
		3	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
10	71—T	3	20	59	74	127	84	91	24	14	21	19	12	7	7	2	1
7	72—T	1	12	20	21	15	7	22	8	1	5	N	N	N	N	N	N
N	91—T	N	N	N	N	N	N	N	Only 4 adult female worms were available and transplanted.								
N	93—T	N	N	N	N	N	N	N	—do—								
4	98—T	29	57	255	146	376	44	N	N	N	N	N	N	N	N	N	N
8	99—T	3	62	201	183	276	257	113	N	N	N	N	N	N	N	N	N
N	192—T	8	14	93	107	84	106	N	N	N	N	N	N	N	N	N	N
1	203—T	2	11	61	76	23	35	16	2	1	N	N	N	N	N	N	N
38	205—T	N	3	9	8	3	5	2	2	5	N	1	N	2	N	N	1
7	219—T	2	17	191	208	400	260	N	N	N	N	N	N	N	N	N	N
N	226—T	2	10	27	40	29	50	20	37	20	18	10	6	3	3	1	2
4	228—T	1	6	11	39	11	14	8	13	15	2	9	9	9	4	2	3

N = Negative for microfilariae in tail-blood.

The average duration of parasitaemia from commencement up to autopsy in the rats into which the worms were transplanted (Tables I, II and III) was 29·2 weeks. This was about 11 weeks more than the average duration of parasitaemia in normal untreated infections (Dalip Singh and Raghavan, 1962 *loc. cit.*). The course of microfilaraemia in the rats into which adult worms from treated animals were transplanted was not dissimilar to that of animals into which worms, taken out of latent but untreated animals, were transplanted (Ramakrishnan *et al.*, 1962).

On an average the normal rats into which worms from treated animals were transplanted showed microfilariae in their peripheral blood for about 7·5 weeks. As against this, on an average, similar rats into which worms from untreated animals were transplanted showed microfilariae in the peripheral blood for about 9·3 weeks. In both the cases the duration of patency was about half that of normal untreated infection ; the explanation, if any, for the difference is not understood at present.

The average number of microfilariae per week in untreated mite-induced infection was comparatively higher than that of the animals with transplantad worms (Table IV). This indicated that the production of microfilariae was higher in the early part of the life span of the adult female worms as compared to the later part of their life.

TABLE IV.

Weekly average number of microfilariae per 2 c.mm. tail-blood of (i) normal untreated infection, (ii) after worms transplant from treated rats to normal rats, and (iii) after worm transplant from untreated latent rats to normal rats.

	Weeks.														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Normal untreated infection (11).	72	119	187	263	307	330	230	94	114	148	76	20	20	29	12
2. *After worm transplant from treated rats to normal rats (12).	21	83	90	134	86	39	15	13	12	13	9	5	5	2	2
3. †After worm transplant from untreated latent rats to normal rats (11).	140	101	87	65	28	41	26	19	25	19	13	‡	‡	9	‡

( ) Figures within brackets show the number of rats for which the data have been averaged.

* Reproduced from Table III of this paper.

† Reproduced from Table I in the publication by Ramakrishnan *et al.* (1962).

‡ Results not recorded.

3. VIABILITY OF MICROFILARIAE IN RATS WITH TRANSPLANTED WORMS.

In order to determine the infectivity of the microfilariae produced by the transplanted worms, mites were fed on rat number 219-T (Table III). A fortnight later, infective larvae were noted in a few of the mites dissected. Eigh

albino rats (20 days old) were exposed to the bites of these infective mites. The peripheral blood of five of these rats was found to be positive for microfilariae 58 days to 65 days after the exposure.

The findings mentioned above clearly showed that diethylcarbamazine had no sterilizing effect *in vivo* on the adult female worms in the treated albino rats; microfilariae were produced by these worms when transplanted into the normal albino rats. The microfilariae from such worms developed normally into infective larvae in the mites. They, in their turn, were able to infect normal albino rats.

DISCUSSION.

The observations described above were contrary to the findings of a number of workers referred to earlier. The adult worms of *L. carinii* were recovered alive after treatment with heavy dosages of diethylcarbamazine and were found to be actively motile. There was no evidence that the drug had any lethal effect on the adult worms. The transplantation procedures required the least amount of handling of the recovered worms. It was, therefore, not possible to be certain that there was no mortality at all of even a small proportion of adult worms attributable to the drug. It, however, seemed unlikely.

In the present series there was no evidence of sterilization of the adult female worms after treatment. These worms, when transplanted into the peritoneal cavities of normal adult rats, produced microfilariae in their peripheral blood for varying periods up to a maximum of 15 weeks (Table III). The mites that fed on one rat (219-T, Table III) into which worms had been transplanted from a treated animal, were infected. In turn they were found to be infective to other rats. Five out of the 8 albino rats exposed to the bites of these infective mites, showed microfilariae in their peripheral blood from 58 to 65 days after exposure.

The data did not lend themselves to form any conclusion on the effect of the drug on the reproductive capacity of the male. Even if the adult male worms were selectively sterilized by the treatment, the reproductive capacity of the female was not affected. Webber (1954) reported that the presence of male worms was not essential for the maintenance of the microfilarial infection produced by implanting fertilized adult female worms. Webber (1954 *loc. cit.*) concluded that repeated insemination of the female worms was not necessary.

A noteworthy feature of the observations was the considerable variation in the effect of the drug (identical dosage schedule) on microfilariae in individual rats. This individual variation was apparent in all the four preparations of the drug used. No correlation was apparent between the duration of patency or the degree of infestation prior to treatment and the final effect of the drug on microfilariae. Whether the variation is attributable to individual differences in the rats to absorb and metabolise the drug or to inherent differences in the characteristics of the microfilariae themselves, should remain a matter for speculation at present.

The heart-blood samples from 8 out of 12 treated animals showed microfilariae at autopsy when the peripheral blood had remained negative for 1 to 6 weeks (Table III). Identical findings were reported by Ramakrishnan *et al.* (1962) in the case of untreated infections. It appeared, as suggested by Hawking and Thurston (1951), that during the process of dying there was a massive liberation of microfilariae from the lungs and these migrated to the heart.

SUMMARY.

Albino rats, infected with *L. carinii*, were treated with various dosages of diethylcarbamazine. The animals were autopsied when their peripheral blood had remained negative after treatment for varying periods up to a maximum of 6 weeks.

The adult worms recovered at autopsy were found alive and actively motile. They were transplanted into the peritoneal cavities of normal albino rats. The recipient animals showed microfilariae in their peripheral blood up to a maximum period of 15 weeks after the worm transplant. The microfilariae were able to infect the mites, *Ornythonyssus bacoti*. The mites, in turn, were able to infect the normal albino rats.

It was concluded that diethylcarbamazine has no sterilizing effect *in vivo*.

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DETERMINATION OF PHYSIOLOGICAL AGE IN THE NATURAL POPULATION OF *ANOPHELES FLUVIATILIS* JAMES, 1902, BY POLOVODOVA'S TECHNIQUE.

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INTRODUCTION.

THE recognition of parous mosquitoes from the nulliparous is important in relation to the survival rates of mosquitoes. Various methods have been in vogue by different workers for age-determination in mosquito populations. Based on Perry's wing-grading, Bhombore *et al.* (1954) tried a morphological method of determining the age composition in *Anopheles*, and established the wing-index in the manner of calculation of the average enlarged spleen. Modern methods aim at determining the physiological age of mosquitoes by qualitative changes in the reproductive organs of a female mosquito (Polovodova, 1949 ; Detinova, 1949, 1962 ; Lewis, 1957, 1958 ; Gillies, 1958 ; Achuthan, 1958 ; Bertram, 1959). A preliminary appraisal of the use of this technique in anopheline mosquitoes has been made by the Division of Malaria Eradication, World Health Organization, Geneva (1960).

During the course of entomological collections at Nuthandi for the susceptibility tests, opportunities arose to try out the Polovodova's technique in wild-caught *A. fluviatilis*, from structures sprayed nearly a year ago. This study was undertaken during the period December 1, 1961 to February 28, 1962. The season of prevalence of this species has been described by Sen *et al.* (1960) as November to February, with a peak of abundance in January, from near about the present situation.

MATERIALS AND METHODS.

Collections were obtained in the early hours of morning (at an overall per man-hour density of 1·7) from a village situated in the vicinity of the headquarters laboratory. Maximum collections of *A. fluviatilis* were aimed at by exhaustive searches, for one of us (S.A.A.) accompanied the junior field staff on several occasions during the period and exhaustive and thorough searches were confirmed.

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The mosquitoes were dissected on the day of collection immediately after they were received in the laboratory. This village had been last sprayed in January, 1961.

The original dissection technique of Polovodova and Detinova as re-described by Gillies (1958 *loc. cit.*) and Achuthan (1958 *loc. cit.*) was followed. Dissection of ovaries was done in physiological saline under a binocular dissecting microscope, using very fine pointed needles. The paired ovaries were pulled out, one ovary was severed and held by the needle in the left hand at the region of the internal oviduct, while with the other needle in the right hand, the intimal sheath of the ovary was torn in longitudinal bits, which enabled the recognition of the individual ovarioles. They were then liberated from the internal oviduct to reveal the full extent of the ovariole. The follicular relics, when present, could be seen *in situ*, at the pedicellar tip of individual ovariole. Under dry high power of microscope, even the yellow granules inside the follicular relics could be discriminated. Successful attempts were made to expose a minimum of 9-10 ovarioles per ovary, while 12 ovarioles have been exposed per specimen on an average.

During the period, 305 *A. fluviatilis* (females) were collected, of which only 171 gorged specimens, that were in abdominal conditions mainly '*B*' and quite a few in '*C*' (Viswanathan and Ramachandra Rao, 1943) and in Stages II and III of Christophers, were dissected and 2030 ovarioles examined in all. The balance of 134 specimens could not be dissected as they constituted a few in abdominal conditions '*A*' and quite a few in '*D*'. Thus 56 per cent of the *A. fluviatilis* collected, were dissected successfully. The details are mentioned in Table I.

TABLE I.

Age-composition of A. fluviatilis *from Nuthandi near Dhanbad, during December, 1961—February, 1962.*

Weeks commencing from December, 1961.	Number of mosquitoes dissected.	Number of mosquitoes found in each follicular group :			
		(0)	(1)	(2)	(3)
1st week.	4	..	4	..	..
2nd week.	16	6	9	1	..
3rd week.	22	1	18	3	..
4th week.	17	3	10	4	..
5th week.	34	11	14	9	..
6th week.	11	6	5	..	..
7th week.	29	8	12	7	2
8th week.	4	1	2	1	..
9th week.	9	5	1	3	..
10th week.	7	3	2	2	..
11th week.	8	4	1	3	..
12th week.	10	4	2	4	..
Total ...	171	52	80	37	2
Per cent parous ...	..	30 Per cent.	47 Per cent.	22 Per cent.	1 Per cent.

Achuthan and Sitaraman (1959) have worked out in eleven Indian anophelines, including 22 *A. fluviatilis*, obtained from an area in Mandya (Mysore) under interruption of spraying, during the period August to October, 1959. A summary of their findings relating to *A. fluviatilis* is noted below.

27 per cent	...	Nulliparous.
36 per cent	..	1—parous.
18 per cent	...	2—parous.
14 per cent	...	3—parous.
5 per cent	...	4—parous.

In Table II are presented the number of mosquitoes collected during the present study, placed according to Christopher's stages of ovary, as also the number of mosquitoes found in various stages of gonotrophic cycle. The exact calendar-age, as deduced by using the formula based on the physiological age of the dissected mosquito-population, is as noted below :—

Expression $A = (C \times G) + a + s$... Achuthan (1958 *loc. cit.*), where :—

'*A*' is the computed age to be derived ;

'*C*' is the number of follicular relics ;

'*G*' is the known gonotrophic cycle in days—3 days in winter—(Viswanathan *et al.*, 1944) ;

'*a*' is the first feeding after emergence—a constant for the species of mosquitoes (2 days) ;

'*s*' is the stage of ovary observed during dissection—

Stage II—roughly 2 days,

Stage III—roughly 3 days (Puri, 1957).

Computed calendar-age of *A. fluviatilis*, based on the number of egg-layings, detailed as the number of mosquitoes in each age-group :—

Ovary in Stage II.				Ovary in Stage III.		
4 days	7 days	10 days.	13 days.	5 days.	8 days.	11 days.
0 (35)	1 (36)	2 (24)	3 (2)	0 (17)	1 (43)	2 (14)

Note : Figures in brackets denote the number of mosquitoes, and those outside relate to their completed gonotrophic cycle.

Altogether :—52 were nulliparous—being in age-group 4-5 days.
79 were 1-parous—being in age-group 7-8 days.
38 were 2-parous—being in age-group 10-11 days.
2 were 3-parous—being in age-group 13 days.

In Table III, is arranged the age composition, weekwise, according to Macan's subdivision of Christopher's classification of ovary. The retained eggs, encountered during the 9th and the 12th week, are also indicated appropriately.

TABLE II.

Dissections for age-grouping of A. fluviatilis *from Nuthandi near Dhanbad, placed according to Christopher's stages of ovary.*

Week commencing from December, 1961.	Number of *A. fluviatilis* collected.	CHRISTOPHER'S STAGE II.						CHRISTOPHER'S STAGE III.						Number of *A. fluviatilis* not dissected.	Remarks.
		Number dissected.	Number of ovarioles examined.	Follicular relics noted and number of mosquitoes.				Number dissected.	Number of ovarioles examined.	Follicular relics noted and number of mosquitoes.					
				0	1	2	3			0	1	2			
1st week	13	3	29		1 (3)			1	17		1 (1)			9	The mosquitoes not dissected account for those that were in abdominal conditions 'A', 'D' and beyond.
2nd week	42	11	157	0 (5)	1 (6)			5	69	0 (1)	1 (3)	2 (1)		26	
3rd week	46	10	99	0 (1)	1 (9)			12	195		1 (9)	2 (3)		24	
4th week	18	10	119	0 (3)	1 (4)	2 (3)		7	91		1 (6)	2 (1)		1	
5th week	42	17	185	0 (7)	1 (5)	2 (5)		17	186	0 (4)	1 (9)	2 (4)		8	
6th week	13	4	47	0 (4)				7	79	0 (2)	1 (5)			2	
7th week	42	19	194	0 (7)	1 (5)	2 (5)	3 (2)	10	96	0 (1)	1 (7)	2 (2)		13	
8th week	7	2	22			2 (2)		2	25	0 (1)	1 (1)			3	
9th week	18	6	77	0 (2)	1 (1)	2 (3)		3	37	0 (3)				9	
10th week	18	5	64	0 (2)	1 (2)	2 (1)		2	24	0 (1)		2 (1)		11	
11th week	31	5	56	0 (1)	1 (1)	2 (3)		3	39	0 (3)				23	
12th week	15	5	66	0 (3)		2 (2)		5	57	0 (1)	1 (2)	2 (2)		5	
Overall	305	97	1,115	0 (35)	1 (36)	2 (24)	3 (2)	74	915	0 (17)	1 (43)	2 (14)		134	

Note : Figures in brackets denote the number of mosquitoes.

TABLE III.

Weekly record of A. fluviatilis *dissections for follicular relics, arranged according to Macan's sub-division of Christopher's stages of ovary.*

Week commencing from December, 1961.	II-EARLY: Yolk granules very little.						II-MIDDLE: Yolk granules fairly developed.					II-LATE: Yolk granules well-developed.				
	Number of mosquitoes dissected.	Number of ovarioles examined.	Follicular relics recorded.				Number of mosquitoes dissected.	Number of ovarioles examined.	Follicular relics recorded.			Number of mosquitoes dissected.	Number of ovarioles examined.	Follicular relics recorded.		
1st week	..	..	..				..	..	..			3	29	1 (3)		
2nd week	6	105	0 (5)	1 (1)			1	10	1 (1)			4	42	1 (4)		
3rd week	5	46	0 (1)	1 (4)			3	36	1 (3)			2	17	1 (2)		
4th week	6	64	0 (2)	1 (2)	2 (2)		..	..	..			4	55	0 (1)	1 (2)	2 (1)
5th week	10	114	0 (5)	1 (4)	2 (1)		3	30	0 (1)		2 (2)	4	41	0 (1)	1 (1)	2 (2)
6th week	3	32	0 (3)				..	..	..			1	15	0 (1)		
7th week	11	119	0 (4)	1 (3)	2 (2)	3 (2)	1	9	0 (1)			7	66	0 (2)	1 (2)	2 (3)
8th week	1	10			2 (1)		1	12			2 (1)	..	..		..	
9th week	3	40	0 (1)		2 (2)		3	37	0 (1)	1 (1)	2* (1)	..	..		..	
10th week	2	22	0 (2)				2	30		1 (1)	2 (1)	1	12		1 (1)	
11th week	1	15	0 (1)				1	12			2 (1)	3	29		1 (1)	2 (2)
12th week	2	27	0 (2)				1	12	0 (1)			2	27			2 (2)
Total	50	594	0 (26)	1 (14)	2 (8)	3 (2)	16	188	0 (4)	1 (6)	2 (6)	31	333	0 (5)	1 (16)	2 (10)

* Retained egg in II-middle and 2 (1) seen.

Note—Figures in brackets denote the number of mosquitoes.

(*Contd.*)

TABLE III. (*Concld.*)

Weekly record of A. fluviatilis *dissections for follicular relics, arranged according to Macan's sub-division of Christopher's stage of ovary.*

Week commencing from December, 1961.	III-EARLY : Yolk granules filling more than ½ the egg.					III-MIDDLE : Yolk granules filling ⅔ of the egg.					III-LATE : Yolk granules filling the egg nearly complete.				
	Number of mosquitoes dissected.	Number of ovarioles examined.	Follicular relics recorded.			Number of mosquitoes dissected.	Number of ovarioles examined.	Follicular relics recorded.			Number of mosquitoes dissected.	Number of ovarioles examined.	Follicular relics recorded.		
1st week	1	17		1 (1)		..	..		..		..	..		..	
2nd week	..	..		..		3	37		1 (2)	2 (1)	2	32	0 (1)	1 (1)	
3rd week	2	30			2 (2)	1	7			2 (1)	9	158		1 (9)	
4th week	1	10		1 (1)		3	39		1 (3)		3	42		1 (2)	2 (1)
5th week	..	..		..		3	40		1 (2)	2 (1)	14	146	0 (4)	1 (7)	2 (3)
6th week	..	..		..		4	52	0 (2)	1 (2)		3	27		1 (3)	
7th week	2	15		1 (1)	2 (1)	2	25	0 (1)	1 (1)		6	56		1 (5)	2 (1)
8th week	..	..		..		1	10	0 (1)			1	15		1 (1)	
9th week	..	..		..		..	..		..		3	37	0 (3)		
10th week	2	24	0 (1)		2 (1)	..	..		..		..	..		..	
11th week	..	..		..		3	39	0 (3)			..	..		..	
12th week	3	40	0 (1)		2* (2)	..	..		..		2	17		1 (2)	
Total	11	136	0 (2)	1 (3)	2 (6)	20	249	0 (7)	1 (10)	2 (3)	43	530	0 (8)	1 (30)	2 (5)

* Retaind egg in one III-early and 2 (1) seen.

Note—Figures in brackets denote the number of mosquitoes.

DISCUSSION.

On a review of this preliminary study, it is gathered that the majority (77 per cent) constituted an admixture of nulliparous, with a preponderance of pauciparous ones. Also encountering of ovary in Stage I, and nulliparous in early Stage II in quite a few mosquitoes in later weeks, was indicative of fresh emergence. Twenty-two per cent of the *A. fluviatilis* population sampled was on the threshold of epidemiologically dangerous age of 10 days and over, indicating 2-follicular relics. Also a little over 1 per cent of the dissected mosquitoes were with 3-follicular relics. Thus this study reveals that 23 per cent of the mosquito population has reached the critical longevity. But from Mandya (Mysore), Achuthan and Sitaraman (1959 *loc. cit.*) have recorded the corresponding older age-group in 37 per cent of *A. fluviatilis*. As also in Mandya area, the maximum number of gonotrophic cycles completed is 4, against 3 during the present study.

It is a point of interest to note that, incidentally in both the areas, the survival of the species has been without the selection pressure of the insecticide.

There were two instances of retained ripe egg (mature egg), one in the 9th week and the other in the 12th week, of collection as indicated in Table III. In them, the ovary was in Stages II and III respectively and depicted 2-follicular relics in either case ; evidently both these specimens had reached the epidemiologically dangerous level.

The authors experienced the following difficulties :—

1. Though Detinova (1962 *loc. cit.*) describes as "Females at any stage of blood digestion and ovarian development are used for dissection", the authors found it difficult to dissect out ovarioles and expose relics in ovaries beyond Stage III, as by that time yolk fills the egg completely and hence the sheath ruptures and the yolk oozes out. This difficulty has also been observed by earlier workers.

2. It was not unlikely to break the follicular relics in this delicate dissection ; as such a good number of ovarioles were separated and the maximum number of relics observed in any ovariole recorded.

3. This was a time-consuming dissection, requiring nearly ½ hour for each specimen to successfully isolate on an average 12 ovarioles.

SUMMARY.

To determine the age composition of anophelines, several workers have tried different methods. The Russian malariologists evolved a method of delicate dissection of ovaries and counting the follicular relics by which the exact age could be established physiologically. This method was followed in case of *A. fluviatilis* (for the season of its maximum prevalence) from a village near Dhanbad and the age-composition of 171 specimens established. The maximum number of relics recorded being 3, in only 1 per cent of the population sampled. Twenty-three per cent of the dissected specimens had reached the potentially dangerous level of 10 days.

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THE SELECTION OF INSECTICIDE-RESISTANT STRAINS OF THE MOSQUITO, *CULEX FATIGANS* WIED., IN THE LABORATORY.

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[October 15, 1962.]

INTRODUCTION.

THE resistance of insects to insecticides is one of the most baffling problems facing entomologists today. Current trends in insecticide-resistance in vectors of diseases have recently been reviewed by Micks (1960), Brown (1960, 1961), and Perti and Ranganathan (1960).

Attempts have been made by several workers to raise laboratory colonies of resistant strains of mosquitoes by selection with DDT, with a view to studying the mechanism of insecticide-resistance. Fay *et al.* (1949) found that exposure of adults of *Anopheles quadrimaculatus* to residues of DDT, through several generations, produced only slight increase in resistance. Pal *et al.* (1952), in preliminary studies to select resistant strain of *A. stephensi*, observed slight tolerance to DDT. Hawkins (1956) reported slight increase in resistance in *A. quadrimaculatus* larvae on selection with DDT. Davidson (1957) and Weyer (1957) found slight increase in tolerance in *A. gambiae* and *A. stephensi* adults on selection with DDT. Sautet *et al.* (1958) attempted to raise resistant strains of *Aedes aegypti* by exposing the fourth-instar larvae, in successive generations, to DDT and obtained appreciable resistance within four generations. Gartrell and Ludvik (1954) made selection by the topical application of DDT solution in ethanol on adults of *A. quadrimaculatus* but did not find an increase in resistance following selection for 18 generations. Mohan (1960) selected adults of *Culex fatigans* by exposing to insecticide residues of DDT for 78 generations and reported considerable resistance in the strain. This paper presents data on the selection of two DDT-resistant strains of the mosquito, *Culex fatigans* Wied., in the laboratory.

MATERIALS AND METHODS.

Insecticides.—The insecticide used for inducing resistance in mosquitoes was DDT, pp'-isomer (m.p. 108°C). Lindane, dieldrin and diazinon were used for tests on cross-tolerance of DDT-resistant strains.

Insects.—The larvae for laboratory selection with DDT were drawn from the progeny obtained from larvae and pupae of *C. fatigans* collected from the field or from a susceptible laboratory-colony of the insect which was raised from a single egg-raft on September 17, 1945. The method of rearing the mosquitoes was as described by Newman *et al.* (1949). The third-instar larvae were used in the larval susceptibility test. Adult female mosquitoes, 40±5 hours old, were used in adult susceptibility tests.

Method of selection.—The larvae obtained in successive generations, both of the field strain (FRS) and the laboratory strains (LRS), were subjected to such dosages of DDT which caused about fifty per cent kill of the larvae. One ml. of alcoholic solution of the insecticide, in requisite concentration, was dispersed in 200 ml. water contained in a petri dish, 15 cm.×5 cm., and 50 second-instar larvae were liberated in each dish. The water in the dishes was changed every second day till all the larvae pupated. The emerging adults were subsequently inbred and the resultant larvae of the successive generations were selected and tested for resistance.

Attempt for inducing resistance in *C. fatigans* was also made by breeding only the late-emerging adults from the normal susceptible laboratory-colony of the insect. The larvae that pupated last, constituting about ten per cent of the total pupation, were used for the selection. This process of selection was continued in successive generations of the strain (LES).

Larval susceptibility tests.—The assessment was carried out by liberating fifty larvae in dishes containing varying concentrations of the insecticide in 200 ml. water (Damodar *et al.*, 1962). The per cent mortality of the larvae was determined after 48 hours. There were two replicates for each concentration investigated. The experiments were carried out at 26±1°C.

Adult susceptibility tests.—'Whatman' filter papers Number 1 (11 cm.) were impregnated with solutions of DDT in liquid paraffin in varying concentrations. For the treatment, a filter paper was immersed in insecticidal solution for one minute. The excess liquid was removed by suspending the treated paper for five minutes by means of a clip. The treated paper was subsequently kept for five minutes between two layers of untreated filter papers under four pound (1·82 kg.) weight. The assessment was carried out by confining twenty insects for varying periods under a clean glass funnel (7·0 cm. diam.) placed over the treated filter papers. Observations on the mortality were recorded 24 hours thereafter. There were two replicates in each assay. The temperature and relative humidity during the experiments were 26±1°C and 57±5 per cent respectively.

RESULTS AND DISCUSSION.

The results of the larval and adult susceptibility/ resistance tests are mentioned in Tables I to IV. The relationship between dosage and mortality, in the range

TABLE I.

Successive increases in larval resistance of C. fatigans *induced by laboratory-selection with DDT.*

Strain*	Generation.	Concentration ppm.	Mortality, per cent.	LC_{50} ppm.
FRS (Field resistant strain).	1	0·04	33	0·047
		0·05	49	
		0·06	75	
		0·07	85	
	5	0·20	46	0·22
		0·30	64	
		0·50	73	
		0·75	83	
	7	0·75	24	1·00
		1·00	49	
		1·25	61	
		2·25	89	
	11	8·0	23	11·3
		10·0	31	
		12·0	55	
		20·0	88	
	31	10·0	23	23·0
		20·0	42	
		30·0	53	
		40·0	69	
	36	25·0†	79	..
		50·0	83	
		75·0	79	
		100·0	65	
	38	25·0†	88	..
		50·0	60	
		75·0	58	
		100·0	42	
LRS (Laboratory resistant strain).	14	0·025	23	0·036
		0·035	38	
		0·045	66	
		0·060	85	
	23	0·06	27	0·066
		0·07	63	
		0·08	81	
		0·09	89	
	27	0·04	28	0·052
		0·05	31	
		0·07	70	
		0·08	76	
	28	0·075	37	1·20
		1·250	43	
		1·500	72	
		2·000	80	
	51	10·0	19	..
		20·0	22	
		40·0	30	
		50·0	45	

(Contd.)

TABLE I. (*Concld.*)

Strain.*	Generation.	Concentration. ppm.	Mortality, per cent.	LC_{50} ppm.
LRS (Laboratory resistant strain).	58	25·0†	93	..
		50·0	85	
		75·0	89	
		100·0	76	
	59	25·0†	73	..
		50·0	75	
		75·0	85	
		100·0	85	

* LC_{50} of the normal laboratory susceptible strain (SS) ranged between 0·03 ppm. and 0·06 ppm. during the experiments.

† 20 ppm. did not effect appreciable mortality of the test larvae; the kill was only 5 to 10 per cent.

TABLE II.

Selection for resistance by breeding late emerging adults of C. Fatigans.

Strain.*	Generation.	Concentration ppm.	Mortality, per cent.	LC_{50} ppm.
LES (Late emerging strain).	10	0·030	15	0·047
		0·035	27	
		0·040	49	
		0·050	58	
	16	0·035	18	0·048
		0·040	14	
		0·045	29	
		0·050	58	
	20	0·030	19	0·042
		0·035	29	
		0·040	41	
		0·045	56	
	25	0·03	23	0·046
		0·05	52	
		0·06	58	
		0·07	77	
	47	0·02	16	0·034
		0·03	42	
		0·04	64	
		0·05	74	
		0·06	84	
	49	0·02	20	0·036
		0·03	28	
		0·04	52	
		0·05	72	
		0·06	84	

* LC_{50} of the normal laboratory susceptible strain (SS) ranged between 0·03 ppm. and 0·06 ppm. during the experiments.

TABLE III.

Development of DDT-resistance in adults of C. fatigans.

Strain.	Generation.	Concentration. per cent.	Period of exposure (hours).	Mortality, per cent.	LC_{50} hours.
FRS	31	3·75	2·00	0	..
			4·00	0	
			6·00	0	
			16·00	38	
SS	..	3·75	1·00	20	1·7
			1·20	33	
			1·40	48	
			2·00	56	
			2·20	75	
FRS	33	3·50	16·00	25	24·0
			20·00	35	
			24·00	45	
			28·00	58	
			32·00	70	
SS	..	3·50	1·40	33	2·0
			2·00	35	
			2·40	50	
			3·00	70	
			3·20	83	
LRS	52	3·75	16·00	3	25·0
			20·00	28	
			24·00	45	
SS	..	3·75	1·00	20	1·7
			1·20	33	
			1·40	48	
			2·00	58	
			2·20	75	

SS = Susceptible strain ; FRS = Field resistant strain ;
LRS = Laboratory resistant strain.

of concentrations of insecticides investigated in the larval susceptibility tests, has been estimated by plotting the results on logarithmic-probability papers, fitting the regression line by eye and reading the LC_{50} from the graph. The LC_{50} represents the insecticide concentration at which fifty per cent of the larvae are killed. Similarly, the relationship between time of exposure and mortality, at the concentration of insecticide investigated in the adult susceptibility tests, has been estimated in terms of LC_{50}.

It will be observed from the data (Table I) that *C. fatigans* collected from field populations, and those from normal susceptible laboratory strain, are capable of developing resistance to DDT in the laboratory. It will also be seen that the selection of larval resistance is much more rapid in the field-strain than in the laboratory-strain. Although the development of resistance in the laboratory-strain

TABLE IV.

Larval susceptibility tests on cross-tolerance of DDT-resistant strains of C. Fatigans *to lindane, dieldrin and diazinon.*

Insecticide.	Strain.	Generation.	Concentration ppm.	Mortality, per cent.	LC_{50} ppm.
Lindane	FRS	31	0·50	34	0·78
			0·75	42	
			1·00	54	
			1·25	81	
	SS	..	0·10	22	0·20
			0·15	36	
			0·30	69	
			0·35	81	
	FRS	38	0·25	34	0·47
			0·50	44	
			0·75	62	
			1·00	82	
	SS	..	0·10	26	0·20
			0·25	60	
			0·50	82	
			0·75	92	
	LRS	53	0·1	30	0·22
			0·2	50	
			0·5	76	
			1·0	84	
	SS	..	0·10	24	0·23
			0·20	35	
			0·25	50	
			0·30	70	
	LRS	59	0·10	28	0·18
			0·25	55	
			0·50	68	
			0·75	95	
	SS	..	0·10	33	0·16
			0·25	57	
			0·50	81	
			0·75	92	
Dieldrin	FRS	31	0·10	28	0·16
			0·15	44	
			0·20	68	
			0·35	83	
	SS	..	0·0100	23	0·014
			0·0150	47	
			0·0175	66	
			0·0200	84	
	FRS	39	0·100	30	0·14
			0·125	42	
			0·250	56	
			0·500	88	
	SS	..	0·0030	26	0·01
			0·0060	48	
			0·0825	68	
			0·0250	90	

(*Contd.*)

TABLE IV. (*Concld.*)

Insecticide.	Strain	Generation.	Concentration ppm.	Mortality, per cent.	LC_{50} ppm.
Dieldrin	LRS	53	0·15	34	0·20
			0·20	54	
			0·30	70	
			0·40	70	
	SS	..	0·0125	23	0·015
			0·0150	47	
			0·0175	67	
			0·2000	84	
	LRS	59	0·100	38	0·13
			0·125	50	
			0·250	70	
			0·500	86	
	SS	..	0·0030	26	0·006
			0·0060	48	
			0·0125	68	
			0·0250	90	
Diazinon	FRS	35	0·050	14	0·08
			0·075	27	
			0·100	43	
			0·125	85	
	SS	..	0·045	32	0·06
			0·050	37	
			0·075	69	
			0·100	83	
	FRS	39	0·070	20	0·11
			0·100	38	
			0·125	56	
			0·150	78	
	SS	..	0·070	31	0·09
			0·100	52	
			0·125	66	
			0·150	79	
	LRS	56*	0·06	34	0·08
			0·08	55	
			0·10	70	
			0·14	84	
	SS	..	0·0175	48	0·02
			0·0200	62	
			0·0225	70	
			0·0250	84	
	LRS	59*	0·0070	24	0·011
			0·0100	40	
			0·0125	57	
			0·0150	70	
			0·0200	94	
	SS	..	0·0070	31	0·009
			0·0100	52	
			0·0125	66	
			0·0150	79	
			0·0175	90	

SS = Susceptible Strain ; FRS = Field Resistant Strain ; LRS = Laboratory Resistant Strain.

* The increase in tolerance to diazinon indicated in the fiftysixth generation is not shown in the fiftyninth generation. The DDT-resistant strain (LRS) cannot, therefore, be regarded as showing cross-tolerance to diazinon.

is much delayed, that is, the larvae do not show resistance to the insecticide up to the twenty-seventh generation, the development of resistance in the subsequent generations is fairly rapid and is about 400 to 1500-fold in the fiftyninth generation. In the field-strain, the resistance appears fairly early, that is, in the fifth generation and then proceeds rapidly in the subsequent generations and is about 400 to 1500-fold in the thirtyeighth generation.

The data in Table III also point to the development of DDT-resistance in the adult *C. fatigans*. This shows that the resistance is carried from the larval stage to the adult stage.

It will be observed from the data in Table II that the late-emerging strain (LES) is not capable of developing resistance to DDT in the laboratory. The selection based on late pupation or late adult development even up to 49th generation of the strain, proved ineffective. The late pupating larvae usually yielded larger and darker pupae and majority of the adults that emerged from these pupae were females. The development of physiological resistance through late pupation has, however, been established in houseflies by many workers (Pimental *et al.*, 1961; McKenzie and Hoskins, 1954 and Kerr *et al.*, 1957).

The results in Table IV of larval susceptibility tests on cross-tolerance of the two DDT-resistant strains (FRS and LRS) developed in the laboratory indicate that the resistant-strains do not show cross-tolerance to lindane or diazinon ; they exhibit cross-tolerance to dieldrin, which is about ten times that of the normal susceptible-strain (SS). Dieldrin resistance is, however, reported to be linked with BHC-resistance (Brown, 1958). The observation that both FRS and LRS show cross-tolerance to dieldrin and not to lindane appears, therefore, to be strain-specific.

SUMMARY.

The selection of two DDT-resistant strains of the mosquito, *Culex fatigans* Wied., in the laboratory has been described. The larvae were drawn from the progeny obtained from the field or from a susceptible laboratory-colony of the insect. The larvae in successive generations in the two strains were subjected to such sub-lethal doses of DDT which caused about fifty per cent kill.

The data on the selection of resistance, both in the field-strain and the laboratory-strain, have been presented. The results showed that the development of larval resistance is much more rapid in the field-strain than in the laboratory-strain. Although the development of resistance in the laboratory-strain is much delayed, that is, the larvae do not show resistance up to the twentyseventh generation, the development of resistance in the subsequent generations is fairly rapid and is about 400 to 1500-fold in the fiftyninth generation. In the field-strain the resistance appears fairly, that is, in the fifth generation, and then proceeds rapidly in the subsequent generations and is about 400 to 1500-fold in the thirtyeighth generation.

The data also show development of DDT-resistance in the adult *C. fatigans*, that is, the resistance is carried from the larval stage to the adult stage.

The larvae of the two DDT-resistant strains do not show cross-tolerance to lindane or diazinon. They, however, exhibit cross-tolerance to dieldrin, which is about ten times that of the normal susceptible strain.

Attempt for inducing resistance in *C. fatigans* has also been made by breeding only the late-emerging adults from the normal susceptible laboratory-colony of the insect. The late-emerging strain, however, is not capable of developing resistance to DDT in the laboratory on selection based on late pupation or late adult development even up to the 49th generation.

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A FILARIASIS SURVEY OF TRICHUR (KERALA STATE).

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[December 11, 1962.]

INTRODUCTION.

A FILARIASIS survey of the Trichur Municipal Town Area was conducted between December 5, 1960 and October 30, 1961. Trichur Town is the headquarters of the Trichur District, and is situated about the middle of the Kerala State, 15 miles east of the Arabian Sea and about 20 miles west of the Western Ghats. It has an area of 4·75 sq. miles and has a population of 83,115 (1951 Census). The town is divided into 31 Municipal Wards.

The district is connected with the neighbouring areas by rail and road.

The town has no protected water-supply. A limited amount of water is being supplied by the municipality in lorries. There is no planned drainage scheme in the town and, therefore, ideal conditions for the breeding of mosquitoes, especially of *Culex fatigans*, are numerous.

FILARIASIS SURVEY.

The survey was conducted by house-to-house visits from 8 p.m. to 12 midnight. Twenty c.mm. of blood was taken from each person and a thick smear was made, and after staining with methylene blue, these smears were examined under the microscope for the presence of microfilariae. Approximately 10 per cent of the population (8,386 persons) from the various wards of the town were thus examined. The filarial history and symptoms, if any, of each person were also noted. All age-groups of both sexes were sampled. A mosquito survey also was carried out to determine the prevalent species, and to detect the vector by dissection of mosquitoes caught from houses and cattle-sheds.

FINDINGS.

Infection rate.—The microfilariae in all the infected persons were of *Wuchereria bancrofti*. The infection (microfilaraemia) rates for the different age-groups are shown in Table I. The gross infection rate was 2·7 per cent. The youngest person in whom infection was found was a boy aged 2 years ; and the oldest, a man of 72 years. The infection rate was maximum (*i.e.*, 3·7 per cent) in the age-group of 41-50 years. The average microfilarial infestation rate was found

to be 11·2 per 20 c.mm. of blood, and the maximum number of microfilariae in any blood smear was 50.

TABLE I.

Infection (microfilaraemia) rate according to different age-groups.

Age-groups (in years.)	Number of persons examined.	Number of persons with microfilariae.	Infection rate, per cent.
0 to 1	43	Nil	Nil
2 to 5	580	3	0·5
6 to 10	1,175	22	0·2
11 to 20	2,476	71	2·8
21 to 30	1,512	46	3·0
31 to 40	1,068	33	3·1
41 to 50	757	28	3·7
Above 50	785	23	2·9
Total :	8,386	226	2·7

Disease manifestations.—Out of 8,386 persons examined, 35 had filarial disease, a gross disease rate of 0·41 per cent. All of them had elephantiasis of either of the lower extremities in mild or moderate form, with adenitis, fever and rigor. No case of hydrocele, elephantiasis of the scrotum, or chyluria was found.

TABLE II.

Disease rate according to age-groups.

Age-groups (in years).	Number of persons examined.	Number of persons showing disease manifestations.	Disease rate, per cent.
0 to 1	43	Nil	Nil
2 to 5	580	Nil	Nil
6 to 10	1,175	1	0·1
11 to 20	2,476	7	0·3
21 to 30	1,512	4	0·3
31 to 40	1,068	5	0·5
41 to 50	757	7	0·9
Above 50	785	11	1·4
Total :	8,386	35	0·4

Table II shows that the disease rate was highest in the age-group "above 50 years", being 1·4 per cent. The youngest case with filarial disease was a boy 10 years of age, and that was the only case in the age-group "6-10 years". The oldest case with disease manifestations was a woman 68 years of age. Table II shows that the disease rate increased with age. Most of the cases with evidence of filarial disease had never left the Trichur Town or had never stayed in any area where filariasis is endemic.

Endemicity rate.—Out of the 8,386 persons examined, 226 persons showed microfilariae and 35 had disease manifestations. Persons having filarial disease

manifestations did not show microfilaraemia. The endemicity rate of Trichur Town is 3·1 per cent.

TABLE III.

Endemicity in different age-groups.

Age-group (in years.)	Number of persons examined.	Persons with microfilariae.	Number with disease manifestation.	Number with disease and microfilariae.	Endemicity rate, per cent.
0 to 1	43	Nil	Nil	Nil	Nil
2 to 5	580	3	Nil	Nil	0·5
6 to 10	1,175	22	1	Nil	1·9
11 to 20	2,476	71	7	Nil	3·1
21 to 30	1,512	46	4	Nil	3·3
31 to 40	1,058	33	5	Nil	3·6
41 to 50	757	28	7	Nil	4·6
Above 50	785	23	11	Nil	4·3
Total ...	8,386	226	35	Nil	3·1

The survey also showed that the gross infection and disease rates are about equal for both sexes (Table IV).

TABLE IV.

Microfilaraemia and filarial disease rates for males and females compared.

Age-group in years.	MALES:						FEMALES:					
	Number of persons examined.	Number with microfilariae.	Percentage.	Number of persons with disease.	Percentage.	Number with both.	Number of persons examined.	Number with microfilariae.	Percentage.	Number of persons with disease.	Percentage.	Number with both.
0 to 1	26	Nil	Nil	Nil	Nil	Nil	17	Nil	Nil	Nil	Nil	Nil
2 to 5	332	1	0·3	Nil	Nil	Nil	248	2	0·8	Nil	Nil	Nil
6 to 10	612	12	1·9	1	1·6	Nil	563	10	1·8	Nil	Nil	Nil
11 to 20	1,356	37	2·7	1	0·1	Nil	1,120	34	3·0	6	0·5	Nil
21 to 30	721	25	3·4	2	0·3	Nil	791	21	2·7	2	0·3	Nil
31 to 40	467	14	3·0	3	0·7	Nil	591	19	3·2	2	0·3	Nil
41 to 50	331	16	4·8	2	0·6	Nil	426	12	2·8	5	1·2	Nil
Above 50	419	15	4·1	3	0·7	Nil	366	8	2·2	8	2·2	Nil
Total ...	4,264	120	2·8	12	0·3	Nil	4,122	106	2·6	23	0·6	Nil

Entomological observations.—During the survey, mosquitoes were collected from human dwellings, cattle-sheds and mixed dwellings. They were identified and dissected in the laboratory. The results of dissections are shown in Table V.

The vector of filariasis in Trichur Town is *Culex fatigans*. The maximum number of filaria larvae found in a single mosquito was six. The infection rate in *C. fatigans* was 0·5 per cent and the infectivity rate 0·3 per cent.

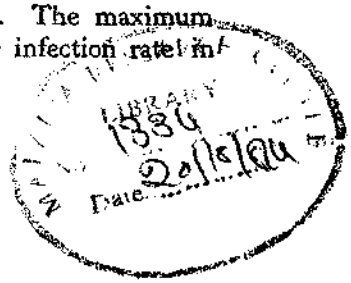

TABLE V.

Results of dissection of mosquitoes.

Mosquito species.	Number collected.	Number dissected.	Number positives.	NUMBER OF MOSQUITOES SHOWING FILARIAL LARVAE :			
				I.	II.	III.	IV.
Culex fatigans ...	2,431	1,808	9	2	1	..	6
Other culicines ...	71	..	..	..	..	..	..
Anopheles species ...	4	..	..	..	..	..	..
Total ...	2,506	1,808	9	2	1	..	6

DISCUSSION.

The filarial endemicity of Trichur Town is quite low, only 3·1 per cent. Most of the cases with clinical filariasis, it was noted, had never stayed in any area where filariasis is endemic and had acquired the infection locally. Apparently filariasis is of recent origin in Trichur Town. Presumably it has been introduced into the town by the floating population or immigrants from the infected coastal areas. The lack of efficient drainage favours the breeding of *Culex fatigans* and the spread of bancroftian filariasis in the town.

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SPLENOMEGALY IN NAGAMANGALA TALUK, MANDYA DISTRICT, MYSORE STATE. A REVIEW IN RETROSPECT.

BY

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[December 12, 1962.]

BRIEF HISTORY.

NAGAMANGALA is one of the *taluks** of Mandya District, Mysore State, with a population of 1,11,253 (1961 census) distributed over an area of 402 square miles, the population density being 276 per square mile. The taluk lies between 12° and 13° latitudes and 76° and 77° longitudes.

The area under cultivation in this *taluk* is 81,956 acres, of which only 6,183 acres (7·5 per cent) are under wet cultivation (paddy and sugar-cane) and the rest under dry cultivation (ragi, maize, navane, avare, castor, etc). The wet cultivation is confined to the tank '*achkats*'† which are far and few. Unlike the rest of Mandya District, this *taluk* is not served by the Visweswaraya Canal (Shama Sastry *et al.*, 1961). The average annual rainfall is 24·69 inches.

The DDT spraying was first commenced during August, 1958, in the whole of the *taluk*; thereafter, the *taluk* received 7 more rounds, the date of last spray being December 5, 1961, and since then interrupted. Spleen and parasite surveys were conducted in the Nagamangala Taluk during 1956-57. The details are set out in Table I. The spleen rate varied from 1·06 per cent to 30·0 per cent; the overall average being 5·6 per cent. The average enlarged spleen ranged from 1·0 to 2·8. The overall parasite rate was 9·8 per cent., all the 3 species of plasmodia being encountered. *P. vivax* was the dominant species. The Infant Parasite Rate and the Child Parasite Rate during the survey period were 3·8 per cent and 10·5 per cent respectively.

Since the commencement of the active surveillance work during the later part of April, 1960, to end of July, 1962, parasite-positives have not been encountered. However, in one of the dispensaries (Nagamangala), cases of enlarged spleen, clinically diagnosed as malaria, were reported. Between June, 1961 and January,

* '*Taluk*' is a revenue division of a district.

† '*Achkat*' is the area commanded by the tanks for wet cultivation.

TABLE I.

Spleen and parasite surveys in Nagamangala Taluk, during 1956-57.

Serial Number.	Village.	Date of survey.	Number examined for spleen.	Number with enlarged spleen.	Spleen rate, per cent.	Average enlarged spleen.	Blood smears collected: Infant. Number taken.	Infant. Number positive.	Child. Number taken.	Child. Number positive.	Fever. Number taken.	Fever. Number positive.
1	Tibbanahalli	October 17, 1956	38	1	2·6	2·0	1	..	20	3	..	..
2	Settihalli	October 17, 1956	42	1	2·4	2·0	2	..	32	2	..	..
3	Devalapura	October 18, 1956	115	3	2·5	2·3	14	..	62	..	..	..
4	Kasalagere	October 18, 1956	33	1	3·0	2·0	1	..	28	4	1	..
5	Kuntankoppal	October 19, 1956	15	4	26·6	2·0	1	..	15	3	1	..
6	Gollarahalli	October 19, 1956	33	5	15·15	2·02	5	2	29	8	1	..
7	Muthsandra	October 19, 1956	18	1	5·5	2·0	..	..	18	3	..	..
8	Arakere	November 14, 1956	49	5	10·2	1·6	6	1	9	4	..	..
9	Bellekere	November 14, 1956	25	4	16·0	1·75	2	..	4	2	..	..
10	Thumbinakere	November 15, 1956	47	2	4·2	2·0	2	1	2	1	..	..
11	Hadamgere	November 15, 1956	50	8	16·0	1·75	3	..	8	..	3	..
12	Bindiganahole	November 16, 1956	74	5	6·75	1·8	.	..	6	..	..	..
13	Nettigere	August 22, 1956	60	9	15·0	2·8	6	..	38	4	1	..
14	Javaranahalli	August 23, 1957	41	1	2·44	1·0	6	..	26	1	..	..
15	Bellur	August 23, 1957	189	2	1·05	1·5	13	..	58	2	..	..
16	Agachahalli	August 23, 1957	33	0	..	..	..	..	10	1	1	1
17	Chunchanahalli	August 24, 1957	39	1	2·56	2·0	2	..	10	2	2	1
18	Kenchanahalli	August 24, 1957	48	1	2·08	1·0	7	..	11	1	..	..
19	Mudigere	August 24, 1957	38	0	0·0	..	3	..	17	..	..	..
20	Vodyarhalli	August 24, 1957	33	10	30·0	2·3	4	..	12	2	5	1
21	Doddajatka	August 25, 1957	67	1	1·5	1·0	7	..	10	1	..	..
22	Chikjatka	August 25, 1957	59	3	5·08	1·6	6	..	19	2	2	1
23	Govindaghatta	August 25, 1957	35	0	..	..	7	..	9	1	..	..
24	Chakanahalli	August 25, 1957	57	0	..	..	7	..	14	2	1	1
		Total	1,238	68	5·4	..	105	4	467	49	18	5

By species, the parasite positives are analysed as follows :—

Source of smear.	*P. vivax.*	*P. falciparum.*	*P. malariae.*	Mixed.	Total.
Infants ...	0	3	0	1	4
Children ...	22	20	5	2	49
Fevers ...	5	0	0	0	5
Total ...	27	23	5	3	58

1962, 67 such cases were encountered by the Medical Officer, Nagamangala (Table II), distributed in 54 villages (Map 1).

TABLE II.

Distribution of cases of splenomegaly by months.

Months.	Number of cases.
1961	
June	7
July	13
August	8
September	10
October	7
November	8
December	9
1962	
January	5

VERIFICATION OF THE FINDINGS.

The distribution of the reported cases of splenomegaly by age-group is as follows (Table III).

TABLE III.

Age composition of cases.

Age-group.	Number of cases.
Below 2 years	1
2—12 years	6
Above 12 years	60

The degrees of spleen enlargements as detected by the Medical Officer are depicted in Table IV.

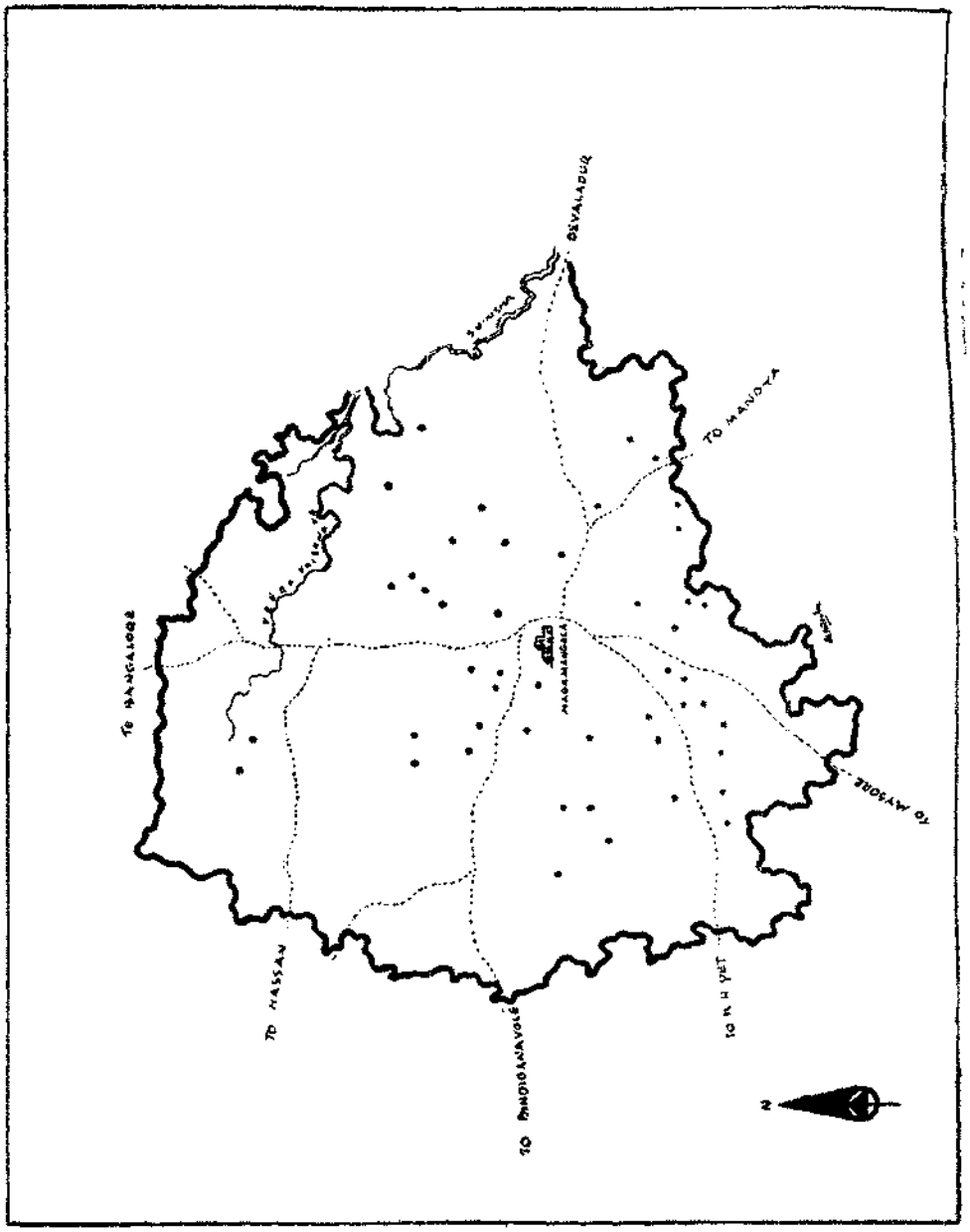
TABLE IV.

Frequency distribution according to spleen size.

Size.	Number of cases.
P_1	35
P_2	23
P_3	9

From Tables III and IV, it could be made out that the majority of the cases were among adults, the spleen size being small. All the 67 cases were blood-filmed at the time of their first visit to the dispensary and were found to be negative for malaria parasites on examination. Obviously, therefore, an investigation was found necessary to determine the incidence of splenomegaly in the community and its relationship, if any, to malaria.

Many of the cases were again contacted about 6 months later in the field, when again their blood-films did not reveal any parasites. Subsequent follow-up



MAP 1.
Nagamangala Taluk, showing villages where splenomegaly was recorded.

smears were also done in some of these cases, when also no parasites were demonstrable in the blood. However, during the field enquiry of these cases, 10 more enlarged spleen cases were encountered, who had not attended the dispensary. Their blood-films also were negative for malaria parasites. Random spleen survey was carried out in some of the villages. The details are set out in Table V. The average spleen rate during this survey was 0·9 per cent and the average enlarged spleen was 1·8.

TABLE V.

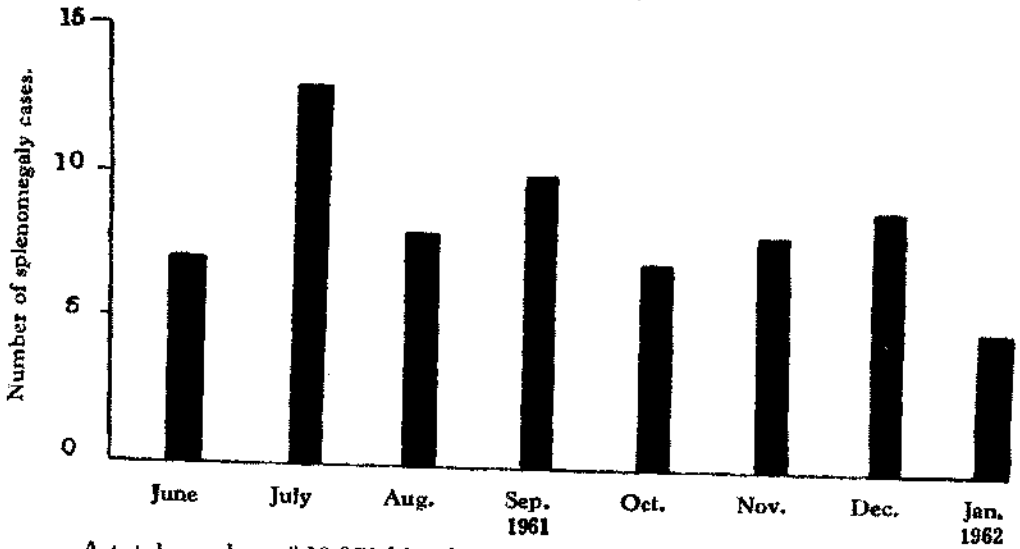
Random spleen survey carried out in some villages.

Village.	Date of visit.	Number examined.	Number with enlarged spleen.	Spleen rate per cent.	Average enlarged spleen.	Number with liver enlargement.
Naragalu	February 12, 1962	30	1	3·3	3·0	..
Mudalakoppal	February 12, 1962	22	0	..	..	..
Saremelanakoppal	February 12, 1962	24	0	..	..	..
Tattahalli	February 12, 1962	37	0	..	..	..
Burudagunte	February 13, 1962	46	1	2·1	1·0	..
Chottanayakanahalli	February 14, 1962	20	1	5·0	2·0	..
Devalapura	July 18, 1962	136	0	..	..	3
Bellur	July 18, 1962	74	0	..	..	..
Kuntanakoppal	July 18, 1962	13	0	..	..	..
Shankanahalli	July 30, 1962	43	0	..	..	5
Halati	July 24, 1962	36	0	..	..	..
Konanur	July 30, 1962	55	2	3·6	1·5	..
	Total	536	5	0·9	1·8	8

During the field visits for the verification of splenomegaly in these cases, a total of 66 fever cases were detected. Smears were also obtained from the inmates in the houses of the reported spleen positive cases, the number being 155. All of them were negative for malaria parasites on examination.

Since the number of cases reported was the highest during July, 1961 (Chart 1), the active surveillance data were analysed for the *taluk* as a whole and are depicted in Chart 2. There are 13 malaria surveillance divisions in this *taluk*. As could be seen from Chart 2, apart from two very minor peaks during May and October, there was no abnormal rise of fever cases either for the fortnights previous or succeeding to July 1961, when the maximum number of spleen cases have been reported by the Medical Officer. This would perhaps suggest that the reported spleens may not be malarial. Further, during the first blood screening of the 67 cases, no malaria parasites were demonstrable. Negative result of a single smear examination may not exclude the possibility of a latent infection, particularly so from persons with chronically enlarged spleens, although probably a certain proportion of the enlarged spleens may be due to causes other than malaria. But, however, the subsequent follow-up smears were also negative for malaria parasites, the number collected being 126.

CHART 1.
Splenomegaly in Nagamangala.



A total number of 19,871 blood smears were collected from all the 13 sections between April, 1960, and July, 1962 (Table VI). All were negative for malaria parasites. Further, a total number of 5,110 blood smears from 6 dispensaries of this *taluk* were collected between the years 1960-1962* and they were also negative for malaria parasites. Before any conclusion was drawn, a mass-blood survey was thought necessary. Four villages of the *taluk*, where a peak in active surveillance figures was discernible during the early part of April, 1962, were screened by mass-blood survey. Besides these, mass-blood survey was carried out in six other villages of the *taluk*. Out of 1,188 blood smears thus collected, none was positive for malaria parasites.

TABLE VI.
Details of the examination of 19,871 blood smears.

Year.	Active.	Passive.	Mass-blood survey.	Contacts.	Follow up smears.	Fevers.
1960	4,385	20	..	..	..	..
1961	9,112	1,324	..	..	..	..
1962	6,374*	3,266	1,118	155	126	66

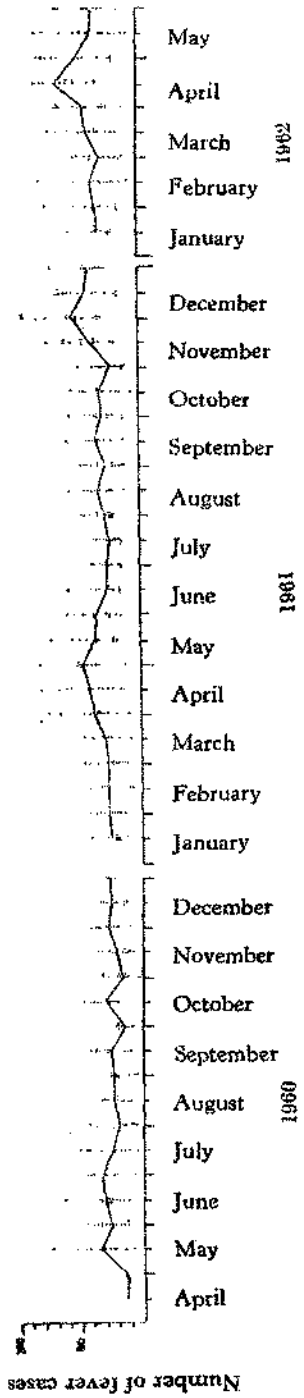
DISCUSSION.

In view of the constancy of the splenomegaly in malaria infections, spleen rate in community was widely used to ascertain the status of this infection, though, with change in the strategy from control to eradication, more stress is laid on blood

* Up to end of July, 1962.
Subsequent to the despatch of this paper to the press, 14,162 blood smears were examined up to the end of June, 1963 (8,463 under active and 5,699 under passive surveillance) and found to be negative for malaria parasites.

CHART 2.

Fortnightly fever surveillance data of Nagamangala Taluk during 1960-1962.



The continuous line depicts the average performance (number of fever cases per surveillance worker per fortnightly visit, whereas the individual vertical dots represent the actual number of fever cases encountered by the workers in that particular fortnight.

smear examination during the later stages of the eradication programme. However, discrepancies in blood smear examinations are not uncommon, since a single negative survey is suggestive of a latent infection, particularly in splenomegaly cases. Further a proportion of these enlarged spleen cases, though small, may be due to other causes, such as infectious mononucleosis, rickets, leishmaniasis, tropical eosinophilia, congenital syphilis, miliary tuberculosis, measles, epidemic relapsing fever, sarcoidosis, etc.

Perhaps the most significant finding reported in this paper is the complete absence of malaria parasitaemia, in the population of this *taluk*, where certain number of enlarged spleens were encountered. Further, these cases are not confined to any one village or season, but somewhat evenly spread out. This, together with the absence of parasite-positive smears over a period of about 2 years past, would challenge their possible malarial aetiology. It, therefore, raises a very important question, namely, what will be the irreducible minimum spleen rate under disappearing malaria, since a small proportion of the enlarged spleens may be due to other causes. As a corollary, it would be necessary, therefore, to determine the contributory factors for this irreducible minimum of spleen rate.

Published reports on the incidence of splenic enlargements, in the absence of malaria, are not extensive. The tabulated data are set out in Table VII.

TABLE VII.

Record of spleen rates in children in the non-malarious areas of the world.

Author.	Locality.	Spleen rate, per cent.	Remarks.
Ross *et al.* (1914)	London	1·06	Palpable spleens
Darling (1920)	Fiji Islands	1·5	..
Boyd (1949)	Lorraine, OHIO	2·7	*P.D.I.
Maxcy *et al.* (1927)	Washington and Hagerstown.	13·2	P.D.I.
Russell (1935)	Massachusetts	3·9	P.D.I.
Smith (1945)	Massachusetts (White)	14·0	P.D.I. in only 10·8 per cent
	Massachusetts (Negro)	4·9	P.D.I. in only 3·8 per cent.

**Note*:— P.D.I means palpable on deep inspiration.

There are no available records in India at present about the status of spleen under conditions of total absence of malaria. However, Christophers *et al.* (1936) have recognised as healthy areas where the spleen rate in children does not exceed 10 per cent. Viswanathan (1950) has quoted reduction of spleen rate from 70 per cent to 7 per cent in North Kanara between 1945-1949, after total interruption of transmission of malaria. Shama Shastry and Narayana Iyengar (1959) have reported in pilot study areas (interrupted areas*), the reduction of spleen rates from

* "*Interrupted areas*" are the areas where for two consecutive years, prior to interruption of spray, the child spleen index, the child parasite index and the infant parasite index were less than 5 per cent, 1 per cent and zero, respectively.

82 per cent to 0·4 per cent. But, there is no literature as to the fate of the spleens under conditions of disappearing malaria. However, in other countries, spleen status of children residing in non-malarious areas has already been cited (Table VII).

The chronically enlarged spleen also occurs in certain of the other diseases. The differential leucocyte counts of blood smears done in a few of these enlarged spleens did not indicate any of the above mentioned diseases. But, however, the anaemia was rather severe in some of the cases. On enquiry, Medical Officers of the 6 dispensaries have reported that 10 to 25 per cent of their daily out-patient statistics, have shown advanced hookworm infestation.

The results of investigations detailed above conclusively showed that malaria has been absent in the area since at least 1960. This continues to be so. The causes of enlarged spleens in about 2 per cent of the population of the *taluk* certainly need investigation. A major contributory cause would seem to be high degree of anaemia, due probably to ankylostomiasis disease.

SUMMARY.

The endemicity of malaria in the *taluk* of Nagamangala varied from low to moderate endemicity during 1956-57. All the 3 species of plasmodia had been encountered, *P. vivax* being the dominant species. The Malaria Eradication operations were commenced in 1958 and there has been no malaria transmission in the areas at least since 1960.

The Medical Officer of the Nagamangala Dispensary reported 67 splenomegaly cases in 54 villages between June, 1961, and January, 1962. But these cases were not confined to any one village or group of villages or concentrated in any one season.

In the absence of malaria in Nagamangala since 1960, an important question arises, namely, what will be the irreducible minimum of spleen rate in areas where malaria is totally absent and what are the contributory factors for the persisting splenomegaly.

ACKNOWLEDGEMENTS.

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OBSERVATIONS ON THE IRRITABILITY OF MOSQUITOES TO DDT.

BY

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AND

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[December 20, 1962.]

THE results obtained with the provisional method for determining the irritability of adult mosquitoes, recommended by the World Health Organization Expert Committee on Insecticides, are satisfactory, in general, to demonstrate the variations of irritability between different populations of mosquito strains or species.

In performing this experiment, we must bear in mind that each mosquito, as every living being, has a different response to the irritant action exerted by the environmental factors. The response to DDT may be influenced by the following factors :—

1. *Behaviour* : (*a*) Mode of life (wild or domestic life).
   (*b*) Mode of the contact of the legs with the surface.
2. *Physiological factors* : Age, food, metabolism, fatigue, sub-intoxication, etc.
3. *Traumatism and microtraumatism.*
4. *Influences from the environment* :
   (*a*) The change from a free life to the captivity.
   (*b*) The degree of adaptation to the experimental condition (light, temperature, humidity).
5. *Morphological structures of the tarsal segments.*
6. *Influences exerted by a previous contact with DDT.*

METHOD AND MATERIAL.

Taking into account that the response to irritant factor is in some way individual, in all our experiments the mosquitoes were exposed individually. The irritability of each mosquito exposed to DDT, was compared with its own irritability on non-impregnated paper. Before determining the irritability of each mosquito to DDT, we determined the normal irritability of the same mosquito on filter paper impregnated with risela oil. In this way, we were able to make a more

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accurate appreciation of the influence exerted by DDT. In order to reduce the influence of handling, we employed conical chambers designed by Ungureanu (1961) which permitted us to change the contact of mosquito without the change of mosquito from the conical chamber.

The normal irritability of mosquito was observed for 3 to 15 minutes and the irritability to the 2 per cent or 4 per cent DDT was established during the following period of 3 to 15 minutes. No mortality was observed in the *A. maculipennis* group during this time of exposure to the 2 per cent or 4 per cent DDT. All exposures, presented in this paper, took place in artificial light obtained with an electric bulb of 60 watts and placed in front of the experimental chambers at a distance of about 60 cm. The temperature and relative humidity during exposure and observation period were 25°—27°C and 60—75 per cent respectively.

The mosquitoes used in our experiments were.— *A. (I) atroparvus, A. typicus, A. messeae* bred in laboratory from eggs obtained from mosquitoes collected in nature ; A. (*I*) *atroparvus* susceptible colony, and *A. typicus* and *messeae* collected in nature.

Some experiments with mosquitoes, collected in nature, were carried out after 1-2 hours of capture ; other experiments were made after 24-48 hours' period of adaptation of mosquito to the laboratory conditions ; during this time the mosquitoes were fed with 10 per cent sugar solution on cotton wool.

RESULTS.

1. The influence on the irritability of mosquito by different concentrations of DDT.—It will be seen that usually a significant difference occurred in the irritability of mosquitoes exposed to 2 per cent and 4 per cent DDT, but this difference was not proportional to the increase of the concentration of DDT.

From practical point of view it was not necessary to determine the phenomenon of irritability on both concentrations ; DDT 2 per cent appeared to be a satisfactory concentration for mosquito species with high irritability. For *A. maculipennis, A. hyrcanus* and the *Culex* group, 4 per cent DDT seemed to be desirable.

2. The results obtained with replicates of 5 mosquitoes were sometimes very similar with those obtained with individual exposure, but the method of exposing in the same conical chamber more than one mosquito is not recommended because in this case we are not able to eliminate the eventual influence of proximity nor to establish the reactivity of each mosquito on normal and impregnated paper. (Table I).

3. The influence of temperature.— *A. maculipennis* collected from nature, exposed at 16°C to 4 per cent DDT, had a reduced irritability than at 25°C. (Table II).

4. The influence of environment.— Mosquitoes collected in nature and tested in the laboratory 1 or 2 hours after capture, were more irritable than the mosquitoes kept 24—48 hours in normal laboratory conditions. *A. typicus,* tested after one hour interval, was more irritable than the mosquitoes kept 24 hours under normal

TABLE I.

Mosquito irritability to DDT. A. typicus (*nature*).

STEP A. TIME LAPSE (MINUTES) BEFORE FIRST TAKE-OFF :					STEP B. NUMBER OF TAKE-OFFS IN 15 MINUTES :					ALTERNATIVE METHOD. (GROUPS OF 5 MOSQUITOES). NUMBER OF TAKE-OFFS IN 15 MINUTES :				
Mosquito number.	Control.	DDT 2 per cent.	Control.	DDT 4 per cent.	Mosquito number.	Control.	DDT 2 per cent.	Control.	DDT 4 per cent.	Mosquito number.	Control.	DDT 2 per cent.	Control.	DDT 4 per cent.
1	15′	1′ 30″	15′	2′	1	0	7	0	30					
2	15′	1′	15′	1′ 36″	2	0	3	0	18	1	4	51	0	60
3	15′	4′	10′	3′	3	0	4	1	16					
4	15′	5′ 30″	15′	1′	4	0	6	0	6	2	0	21	4	84
5	15′	2′	3′	1′	5	0	4	4	18					
6	5′	2′	15′	1′	6	9	13	0	33					
7	15′	3′	15′	8′	7	0	9	0	3	3	2	53	0	58
8	15′	1′	7′	2′	8	0	8	3	12					
9	15′	5′	15′	3′ 30″	9	0	6	0	15	4	0	49	2	76
10	15′	3′	3′	3′	10	0	6	5	10					
11	15′	5′	15′	2′	11	0	13	0	3	5	1	40	0	62
12	5′	8′	15′	1′	12	3	2	0	36					
13	15′	8′	4′	2′ 30″	13	0	1	3	10					
14	3′	2′	15′	3′	14	1	34	0	8	6	0	50	5	87
15	6′	1′	15′	2′	15	3	3	0	13					
16	15′	2′	15′	1′ 30″	16	0	4	0	4					
17	3′	1′ 30″	6′	2′ 30″	17	1	6	4	11	7	3	48	2	48
18	15′	6′	15′	3′	18	0	3	0	6					
19	3′	2′	7′	5′	19	3	8	0	7	8	2	55	0	42
20	15′	3′ 30″	5′	4′	20	0	14	0	12					
Average	11′ 45″	3′ 21″	11′ 15″	2′ 37″		1·0	7·6	1·1	13·5		0·3	9·1	0·3	12·9

conditions. Mosquitoes, recently collected and kept in darkness for one hour before test, were less irritable than the mosquitoes kept for a similar period of time in the same light as during the experiments. But the mosquitoes kept 24 hours in darkness were not less irritable than the mosquitoes kept under normal laboratory conditions (Table III).

TABLE II.

Influence of temperature on mosquito-irritability to DDT. A. typicus (*nature*)*.

Concentration.	Number of mosquitoes.	Number of take-offs per mosquito.	Time of the first take-off.	Temperature during exposure.	I†—N‡
Control ... ... DDT 4 per cent. ...	20 20	4, 9 9, 5	10′ 25″ 4′ 30″	16·5°C } 16·5°C }	4, 6
Control ... ... DDT 4 per cent. ...	20 20	21, 2 29, 6	2′ 54″	25°C } 25°C }	8, 8

* Tests carried out after 1 hour from capture.

† I = irritability to DDT. ‡ N = normal irritability.

TABLE III.

Variability of mosquito-irritability to DDT under different environmental conditions: A. typicus.

Conditions.	Concentration.	Number of mosquitoes tested.	Number of take-offs per mosquito.	Time of the first take-off	Temperature during exposure for 24 hours.	Humidity during exposure for 24 hours.	I*—N†
Collected in nature; kept for one hour in light.	Control I	10	20,5	..	25°C	60—70 per cent	10,3
	Control II	10	10,3	..			
	DDT 4 per cent.	10	20,6	2′6″			
Tested after 1½ hours from capture; kept for one hour in darkness.	Control I	10	9,5	..	25°C	60—70 per cent	14,7
	Control II	10	1,8	..			
	DDT 4 per cent.	10	16,5	1′33″			
Kept for 24 hours in darkness.	Control I	15	1,9	..	25°C	60—70 per cent	9,0
	Control II	15	2,4	..			
	DDT 4 per cent.	15	11,4	3′6″			
Collected in nature; kept for 24 hours in normal laboratory conditions.	Control I	20	3,8	..	26°C	65 per cent	7,6
	Control II	20	1,9	..			
	DDT 4 per cent.	20	9,5	5′			

* I = irritability to DDT. † N = normal irritability.

Mosquitoes from laboratory colony were less irritable than the mosquitoes caught in nature. Keeping the mosquitoes recently collected from nature, for a quarter of an hour in the conical chambers on non-impregnated paper, we noticed that a reduction of irritability occurred in the next period of 15 minutes' observations on paper impregnated with risela oil (Table IV).

The reduction of irritability under the above mentioned laboratory conditions demonstrated the phenomenon of adaptation of mosquitoes to the conditions of experiments.

TABLE IV.

Reduction in the irritability of the wild-caught A. typicus *when held for 15 minutes before exposure on treated papers.*

Mosquito number.	NUMBER OF TAKE-OFFS IN 15 MINUTES :			Time lapse (minutes) before first take-off DDT 4 per cent W.H.O.
	Roneograph paper.	Paper with risela oil.	DDT 4 per cent W.H.O.	
1	2	2	32	2′ 5″
2	1	0	11	3′
3	10	1	19	2′ 5″
4	1	6	16	3′
5	7	0	0	15′
6	2	1	14	2′
7	0	0	0	15′
8	5	2	6	2′ 5″
9	2	1	2	10′
10	2	4	7	2′
11	25	11	16	2′ 5″
12	0	0	5	1′ 5″
13	0	0	3	2′
14	0	0	10	3′ 5″
15	0	0	5	2′
16	1	1	16	2′
17	3	0	7	3′ 5″
18	0	0	6	1′ 5″
19	3	0	8	1′ 8″
20	0	2	6	2′
21	6	2	14	7′
22	1	2	7	7′
23	8	5	6	3′
24	13	7	26	1′ 5″
Average	3·8	1·9	10·0	3′ 54″

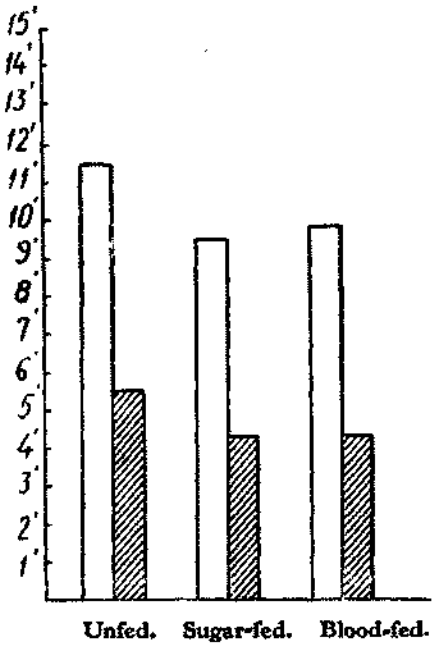
In order to reduce the irritability of mosquitoes recently caught in nature on non-impregnated paper, a period of 15 minutes in conical chambers under the same intensity of artificial or natural light was necessary, before establishing the normal irritability of individual mosquito. This period was not necessary for the mosquitoes kept under laboratory conditions for about 24 hours, or for the laboratory-bred mosquitoes.

5. *The influence of food.*—Unfed or sugar-fed mosquitoes were more irritable to DDT, than the blood-fed mosquitoes. The differences of irritability to 2 per cent DDT were more accentuated than to 4 per cent DDT (Graphs 1 and 2).

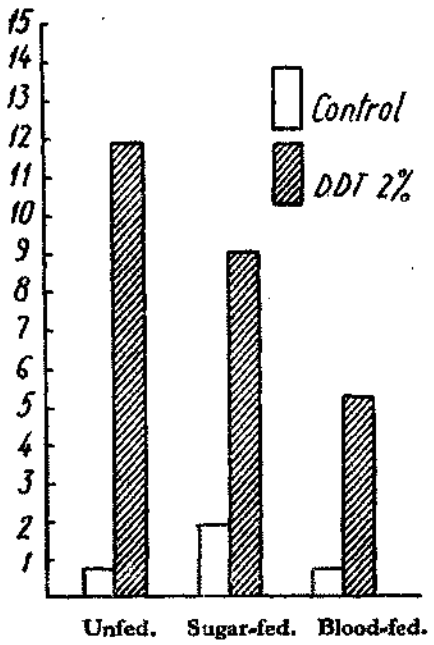
6. *Influence of the repeated contact to DDT.*—We have demonstrated that the repeated contact to DDT of *A. maculipennis* species produced a considerable reduction of irritability to DDT. The irritability of mosquitoes exposed to 1 per cent, 2 per cent or 4 per cent DDT, three times, every 3 hours or every 48 hours, has diminished to a very low level after each exposure. The phenomenon can be explained not by differences of age or food, but by a desensitisation of tarsal segments, (Ungureanu and Teodorescu, 1961). The phenomenon was confirmed by Culen and Zulueta (1962) for *A. gambiae.*

GRAPH 1.

Time lapse (minutes) before first take-off.

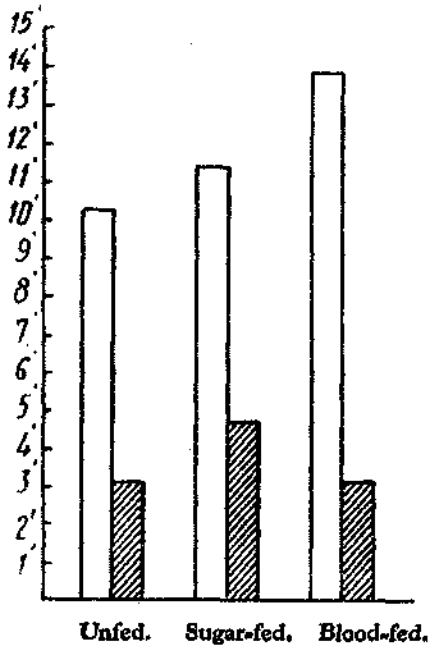


Number of take-offs in 15 minutes.

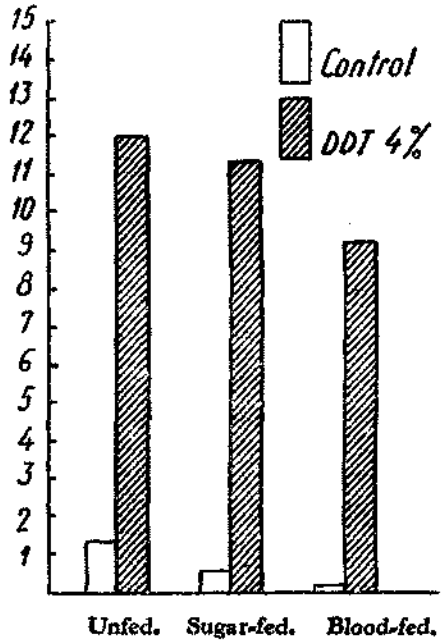


GRAPH 2.

Time Lapse (minutes) before first take-off.



Number of take-offs in 15 minutes.



This very important fact changed the importance, accorded initially to the study of DDT irritability, for all endophilic species of mosquitoes at least in the temperate zones. This phenomenon explains why, in spite of the irritability to DDT of *A. maculipennis* species, we have obtained spectacular results in the practice of malaria eradication.

CONCLUSIONS.

The results of the investigations presented in this paper indicated that the irritability to DDT was influenced by biological and morphological factors and by the conditions of the experiment.

Laboratory-bred mosquitoes were less irritable to DDT than the mosquitoes caught in nature. Mosquitoes collected in nature and kept for 24 hours under laboratory conditions, became less irritable. The blood-fed mosquitoes were less irritable than the unfed or sugar-fed mosquitoes. The importance of the irritability to DDT of mosquitoes, must be evaluated in the light of the natural behaviour and the influences of environmental biotic and abiotic factors.

A reduction of the irritability occurred especially during the control period if the mosquitoes were kept for about at least 15 minutes in the conical chambers under the same light as during the contact with the DDT impregnated paper. The authors employed as control the same mosquitoes exposed individually on non-impregnated paper before the exposure to 2 per cent or 4 per cent DDT. A great individual variability of the irritability was observed.

For the study of the irritability to DDT of *A. maculipennis*, *A. hyrcanus* and *Culex* group, exposures to 4 per cent DDT were found suitable. For very irritable species, 2 per cent DDT gave satisfactory results. From practical point of view, 3 minutes as "settling period" and 15 minutes for observations on normal paper, and the same period of time for observations on the DDT-impregnated paper, was found to be entirely satisfactory for the study of irritability.

Repeated contact with the DDT sprayed surfaces, produced a considerable decrease of the irritability. This very important phenomenon explained why, in spite of the irritability to DDT observed in our country by one of us on the first day of spraying with DDT (July, 1947), we obtained, in practice, spectacular results with this insecticide. Consequently the study of irritability proved to be of minor importance from the practical point of view, at least for the intra-domestic species from temperate zones.

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STUDIES ON THE BLOOD-PARASITES OF THE WHITE-THROATED MUNIA, *UROLONCHA MALABARICA* LINNAEUS.

(*A*) *Trypanosoma avium* DANILEWSKY, 1885.

(*B*) *Trypanosoma delhiense* SP. NOV.

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INTRODUCTION.

In connection with the studies on blood parasites of the white-throated munia, *Uroloncha malabarica* Linnaeus (Grewal, 1961), 69 birds, during a period of over 2 years, were examined out of which 28 (40·6 per cent) showed natural infection with trypanosomes. Out of the infected lot, 10 birds showed mixed infection of the two species of trypanosomes described in this paper ; while in 18 only *T. avium* was encountered. No bird with the infection of *T. delhiense* only was found.

Trypanosomes of birds are very interesting as a wide range of animals, both birds and mammals, can be infected with them (David and Nair, 1955 ; David and Satya Parkash, 1957 ; Grewal *et al.*, 1957).

On studying the detailed morphology of the trypanosomes, met with in the blood and the bone-marrow of the white-throated munia, *Uroloncha malabarica*, I came across two distinct species of trypanosomes. By studying their morphological characteristics only, I have been able to show the relationship of one of them with *T. avium* Danilewsky, 1885 ; while the other one appears to be a distinct species having no relationship with any of the trypanosomes described from the birds uptil now. I have called it *Trypanosoma delhiense* sp. nov.

In this paper, it is proposed to deal with the morphological study of the two species of trypanosomes met with in this bird and their behaviour in this vertebrate host. These observations are based on the preparations made at intervals from the blood of some infected birds, which remained alive in captivity for a long time ; while other preparations are made from the blood and bone-marrow of birds sacrificed after they were kept under observation for more than three months.

So far as working out the life cycle of these trypanosomes in the invertebrate host is concerned, I have tried two species of mosquitoes, *Culex fatigans* and

Aëdes aegypti, but there was no development. Further observations are in progress, and, as soon as some convincing results are obtained, they will be communicated in the future publications.

EXPERIMENTAL DATA.

Description of the trypanosomes, as observed in the blood of the white-throated munia, U. malabarica.— In fresh preparations of blood, the flagellates are large, elongated and slender in appearance. In one of the forms, the free flagellum is active, comparatively short and directed forwards. According to my views, it is *Trypanosoma avium* Danilewsky, 1885. In the second form, there is no free flagellum, the absence of which is confirmed by examining the flagellate under stained preparations. I have named it *Trypanosoma delhiense* sp. nov.

The parasites move slowly in the plasma, remaining under the same microscopical field for a long time, unlike *T. lewisi*, which is very quick in its movements. The posterior aflagellar end is long and fairly tapering, but differs considerably in the two species. It appears to be comparatively stiffer than the anterior end, which moves about freely as the anterior end lashes. With the help of their anterior end, they throw the red blood corpuscles in all directions.

The only internal structure visible, in the living specimens, is the nucleus which is oval or circular. The kinetoplast, usually, is not seen. The trypanosomes possess a well-developed undulating membrane which has four to seven folds or pleats. They are comparatively more in *T. avium* than in *T. delhiense*.

Description of the trypanosomes, as observed, after fixing and staining.—In dry-fixed blood films, stained with Giemsa or J.S.B. (Jaswant Singh, 1956) stains, the general appearance of the organisms is the same as seen in the living specimens. The cytoplasm takes a deep blue stain, particularly in the middle of the body. It appears in some specimens as finely alveolar. Occasionally, a few small vacuoles or spaces are to be seen in the cytoplasm. In a few other specimens, cytoplasm appears patchy, taking light-blue stain with a few dark-blue patches. Longitudinal striations are seen in the majority of the specimens.

(A) *TRYPANOSOMA AVIUM* DANILEWSKY, 1885 (A trypanosome with free flagellum). (Plate I, Figs. 1 to 4).

Danilewsky (1885) described *T. avium* from owls and roller birds. Stephens and Christophers (1908) found a trypanosome in the Indian crow, *Corvus splendens* and called it *T. corvi*. From the description given by the authors, the latter species of trypanosome appears a synonym of the former species. Das Gupta and Siddons (1941) gave a brief description of a trypanosome from the blood of white-throated munia, *U. malabarica*. They mentioned that the possession of a long caudal process is one of the characteristics of the trypanosome from this bird and so gave it a new name, *T. knowlesi*. I have compared it with *T. avium*, described by me from the white-throated munia, *U. malabarica* and *T. avium*, described by Baker (1956) from canary, black bird, rooks and jackdaw. I strongly feel that

T. knowlesi is morphologically in no way different from *T. avium*, until and unless it does not show some biological differences ; hence considered its synonym. Das Gupta and Siddons (1941) differentiated *T. knowlesi* (*T. avium*) from a trypanosome described by Hanna (1903) from the blood of domestic pigeon in India. It was named *T. hannai* by Pittaluja (Wenyon, 1926). A few differences given by Das Gupta and Siddons (1941) are not sufficient enough to separate the two trypanosomes (*T. knowlesi* and *T. hannai*) from each other to such an extent that a separate specific name be given to them. The two trypanosomes resemble *T. avium* on various points, hence both of them are considered as synonyms of *T. avium* Danilewsky, 1885. This is further made more evident by seeing Table II, and Figs. 1, 2, 3 and 4 (Plate I). By taking careful measurements of the trypanosome (with a free flagellum), described here, I have compared it with *T. avium* Danilewsky, and have noted certain differences. However, I am of the opinion that, at present, there is not, as yet, sufficient data, at my disposal, for the adoption of a different specific name for it.

Although *T. avium* Danilewsky is a "posterior station" trypanosome and does not develop in the gut of the mosquitoes, *C. molestus* and *A. aegypti* (Baker, 1956), the flagellate under discussion, also, does not take to the insects (*C. fatigans* and *A. aegypti*). Some soft ticks were collected from the body of infected birds. None of them was found to harbour the developmental stages of the flagellates. It is, thus, very difficult to place it amongst any type of trypanosomes, and as such, for time being, I have taken only the morphological characters into consideration and have called it *T. avium*. At a later stage, I will study the life cycle in some invertebrate host, the cross-immunity tests and the host specificity ; only then, *if need be*, I will be in a position to raise it to an independent specific level.

The average measurements of 25 trypanosomes from blood and bone-marrow are taken. The trypanosome varies from 42·9 μ to 53·7 μ with an average of 47·64 μ in length, and 4·0 μ to 7·0 μ, with an average of 6·2 μ in length (width being taken from the middle of the body, where it is the largest).

The *nucleus* is situated more towards the posterior extremity of the body. It appears as a solid, red-stained mass, and is more frequently ovoid in shape, but it is rarely round. It measures from 3·2 μ to 7·0 μ in length, and 2·0 μ to 4·0 μ in breadth, with an average of 4·7 μ by 3·1 μ ; while when round it has a diameter of 4·0 μ.

The *kinetoplast* (*parabasal body* + *blepharoplast*) is prominent and stains deep red and shows no structural details. The parabasal body appears relatively small, ovoid or round in shape. In almost all the specimens, there is a large flagellar vacuole beside the kinetoplast. The kinetoplast varies from 0·5 μ to 1·0 μ, with an averge of 0·65 μ by 0·55 μ. Distance between the posterior end and the middle of the kinetoplast varies from 7·5 μ to 15·4 μ, with an average of 10·7 μ.

The red-staining *flagellum*, either runs along one side of the body, or crosses over once or twice, and finally becomes the *free flagellum*. At its proximal end,

it nearly always stops short of the kinetoplast. It is only very seldom that it appears to come into contact with the kinetoplast. At the origin of the flagellum, there is another small dot, the *blepharoplast*, smaller than the *parabasal body*. The length of the free flagellum varies from 4·5 μ to 8·6 μ, with an average of 7·0 μ, and the length of the entire flagellum varies from 37·5 μ to 48·4 μ, with an average of 43·5 μ.

The *undulating membrane* is conspicuous, but is not very well developed. It does not take any stain, and forms 6-7 undulations, which tend to become smaller towards the anterior end.

The measurements, stated in Table I, are taken from dried preparations of 25 trypanosomes, either from the blood or from the bone-marrow.

TABLE I.

Measurements, in microns, of Trypanosoma avium *from the white-throated munia,* Urolonchа malabarica.

Number.	Measurements.	Extremes.	Average.
1	Length of the body (including free flagellum)	42·9 — 5·37	47·64
2	Breadth (excluding undulating membrane)	4·0 — 7·0	6·2
3	Distance from posterior end to the centre of kinetoplast	7·5 — 15·4	10·7
4	Distance from the centre of kinetoplast to the centre of nucleus	7·1 — 11·0	8·9
5	Distance from the centre of nucleus to anterior end (without free flagellum)	16·6 — 31·1	21·1
6	Dimension of kinetoplast	0·5 — 1·0 × 0·5 — 0·8	0·65 × 0·55
7	Dimension of nucleus	3·2 — 7·0 × 2·0 — 4·0	4·7 × 3·1
8	Breadth of undulating membrane	0·5 — 2·0	0·86
9	Length of free flagellum	4·5 — 8·6	7·03
10	Total length of flagellum	37·5 — 48·4	43·5

In order to do the comparative study of *T. avium* Danilewsky (Baker, 1956); *T. knowlesi* = *T. avium* (Das Gupta and Siddons, 1941); *T. hannai* = *T. avium* Pittaluja, 1912 (Wenyon, 1926) and *T. avium* (described by me), Table II shows clearly that they all resemble each other morphologically, to such an extent that they can be considered as one species.

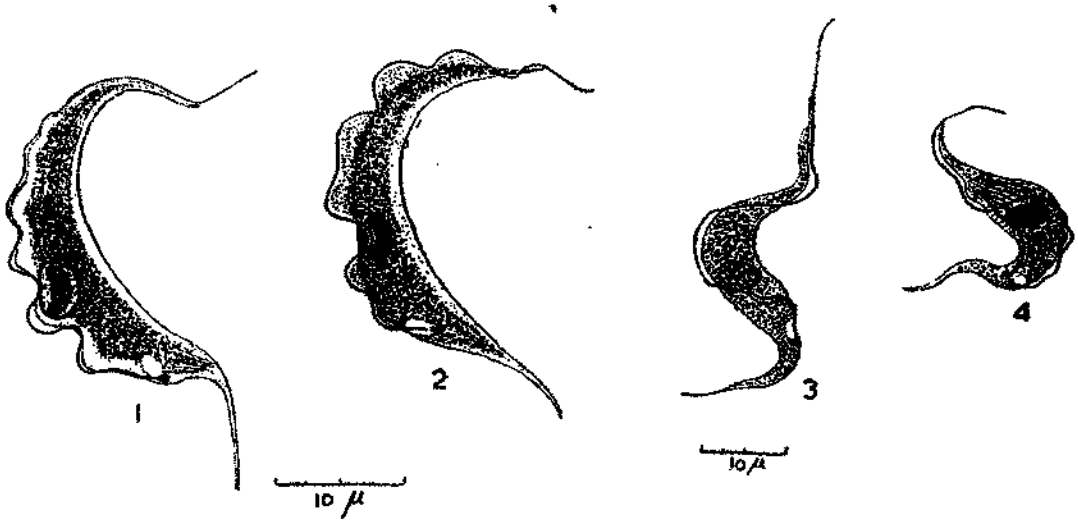
(B) *TRYPANOSOMA DELHIENSE* sp. nov. Grewal, 1962 (a trypanosome without free flagellum). (Plate II, Figs. 1, 2).

From the data available on the trypanosomes of birds, I have not come across any flagellate that is hitherto been described, which is without a free flagellum.

I have taken measurements of the stained specimens, under discussion. The differences between the two trypanosomes, *T. avium* and *T. delhiense* sp. nov. (Table IV) provide me with a convincing evidence about the latter, undoubtedly, a distinct species, to which the name *Trypanosoma delhiense* sp. nov. is given. The present record is strictly on the morphological characteristics of this trypanosome and no other investigations are possible at present as the two strains have not been separated either in birds or in insects.

PLATE I.

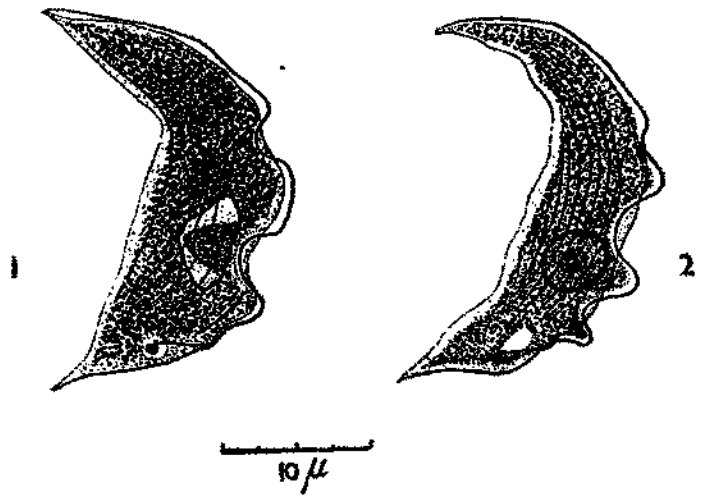
Trypanosoma avium Danilewsky, 1885.



FIGS. 1, 2. Camera lucida drawings of *T. avium* (described by Grewal, 1962) from dried, Giemsa stained films of peripheral blood (Fig. 1) and of bone-marrow (Fig. 2).
FIG. 3. Reproduction of *T. avium* (described by Baker, 1956).
FIG. 4. Reproduction of *T. avium* (described by Das Gupta and Siddons, 1941). ×1300.

PLATE II.

Trypanosoma delhiense sp. nov. Grewal, 1963.



FIGS. 1, 2. Camera lucida drawings of *T. delhiense* sp. nov. from dried, Giemsa stained films of peripheral blood (Fig. 1) and of bone-marrow. (Fig. 2).

TABLE II.

Comparison between T. avium *Danilewsky, 1885 (Baker, 1956),* T. knowlesi = T. avium *(Das Gupta and Siddons, 1941),* T. hannai = T. avium *Pittaluja, 1912 (Wenyon, 1926) and* T. avium *Danilewsky (Grewal). Measurements are in microns.*

Name of the trypanosome :—	*T. avium* (Plate I, Figures 1, 2)		*T. avium* (Plate I, Figure 3)		*T. knowlesi* = *T. avium* (Plate I, Figure 4)		*T. hannai* = *T. avium*	
Described by :—	Grewal, 1962.		Baker, 1956.		Das Gupta and Siddons, 1941.		Pittaluja, 1912 (Wenyon, 1926).	
Name of the host :—	The white-throated munia, *Uroloncha malabarica.*		Canary, *Serinus canarius.*		The white-throated munia, *Uroloncha malabarica.*		Domestic pigeon, *Columba livia.*	
	Extremes.	Average.	Extremes.	Average.	Extremes.	Average.	Extremes.	Average.
Length (in microns) of the body :—	42·9—53·7	47·64	34·9—54·3	47·7	44·0—63·0	..	45·0—60·0	..
Distance (in microns) between kinetoplast and posterior end :—	7·5—15·4	10·7	7·8—19·8	14·6	12·0—18·0	..	19·0—22·0	..
Distance (in microns) between kinetoplast and nucleus :—	7·1—11·0	8·9	7·4—10·2	10·0	8·0—11·0	..	..	4·65
Length (in microns) of free flagellum :—	4·5— 8·6	7·03	3·7—12·7	7·2	7·0—11·0	..	6·0— 8·0	..
Breadth (in microns) :—	4·0— 7·0	6·2	3·5— 9·0	5·7	5·0— 8·0	..	6·0— 8·0	.
Number of trypanosomes measured :—	25		25		40		..	
Other peculiarities :—								
(*a*) Long thin caudal process :—	Present.		Present.		Present.		Present.	
(*b*) Marked longitudinal striations on the surface of the body :—	Present in some, absent in the others, of various sizes also.		Present.		Present; extending throughout the entire length.		In some specimens distinct evidence of long striations.	
(*c*) Granules of dark violet colour :—	Scattered throughout the substance in some specimens, especially towards each end.		Same as in *A*.		..		Same as in *A*.	

Average measurements of 25 trypanosomes from blood and bone-marrow are taken. The trypanosome varies from 35·4 μ to 39·6 μ in length, with an average of 37·65 μ, and 7·4 μ to 9·8 μ in breadth, with an average of 8·4 μ.

Round or ovoid, red-stained mass of chromatin, the *nucleus*, is situated roughly in about the middle of the body. It measures, when oval, from 4·0 μ to 6·6 μ in length, with an average of 5·4 μ, and from 3·4 μ to 4·4 μ in breadth, with an average of 3·9 μ ; while in the forms with round spherical nucleus, it has a diameter of 4·2 μ.

The *kinetoplast* is prominent and stains deep red. It measures from 1·0 μ to 1·5 μ in length, with an average of 1·2 μ, and 0·8 μ to 1·0 μ in breadth, with an average of 0·85 μ. Distance between the posterior end and the kinetoplast varies from 6·9 μ to 8·0 μ, with an average of 7·5 μ.

The *flagellum* runs along one side of the body and appears to be originating from the kinetoplast. In some of the forms, it seems to go up to the anterior end of the body, which is very thin and looks like a flagellum ; while in others the flagellum stops short just a little before the tip of the anterior end. The length of the entire flagellum varies from 36·4 μ to 42·4 μ, with an average of 39·6 μ.

The *flagellar vacuole* is large and prominent, situated in front of the kinetoplast.

The *undulating membrane* forms 4 to 6 undulations which become smaller towards the anterior end.

TABLE III.

Measurements (in microns) of T. delhiense *sp. nov. from the white-throated munia,* U. malabarica.

Number.	Measurements (in microns).	Extremes (microns).			Average (microns).
1	Length of the body	35·4	—	39·6	37·65
2	Breadth (excluding undulating membrane)	7·4	—	9·8	8·4
3	Distance from posterior end to the centre of kinetoplast	6·9	—	8·0	7·5
4	Distance from the centre of kinetoplast to the centre of nucleus	8·2	—	8·7	8·3
5	Distance from the centre of nucleus to anterior end	19·5	—	22·9	21·8
6	Dimension of kinetoplast	1·0 — 1·5×0·8 — 1·0			1·2 × 0·85
7	Dimension of nucleus	4·0 — 6·6×3·4 — 4·4			5·4 × 3·9
8	Breadth of undulating membrane	1·2	—	2·2	1·5
9	Total length of flagellum	36·4	—	42·4	39·6

Note : Measurements stated in Table III are taken from dried preparations of 15 trypanosomes from bone-marrow and 10 individuals from blood.

By comparing Tables I and III, and looking through Table IV, it becomes quite evident that the two trypanosomes are wide apart from each other. The differences between the two are sufficient enough to raise this trypanosome up to the standard of a new species. It is proposed to call it *Trypanosoma delhiense* sp. nov.

TABLE IV.

Comparison between T. avium *and* T. delhiense *from the white-throated munia,* U. malabarica.

Number.	Measurements (in microns)	*T. delhiense* sp. nov.	*T. avium.*
1	Length of the body	37·65 μ	40·61 μ (excluding free flagellum).
2	Breadth of the body	8·4 μ	6·2 μ
3	Distance from the posterior end to the centre of kinetoplast	7·5 μ	10·7 μ
4	Distance from the centre of kinetoplast to the centre of nucleus	8·3 μ	8·9 μ
5	Distance from the centre of nucleus to anterior end	21·8 μ	21·1 μ
6	Dimension of kinetoplast	1·2 μ × 0·85 μ	0·65 μ × 0·55 μ
7	Dimension of nucleus	5·4 μ × 3·9 μ	4·7 μ × 3·1 μ
8	Breadth of undulating membrane	1·5 μ	0·86 μ
9	Free flagellum	Absent	Present, 7·03 μ in length.
10	Total length of the flagellum	39·6 μ	43·5 μ

Two attempts were made to prepare the culture of *T. avium* and *T. delhiense* in the NNN medium, but with no success.

Regarding the isolation of the two trypanosomes, various attempts are in progress, and as soon as I am able to infect any invertebrate host with the two trypanosomes, a detailed and comparative study will be made, the results of which will be given in the future communications. Since no insect vector could be infected with any of the two trypanosomes, it was not possible to infect any 'clean' bird with any of the two trypanosomes described herewith.

The majority of the birds are infected with *T. avium*; but the birds which have the mixed infection, the ratio between the two trypanosomes *viz., T. avium* (with a free flagellum) and *T. delhiense* sp. nov. (without a free flagellum) is 10 : 1. Both trypanosomes prefer bone-marrow, but it is observed that the non-flagellated form, *T. delhiense*, can be more easily seen in the bone-marrow than in the blood preparations. Since the infection of birds with the flagellated form, *T. avium*, is much more, it is easy to locate it in any of the preparations made from infected birds. The non-flagellated form, *T. delhiense*, can easily be missed, unless the preparations are carefully examined, and a long search is required before one individual is seen.

Bird to bird infection by the inoculation of blood, harbouring trypanosomes, was not possible, probably, due to the cryptic infection.

Preparations were carefully examined to determine the number of trypanosomes seen in the blood as well as in the bone-marrow. By counting them carefully, it was found that roughly for each one of *T. avium* in blood there were 3 in bone-marrow; while for each one of *T. delhiense* in the blood there were 5 in the bone-marrow.

HABITAT.

In general, both the trypanosomes are more numerous in the bone-marrow, but the infection with *T. avium* is comparatively more. The exception was only in the case of 5 birds in which one or two parasites, after every 10 to 15 fields (high power objective and X 6 occular), could be seen in a fresh cover-slip preparation from the blood ; while in one of them, after post-mortem examination, infection of the bone-marrow was not comparatively heavy. This signifies that it is the same parasite which has come in the blood from the bone-marrow. Such results were usually obtained in summer (mostly in May to July); while in winter the preferable site for the trypanosomes appears to be bone-marrow. In a few cases, the infection, both in blood and in bone-marrow, was so low that a long tiresome search was needed before a bird could be called as 'clean'. This is the reason why a special capsule, to determine the infection in birds, was devised (Grewal, 1961).

Why is the bone-marrow a preferable site in winter and not in summer, I am not yet competent enough to give reasons.

SUMMARY.

Natural infection of blood and bone-marrow of the white-throated munia, *Uroloncha malabarica* with *Trypanosoma avium* Danilewsky 1885, and *Trypanosoma delhiense* sp. nov., is described.

The detailed morphological study of the two trypanosomes, *T. avium* (*with a free flagellum*) and *T. delhiense* (*without a free flagellum*) is made. It is shown that morphologically *T. avium*, (42·9 μ to 53·7 μ in length with an average of 47·64 μ; and 4·0 μ to 7·0 μ in breadth with an average of 6·2 μ) resembles *T. avium* (described by Baker, 1956) ; while *T. delhiense* (35·4 μ to 39·6 μ in length with an average of 37·65 μ ; and 7·4 μ to 9·8 μ in breadth with an average of 8·4 μ) is altogether a new species and does not resemble any trypanosome described from birds uptil now, hence it is given a new specific name.

T. knowlesi Das Gupta and Siddons, 1941, and *T. hannai* Pittaluja, 1912, are proved as synonyms of *T. avium* Danilewsky, 1885.

Both the trypanosomes (*T. avium* and *T. delhiense*) did not develop in the body of any insect or in NNN medium. It was not possible to infect 'clean' birds, by the inoculation of blood from infected birds, with any of the two species of trypanosomes, probably, due to a cryptic infection. Majority of the birds showed mixed infection, but those which harboured single species of parasites were found so only with *T. avium* and not with *T. delhiense*. They prefer bone-marrow in general, but are also seen in blood. Their ratio is 10 : 1 in birds showing mixed infection.

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FRESH FOCAL FILARIA TRANSMISSION RESULTING FROM RAPID URBANISATION.

BY

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[July 24, 1962.]

In a review on filariasis and its control in India, Jaswant Singh and Raghavan (1953) recorded that endemic areas existed in many States, the exceptions being Delhi, Himachal Pradesh, Jammu and Kashmir, Punjab, large parts of Mysore State, Rajasthan and Western areas of Uttar Pradesh. This statement was based on information collected from the Health Authorities of the different States in India. The presence or absence of filarial disease in the local communities formed the basis of the information received from the States. The studies of Acton and Rao (1930) and Rao and Iyengar (1930) on the effect of climate and the need for repeated reinfections for the establishment of the infection and development of the disease lent additional support to such an inference.

Filaria surveys carried out under the National Filaria Control Programme (Raghavan, 1955), however, revealed a number of areas such as Kanpur (Singh, 1960) with high infection rate (10·3 per cent) but negligible or no disease. From the data that became available, it was apparent that mere absence of filarial disease in the community was no matter for complacency. Filariasis transmission can be in progress for a period of years before the disease is established. The rapid industrialisation and urbanisation without adequate concomitant arrangements for disposal of human, animal and trade wastes, leading to increased culicine breeding, combined with the immigration of people from different parts of the country—some of them being symptomless but infected persons from endemic areas—appeared to be the contributing factors.

Reconnaissance surveys were carried out in different areas in the country, particularly those which were presumed to be non-endemic for filariasis so far. The results of these studies, carried out during 1960-61, are presented in this paper.

Reconnaissance to determine filariasis transmission is a new concept and has not been perfected by any means. There are a number of inherent difficulties like the identification of the infective larvae. Rapid human and mosquito surveys were carried out by special teams from the Malaria Institute of India. Local health organisations collected *C. fatigans* from their areas and sent them to the Institute by air for dissection.

Blood examinations were made from Amritsar, Panipat, Karnal and Sonepat in Panjab State ; Vrindavan and Hardwar in Uttar Pradesh ; Jaipur, Udaipur, Jodhpur and Ajmer in Rajasthan and from various localities in Delhi. The blood-smears were air dried, stained with J.S.B. Stain I (Jaswant Singh and Bhattacharjee, 1944), and examined for microfilaria. Mosquito collections and dissections were also carried out from most of the above mentioned areas. When mature infective larvae were found in the mosquitoes, they were identified according to the descriptions given by Iyengar (1957) and Nelson (1959).

RESULTS.

An area-wise analysis of the results of blood-smears examined is presented in Table I. The blood examinations brought out the following facts :—

(1) Of 10,134 persons examined, 88 were positive for *W. bancrofti*, one for *B. malayi* and one for *A. perstans*. The last was in an East African student at the Delhi University. Enquiries revealed that all the positive persons were from areas known to be endemic for filariasis and had settled in the areas surveyed for varying periods up to a maximum of 25 years. These persons had paid occasional short visits to their homes in endemic areas. Reinfections during such visits cannot be eliminated. It would, however, appear that filarial infection can persist in man for long periods even in the absence of any significant local transmission.

(2) No indigenously acquired infection was noted in the limited survey carried out. The mosquito dissections (Table II) revealed that filaria transmission, though of a low degree, was in progress in some of these towns. The infections were of *W. bancrofti* wherever the larval stages were identified.

CONCLUSIONS.

The reconnaissance surveys carried out gave unequivocal evidence regarding the presence of microfilaria-positive persons and of active transmission in many towns which were hitherto considered free from the risk of filariasis. In all the surveyed areas, rapid and improper urbanisation and industrialisation are in progress, leading to increased culicine breeding. Introduction of microfilaria carriers into such areas is inevitable through immigrating workers who are attracted from different parts of the country. This inevitably results in some degree of local transmission. There is an imminent risk of such areas becoming endemic for filariasis in the course of years.

TABLE I.

Results of examination of night blood-smears.

State/Town.	Number of persons examined.	Number positive for Mf.	Species of Mf.	Duration of stay in the town surveyed	Native district or the State of the positive persons.
PUNJAB					
Amritsar	1,053	6	*W. bancrofti*	8 to 25 years	Azamgarh (Uttar Pradesh) and Amritsar*.
Karnal	840	1	*W. bancrofti*	..	Uttar Pradesh.
Panipat	643	1	*W. bancrofti*	..	Sultanpur (Uttar Pradesh).
Sonepat	497	12	*W. bancrofti*	..	Ballia, Gorakhpur, Bareilly, Gonda and Durbasa (Uttar Pradesh) and Kerala.
DELHI					
Delhi and New Delhi	1,892	26†	*W. bancrofti*		Bihar, Uttar Pradesh, Calcutta, Madras, 24-Parganas, East Pakistan, Cuttack, Gorakhpur, Nawadip, Uganda (East Africa).
UTTAR PRADESH					
Vrindavan	40	4	*W. bancrofti*	..	Priests from endemic areas of South India.
Hardwar	193	4	3-*W. bancrofti* 1-*B. malayi*	2 to 6 years (20 years)	Azamgarh, Ghazipur, Chhapra (Uttar Pradesh) and Kerala.
RAJASTHAN					
Jaipur	762	6	*W. bancrofti*	..	Uttar Pradesh, Madras, Gujarat and Bihar.
Udaipur	1,037	12	*W. bancrofti*	..	Chittor, Madras, Gujarat, Surat, Banaras, Porbandar, Jamnagar, Junagarh, Jaunpur, Unnao (Uttar Pradesh).
Jodhpur	2,324	16	*W. bancrofti*	..	Kerala, Calcutta, Hyderabad, Warrangal, Ghazipur, Azamgarh, Bihar and Secunderabad.
Ajmer	853	2	*W. bancrofti*	½ to 1 year	Faizabad (Uttar Pradesh).

* The positive person from Amritsar was in Gaya and Gonda for one month.

† Includes one positive for *A. perstons* from Uganda (East Africa).

TABLE II.

Results of mosquito collections and dissections.

State/Town.	Number of *C. fatigans* dissected.	Number positive, stage and location of parasites.	Infection rate, per cent.	Infectivity rate, per cent.
PUNJAB				
Amritsar	2468	4 { 1-IV (2) Head and thorax 1-III (1) Thorax. 1-IV (10) Thorax. 1-III* (3) Malpighian tubules.	0·16	0·08
Karnal	707	4 { 1-IV (2) 1-III (6), II (2) Thorax. 1-III (6), II (5) Thorax, 1-IV (1) Head.	0·56	0·28
Panipat	381	1 { IV (3) Thorax. III (4) Abdomen.	0·26	0·26
Sonepat	500	4 { 1-III (5), II (2) 1-II (8) 1-II (23) 1-II (18) } Thorax	0·8	..
DELHI	2676	7 { 1-III (2), IV (2) Thorax. IV (4) Head. 1-III (4) Thorax. 1-IV (1) Thorax. 1-I (1), II (4) Thorax. 1-I (6) Thorax. 1* { IV (4) Abdomen. IV (3) Thorax. IV (5) Head. 1* { IV (4) Abdomen. IV (7) Thorax. IV (15) Head,	0·26	0·15
UTTAR-PRADESH				
Modinagar	105	..	..	..
Mathura	93	3 { 1-IV (1) 1-IV (27), III (1) 1-III (3) Thorax.	3·22	2·15
Hardwar	411	1 { 1-III (16) Thorax.	0·24	..
RAJASTHAN				
Jaipur	1084	2 { 1-IV (1) Head. 1-IV (2) Thorax and Head.	0·18	0·18
Udaipur	630	2 { 1-II (3) Thorax. 1-I (1) Abdomen	0·32	..
Jhodhpur	772	..	..	..
Ajmer	394	..	..	..
Total	10221	28	0·27	0·13

* Of animal origin.

I=Microfilaria. II=Sausage form. III=Pre-infective larva. IV=Infective larva.

Arabic numericals after Roman numericals (in brackets) indicate numbers of respective forms noted.

SUMMARY.

Filaria transmission and human cases were recorded in a number of areas in North-Western India, hitherto believed to be free from the risk of filariasis.

The hazards due to rapid and improper urbanisation leading to filariasis transmission are discussed.

From the limited blood surveys and histories obtained, it would appear that the infection in man can persist for long periods, even as long as two decades, in the absence of re-infection.

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THE SYNERGISM OF DDT BY SYNTHETIC PINE OIL.

BY

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[January 19, 1963.]

INTRODUCTION.

THE importance of synergists for insecticides, particularly for use in the fly and mosquito sprays, is well recognised (Blackith, 1953 ; Sumerford, 1954). In recent years, there have been attempts by several workers (Perry and Hoskins, 1951 ; Tahori, 1955 ; Zhuravelva, 1960 ; Fales and Bodenstein, 1961) to synergize the activity of synthetic contact insecticides in view of the spectacular development of insecticide-resistance in several species of insects of medical importance (Perti and Ranganathan, 1960 ; Brown, 1961). It appeared of interest, therefore, to investigate the feasibility of using certain substances which could increase the activity of insecticides. Pine oil and its products—thanite and terpin diacetate—have been reported (Brown, 1951) to act as synergists for pyrethrins. It is the object of this paper to present results on the activation of insecticidal properties of DDT, when used in combination with synthetic pine oil, as a space spray or when topically applied, on normal laboratory strains of the housefly, *Musca nebulo* Linn., and the mosquito, *Culex fatigans* Wied.

MATERIALS AND METHODS.

Synthetic pine oil, obtained from Messrs Prabhat General Agencies, Bombay, and DDT (Tecnnical grade) were used as solutions in acetone.

Susceptible strains* of houseflies, *M. nebulo*, and mosquitoes, *C. fatigans*, drawn from laboratory cultures, were used as test insects. The flies were reared by the method described by Basden (1949) for *M. domestica* and the age of the

* The authors have observed synergistic action of synthetic pine oil on DDT against susceptible insects. From the data presented it appears that both the strains of house-fly and *C. fatigans* are tolerant to DDT. It was confirmed by writing to the authors that the strains of insects used were normal colony strains which had no experience of exposure to any insecticide previously. It appears likely that inadvertant selection in the process of colonisation might have resulted in the development of enhanced tolerance to DDT in the strains of insects used.

—EDITOR.

insects used was four to five days. The mosquitoes were reared by the method described by Newman *et al.* (1949) and adult females, forty to sixty hours old, were used in the tests.

The synergistic activity of synthetic pine oil with DDT, when used as space spray against flies and mosquitoes, was assessed by the method described by Dixit and Perti (1962). There were four replicates in each assay. The bioassay of DDT and mixtures of DDT and pine oil, when topically applied, by means of a micro-syringe, on the dorsal thoracic region of the insects, was carried out by the method described by Wal *et al.* (1962). There were fifty insects in each assay. The temperature and relative humidity during the experiments were 26 ± 1°C and 70 ± 5 per cent respectively.

RESULTS AND DISCUSSION.

The results obtained in the various experiments on the toxicity of DDT and the synthetic pine oil-DDT mixtures against *M. nebulo* and *C. fatigans* are summarised in Tables I and II. It will be noted from these data that the toxic action of DDT as a space spray or on topical application was increased both against flies and mosquitoes when the insecticide was admixed with pine oil (1 : 5). The synergism of DDT by pine oil was, however, more pronounced against mosquitoes than against flies.

TABLE I.

Toxicity of DDT, and mixtures of DDT and pine oil, as space sprays.

Test insect.	Concentration of spray : DDT. Per cent (w/v).	DDT. mg./cu.m.	Pine oil. Per cent (w/v),	Pine oil. mg./cu.m.	Knockdown in 10 minutes, per cent.	Mortality in 24 hours, per cent.
C. fatigans	0·25	30	..	..	32	42
	..	..	1·25	150	5	0
	0·25	30	1·25	150	93	88
M. nebulo	0·25	30	..	..	27	39
	..	..	1·25	150	0	3
	0·25	30	1·25	150	30	55

TABLE II.

Toxicity of DDT, and Mixtures of DDT and pine oil, applied topically on insects.

Test insect.	Dosage (μg/insect) : DDT.	Pine oil.	Mortality in 24 hours, per cent.
C. fatigans	1·0	..	32
	..	5·0	0
	1·0	5·0	90
M. nebulo	16·0	..	35
	..	80·0	0
	16·0	80·0	77

As space sprays (Table I) against *C. fatigans*, DDT-pine oil mixtures effected three-fold increase in knockdown and more than two-fold increase in kill of the insects. The mixtures, however, did not increase the knockdown of *M. nebulo* although the kill was slightly increased (1·4 times). When applied topically on the insects (Table II), pine oil increased the activity of DDT by about three-fold against *C. fatigans* and two-fold against *M. nebulo*.

SUMMARY.

The synergism of DDT by synthetic pine oil was investigated against the housefly, *Musca nebulo* Linn., and the mosquito, *Culex fatigans* Wied. It was found that pine oil increased the toxic action of DDT both against flies and mosquitoes when the mixtures were used as space sprays or were topically applied on the insects. The synergism was, however, more pronounced against *C. fatigans* than against *M. nebulo*.

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STUDIES ON CULICINE MOSQUITOES.

3. Laboratory studies on the effects of the blood of different vertebrates on egg-production and biology of *Culex fatigans* Wied.

BY*

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[January 10, 1963.]

ABSTRACT.

THE natural populations of *Culex fatigans* show different host preference over the range of their distribution. In South-East Asia (including India) and Western Pacific, man seems to be the preferred host. Laboratory studies on the effects of the blood of different vertebrates (man, sparrow, bulbul, rabbit and rat) on the egg production and biology of the Delhi-strain of *C. fatigans* confirmed the species preference for human blood in the presence of other sources of blood offered. The number of eggs laid was found to be proportional to the quantity of blood ingested. The source of blood meal did not have any significant effect on the period of gonotrophic cycle, period of development from egg to adult, percentage of adult emergence and the sex-ratio. The species showed 30 per cent or more gonotrophic discordance irrespective of the source of blood meal.

INTRODUCTION.

Like other blood sucking arthropods, *Culex fatigans* requires blood-feed for development of eggs. However, reports on the host preferences, based on the precipitin tests of blood-meals of wild-caught populations, have failed to point out to any uniform host preferred over the range of the distribution of this species. Mathis (1935) in Dakar (Senegal) and Hammon *et al.* (1945) in California observed a high proportion (up to 88·0 per cent) of wild-caught females to have fed on birds. On the other hand, Toumanoff (1935) in Indo-China and Reeves and Rudnik (1951) in Guam observed a distinct preference for human blood. They observed 84·7—100·0 and 67·0 per cent mosquitoes respectively having had a human blood-meal. Recently Satya Prakash *et al.* (1962), while reporting the results of 8,228 blood-meals tested from different parts of India, observed that 77·4 per cent of the specimens were found to have taken a human blood-meal.

* The assistance of Shri H.L. Dhingra, M.A., Stat. Dip. (I.S.I.) for statistical analysis of the data is acknowledged.

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A population of mosquitoes caught in houses in the Congo did not show a clear-cut preference for any host. The proportion of mosquitoes with human blood varied between 50·0 and 70·0 per cent while the rest had fed on birds (Wanson and Nicolay, 1937).

Notwithstanding the results of field observations, it has been the practice to maintain the laboratory colonies of *C. fatigans* in this Institute on avian blood. This has been adopted after experimenting with different laboratory animals. Among birds, the common sparrow and the bulbul proved to be better hosts than the pigeon in that the former hosts allowed better feeding of the insect, resulting in a proportionately higher number of eggs. Recentty, however, Krishnamurthy and Pal (1959) observed that the Delhi-strain of *fatigans* showed gonotrophic dissociation when fed on man. These workers also indicated that the average number of eggs with human blood-feed were much less than those obtained with the avian blood-feed.

The present experiments were carried out during 1961-62 to study the host preference and the effect of quality and quantity of different sources of blood feeds on the egg production, viability of eggs, developmental period from egg to adult and sex ratio of the Delhi strain of *Culex fatigans* maintained in the Entomology Laboratory of the Central Institute for Communicable Diseases.

MATERIAL AND METHODS.

The experiments reported in this paper were carried out in the insectary under constant temperature (24·0°—25·6°C) and relative humidity (78 — 82 per cent). The adult females were obtained from the eggs of the laboratory strain of *Culex fatigans* maintained on the blood of sparrow and bulbul since 1948. The immature stages were raised on hay infusion and yeast. Adults, emerging during a period of 12 hours, were isolated and held for 24 hours after which they were used for experimental feeding. This ensured uniformity of age in the females used for experimentation.

To observe the effect of different blood-feeds on the egg production, viability of eggs, developmental period, sex ratio of emerging adults and the ratio of gonotrophic concordance, 100 females (each of the same age) were confined in five 12×12×12 inches wire-frame muslin cages (Wattal *et al.*, 1959) for night feeding. In four cages, a different host was introduced in each cage at 7 p.m. and removed at 7 a.m. The hosts used were (*i*) sparrow (*Passer domesticus*) ; (*ii*) bulbul (*Malpastes haemorrhous haemorrhous*) ; (*iii*) albino rat ; and (*iv*) rabbit (*Lepus cuniculus*). All the animals were confined in wire-frame cages. In the fifth cage, two volunteers (men) put in their hands and part of the forearms in the cage alternately after every third hour during the period of 12 hours feeding. Mosquitoes that had taken a blood-feed were isolated in specimen tubes of 3 inch×1 inch size. Water was kept in the tubes for egg-laying. The open end of the tube was plugged with cotton-wool or closed with a piece of mosquito-netting.

The eggs obtained were counted on a moist filter paper under a stereoscopic dissection microscope under 27 magnification. The egg-rafts were then transferred

to enamelled trays of 8 inches diameter @ 100 larvæ per tray. The larvæ were maintained on dry powdered brewers' yeast. The water temperature of the larval pans varied from 26 to 28°C. These experiments were repeated three times.

To find the feeding preference of *Culex fatigans*, 500 females of the same age were released in a 18×18×18 inches wire-frame muslin cage and the five hosts mentioned above were offered simultaneously. The mosquitoes were allowed to feed from 7 p.m. to 7 a.m. Freshly-fed mosquitoes were isolated, and their gut contents were taken on a filter paper. The blood-meals thus obtained were subjected to the tests against antisera of the host tried. This experiment was repeated twice.

To get a quantitative estimate of the amount of blood ingested from different hosts and its relationship with the number of eggs produced, 50 females (each of known age) were isolated in card-board boxes of $1\frac{3}{4}\times1\frac{3}{4}\times1\frac{1}{2}$ inches size with a nylon-netting sleeve on one side and the same netting fixed on the other side. The mosquitoes were introduced through the sleeve and were made to feed on man, bulbul and rabbit for 12 hours at night. The feeding was effected through netting held against the exposed parts of the host. The card-board boxes were weighed in a torsion balance before putting the insect and before and after feeding. The fed mosquitoes were isolated and processed in the manner mentioned earlier. In this way, the average amount of blood ingested by a fed mosquito was determined. The fed mosquitoes were isolated and kept for observation as described above.

RESULTS.

One hundred female *C. fatigans* each were allowed to feed on man, sparrow, bulbul, rabbit and rat in separate cages. The summary of the data obtained is presented in Table I.

TABLE I.

Data indicating per cent feeding, per cent oviposition, and egg-production of Culex fatigans *females following ingestion of blood-meals from different hosts : Mean values of three replicates.*

Host.*	Per cent fed.†	Per cent oviposited.‡	Average number of eggs laid per female.§
Man	33·0	65·6	111·1 (33—227)§§
Sparrow	36·3	71·5	174·7 (63—232)
Bulbul	37·3	58·0	160·1 (42—286)
Rabbit	15·0	70·3	149·0 (54—213)
Rat	1·6	80·0	155·0 (25—212)

* 300 females were used with each host in three replicates.
† Per cent fed during 12 hours.
‡ Per cent of the total fed.
§ For the number of females oviposited after single feeding.
§§ Range per female.

Analysis of variance for per cent fed, per cent oviposited and average number of eggs laid per ovipositing female was carried out separately and is contained in

Table II. It was found that the hosts differed significantly for the proportion fed and the average number of eggs laid per female. By applying the 'least significant difference' test it was found that only rat differed significantly from other hosts with regard to the per cent *fatigans* feeding on different hosts. The differences in feeding on other hosts, apparent in Table I, were not found to be significant.

TABLE II.*

Analysis of variance table for per cent fed, per cent laid eggs and average number of eggs of Culex fatigans *females following ingestion of blood meals from different hosts.*

Source of variation	Degrees of freedom.	Sum of squares.	Mean sum of squares.	F/Ratio	Remarks.
(A) PER CENT FED.†					
Between the replicates	2	332·17	166·08	3·53	
Between the hosts ...	4	2374·47	593·63	12·51	Significant
Error	8	379·50	47·44		
Total	14	3086·14			
(B) PER CENT LAID EGGS.					
Between the replicates	2	522·90	261·45		Not
Between the hosts ...	4	50·38	12·59	0·07	Significant
Error	6	1021·21	170·20		
Total	12	1594·49			
(C) AVERAGE NUMBER OF EGGS LAID.					
Between the replicates	2	55172	27586·0		
Between the hosts ...	4	152852	38213·0	38·186	Significant
Interaction between hosts and replicates	8	278267	34782·1		
Error	230	230180	1000·7		
Total	244	716471			

* The significance of difference between the per cent fed, per cent laid eggs and the average number of eggs was tested by 'analysis of variance' technique. The percentages of fed and laid eggs were first converted into arcsin $\sqrt{\text{percentage}}$ and wherever the data was missing, it was estimated by Yates technique.

† The critical difference for arcsin $\sqrt{\text{percentage}}$ is 21·5.

The significance of differences in average number of eggs laid were tested in pairs. The results are presented in Table III.

These observations (Table I) showed that *fatigans* would readily feed on man or birds (sparrow and bulbul) but would not feed to the same degree on rabbit. The rat proved to be the least preferred host and the degree of feeding on this host was found to be significantly lower than on other hosts. It was also evident that a single blood-meal taken by the females does not always result in oviposition. Out of the females fed, only 58·0, 65·6, 70·3 and 71·5 per cent females oviposited after a single blood-meal when fed on bulbul, man, rabbit and sparrow respectively. The proportion of females ovipositing were not found to show significant difference

when fed on different hosts. Therefore, it was apparent that a fair proportion of the population of the Delhi-strain of *Culex fatigans* showed gonotrophic discordance, irrespective of the source of blood-feed, but did not show gonotrophic dissociation when fed on man or any other vertebrate used in the present observations.

TABLE III.

Comparative statement of the actual differences of the means with their standard deviations for all possible combinations.

Serial number.	Hosts compared.	Differences in mean egg production.	Standard deviation of the differences.	Differences in mean egg production (column 3), divided by standard deviation of the differences (column 4),	Remarks.
(1)	(2)	(3)	(4)	(5)	(6)
1	Man/sparrow	63·6	± 8·2	7·76	Significant
2	Man/bulbul	49·0	± 9·0	5·44	Significant
3	Man/rat	43·9	±29·8	1·47	Not significant
4	Man/rabbit	38·3	±11·0	3·48	Significant
5	Sparrow/bulbul	14·6	± 7·28	2·0	Significant
6	Sparrow/rat	19·7	±21·6	0·91	Not significant
7	Sparrow/rabbit	25·3	± 9·6	2·6	Significant
8	Bulbul/rat	5·1	±25·6	0·2	Not significant
9	Bulbul/rabbit	10·7	±10·3	1·03	Not significant
10	Rat/rabbit	5·6	±28·7	0·19	Not significant

There was great variation in the number of eggs laid by a female *fatigans* after a single blood-feed from any of the hosts (Table I). The least number of eggs laid by a female was 25 when fed on rat and the highest number was 286 after a blood-feed from bulbul. The average number of eggs laid by females fed on different hosts showed significant differences. The number of eggs laid after feeding on sparrow was found to be significantly higher than the eggs obtained after feeding on man, bulbul and rabbit (Table III). Sparrow did not differ from rat in this respect. However, in view of the rat being the least preferred host for feeding, this similarity in egg production could not be considered of any consequence.

In the experiments reported above, the mosquitoes were confined with a single host and thus did not have a choice of feeding. Therefore, to find out the host preference of *fatigans*, 1000 females in two lots of equal numbers were offered the choice of feeding on man, bird (bulbul), rabbit and rat in a cage described earlier. The blood-meals of 272 mosquitoes fed during 12 hours were examined for the source of blood by precipitin tests. The data thus obtained are shown in Table IV.

These observations showed that the Delhi-strain of *C. fatigans* would prefer to feed on man if available.

The data obtained (Tables I and III) showed that fewer eggs were obtained after human blood-feed as compared with the eggs obtained after ingestion of avian

and rabbit blood-feeds. To understand the cause of these differences in the egg-production, following ingestion of blood from different hosts, 50 females each were made to feed in special card-board boxes, referred to earlier, on man, bird (bulbul) and rabbit. There was no feeding on rabbit but 42 per cent fed on man and 50 per cent fed on the bird during a period of 12 hours. The data obtained from this observation are shown in Table V.

TABLE IV.

Host preference shown by C. fatigans *females when confined with different verteberate hosts in a single cage : Results of precipitin tests.*

Hosts.	Man.	Bird.	Rabbit.	Rat.	Man and bird.	Man and rabbit.	Rabbit and rat.	No reaction*	Total.
Number fed.	217	6	8	4	2	1	1	33	272
Per cent fed.	79·7	2·2	2·9	1·5	0·8	0·4	0·4	12·1	100·0

* The reasons for this could not be established.

TABLE V.

Average number of eggs laid per milligramme of blood ingested by female C. fatigans.

Hosts.*	Per cent fed.†	Per cent oviposited.‡	Eggs laid per female.§	Blood ingested mg./female.§§	Eggs laid per mg. of blood ingested.††
Man	42·0	33·3	42·4	1·85	23·0
Bird	50·0	40·0	118·6	5·03	23·6

* 50 females were used with each host.
† Per cent fed during 12 hours.
‡ Per cent of the total fed.
§ Average for the females that oviposited after single feeding.
§§ Average determined from increase in weight after blood-feed.
†† Eggs laid per mg. blood ingested per female.

These observations (Table IV) showed that the quantity of blood ingested by a female *fatigans* was more when fed on bird than when fed on man. The amount of blood ingested seemed to directly influence the number of eggs laid. When the number of eggs laid was calculated on the basis of a comparable unit (per milligramme) of blood ingested, there was not any significant difference between the number of eggs laid when the mosquito had ingested blood from man or bird.

From these experiments, data were also collected to find the effect of different blood-meals on the gonotrophic cycle, period of development from egg to adult, per cent adult emergence and the sex ratio at emergence of adult C. *fatigans*.

For studying the variations in developmental period, etc., with regard to different hosts, 900 first-instar larvæ, hatched from eggs obtained after ingestion of blood from man, sparrow, bulbul, and rabbit, were reared into adults. Since there was poor feeding on rat, only 300 first-instar larvæ were taken from eggs obtained

after the females had fed on this host. The relevant data on these aspects of the experiments are presented in Table VI below :—

TABLE VI.

Data indicating gonotrophic cycle, developmental period from egg to adult, per cent emergence and sex-ratio at adult emergence of C. fatigans *when fed on different vertebrates.*

Hosts.*	Gonotrophic cycle (days).†	Developmental period (days).‡	Per cent adult emergence.§	PER CENT SEX RATIO :	
				Male.§	Female.§
Man	3—7** (56) 4—10†† (9)	8—17** 12—18††	96·6	51·5	48·5
Sparrow	3—7 (60) 4—10 (18)	8—17 12—18	96·0	57·5	42·5
Bulbul	3—7 (52) 4—10 (13)	8—17 12—18	97·2	54·4	45·6
Rabbit	3—6 (24) 4—10 (9	8—17 12—18	98·0	53·1	46·9
Rat	3—5** (4)	8—16**	98·0	54·8	45·2

* 300 females were used with each host in three replicates.

† Observations made on females that oviposited after a single blood-feed. The actual numbers are indicated in parenthesis. The gonotrophic cycle indicates the time taken for digestion of blood-feed followed by oviposition.

‡ 900 first-instar larvae were used except in case of rat where only 300 larvae were used in this observation. The time required for larval hatching has been added to these figures.

§ There was no seasonal variation.

** Results of experiments carried in August. 50·0 per cent laid eggs after 4 days and 70·0 to 100·0 per cent laid eggs after 5 days following the intake of blood feed.

†† Results of experiments carried in January. 60·0 to 90·0 per cent laid eggs after 8 days following the intake of blood feed.

It was evident (Table VI) that the source of blood-meal obtained by the female *C. fatigans* did not affect the time required for completion of the gonotrophic cycle, period of development from egg to adult in the progeny, per cent adult emergence or the sex ratio of adults. However, some seasonal influence was observed on the time required for completion of the gonotrophic cycle and the period of development from egg to adult. More time was required for both when the observations were made in January than in August. The former month corresponds to winter season and the latter to summer. This difference between the experiments occurred in spite of the constant temperature and humidity maintained in the insectary where these observations were made. These differences probably reflect the inherent biological qualities of the population used for these experiments.

DISCUSSION.

Culex fatigans is known to require blood-feed for development of its eggs. The natural populations seem to fall in two broad groups for the choice of the host. The results of precipitin tests with the blood meals in South-East Asia and Western Pacific point out to the presence of a race in this area showing a preference for human blood whereas the American race seems to show preference for avian blood (*see* 'Introduction'). In Africa the populations of this species seem to show no preference between human and avian blood.

Among the wild-caught populations of this species in India, a high proportion (77·4 per cent) has been found to feed on human blood (Satya Prakash *et al.*, 1962 *loc. cit.*). The present observations also showed that the laboratory-bred Delhi-strain of *fatigans* prefer to feed on man more than on birds, rabbit and rat when all these vertebrates were simultaneously offered as hosts (Table IV). Of the blood specimens which reacted against the antisera, as many as 90·8 per cent were found positive for man. This overwhelming preference for man indicates a strain characteristic as it would not readily feed on other vertebrates offered as hosts.

The stimuli that were likely to govern the feeding preference of this species in the present experiments would be body temperature, smell and the surface area of the host. The body temperature of the hosts offered was as follows :—

Host :—	Man	Bird	Rabbit	Rat
Body Temperature (°C) :—	36·9	41·5	39·5	37·9

The body temperature of man and rat being nearly the same, temperature as such could not have been a factor in selecting the host. As regards the surface area, the results of the precipitin tests carried out in India* on the blood-meals of natural populations showed that the species fed largely on man even in the presence of cattle with decidedly a bigger surface area. The influence of smell was not determined in these experiments and will be elucidated by further experiments.

Various workers (*see* Woke, 1937 ; Krishnamurthy and Pal, 1959) have mentioned about the value of blood-meals from different hosts on the egg production of mosquitoes (*Culex pipiens, Culex fatigans, Aedes aegypti* and *Anopheles stephensi*). It has been generally concluded that fewer eggs were obtained after ingestion of human blood as compared to mosquitoes fed on avian blood. In the present experiments also (Table I), it was observed that avian blood resulted in significantly higher egg production than the blood of man or rabbit. Rat appeared to be the least attractive host and therefore any similarity in egg production could not be considered comparable. However, in the subsequent experiments (Table V) it was observed that a female *fatigans* on average ingested 5·03 mg. blood when fed on bird as compared to 1·85 mg. ingested from man. The egg-production for a comparable unit of 1 mg. blood ingested from man and bird was found to be nearly equal (23·0 and 23·6 respectively). Thus the number of eggs laid was found to be proportional to the quantity of blood ingested and not dependent on the quality of blood of the host.

In these observations the laboratory-bred strain of *C. fatigans* showed a fair proportion of gonotrophic discordance irrespective of the source of blood-meal (Table I). After a single blood-meal from man and the birds, only 65·6 and 58·0 to 71·5 per cent respectively oviposited. This phenomenon of gonotrophic discordance in the population most probably could explain why Krishnamurthy and Pal (1959 *loc. cit.*) did not get eggs after the initial human blood-feed in the Delhi strain of *fatigans*.

* In Indian rural and semi-urban areas, the cattle are invariably found near human dwellings.

From these experiments, information was also collected on the effect of different blood-meals on the time required for completion of the gonotrophic cycle, period of development from egg to adult, per cent adult emergence (Table VI). It was found that blood-meals from man, sparrow, bulbul, rabbit and rat did not significantly alter the course of any of these biological processes.

SUMMARY.

Laboratory experiments were carried out during 1961-1962 to study the effects of the blood of different vertebrates (man, sparrow, bulbul, rabbit and rat) on the egg-production, viability of eggs, developmental period from egg to adult and sex-ratio at adult emergence of the Delhi-strain of *Culex fatigans*. The observations were made under constant temperature (24·80—25·6°C) and humidity (78-82 per cent). The observations revealed that :—

1. *Culex fatigans* fed readily on man, sparrow, bulbul and rabbit but not readily on rat.

2. When given a choice, the females preferred to feed on man than on any other vertebrate mentioned above. Of the blood specimens from mosquito gut which reacted against the antisera, as many as 90·8 per cent were found positive for man. This preference was shown to be comparable to the high proportion of human-fed specimens among natural populations of *fatigans* in India reported by Satya Prakash *et al.* (1962).

3. The preference for human blood seemed to be a strain characteristic and was not influenced by the body temperature and surface area of the host. The influence of smell of host selection was not determined.

4. The laboratory-bred strain of *fatigans* showed 30 per cent or more gonotrophic discordance irrespective of the source of blood-meal.

5. The higher number of eggs obtained after ingestion of avian blood-meal was found to be proportional to greater amount of blood ingested from this host. The number of eggs obtained per mg. of blood ingested from man and bird were almost identical (23·0 and 23·6 respectively).

6. The source of blood-meal did not affect the period of gonotrophic cycle, period of development from egg to adult, per cent adult emergence and the sex-ratio of adult *fatigans* at emergence.

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THE SUSCEPTIBILITY OF MALAYAN ANOPHELINES TO *PLASMODIUM CYNOMOLGI BASTIANELLII*.

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THE history of human malaria investigations records a number of efforts to determine if lower primates are actually reservoirs of human malaria parasites. Although some cross susceptibility had been reported, the specific transmission of human malaria strains from man to monkey or monkey to man in nature has been generally discounted. However, Eyles *et al.* (1960) reported the transmission of *Plasmodium cynomolgi bastianellii*, a *vivax*-like parasite of *Macaca irus*, to man by the bite of a mosquito, and later a strain of *P.c. cynomolgi* was also transmitted to man (Coatney *et al.*, 1961). *Plasmodium cynomolgi* was originally described from a *Macaca irus* from Java by Mayer (1907). *Plasmodium cynomolgi bastianellii* was isolated from *Macaca irus* from Malaya and described by Garnham (1959).

The report of the natural transmission of *P. c. bastianellii* to man graphically demonstrated the necessity for intensive investigations into simian malarias. The laboratories of the Far East Research Project of the U.S. Public Health Service and the Institute for Medical Research of the Federation of Malaya have been working on this problem as a joint program in Malaya for more than two years. It is the purpose of this paper to report the results of studies on the susceptibilities of Malayan anophelines to *P. c. bastianellii*. From a practical point of view, it is essential to demonstrate the potential of local mosquitoes to harbour and proliferate this particular parasite.

MATERIALS AND METHODS.

Mosquitoes, used for experimental infections, were obtained partly from wild populations. Catches of adult mosquitoes were made in a variety of habitats, ranging from brackish water areas in mangrove swamps along the western coast of the Malayan peninsula through coconut, rubber and rice cultivation areas of the coastal plain and inland valleys to jungle swamps and hill forests of the inland areas.

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Laboratory-reared mosquitoes were used whenever possible. These were obtained through : (*i*) Field collected larvae ; (*ii*) The progeny from adults collected in the field and allowed to oviposit in the laboratory ; (*iii*) Adults reared from larvae obtained from eggs oviposited by artificially mated adults (Ow Yang *et al.*, 1963).

The experimental animals consisted of *Macaca mulatta* obtained from Calcutta and New Delhi. Large numbers of monkeys from these areas have been used in this laboratory and none has shown malaria parasites. Confirmation of the uninfected status of these experimental animals was made by blood film examination prior to inoculation.

The strain of *P. c. bastianellii* was the one originally isolated by Garnham, and sent to the U.S. Public Health Service laboratory in Memphis, Tennessee (U.S.A.) for study. It was during the process of studies on this *Plasmodium* that the accidental transfer to man via mosquito occurred.

The strain was maintained in Malaya primarily by blood transfer to an uninfected animal once each week. Under these conditions, gametocytemias, sufficient for the purposes of this study, could be expected approximately seven days after the inoculation. This first peak would usually last from three to four days, and a second peak with infective gametocytes would usually appear about seven days later. In addition, sporozoites from several species of experimentally infected mosquitoes were inoculated and produced infections that were used for mosquito studies.

Preliminary experiments indicated that *Anopheles philippinensis* and *Anopheles maculatus* were quite susceptible to *P. c. bastianellii*. Therefore, in all of the experiments reported in this paper, one of these species was used as the control on the infectivity of the donor at the time of the feeding.

Gametocyte counts were made by determining the number per 100 white blood cells on giemsa stained thin blood films made at the time of feeding. Although there is a considerable margin of error in such a procedure due to variation of white blood cell count, a relationship between the male gametocyte count and the intensity of infection in the mosquitoes was found and is presented in Table I.

The relationship between these two factors as reflected by *A. maculatus* is a linear one for observations on the first three gametocyte count groups. In the last two gametocyte count groups (11-20 and over 20) there is a decrease in the median number of oocysts observed in each lot. The range of the mean number of oocysts for each gametocyte group is also presented in Table I, and it can be seen that the specific relationship between gametocyte count and intensity of infection is only apparent when a considerable number of lots of mosquitoes have been analysed. We have no explanation for the fact that at certain times a very high gametocyte count caused intensive infections in mosquitoes while at other times an equally high gametocyte count would result in a low level response in the mosquito. Similar, variable results are seen with very low gametocyte counts. The same response has been observed in human malarias, particularly *P. falciparum*.

TABLE I.

Relationship between male gametocyte count and intensity of oocyst infection in the guts of Anopheles maculatus *and* Anopheles philippinensis.

Male gametocyte count/100 WBC.	*Anopheles maculatus*:			*Anopheles philippinensis*:		
	Number lots.	Median number oocysts from all lots.*	Range of mean number of oocysts.	Number lots.	Median number oocysts from all lots*.	Range of mean number of oocysts.
1 — 2	11	24	0 — 235	3	0	..
3 — 5	18	46	0 — 582	4	5	1 — 44
6 — 10	13	125	0 — 696	2	67	57 — 77
11 — 20	10	119	2 — 660	3	33	17 — 82
21	6	82	3 — 1000	..	..	..

* The mean number of oocysts was calculated for each lot and the median of the series of means was then determined. Lots on the average consisted of eight mosquitoes.

A. philippinensis, although showing a similar relationship between the number of male gametocytes and the number of oocysts present in the gut, is obviously not as responsive as *A. maculatus*. As will be seen later in this paper, this lower level of susceptibility is also reflected in the percentage of which become infected in a given experimental feeding. Due to the lower level of susceptibility in *A. philippinensis*, the experimental work employed *A. maculatus* as controls whenever possible.

Monkeys were sedated with 10 to 50 mg. of promazine hydrochloride injected intramuscularly. The belly of the animal was clipped and then the animal was tied to a large board with a hole in the centre. The animal was then applied directly to mosquito-netting cages of selected mosquitoes, and usually allowed to feed undisturbed for one hour. Most feedings were done from 7.00 p.m. to midnight.

Blood-fed mosquitoes were removed from the cages and placed in paper cups with gauze tops and maintained on raisins until dissected.

Routine dissections were begun on the fifth post-feeding day, and continued at intervals through the 14th day. Gland dissections for sporozoites were carried out on all examinations after the 8th post-feeding day. Mosquito gut preparations were placed in mercurochrome (Eyles, 1950) and both counts and measurements of cysts were done immediately after dissection. Representative samples of positive guts were mounted in formol glycerine (Wharton, 1959). Salivary glands were prepared in 10 per cent serum in physiological saline. Positive glands for subinoculation were pooled and injected intravenously into clean rhesus monkeys.

RESULTS.

SUB-GENUS *ANOPHELES*

The sub-genus *Anopheles* is well represented in Malaya and most of the species are included in species groups. The majority of the important species were studied but forest species such as *A. aitkeni* and *A. montanus* could not be tested.

The *Anopheles barbirostris* group

This group currently includes ten distinct species, of which six are known from Malaya (Reid, 1962). For many years all of the Malayan species, except *A. barbumbrosus*, were included in the single species, *A. barbirostris*, and it is therefore difficult to evaluate the previous work concerning their relationship to the transmission of malaria. It is presently known that *A. campestris* is an important vector in Malaya and that *A. donaldi* is considered to be a vector in Borneo.

The susceptibility of three species has been evaluated: *A. barbirostris*, *A. campestris*, and *A. donaldi*. In addition, a small amount of information was obtained on *A. hodgkini*. The two Malayan species of the group not evaluated, *A. barbumbrosus* and *A. pollicaris*, are uncommon.

The susceptibility data for all species are summarized in Table II, and each species is discussed separately below :—

TABLE II.

Susceptibility of species of the Anopheles barbirostris *group to infection with* Plasmodium cynomolgi bastianellii *as compared with* A. maculatus *and* A. philippinensis.

Species.	Number of paired feedings.	Number dissected.	Number positive.	Per cent positive.	Average cysts per infected gut.
A. barbirostris	9	84	11	13	29
A. maculatus		60	52	87	260
A. barbirostris	3	71	9	13	14
A. philippinensis		43	38	88	32
A. campestris	5	21	4	19	10
A. maculatus		32	27	84	227
A. donaldi	5	56	2	4	1
A. maculatus		32	32	100	280
A. donaldi	1	6	0	0	..
A. philippinensis		18	13	72	17
A. hodgkini	1	2	1	50	3
A. maculatus		10	9	90	34

Anopheles barbirostris.—This is a common and widely distributed species found in stagnant water with some vegetation, rice-fields, swamps, pools, and drains. It is predominantly zoophilic, and since the natural infection rate has been found to be nil (none positive of 1,981 reported by Reid, 1962), it is believed to be of no importance as a human malaria vector.

The mosquitoes were obtained from a rice-field area at Kampong Tengah near Kuala Lumpur, and included wild-caught and laboratory-reared mosquitoes.

The proportion of *A. barbirostris* susceptible to *P. c. bastianellii* was much lower than that of *A. maculatus* or *A. philippinensis*. The intensity of infection was much less as measured by the average number of oocysts per infected gut, and gland infections with sporozoites did not develop even when a moderate infection had

been observed. In one experiment in which the proportion of *A. barbirostris* infected was 22 per cent, salivary glands from 17 mosquitoes, examined 11·5, 12·5 and 14·5 days after infection, were all negative. Fifteen sets of salivary glands from these dissections were inoculated into an uninfected rhesus monkey, but the animal did not develop an infection.

In most lots of *A. barbirostris*, black spores of Ross were seen very frequently and were clearly associated with an abnormal development of the parasite, indicating an unfavourable environment for development.

Anopheles campestris.—This species is found primarily in the coastal regions of Malaya in fresh water pools and drains with considerable amounts of shade and the larvæ will tolerate small amounts of salinity. It enters houses freely and feeds readily upon man and is responsible for the transmission of human malaria in certain parts of the west coast of Malaya. The natural infection rate has been found to be 0·7 per cent (sporozoite rate, 0·3 per cent) based on 14,950 dissections (Reid, 1962). The mosquitoes used were reared in the laboratory, so that there is no risk of confusion with natural infections.

As Table II shows, *A. campestris* demonstrated a very low level of susceptibility as compared with *A. maculatus*. There have been too few mosquitoes available to determine whether or not sporozoites will develop. The finding of black spores of Ross in this species, as in *A. barbirostris*, indicated a poor adjustment of parasite to host.

Anopheles donaldi.—This species breeds in pools adjacent to swamp forest. Its recent separation from other mosquitoes of the group has given little opportunity to determine its relationship with human malaria, but Reid (1962) presented evidence that this species is a vector in Borneo. There are no published records of *A. donaldi* dissections in Malaya, but an infection rate of 0·9 per cent was found recently in Selangor. (Wharton *et al.*, 1963*a*).

The source of the mosquitoes was from Bukit Kelubi, Selangor, and some of the mosquitoes were reared in the laboratory.

As is shown in Table II, *A. donaldi* is quite refractory to *P.c. bastianelli*. Only two of a total of 62 mosquitoes became infected (both of these were laboratory-reared), and many were fed when the donor was highly infective as is indicated by the *A. maculatus* controls. Again, black spores of Ross were seen frequently.

Anopheles hodgkini.—This species was established by Reid (1962) and breeds in shaded pools in or at the edge of the hill forest. It is widespread but usually not numerous. *A. hodgkini* have been caught with human bait in the forest at night, but there is no evidence to indicate its involvement in the transmission of human malaria, and there is no information available on infection rates of wild mosquitoes. The few mosquitoes used in the present study were reared in the laboratory.

Table II shows that one of two *A. hodgkini* fed became infected, but it should be noted that the donor monkey, as evidenced by the infection in *A. maculatus*, was not highly infective.

The *Anopheles hyrcanus* group.

For many years two species, *A. nigerrimus* and *A. sinensis*, were recognized in this group in Malaya ; however, Reid (1953) established no less than seven separate species within this group that are indigenous to Malaya. The larvae of this group can be found in pools, swamps and rice-fields, and two species will breed in brackish water.

Mosquitoes in the *A. hyrcanus* group are predominantly zoophilic, but will occasionally bite man. *A. nigerrimus* is regarded by Reid (1953) as a potentially important vector of human malaria in Malaya. *A. sinensis* is important in the transmission of human malaria in parts of China, but does not contribute to this problem in Malaya. *A. lesteri* was at one time regarded as a vector of filariasis in Malaya and has recently been recognized as a malaria vector in China (Reid *et al.*, 1962 ; and Ho *et al.*, 1962).

In this study, the susceptibility of all of the Malayan species, except *A. nigerrimus*, was evaluated. This last species could not be obtained in numbers at the time of our work, and the number of *A. crawfordi* obtained was small. The species are discussed individually below and the susceptibility data for all species are presented in Table III.

TABLE III.

Susceptibility of species of the Anopheles hyrcanus *group to infection with* Plasmodium cynomolgi bastianellii *as compared with* A. maculatus *and* A. philippinensis.

Species.	Number of paired feedings.	Number dissected.	Number positive.	Per cent positive.	Average cysts per infected gut.
A. argyropus	7	108	3	3	5
A. maculatus		55	41	75	300
A. crawfordi	4	5	0	0	..
A. maculatus		33	26	79	134
A. indiensis	3	19	0	0	..
A. maculatus		20	20	100	54
A. indiensis	2	27	1	4	1
A. philippinensis		35	25	71	40
A. lesteri	2	36	21	58	130
A. maculatus		10	6	60	349
A. peditaeniatus	6	79	1	1	1
A. maculatus		40	27	68	71
A. peditaeniatus	3	41	1	2	1
A. philippinensis		39	26	67	41
A. sinensis	2	54	12	22	28
A. maculatus		60	51	85	291
A. sinensis	2	14	2	14	2
A. philippinensis		35	25	71	40

Anopheles argyropus.—This species is usually uncommon, but may become locally abundant in areas where there are extensive marshes produced by tin

dredging. It prefers swampy pools where bushes and reeds grow in the water providing considerable shade. Although no dissection data are available for this species, it is not believed to be involved in the transmission of human malaria. The mosquitoes used were caught as adults from the Puchong area near Kuala Lumpur.

A. argyropus was virtually non-susceptible to *P. c. bastianellii*. Only three of 108 mosquitoes became infected. The size of the oocysts indicated that they resulted from the experimental feeding. The number of oocysts in the few which became infected was much lower than in the control *A. maculatus*.

Anopheles crawfordi.—This species of the *A. hyrcanus* group is widely distributed, but is not usually common. It prefers breeding places similar to those described for *A. argyropus*. There is no evidence to incriminate *A. crawfordi* as a human malaria vector in Malaya. No malaria infections were found in 470 dissections of this species. (Wharton *et al.*, 1963*b*).

The mosquitoes used were reared in the laboratory. Only five mosquitoes were fed and none became infected, as compared with 79 per cent of the control *A. maculatus*. Even though the oocyst number in the *A. maculatus* indicated the donor monkey was only moderately infective, the absence of any infection in the five *A. crawfordi* would indicate that it is not susceptible.

Anopheles indiensis.—This species also is a swamp breeder and is quite common in rice-fields, in sites partially shaded by emergent vegetation. There is no evidence that *A. indiensis* is a vector of human malaria in Malaya. Wharton *et al.* (1963*b*) listed 199 wild mosquitoes dissected without finding malaria parasites.

The mosquitoes used were in part laboratory-bred and in part caught as adults from rice-field area at Kampongs Tengah and Kerdas near Kuala Lumpur.

Only a single infection was found in the 46 dissected, and this was in a wild-caught mosquito. It is apparent that *A. indiensis* has an extremely low level of susceptibility, even though some of the feedings were made under suboptimal conditions.

Anopheles lesteri.—In Malaya, this species is virtually confined to the coastal areas breeding in brackish water. It is sometimes found in shady areas in association with *A. baezai*, and at other times in more open areas breeding with *A. sundaicus*. The *A. lesteri* adults apparently prefer animals to man as a source of blood-meals, but will attack man outside houses. There is no evidence that *A. lesteri* is a vector of human malaria in Malaya. All of 350 dissections reported by Reid (1953) and 139 dissections recorded by Wharton *et al.* (1963*b*) were negative. The mosquitoes used were wild-caught adults captured from Telok Piah near the Selangor Coast.

As Table III shows *A. lesteri* is much more susceptible to *P. c. bastianellii* than the other members of the *A. hyrcanus* group. Although the oocyst number was much lower, nearly as large a proportion of mosquitoes became infected as in

the control lots of *A. maculatus*. Sporozoites were seen in the glands 11-5 days after feeding, and glands injected into an uninfected rhesus monkey produced infection after a prepatent period of only eight days. Although it was demonstrated that this species can transmit *P. c. bastianellii*, only a few of the mosquitoes dissected proved to have sporozoites whereas most had had heavy gut infections.

Anopheles peditaeniatus.—This species is commonly found in rice-fields and in other areas where there are partially shaded collections of fresh water. It will attack man occasionally, but is believed to be primarily zoophilic. There is no evidence that it is involved in the transmission of human malaria in Malaya, and 146 dissections listed by Wharton *et al.* (1963*b*) were all negative. The mosquitoes were in part laboratory-reared and part wild-caught from Kampongs Changket and Tengah and from Puchong near Kuala Lumpur. *A. peditaeniatus* demonstrates an extremely low level of susceptibility. Of 110 mosquitoes dissected, only two were infected and one of these was wild-caught.

Anopheles sinensis.—This species prefers open, grassy ponds; and in areas close to the coast may be found breeding with *A. sundaicus*, thus indicating a tolerance for salinity. Although a vector in China, there is no evidence to incriminate this species in Malaya [no infections in 185 dissections (Wharton *et al.*, 1963*b*)] but there are few dissections available upon which to base this conclusion.

The mosquitoes were obtained from a number of localities near Kuala Lumpur and also from near the Selangor coast. Some laboratory-reared females were utilized. Less than 20 per cent of the *A. sinensis* population was susceptible to *P. c. bastianellii*. Certain individual mosquitoes were highly susceptible (one mosquito showed 132 oocysts), and it was the most susceptible of the *A. hyrcanus* group except for *A. lseteri*.

The *Anopheles umbrosus* group.

This group is at present a complex of seven species of which five will be discussed. No data are available for *A. brevipalpis* and *A. brevirostris* which are not common. Breeding habitats range from brackish water swamps along the coastal sections of Malaya to the fresh water areas of the deep jungle swamps. Information from the literature is difficult to evaluate since for many years most were called *A. umbrosus*. Epidemiological work has indicated that *A. letifer* is an important vector of human malaria in certain areas (Hodgkin, 1956). Other species, specifically *A. baezai*, *A. roperi* and *A. umbrosus* demonstrate high sporozoite rates, but recently Wharton *et al.* (1963*a*) reported that the sporozoites found in *A. umbrosus* were *Plasmodium traguli* from the very common mouse-deer. The source of infections in *A. baezai* and *A. roperi* is still unknown but is suspected to be mouse-deer also. The susceptibility data for all species are summarized in Table IV.

Anopheles letifer.—Partially shaded pools and stagnant agricultural drains in areas formerly covered by jungle swamp are typical areas preferred by this species; however, it does not breed to any great extent in the virgin swampy jungle (Reid and Hodgkin, 1950). *A. letifer* is wide-spread on the coastal plain and is found in small numbers in the hills.

TABLE IV.

Susceptibility of species of the Anopheles umbrosus *group to infection with* Plasmodium cynomolgi bastianellii *as compared with* A. maculatus *and* A. philippinensis.

Species.	Number of paired feedings.	Number dissected.	Number positive.	Per cent positive.	Average cysts per infected gut.
A. letifer	9	148	29	20	40
A. maculatus		401	360	90	220
A. letifer	2	11	0	0	..
A. philippinensis		26	15	58	15
A. baezai	3	5	0	0	..
A. maculatus		34	31	91	442
A. roperi	1	2	0	0	..
A. maculatus		5	5	100	696
A. separatus	2	8	2	25	16
A. maculatus		8	8	100	573
A. umbrosus	5	151	29	19	20
A. maculatus		30	30	100	606

Mosquitoes were laboratory-reared and wild-caught from Bukit Kelubi and Bukit Mandul which are swamp forest areas in Selangor. The natural infection rate in this species summarized by Hodgkin (1956) is 0·9 per cent (64 guts and 61 glands positive of 13,986 dissected). Recent dissections from or near the source areas have shown a rate of about 0·4 per cent (8 oocyst and 7 sporozoite infections in 4,019 dissected), but a part of these represented non-primate infections (Wharton *et al.*, 1963*a*).

A. letifer is susceptible at the oocyst level and certain individuals had more than 300 oocysts. However, the potential ability of this mosquito to transmit this *Plasmodium* in nature is considered to be virtually nil. Many are refractory to the parasite (only 20 per cent became infected under optimum conditions), and dissections on the 13·5 post-infection day demonstrated oocysts that were small (comparable to 4·5 days old oocysts in *A. maculatus*) and apparently degenerate. No sporozoite differentiation was seen and the glands were all negative. It is assumed that this mosquito presents a physiological barrier which acts to supress the development of the *Plasmodium* beyond the early oocyst stage.

Anopheles baezai.—This species is typically a brackish water breeder. This mosquito will breed in virtually any collection of brackish water which is not flushed daily and where there is some shade and decaying vegetable matter. However, it is never as widespread or as abundant as its fellow brackish water breeder, *A. sundaicus*. *A. baezai* is attracted to human bait traps which are established in the open but is reluctant to enter houses. Precipitin tests indicate that goats and cattle are the preferred source of blood meals for this mosquito (Reid and Weitz, 1961). This mosquito has been found to be infected in the Rantau Panjang area but Reid and Weitz (1961) concluded that it was not a vector of primate malaria.

Recent evidence suggests that the infections might be from mouse-deer. Dissection records have shown a natural infection rate of 1·5 per cent [10 guts and 17 glands positive of 1865 dissections (Hodgkin, 1956)]. Both wild-caught mosquitoes from Kampong Pandam and laboratory-reared specimens were used for experimental feedings with this species.

Though the numbers are small, the experimental conditions were optimum as is indicated by the *A. maculatus* controls ; and Table IV indicates that *A. baezai* is refractory to *P. c. bastianellii.*

Anopheles roperi.—This member of the *A. umbrosus* group prefers shaded jungle streams in low hill country. It is frequently found in shallow, temporary pools containing decaying leaves and is occasionally found in shaded drains on rubber estates. Although Reid and Hodgkin (1950) suggested that *A. roperi* might be a human malaria vector, recent work indicates that it probably transmits mouse-deer malaria. A natural infection rate of 11·4 per cent of 273 mosquitoes was observed but none resembled primate parasites. Nine sporozoite inoculations into monkeys and one into man were made, but no patent infections resulted (Wharton *et al.*, 1963*a*).

The mosquitoes used were wild-caught at Pacific Tin, a swamp forest area just north of the Selangor river.

Although the number of experimental dissections is small, the response of the controls fed with the two *A. roperi* (Table IV) indicate that the feedings were carried out when the donor was highly infective. Since neither of these mosquitoes became infected there is serious doubt as to the potential of this species to transmit *P. c. bastianellii.* Other experimental work must be done before final conclusions can be drawn.

Anopheles separatus.—This mosquito breeds in drains, pools and swamps where light shade is available. There is no epidemiological evidence that this species is involved in the transmission of human malaria in Malaya, and only one infected mosquito out of 666 dissected was reported by Hodgkin (1956). Recent dissections on this species have shown no infecti ns of 208 examined (Wharton *et al.*, 1963*a*).

Experimental mosquitoes were wild-caught in the Pacific Tin area and some were laboratory-reared.

Certain individuals are quite susceptible to *P. c. bastianellii.* One experimentally infected mosquito demonstrated 225 oocysts ; however, only 25 per cent showed any susceptibility under laboratory conditions.

Anopheles umbrosus.—Early records in Malaya are confused since several species were included under the name *A. umbrosus.* It breeds in dense, swampy jungle of the coastal region of Malaya and is seldom found outside the forest. This species has in the past been considered to be a vector of human malaria in Malaya due primarily to the finding of a considerable number of infections in wild-caught mosquitoes from several different sections of the country. However, Wharton *et al.* (1963*a*) have found that these infections are almost certainly *P. traguli*

from the mouse-deer. The mosquitoes used in these experiments were wild-caught from Bukit Mandul in south-central Selangor and from Pacific Tin. Previously (Hodgkin, 1956) reported a natural infection rate from this species of 1·75 per cent (65 guts and 43 glands positive of 6,097 dissections), and recent dissections showed 4·6 per cent out of 2,661 infected. The data presented in Table IV indicate that *A. umbrosus* is moderately susceptible since 19 per cent had oocysts, but it was not determined if sporozoites would develop.

SUB-GENUS CELLIA.

The sub-genus *Cellia* (= *Myzomyia*) contains only one species group which is considered first. Data were obtained on most of the other species with the notable exception of *A. karwari* and some forest species such as *A. watsoni*.

The *Anopheles leucosphyrus* group

This group of mosquitoes has recently been revised by Colless (1956 : 1957) and now contains seven distinct species and several subspecies. These are distributed broadly from Assam through Burma, Thailand, Indo-china and Formosa south through the Malayan Peninsula and Sumatra east through Borneo and Celebes to the Philippines. Two species of the group are also found in Ceylon and in the tropical rain-forest of south-west India. Six members of the group have been identified in Malaya, *A. balabacensis introlatus*, *A. balabacensis balabacensis*, *A. hackeri*, *A. leucosphyrus*, *A. pujutensis* and *A. riparis macarthuri*. *A. b. balabacensis* is confined to the extreme northwest of Malaya, adjacent to the Thailand border.

Three species found in Malaya have so far proved to be natural vectors of monkey malaria, including *A. hackeri*, *A. b. introlatus* and *A. leucosphyrus* (Wharton and Eyles, 1961 ; Wharton *et al.*, 1962 ; Eyles *et al.*, 1963). *A. leucosphyrus* is a vector of human malaria in Sarawak, parts of Indonesia, Borneo and Sumatra. *A. b. balabacensis* seems to be a vector wherever it occurs. Primarily forest breeders, they are rarely encountered in large numbers in Malaya.

Anopheles balabacensis introlatus.—This jungle breeding mosquito prefers shaded seepages and pools, particularly animal wallows. This sub-species has not been incriminated in the transmission of human malaria, but has recently been identified as a vector of monkey malaria. It seems to be widely distributed through the hill forest regions of central Malaya, but our work has been hampered by difficulties in obtaining large numbers.

The mosquitoes used were derived from laboratory-reared larvae from Ulu Gombak, a primary rain forest area just north of Kuala Lumpur. Dissection records are rather limited with this sub-species, but recent observations in the Institute for Medical Research have shown a 1·5 per cent natural infection rate (3 glands positive of 210 dissections). The sporozoites from these positive glands were inoculated into both man and monkey. One monkey became infected with *P. cynomolgi* but none of the human volunteers demonstrated any parasites (Eyles *et al.*, 1963).

TABLE V.

Susceptibility of species of the Anopheles leucosphyrus *group to infection with* Plasmodium cynomolgi bastianellii *as compared with* A. maculatus.

Species.	Number of paired feedings.	Number dissected.	Number positive.	Per cent positive.	Average number of cysts per infected gut.
A. balabacensis introlatus	4	12	1	8	8
A. maculatus		31	28	90	22
A. hackeri	1	1	1	100	156
A. maculatus		24	24	100	213
A. leucosphyrus	1	1	1	100	1
A. maculatus		11	10	91	37
A. pujutensis	1	1	0	0	0
A. maculatus		12	12	100	78
A. riparis	4	6	5	83	335
A. maculatus		47	40	85	269

The surprisingly low level of susceptibility of this species to *P. c. bastianellii* (Table V) is of considerable interest. It is true that the *A. maculatus* controls demonstrated comparatively light infections, but even under these conditions 90 per cent became infected. In the light of the proven ability of this species to transmit monkey malaria in nature this response to *P. c. bastianellii* is puzzling, but may be due to the fact that the strains of mosquitoes and malaria were not co-indigenous. One co-indigenous *P. cynomolgi* type parasite has been isolated from a wild-caught mosquito of this sub-species, and it is highly infective in the laboratory to *A. b. introlatus*.

Anopheles hackeri.—This member of the *A. leucosphyrus* group has an interesting distribution pattern in Malaya. It has been found breeding in fallen bamboos, water pools in rotton logs and recently in ground pools in the primary hill forest in central Malaya ; this was the area originally considered to be the breeding habitat of this species. A new breeding habitat was recorded for *A. hackeri* when it was discovered to be breeding in the cut bases of nipah palms near the coast (Reid and Weitz, 1961).

A. hackeri is a proven natural vector of several species of monkey malaria, including *P. cynomolgi, P. fieldi, P. knowlesi, P. inui* and *P. coatneyi* (Warren *et al., in preparation*). This mosquito feeds readily on monkeys in the canopy but is rarely attracted to man. There is strong evidence that *A. hackeri* is not involved in the transmission of human malaria in Malaya (Reid and Weitz, 1961). Recent dissections have shown an infection rate of 1·5 per cent of 1,823 specimens examined (Warren *et al., in preparation*). This species has been extremely difficult to work with in the laboratory. Many adults were reared but only one could be induced to feed under experimental conditions. This individual became heavily infected.

Anopheles leucosphyrus.—This species is a pool breeder and is found in small numbers primarily in the deep jungle. Although this mosquito has been caught biting man, there is no evidence that this species is involved in the transmission of human malaria in Malaya ; but has recently been proved to be a vector of monkey malaria. A natural infection rate of 0·7 per cent (1 gut and 1 gland positive of 281 dissections), has been recently recorded (Wharton *et al.*, 1962). Mosquitoes used were laboratory-reared from larvae collected at Ulu Gombak. Only one mosquito was fed under laboratory conditions and it became lightly infected (Table V).

Anopheles pujutensis.—This species breeds in temporary pools in swamp forests and has also been found in secondary forest near the coast. It has never been incriminated as a vector of human malaria, but has been suspected of being a vector of monkey malaria at Rantau Panjang by Reid and Weitz (1961) where they recorded an infection rate of 2·4 per cent of 83 dissected. In recent dissections, an infection rate of 2·5 per cent was found (Warren *et al., in preparation*). One laboratory-reared mosquito was fed but failed to develop an infection.

A. riparis.—This mosquito is found in relatively still pools at the edge of streams in the jungle and in overgrown rubber plantings. Larvae collections would indicate that it is the most common member of the group in hill forest but has only been collected twice as adults, both times on monkey bait. One of the two adults had sporozoites which failed to produce an infection in a rhesus monkey.

The mosquitoes used were reared in the laboratory from larvae collected at Ulu Gombak. Five of six mosquitoes fed became heavily infected (Table V).

SUB-GENUS CELLIA.

Other Species.

The remaining species of the sub-genus are discussed in alphabetical order and the susceptibility data are included in Table VI.

Anopheles aconitus.—This species is widely distributed in Malaya and is particularly associated with inland rice-fields. There is no evidence to incriminate *A. aconitus* in the transmission of human malaria in Malaya, but is a vector in Java. A natural infection rate of 0·04 per cent (one gut positive of 2,063 dissections) was recorded by Hodgkin (1956).

The mosquitoes used were wild-caught at Bukit Kelubi north of Kuala Lumpur. Only two mosquitoes fed, of which one became lightly infected.

Anopheles kochi.—This mosquito will breed in almost any collection of stagnant water that is sunlit or in only light shade. It breeds in rice-fields during certain stages of the crop and can frequently be found with *A. vagus* in muddy pools. Adults of *A. kochi* will enter human bait traps but prefer to feed on animals other than man. There is no evidence, either parasitologically or epidemiologically, that this mosquito is a vector of human malaria in Malaya. It should be noted, however, that *A. kochi* shows a high level of susceptibility to human malaria under

experimental conditions (Hodgkin, 1956). No infections have been recorded in 3,814 dissections (Hodgkin, 1956). The mosquitoes used were laboratory-reared.

TABLE VI.

Susceptibility of Anopheles aconitus, A. kochi, A. subpictus, A. sundaicus, A. tessellatus *and* A. vagus *of the sub-genus* Cellia *as compared with* A. maculatus *and* A. philippinensis.

Species.	Number of paired feedings.	Number dissected.	Number positive.	Per cent positive.	Average cysts per infected gut.
A. aconitus	2	2	1	50	7
A. maculatus		23	18	78	365
A. kochi	6	25	21	84	213
A. maculatus		50	39	98	311
A. kochi	2	7	1	14	30
A. philippinensis		35	25	71	40
A. subpictus	1	1	0	0	0
A. philippinensis		4	1	25	1
A. sundaicus	4	42	30	71	764
A. maculatus		20	16	80	321
A. sundaicus	1	9	7	78	33
A. philippinensis		4	1	25	1
A. tessellatus	1	1	0	0	0
A. maculatus		10	9	90	34
A. philippinensis	5	29	26	90	44
A. maculatus		24	24	100	100
A. vagus	10	62	15	24	116
A. maculatus		61	51	84	392
A. vagus	3	7	0	0	0
A. philippinensis		16	7	44	6

It is apparent from the data presented in Table VI that *A. kochi* is susceptible to this species of *Plasmodium*, and certain individuals showed extremely high oocyst counts. Transmission was successfully accomplished in the laboratory.

Anopheles maculatus.—This mosquito is the most important vector of human malaria in Malaya, but is not a vector in most parts of its range. A natural infection rate of 0·9 per cent (16,239 dissections) has been recorded for this species in Malaya (Hodgkin, 1956). It is a seepage breeder and prefers open sunlight or very little shade, and is rarely found in water which does not have at least some movement. Seepages created by clearing the jungle-covered hills for rubber cultivation particularly encourage prolific breeding. Since *A. maculatus* was found to be very susceptible to *P. c. bastianellii*, it was employed as a control of the infectivity of the donor on most of the experimental feedings.

The *A. maculatus* mosquitoes used were primarily laboratory-reared, however some wild specimens from Ulu Lui, south of Kuala Lumpur, were employed as

well. The summary of the response of *A. maculatus* to *P. c. bastianellii* is seen in Table VII.

A. maculatus is very susceptible to *P. c. bastianellii*; 76 per cent of 450 mosquitoes becoming infected, with an average number of oocysts per infected gut of 180. Transmission was successfully accomplished in the laboratory. On several occasions, when extremely heavy oocysts infections were observed, many of the oocysts degenerated and very few sporozoites were seen in the salivary glands. Reference has already been made to the relationship between gametocyte count and degree of infection in this mosquito.

Anopheles philippinensis.—This species is an open swamp breeder and is particularly numerous in rice-fields. *A. philippinensis* is strongly zoophilic but it will enter human bait traps and feed on man. It is not a vector of human malaria in Malaya, although it is known to be a vector in India. No infections have been recorded in 6,815 dissections from Malaya (Hodgkin, 1956).

The mosquitoes used were both wild-caught (Kampong Kerdas and Kampong Tengah) and laboratory-reared.

As shown in Table VI, *A. philippinensis* is quite susceptible to *P. c. bastianellii*. It was used as a control when *A. maculatus* was not available but was not as susceptible. Transmission was successfully accomplished in the laboratory.

Anopheles subpictus.—This species prefers ponds and swamps with emergent vegetation and open sunlight or light shade. It is rarely a common mosquito and is not involved in the transmission of human malaria in Malaya. No natural infections have been recorded in 54 dissections (Hodgkin, 1956). One mosquito, which fed in the laboratory, failed to develop an infection.

Anopheles sundaicus.—This species is a brackish water breeder and is particularly common where the mangrove has been cleared. *A. sundaicus* is a vector of human malaria in Malaya and other parts of South-East Asia. A natural infection rate of 0·1 per cent (12,474 dissections) has been recorded for this species in Malaya (Hodgkin, 1956).

The mosquitoes used were wild-caught from Telok Piah and Pasir Penambang.

A. sundaicus is very susceptible to *P. c. bastianellii*. The proportion infected (71 per cent) was only slightly lower than that seen in *A. maculatus* (Table VI); but the number of oocysts per infected gut was much higher in *A. sundaicus*. Transmission was successfully accomplished in the laboratory.

Anopheles tessellatus.—This species is widely distributed in Malaya but is seldom abundant. It prefers lightly shaded pools and drains as breeding sites. There is no evidence that this species transmits human malaria in Malaya and no natural infections have been found in 194 dissections. No infection developed in the single mosquito of this species which fed in the laboratory.

Anopheles vagus.—This is a very common species that is widely distributed throughout Malaya. Small muddy pools, which are exposed to sunlight, are

preferred by this mosquito. This species is strongly zoophilic and does not transmit human malaria in Malaya. No natural infections have been observed in 9,399 dissections. Mosquitoes used were obtained from a variety of sources including wild-caught specimens from Kampong Kerdas, Telok Piah, Puchong and Pasir Penambang. Some laboratory-reared mosquitoes were also used.

Only 24 per cent of the *A. vagus* became infected but individual mosquitoes had up to 325 oocysts. It is not known if sporozoites will develop in this species.

TABLE VII.

Comparative susceptibility of Malayan Anopheles to Plasmodium cynomolgi bastianellii.

Species.	Number of experiments.	Number examined.	Number positive.	Per cent positive.	Average Number of cysts.
Sub-genus *Anopheles*					
A. barbirostris group					
A. barbirostris	9	155	16	10	27
A. campestris	5	21	4	19	10
A. donaldi	6	62	2	3	1
A. hodgkini	1	2	1	50	3
A. hyrcanus group					
A. argyropus	7	108	3	3	15
A. crawfordi	4	5	0	0	0
A. indiensis	5	46	1	2	1
A. lesteri	3	37	21	57	130
A. peditaeniatus	9	110	2	2	1
A. sinensis	11	68	14	21	25
A. umbrosus group					
A. letifer	11	159	29	18	4
A. baezai	3	5	0	0	0
A. roperi	1	2	0	0	0
A. separatus	2	8	2	25	16
A. umbrosus	5	151	29	19	20
Sub-genus *Cellia*					
A. leucosphyrus group					
A. balabacensis	4	12	1	8	8
A. hackeri	1	1	1	100	156
A. leucosphyrus	1	1	1	100	1
A. pujutensis	1	1	0	0	0
A. riparis	4	6	5	83	335
A. aconitus	2	2	1	50	7
A. kochi	8	32	22	69	205
A. maculatus	53	450	344	76	180
A philippinensis	9	86	65	76	36
A. subpictus	1	1	0	0	0
A. sundaicus	5	51	37	73	676
A. tessellatus	1	1	0	0	0
A. vagus	13	69	15	22	185

DISCUSSION.

There are a great number of species of *Anopheles* in Malaya available as potential vectors, which renders a study of the epizootiology of monkey malarias

a complex problem. Laboratory experiments were undertaken to determine which of the many species were susceptible to *Plasmodium cynomolgi bastianellii*, in order to point the way toward a more logical development of field studies. The result was that it was found that many species possessed some degree of susceptibility to this malaria parasite, as is shown in the summary (Table VII). Monkeys were selected for feeding when gametocyte counts were high, and when, according to previous observations, these gametocytes had the greatest infectivity to mosquitoes. Results were presented in terms of the percentage of mosquitoes infected and the intensity of infections as measured by the number of oocysts present. Evaluation, based on oocyst infection, must be viewed with caution as far as final significance is concerned.

The most important aspect of susceptibility, is the ability to develop sporozoites and to transmit the parasite to another vertebrate host. There are apparently biological barriers which may inhibit the development of the mosquito infection at various stages. For instance, in members of the *A. barbirostris* group, the development of black spores indicated an inhospitable environment for the parasite early in the oocyst stages. In *A. letifer*, oocyst development proceeded normally through the fifth or sixth day, then growth slowed or ceased and degeneration of the oocysts followed. No oocysts with differentiating sporozoites were seen, nor did sporozoites appear in the salivary glands. In the case of *A. maculatus*, development usually proceeded normally until sporozoites were present; however in some instances, usually associated with extremely high oocyst infections, development of the majority of oocysts ceased when about 45 microns in diameter and very few sporozoites reached the glands. Because of these barriers to development, actual transmission by the mosquito was attempted whenever possible.

Additional qualifications must be kept in mind in interpreting the experimental susceptibility data. First, the feedings were made on monkeys with extremely high gametocyte counts, and in nature such high counts are probably rarely encountered. Since the percentage of mosquitoes which becomes infected is lower when the gametocyte counts are lower, the experimental study may give an exaggerated idea of the susceptibility of the mosquitoes.

Secondly, the experiments employed rhesus monkeys, which are not the natural hosts of *P. cynomolgi bastianellii*. It is possible that similar experiments, using *Macaca irus*, might show a different relationship between gametocytemia and infection.

The information in this paper applies to *P. c. bastianellii* and so cannot be related directly to the other species of monkey malaria. Generally, susceptibility to infection seems to be related more to the mosquito host than to the parasite and if a mosquito is susceptible to one malaria parasite, then it probably will be susceptible to others. In nature, it is likely that transmission of species of monkey malaria in a given habitat will be by the same vector. For example, five species of monkey malaria have been isolated from *A. hackeri*. At the same time, in so far as

comparison can be made, the susceptibility of the *Anopheles* to *P. c. bastianellii* roughly parallels their susceptibility to human malarias (Hodgkin, 1956). *A. maculatus* and *A. kochi* are both good hosts for human malaria, while the *A. barbirostris* group are poor hosts. Due to the newer taxonomy, comparison cannot be made too strictly, and further studies are necessary to evaluate the susceptibility of the newly differentiated species in the *A. hyrcanus*, *A. barbirostris* and *A. umbrosus* groups to human malarias. Parasite strains must also be important, as was shown by the apparent failure of *A. b. introlatus* as a host for *P. c. bastianellii*, even though it is a vector in nature of a *P. cynomolgi* strain from a different ecological area of Malaya than the type locality of *P. c. bastianellii*.

With the qualifications mentioned kept in mind, the following tentative evaluation of the potential ability of the species studied to transmit *P. cynomolgi bastianellii* in nature can be made.

In the sub-genus *Anopheles*, fifteen species have been studied and most showed a low degree of susceptibility. Four species of the *A. barbirostris* group were either refractory or only slightly susceptible, and the evidence available indicates none would be efficient vectors in nature.

In the *A. hyrcanus* group, six species have been investigated and all, except *A. lesteri* and *A. sinensis*, were practically insusceptible. *A. lesteri* is moderately susceptible and transmission has been accomplished in the laboratory. Depending upon its habits in nature, it is conceivable that it could be a vector. *A. sinensis* is much less susceptible. Even though under optimum conditions about 22 per cent develop infections, it is unlikely that this degree of susceptibility would allow it to be a vector in nature. A deficiency in the present study is that *A. nigerrimus* of the *A. hyrcanus* group could not be studied due to non-availability of mosquitoes. Under some circumstances it is considered to be a vector of human malaria, so a study of its susceptibility to simian malaria would have been of interest.

Five species of the *A. umbrosus* group have been studied, but only on *A. letifer* and *A. umbrosus* were sufficient data obtained to allow conclusions to be drawn. Both are only slightly susceptible to infection and there is apparently a physiological barrier which prevents the development of sporozoites. The small amount of information available indicates the other species of the group are also of low susceptibility and all, but *A. baezai*, are either rare or locally distributed. The evidence available would seem to be that no member of the *A. umbrosus* group is likely to be involved in the transmission of *P. cynomolgi bastianellii*.

Thirteen species of the sub-genus *Cellia* have been studied, and in general they have been found to be much more susceptible to *P. cynomolgi bastianellii* than the members of the sub-genus *Anopheles*.

More experimental data are required for *A. aconitus*, *A. subpictus*, and *A. tessellatus* before their response can be evaluated. *A. vagus*, though some individuals are susceptible, is probably not sufficiently susceptible to be a vector in nature.

A. sundaicus, *A. maculatus* and *A. kochi* are all highly susceptible experimentally, and *A. philippinensis* is moderately susceptible. Transmission has been accomplished in the laboratory with all of them, and all are sufficiently susceptible that they could transmit in nature if their habits permitted. This dramatically illustrates the point that the susceptibility data are only useful when used in conjunction with information on the habits, as all evidence to the present indicates that these species are not monkey malaria vectors in nature, due to the fact that they are not frequently attracted to monkeys in the canopy (Wharton *et al.*, 1963*b*). Even so, *A. sundaicus* and *A. maculatus* could, over the long period, supply a link between man and monkey as both occasionally bite monkeys and both bite man quite frequently.

The *A. leucosphyrus* group of the sub-genus *Cellia* is the most interesting as far as simian malaria is concerned for three members have been proved to be vectors in nature, and several members are attracted to both man and monkey, and not to any great degree to other animals studied. Unfortunately, study of this group has been frustrated by technical difficulties. They are difficult to obtain in numbers in Central Malaya, and are reluctant to feed under experimental conditions. For instance, it has been virtually impossible thus far to induce *A. hackeri*, which feeds almost wholly on monkeys in nature (Reid and Weitz, 1961), to take monkey blood in the laboratory. The single mosquito fed became infected and the fact that it is a proved vector, indicates that this finding is reliable.

The data on *A. leucosphyrus* are so meagre as to be practically meaningless, but this also is a proved vector. *A. balabacensis introlatus*, as previously discussed, is relatively insusceptible to *P. cynomolgi bastianellii*, but it is also a proved monkey malaria vector, and is quite susceptible to a coindigenous strain of *P. cynomolgi*. The only individual of *A. pujutensis* fed did not become infected, but this does not fit in with circumstantial evidence from field studies that it may be a simian malaria vector in certain areas of Malaya (Reid and Weitz, 1961). *A. riparis* is of particular interest as up to the present, it had never been observed to bite any vertebrate, but two have been caught recently attracted to monkeys (Wharton *et al.*, 1963*b*). It is a jungle mosquito, widely distributed in the hilly part of Malaya and is quite susceptible to *P. cynomolgi bastianellii*.

Our conclusions, in spite of the fragmentary data, are that all members of the *A. leucosphyrus* group must be regarded with suspicion and studied carefully. These are mosquitoes of the forest and jungle and are probably primarily primate biters. The overall range of the group coincides remarkably with the known distribution of monkey malaria in the Far East.

SUMMARY.

The experimental susceptibility of twenty-seven species of Malayan anophelines to *Plasmodium cynomolgi bastianellii* has been studied. A large number demonstrated at least a moderate level of susceptibility to this malaria parasite.

Members of the sub-genus *Anopheles* were generally poor hosts. Only *A. lesteri* of the *A. hyrcanus* group was sufficiently susceptible to be a potential vector in nature.

Several species in the sub-genus *Cellia* were good hosts for this parasite including *A. maculatus, A. sundaicus, A. kochi* and *A. philippinensis*. The *A. leucosphyrus* group, which contains three proven vectors of monkey malaria, could not be extensively studied due the unavailability of sufficient numbers of mosquitoes.

Transmission was successfully accomplished in the laboratory with one species from the sub-genus *Anopheles* (*A. lesteri*) and four species from the sub-genus *Cellia* (*A. kochi, A. maculatus, A. philippinensis* and *A. sundaicus*).

The broad patterns of susceptibility of the Malayan anophelines to this species of monkey malaria were similar to those previously recorded for human malaria.

The relationship between gametocytemia in the vertebrate host and infectivity to mosquitoes is discussed.

The necessity for using caution in the interpretation of experimental data on the susceptibility of anopheline mosquitoes to malaria parasites, without correlated epidemiological studies, is also discussed.

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A NOTE ON RESIDUAL EFFECTIVENESS OF INSECTICIDES APPLIED ON MUD SURFACE

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THE selection of resistant strains of vector species to chlorinated hydrocarbon insecticides (Perti and Ranganathan, 1960; Brown, 1961) has resulted in the search for more potent methods of insect control. The residual effectiveness of several newer synthetic contact insecticides applied on mud surface has recently been studied both against culicine mosquitoes (Sharma *et al.*, 1957 ; Ramakrishnan *et al.*, 1960) and houseflies (Sharma and Kalra, 1962), and the use of organophosphorus compounds has been suggested for residual spraying to counteract resistance. However, in view of the wide application of chlorinated hydrocarbon insecticides in vector control programmes, particularly where susceptible strains of insects are encountered (World Health Organization, 1960) and considering the toxic hazards (Hayes, 1960) associated with the use of organophosphorus insecticides, it appeared of interest to investigate the comparative residual efficiency of certain chlorinated hydrocarbon insecticides and organophosphorus compounds applied on mud surface against susceptible strains of mosquitoes and houseflies. The results are presented in this note.

MATERIALS AND METHODS

The mud panels (15×15×1·3 cm.), used in these investigations, were prepared from clay and treated by spraying requisite concentrations of the insecticidal formulation by the methods described by Paul and Perti (1962). The amount of insecticidal deposit was estimated (World Health Organization, 1956) from filter papers similarly treated with the insecticide. The treated panels were dried in the air for 48 hours before exposure to the insects. Adult female mosquitoes, *Culex fatigans* Wied., 40 to 60 hours old, and houseflies, *Musca nebulo* Linn., 4 to 5 days old, drawn from susceptible laboratory strains were used as test insects. The water wettable formulations of DDT, gamma BHC, dieldrin, chlordane, heptachlor, diazinon and malathion obtained from the trade were used in the various experiments. For assessment of toxicity, the insects were confined on the treated mud panels under inverted glass funnels (7·5 cm. diameter) for two hours. Twenty adult

females were used in each experiment and there were two replicates for each assay. Observations on the mortality of the insects were recorded 24 hours after the exposure. Subsequent exposures of the insects on the treated mud panels were carried out at intervals and finally discontinued when the mortality effected in two successive exposures was below 20 per cent. The temperature and relative humidity during the investigations were 25 — 33°C and 36 — 98 per cent respectively.

RESULTS AND DISCUSSION.

The results obtained in the various experiments are presented in Tables I to III. The index of residual activity of an insecticide has been considered here as the ratio of period (in days) during which not less than 50 per cent mortality is effected and the dosage applied on mud surface as gm./sq. metre. It will be noted from the data in Table I that as residual insecticides applied as water wettable powder on mud surface, at minimum dosages effecting high initial kill of *C. fatigans*, gamma BHC, dieldrin, chlordane and DDT are the most effective in the order stated. These are followed by heptachlor and malathion ; diazinon is the least effective. However, when applied in certain specific dosages (Table II) it will be seen that gamma BHC, dieldrin, DDT, chlordane and heptachlor are the most effective in the order stated and are followed by diazinon ; malathion is the least effective. Against *M. nebulo* (Table III), gamma BHC, dieldrin, diazinon, heptachlor and chlordane are the most effective in the order stated and are followed by malathion ; DDT is the least effective.

TABLE I.

Residual activity of insecticides against C. fatigans *when applied as water wettable powder on mud surface (using minimum dosages for effecting high initial kill of insects).*

Insecticide.	Dosage (gm. per sq. metre) The figures within brackets indicate the dosage in mg. per sq. ft.	Per cent mortality in successive exposures (The figures within brackets indicate the number of days after treatment).										Period (in days) up to which 50 per cent mortality was recorded.	Index of residual activity.
DDT	0·40 (37·6)	98 (2)	23 (15)	0 (26)	0 (37)							10	25
Gamma BHC	0·044 (4·10)	100 (2)	100 (4)	100 (7)	95 (9)	67 (20)	45 (30)	65 (40)	63 (50)	8 (60)	3 (71)	52	1,182
Dieldrin	0·53 (49·6)	90 (2)	70 (13)	55 (23)	10 (35)	23 (46)						22	42
Chlordane	1·71 (158·6)	98 (2)	98 (13)	95 (23)	98 (33)	18 (43)	48 (53)	63 (62)	0 (79)	0 (89)		65	38
Heptachlor	0·99 (92·4)	95 (2)	98 (12)	18 (22)	38 (32)	30 (43)	3 (60)	5 (71)				16	16
Diazinon	0·58 (54·0)	98 (2)	60 (5)	28 (7)	8 (9)	0 (13)						6	10
Malathion	2·12 (197·6)	98 (2)	85 (12)	23 (23)	48 (35)	43 (45)	3 (62)	0 (73)				32	15

TABLE II.

Residual activity of insecticides against C. fatigans *when applied as water wettable powder on mud surface. (Using certain specific dosages*).*

Insecticide.	Dosage† (gm./sq. metre). The figures within brackets indicate the dosage in mg./sq. ft.	Per cent mortality in successive exposures (The figures within brackets indicate the number of days after treatment)															Period (in days) upto which 50 per cent mortality was recorded.	Index of residual activity.
DDT	2·22 (206·0)	98 (2)	100 (12)	93 (22)	86 (47)	78 (54)	85 (85)	95 (97)	83 (122)	76 (132)	68 (142)	52 (153)	27 (166)	20 (176)	8 (187)	12 (198)	152	69
Gamma BHC	0·67 (62·5)	100 (2)	100 (12)	98 (25)	92 (42)	90 (49)	88 (69)	86 (79)	96 (103)	82 (113)	65 (123)	46 (135)	25 (145)	48 (159)	13 (169)	8 (179)	132	107
Dieldrin	0·82 (76·12)	100 (2)	100 (12)	63 (26)	75 (36)	78 (53)	66 (77)	68 (86)	82 (96)	68 (118)	45 (131)	58 (141)	17 (152)	23 (163)	13 (173)	3 (183)	104	128
Chlordane	2·17 (202·0)	96 (2)	90 (12)	100 (22)	100 (32)	95 (43)	63 (53)	56 (63)	52 (73)	13 (86)	8 (100)						74	34
Heptachlor	2·58 (240·0)	100 (2)	98 (12)	100 (22)	100 (32)	100 (43)	73 (53)	63 (63)	23 (73)	18 (83)	23 (95)	3 (107)	2 (117)				67	27
Diazinon	0·30 (27·5)	63 (2)	25 (3)	13 (5)	5 (7)	3 (18)											3	10
Malathion	0·32 (29·8)	38 (2)	0 (3)	0 (6)													†	..

* The dosages for DDT, gamma BHC and dieldrin approximate those recommended by the World Health Organization. [*Wld. Hlth. Org. Tech. Rep. Ser.*, **123**, p. 36. (1957)].
The dosages for malathion and diazinon are approximately those recommended by Messrs. Lederle Laboratories, Bombay. [Puri, I.M. (Insecticide Consultant, Lederle Laboratories, Bombay, India)—*Personal communication* (1960)].
The dosages for chlordane and heptachlor approximate those used for DDT in these experiments.

† The initial mortality registered on mud panels, treated with malathion, was only 38 per cent. Hence no figure is given.

TABLE III.

Residual activity of insecticides against M. nebulo when applied as water wettable powder on mud surface. (Using certain specific dosages).*

Insecticide.	Dosage (gm./sq. metre), The figures within brackets indicate the dosage in mg./ sq. ft.	Per cent mortality in successive exposures. (The figures within brackets indicate the number of days after treatment).	Period (in days) up to which 50 per cent mortality was recorded.	Index of residual activity.
ODT	2·22 (206·0)	100 (2) 98 (12) 28 (22) 32 (36) 3 (46) 12 (53)	18	8
Gamma BHC	0·67 (62·5)	100 (2) 100 (13) 90 (35) 70 (42) 92 (49) 60 (71) 98 (81) 83 (104) 86 (114) 58 (124) 78 (134) 46 (144) 18 (158) 7 (171) 7 (181)	142	212
Dieldrin	0·82 (76·12)	100 (2) 100 (12) 96 (19) 100 (36) 100 (63) 100 (78) 100 (108) 98 (132) 100 (144) 95 (154) 43 (164) 43 (174) 12 (186) 10 (196)	162	200
Chlordane	2·17 (202·0)	96 (2) 90 (12) 100 (22) 98 (32) 100 (43) 100 (54) 100 (65) 53 (75) 50 (86) 23 (99) 40 (109) 18 (121) 13 (131) 2 (141)	86	39
Heptachlor	2·58 (240·0)	100 (2) 100 (12) 100 (22) 100 (32) 100 (43) 100 (54) 100 (65) 75 (75) 73 (86) 60 (99) 38 (109) 0 (121) 2 (131)	104	42
Diazinon	0·30 (27·5)	100 (2) 100 (3) 100 (9) 83 (19) 36 (27) 30 (3) 8 (49) 3 (59)	25	86
Malathion	0·32 (29·8)	80 (2) 17 (12) 0 (19)	7	22

* These dosages are the same as the dosages investigated against *C. fatigans* (See Table II).

The grading of the efficiency of the insecticides for residual effectiveness against *C. fatigans* was carried out by the method described by Wal *et al.* (1962). The results on the grading are depicted in Table IV. It will be seen from the data that in the overall assessment the most effective insecticides are gamma BHC, dieldrin, DDT and chlordane. These are followed by heptachlor and diazinon ; malathion is the least satisfactory.

TABLE IV.

Grading of the residual effectiveness of insecticides against C. fatigans.

Insecticide.	Marks for minimum dosage for high initial kill. (Marks obtained on the basis of minimum dosages of insecticides effecting high initial kill of insects.)	MARKS FOR RESIDUAL ACTIVITY :		Total number of marks.	Average (Total number of marks scored divided by the number of techniques employed).
		Marks obtained on the basis of index of residual activity of insecticides using minimum dosages for effecting high initial kill of insects (Table I).	Marks obtained on the basis of index of residual activity of insecticides using certain specific dosages (Table II).		
Gamma BHC	7	7	7	21	7·0
Dieldrin	5	6	6	17	5·7
DDT	6	4	5	15	5·0
Chlordane	2	5	4	11	3·7
Heptachlor	3	3	3	9	3·0
Diazinon	4	1	2	7	2·3
Malathion	1	2	1	4	1·3

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ANNOUNCEMENT

NETHERLANDS UNIVERSITIES FOUNDATION FOR INTERNATIONAL CO-OPERATION.

INTERNATIONAL COURSES IN HEALTH DEVELOPMENT

"The desire of the developing countries to achieve a higher level of prosperity and improve their social structure is at present attracting world-wide attention. The promotion of health, which is closely associated with these goals, requires the institution of Healh Services on international, national, regional and local levels. An integrated development of health demands the co-operation of all medical facilities and every clinician. The essential members of the Health Services are doctors graduated from national medical faculties.

"The Public Health system presumes knowledge of a wide number of subjects, based on socio-economic and cultural insight. In many tropical countries, the development of health is geared to the present-day rapid socio-economic transition. But such subjects are not normally included in the western or western-type medical training.

"It is entirely in keeping with the policy of the World Health Organisation that much attention should be devoted to the promotion of Public Health. The 15th Assembly adopted a resolution authorizing the Director-General to implement an accelerated programme for assisting newly independent and emerging States, concentrating on national health planning and related training and on expanding and accelerating medical education and training of national staff.

"With the reality of this situation in mind, a number of institutions in Belgium and the Netherlands have joined forces to organise an international course on the development of health under conditions of rapid social change, combined with an introduction to health and diseases in the tropics.

"The course is not meant to be merely one of instruction or indoctrination. Although it is not feasible to omit a considerable number of formal lectures covering the large field to be explored, emphasis will be laid on group discussions and the exchange of experience. Participants from developing countries are, therefore, requested in advance to provide themselves with documentation regarding some of their national health problems.

"The programme reflects the conviction of the co-operating institutes, that even the clinician, in an environment of socio-economic change, should be thoroughly conversant with the concepts of integrated health, and, on the other hand, that the public health official needs detailed introduction into the notions, facts and techniques of clinical medicine. During the course many aspects, which are included in or omitted from the normal medical curriculum given in the

developed countries, will be scrutinized, completed and synthesized. The emphasis will be on conceptual and creative thinking supported by factual knowledge. The organizers believe that the course should be truly international in order to promote the exchange of views and experience.

"The course will cover a period of five months and will include a final examination. Successful candidates will obtain a Diploma in the Development of Tropical Health. Two courses will be given simultaneously with either French or English as the medium of instruction. In 1964 the courses will be held in the Netherlands, in 1965 in Belgium. The first courses will be held at the Royal Tropical Institute at Amsterdam between February and July, 1964, those in Belgium will be held at the Institute for Tropical Health 'Prins Leopold' at Antwerp from February to July, 1965".

The subjects to be dealt with during the course include problems of comprehensive development, principles of health development, public health and diseases in the tropics and their clinical and therapeutic aspects and practical exercises on Bacteriology, Mycology, Medical Zoology, Hematology and Nutrition.

There is also an optional possibility for further specialization in statistics, malaria eradication and clinical pathology.

The programme of the course, as well as further information, will be gladly supplied by : Netherlands Universities Foundation for International Co-operation (NUFFIC), Molenstraat 27, The Hague ; and by the Belgian Office of Co-operation and Development, Gulden Vlieslaan 55, Brussels.

ANNOUNCEMENT

MEDICAL COUNCIL OF INDIA

SILVER JUBILEE RESEARCH AWARD—1964

To commemorate its Silver Jubilee, the Medical Council of India has created a Silver Jubilee Research Award Fund. The first award will be made in November/December, 1964. This award would be open to all citizens of India and foreign nationals who have spent considerable time for research in India, male or female, and who have distinguished themselves by outstanding original research in field of medical and allied sciences. The value of the award would be Rs. 15,000 and a Gold Medal which may be of the value of up to Rs. 1,000. These awards for the present will be presented once in two years at a ceremonial function at which the successful candidates would be required to make an oration.

The award will be made on the basis of nominations of candidates to be submitted alongwith copies of monographs or reprints of nominees' special study and research. These would be scrutinized by an Expert Committee which will be constituted by the Committee of Management as and when necessary and then it would make recommendations in due course. The decision of the Committee of Management of the Silver Jubilee Research Award Fund of the Council shall be final.

In the case of a joint research work the award shall be divided between the workers in such proportion as may be decided. The role of the person nominated for the award should be clearly indicated so as to make it easy to determine whether the major part of the work has been done by that person.

Nominators are requested to forward nomination forms complete in all respects as indicated in the instructions.

Blank nomination forms can be had from the Secretary, Medical Council of India, Kotla Road, New Delhi, and completed Nomination Forms should reach him not later than 1st May 1964 through Registered Post A.D.

V.V. PURI
Secretary
Medical Council of India.

Indian Journal of Malariology, **17**, 2-3, *June-September 1963*.

A NOTE ON THE TOXICITY OF 'DICHLOROVOS' TO INSECTS OF PUBLIC HEALTH IMPORTANCE.

BY

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AND

R. L. KALRA.

(*Central Institute for Communicable Diseases, Delhi.*)

[May 27, 1963.]

QUARTERMAN (1955) suggested the possibility of the use of the organophosphorus insecticide dichlorovos* with a high vapour pressure, in the public health field. Mathis *et al.* (1959) explored its potential in vector control programmes as a residual fumigant.

Preliminary trials were carried out at the Central Institute for Communicable Diseases, Delhi, India, to evaluate the toxicity of dichlorovos as a contact insecticide as well as a persistent fumigant, against house-flies (*M. nebulo/vicina*), bed-bugs (*C. hemipterus*) and rat-fleas (*X. cheopis*). The results of these investigations are presented in this note.

TOXICITY OF DICHLOROVOS AS A CONTACT INSECTICIDE.

Toxicity of dichlorovos to house-flies and bed-bugs was determined using topical application method. Varying concentrations of technical grade† dichlorovos as alcoholic solutions were applied to the thorax of anaesthetised insects at the dosage of one microlitre per insect, using a microsyringe‡. The test insects were then held under observation for 24 hours after which mortality in them was recorded. The data obtained were analysed by Probit analysis method and summary of the results is given in Table I. Rat-fleas, however, could not be tested by this technique due to the inherent difficulties of handling these insects. The results of these investigations indicated that amongst house-flies tested, males were more susceptible than the females, whereas no difference in susceptibility between the sexes was observed with bed-bugs.

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* Chemical name :— O-O-dimethyl-2-2-dichlorovinyl-phosphate.

† Technical grade dichlorovos supplied by Messrs. Shell Chemicals containing 93·0 per cent active ingredient was used.

‡ 'AGLA' microsyringe with vernier scale micrometer marketed by Messrs. Burroughs Wellcome & Co., London, was employed.

TABLE I.

Summary of results depicting the toxicity of dichlorovos by topical application method against certain insect species.

Insect species.	Source of supply.	Equation of the fitted *pr-ld* regression line.	Chi square (*df*)*.	LD50 (μg./insect) with fiducial limits for 95 per cent probability.	Variance of *b*.	Factor by which dosage was multiplied.
Musca spp. (females)	Laboratory-reared.	$Y = 2{\cdot}3983 + 3{\cdot}79x$	1·08 (2)	0·048 (0·036—0·065)	0·10240	100
Musca spp. (males)	Laboratory-reared.	$Y = 1{\cdot}8877 + 2{\cdot}26x$	0·32 (1)	0·024 (0·018—0·034)	0·29271	1,000
Cimex hemipterus (females)	Field-collected (DDT and DLN resistant).	$Y = 2{\cdot}5584 + 1{\cdot}49x$	0·91 (1)	0·044 (0·022—0·083)	0·37206	1,000
Cimex hemipterus (males)	Field-collected (DDT and DLN resistant).	$Y = 1{\cdot}9939 + 1{\cdot}92x$	0·16 (1)	0·037 (0·027—0·05)	0·32892	1,000

* (*df*) = Figures in parenthesis indicate the degree of freedom.

TOXICITY OF DICHLOROVOS AS A FUMIGANT.

The toxicity of dichlorovos as a fumigant was determined against house-flies, cockroaches, bed-bugs and rat-fleas. House-flies and cockroaches were exposed in mosquito net and wire mesh cages, respectively, to dichlorovos vapours released from a dispenser* in a Peet-grady chamber (6 ft. cube). Bed-bugs were exposed to dichlorovos vapours in the trap used by Wattal and Kalra (1961) for rearing bed-bugs. Oriental rat-fleas, *X. cheopis*, reared in the laboratory were exposed to the fumigant effect of dichlorovos kept in a 500 ml. cylinder† (without any net covering at the top). Albino rats were also infested with the fleas and the caged rats were kept in a large size desiccator without the lid. The house-flies were given one hour exposure. The cockroaches, bed-bugs and rat-fleas (in cylinder) were given 2, 4 and 5 days exposure respectively. Fleas on the rats were, however, given only six hours' exposure. The percentage mortality, as observed at different time intervals in the test insects, is given in Table II.

* Dichlorovos dispensers formulated by Messrs. Shell Chemicals were received from Dr. K.D. Quarterman of the Communicable Diseases Centre, Savannah (Ga.), U.S.A.

† To eliminate any doubts regarding the penetration of dichlorovos vapours into a narrow mouthed 500 ml. cylinder, mosquitoes (*C. fatigans*) were caged in a specially prepared cage which could be put into the cylinder, thus limiting the mosquitoes to the same level as the fleas would reach on jumping and were exposed to the dichlorovos vapours in the Peet-grady chamber. Complete mortality among the mosquitoes thus confined was obtained with one hour exposure. The fleas, however, remained alive.

TABLE II.

Percentage kill in insects after varying intervals of time (in hours) when exposed to the fumigant effect of dichlorovos in Peet-grady chamber.

Species of insects.	Source of supply.	Condition of keeping.	PERCENTAGE KILL AFTER :						
			1 hour.	2 hours.	24 hours.	48 hours.	72 hours.	96 hours.	120 hours.
Musca spp. (females)	Laboratory-reared.	Kept in a cloth cage.	100·0	..	..	..	..	..	..
Periplaneta americana (both sexes)	Field-collected.	Kept in an iron mesh cage.	0·0	0·0	45·0	100·0	..	..	..
Cimex hemipterus (both sexes)	Field-collected.	Kept in grooved wooden slats.	0·0	0·0	20·0	72·0	84·0	100·0	..
*X. cheopis** (both sexes)	Laboratory-reared.	Kept in a cylinder (500 ml.) with filter paper strips.	0·0	0·0	0·0	0·0	20·0	40·0	45·0

* *X. cheopis* (both sexes) were also exposed infested on an albino rat for six hours after which they were kept under observation for 48 hours with no mortality observed.

From the results, it would be seen that dichlorovos was effective as a fumigant against caged house-flies, producing 100 per cent mortality in one hour exposure only, whereas it took 48 and 96 hours respectively to obtain the same kill amongst cockroaches and bed-bugs. It was of interest to note that rat-fleas (*X. cheopis*) were uneffected for the first 48 hours after which low mortality occurred during further exposure period and with 120 hours' exposure only 45·0 per cent fleas were found to be dead after which the exposure was discontinued.

SUMMARY AND CONCLUSIONS.

The toxicity of dichlorovos as a contact toxicant and fumigant to house-flies, bed-bugs, cockroaches and rat-fleas was compared. The results showed that although dichlorovos has high contact toxicity to bed-bugs, comparatively it is less toxic to these insects as a fumigant. Vashkov and Shnaider (1961) also observed high contact toxicity of dichlorovos against these insects.

It was interesting to note that dichlorovos vapours were practically ineffective against rat-fleas.

ACKNOWLEDGEMENT.

Our grateful thanks are due to Dr. K.D. Quarterman, Chief, Technical Development Laboratories, Communicable Diseases Centre, Savannah (Ga.), U.S.A for the supply of the dispensers.

Assistance rendered by Shri Gurdial Singh and Shri Sunder Singh during the investigations is duly accredited.

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A SIMPLE TECHNIQUE FOR RAPID SEPARATION OF MOSQUITO PUPAE BY SUDDEN CHILLING

BY

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[June 25, 1963.]

THE time-honoured 'pipette method' (Trembley 1955 ; Christophers, 1960) for separation of pupae from larvae is suitable only for small laboratory cultures. Large scale rearing of adult mosquitoes in the laboratory involves separation of thousands of pupae from the breeding pans. Hand-picking becomes impracticable and a suitable technique for rapid separation of pupae would be a necessity. The mechanical device developed by Fay and Morlan (1959), as modified by Macray (1961), and the 'magnetic method' suggested by Barzeer and Galun (1961) are some of the recent techniques available for rapid separation of pupae.

Both the recent techniques need complicated apparatus to work with. The 'mechanical' larval separator needs accurate adjustment for its successful operation. Further, its efficiency goes down if the pupae vary in their sizes due to differences in the rearing conditions. The 'magnetic' method needs careful adjustment of the amount of iron dust to be fed to the larvae and the period between feeding and separation. When the amount of dust exceeded the optimum, high larval and pupal mortality was observed. Even after proper adjustment of these factors, 4 to 15 per cent larvae were reported to remain unseparated from pupae (Barzeer and Galun, 1961 *loc. cit.*). The 'chilling' method, using iced water for separation of pupae from larvae,* developed by the Central Institute for Communicable Diseases, Delhi, was found to be an easy and practical method with high efficiency. Details of this technique are reported in this note.

In the 'chilling' method, larvae and pupae, from the culture pans, are sieved through a larval net. This is done by slowly pouring the entire contents of the rearing pan into a larval net held over an empty basin. The filtered culture water is preserved for the larvae after separation. The net containing the sieved larvae

* This technique was developed following an accidental discovery of the chilling effect of iced water on larvae, which made them to sink to the bottom of the culture pan leaving the pupae afloat, by one of the technicians of the Insecticide Laboratory.

and pupae is inverted into another basin containing iced water at a temperature of 3° to 4°C. Both larvae and pupae get immediately immobilised. The pupae keep floating on the surface and the larvae sink to the bottom of the basin. All the floating pupae are collected by slowly decanting the water into the larval net held over an empty basin. Thus only the pupae are collected in the net while the larvae are left behind. The residual water, containing the larvae only, is put back into the original larval culture pan. The pupae thus isolated are transferred into bowls containing tap water.

This process of pupal separation was found to work very efficiently with *C. fatigans A. stephensi and A. subpictus*, but with *A. aegypti* some (a very small number) pupae were found to remain with the larvae while a few larvae also came up with the pupae.

The larval developmental period and the larval and adult mortality rates were compared between larvae and pupae separated by 'chilling' and 'pipette' methods. Approximately equal number of eggs of *C. fatigans* were floated in 6 basins. Three such basins were earmarked for 'chilling' method and the other three for 'pipette' method for the separation of pupae. The data collected are summarised in Table I.

TABLE I.

Data regarding effect of 'chilling' and 'pipette' methods for separating pupae.

Method.	Number of larval basins.	Total number of larvae.	Mean number of larvae per basin.	Mean period between commencement to completion of pupation (in days).	Mortality rate in larvae.	Mortality rate in pupae.
'Pipette' method.	3	5,793	1,931	8·0	0·0	0·0
'Chilling' method.	3	6,698	2,233	8·7	0·0	0·0

The average period between commencement and completion of pupation was 8·7 days when larvae were subjected to chilling against 8·0 days when the larvae were never chilled. The difference is insignificant. Chilling of larvae also did not cause any larval and pupal mortality. Mortality rate, up to 20 days of age, in adults hatched from larvae which were chilled was the same as amongst those hatched from larvae not subjected to the chilling effect.

The total time spent by two technicians for separation of 5,000 pupae was 3½ hours when 'pipette' method was used as against only about quarter of an hour when 'chilling' method was used. This indicates that the technique of separating pupae from larvae using the 'chilling' method is convenient, quick, efficient, practicable and also inexpensive.

SUMMARY

A simple technique, used for separating large number of *C. fatigans* pupae from mass cultures, is described. Advantage is taken of the fact that exposure of the culture (mixture of larvae and pupae) to iced water makes the larvae sink to the bottom of the pan, leaving pupae afloat. Chilling the larvae has no adverse effect either on their hatching or on their longevity of the larvae or the adults. The technique is found to be quick, efficient and inexpensive. The technique was extended for the separation of pupae of *A. subpictus* and *A. stephensi*.

ACKNOWLEDGEMENT

The assistance rendered by Sarwashree Sita Ram, Gurdial Singh and Wazir Chand is accredited.

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SOME OBSERVATIONS ON THE EVALUATION OF DICHLOROVOS FOR THE CONTROL OF MOSQUITOES.

BY

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AND

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(Central Institute for Communicable Diseases, Delhi.)

[July 17, 1963.]

Use of residual fumigants such as dichlorovos† (DDVP) offer a new technique of vector control which is potentially capable of revolutionizing the insecticidal approach to the control of insect-borne diseases (Mathis *et al.*, 1959). The ease with which it could be used and its varied application has, therefore, stimulated a large volume of work on this material during recent years (Mathis and Maddock, 1961; Mathis *et al.*, 1961; Miles *et al.*, 1962; Quarterman *et al.*, 1962; Gratz *et al.*, 1962; Smith *et al.*, 1962). The results of investigations carried out at the Central Institute for Communicable Diseases, Delhi, with dichlorovos as a contact insecticide as well as a residual fumigant against mosquitoes, *C. fatigans*, *Ae. aegypti* and *A. subpictus*, are presented in this note.

TOXICITY OF DICHLOROVOS AS A CONTACT INSECTICIDE AND AS A PERSISTENT FUMIGANT.

The toxicity of dichlorovos as contact insecticide was determined by topical application to mosquitoes, *C. fatigans* and *Ae. aegypti*. Various concentrations of alcoholic solution of dichlorovos‡ were applied on the thorax of anaesthetised insects at the dosage of one microlitre per insect, using a microsyringe§. The insects were then held under observation for 24 hours after which mortality in them was recorded. The results thus obtained were analysed by Probit analysis method and the summary of the results is given in Table I.

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* Dr. P.G. Keshavalu is the State Malariologist, In-charge National Malaria Eradication Programme, Madras State. He suggested the trials carried out in wells as a possible solution of a practical problem referred to in the foot-note on page 127.

† Chemical name:— 0-0-dimethyl-2-2-dichlorovinyl phosphate.

‡ Dichlorovos technical grade supplied by Shell Chemicals Company, containing 93·0 per cent active ingredient, was used.

§ 'AGLA' microsyringe marketed by Messrs Burrough's Wellcome & Co., London, was employed.

TABLE I.

Summary of results depicting the toxicity of dichlorovos by topical application method against certain insect species.

Insect species.	Source of supply.	Equation of the fitted *pr-ld* regression line.	Chi square (*df*)*.	LD50(*µ*g./insect) with fiducial limits for 95 per cent probability.	Variance of *b*.	Factor by which dosage was multiplied.
C. fatigans (females)	Laboratory-reared.	$Y = 2{\cdot}0223+2{\cdot}02x$	0·70 (1)	0·003 (0·0024—0·0039)	0·06576	10,000
Ae. aegypti (females)	Laboratory-reared.	$Y = 1{\cdot}8107+2{\cdot}27x$	1·57 (2)	0·0025 (0·002—0·0032)	0·18631	10,000

* (*df*) = Figures in parenthesis indicate the degree of freedom.

The data obtained indicated that both the species of mosquitoes tested were equally susceptible to DDVP.

The toxicity of dichlorovos as a fumigant was also determined against mosquitoes, *C. fatigans*, *Ae. aegypti* and *A. subpictus*. Caged insects were exposed to dichlorovos dispenser* in a Peet-Grady chamber (1·83 metre cube with one dispenser). Two days old glucose-fed adults of *Ae. aegypti*, *C. fatigans*, *A. stephensi* and *A. subpictus* were exposed in Barraud cages to dichlorovos vapours. The percentage mortality, as observed after one hour exposure in all the test insects, was 100 per cent.

PRELIMINARY OBSERVATIONS ON THE EVALUATION OF DICHLOROVOS DISPENSER AS A RESIDUAL FUMIGANT TO MOSQUITOES.

Dichlorovos (DDVP) dispensers received from the Communicable Diseases Centre, Savannah (U.S.A.), were evaluated in a Peet-Grady chamber, a disused well and in a room, for their effectiveness as a residual fumigant against mosquitoes. In all cases, 2-3 days old glucose-fed female mosquitoes, *Ae. aegypti* and *C. fatigans*, were used. These mosquitoes in Barraud cages were exposed to the insecticide vapour. The results obtained are contained in Table II(*a*). The temperature during the experiment was found to vary from 24°C to 27°C in Peet-Grady chamber, 25°-30°C in the well and 28°-33°C in the room.

A single dipenser, when suspended in a Peet-Grady chamber (1·83 metre cube), was found initially to cause complete kill in the caged mosquitoes, *C. fatigans*, within a exposure period of 30 minutes. Some loss in the effectiveness was, however, observed after a lapse of 2 weeks as it took slightly longer time (45 minutes) to obtain the same kill in the mosquitoes exposed. The mortalities obtained in the test insects with the dispenser in the Peet-Grady chamber was found

* Dichlorovos dispensers formulated with Montan wax by Shell Chemicals Company were received from Dr. K.D. Quarterman of the Communicable Diseases Centre, Savannah (Ga.), U.S.A. The air concentrations of the vapour could not be estimated due to lack of facilities.

to be 100 per cent with almost the same exposure period (45 minutes) for about 3 weeks since the experiment was started. After the lapse of 12 weeks the same dispenser, however, took 3½ hours to cause 100 per cent kill in the test insects.

TABLE II (*a*).

Persistence of 'dichlorovos' dispenser against caged C. fatigans (females) under varied conditions.

Place.	Ratio of dispenser to space.	Weeks after the installation of dispenser.	LT_{100} (in hours).
Peet-grady chamber	1 dispenser per 6·12 cubic metres.	0	0·5
		1	0·6
		2	0·75
		4	0·83
		6	0·75
		8	0·5
		10	1·5
		12	3·5
Well (diam. 3 m., depth up to water level 3 m.)	1 dispenser (200·09 cubic metres)	0	2·0
		1	2·0
		2	3·0
		3	5·0
		4	4·0
		5	5·0
		6	5·0
		8	5·0
		12	24·0
Room (4·25 m. × 4·25 m. × 3·66 m.)	1 dispenser per 33 cubic metres (approx.)	0	2·0
		2	4·0
		4	4·0
		8	4·0

A single dispenser, when suspended at the level of one metre above the water surface in a disused well (diam. 3 m., depth of the well up to the level of water= 3 m.), was found to cause 100 per cent mortality in both *C. fatigans* and *Ae. aegypti*, within an exposure period of 2 hours. The mosquitoes for these tests were kept in the cages at the same level as that of the dispenser. The effect was significantly less when the mosquitoes were kept at the level of 3·5 m. above the water level. However, at the end of 24 hours complete kill was observed in these mosquitoes also. The dispenser was found to produce similar mortalities even after the lapse of 2 weeks in the well where partial ventilation takes place. After the lapse of 8 weeks, the dispenser was found to be still effective and was found to give 100 per cent kill within an exposure period of approximately 5 hours. After a lapse of 12 weeks, even 24 hours' exposure caused only 58 per cent kill in the mosquitoes.

The dispensers (at the rate of about one dispenser per 33 cu. metres), when suspended in a closed room*, were found to cause 100 per cent kill in the

* Size of room where all windows, ventilators and doors were closed was 4·25 m. × 4·25 m. × 3·66 m. (66·11 cu. metres). The door was opened twice during the first hour and subsequently every hour for a few minutes. Two dispensers were used in this room.

mosquitoes, within an exposure period of 2 hours. In a partially ventilated room*, however, only 50 per cent kill was obtained with an exposure period of 6 hours and it took about 12 hours for 100 per cent kill. Further, the kill produced in the caged mosquitoes near the ceiling was more than in the mosquitoes kept at the ground level (Table II*b*). The dispensers were found to be still effective in a closed room after the lapse of 8 weeks and were found to give 100 per cent kill within an exposure period of 4 hours.

TABLE II (*b*).

Relative effectiveness of 'dichlorovos' dispenser to caged C. fatigans *(females) kept at different levels in the room (dispensers suspended in the middle of the room).*

Level of mosquitoes.	PERCENTAGE KILL AFTER HOURS :					
	1	2	3	4	5	6
(*a*) Mosquitoes kept near the ceiling.	0·0	0·0	96·0	100·0	..	..
(*b*) Mosquitoes kept in the middle almost at the level of the dispenser.	0·0	0·0	90·0	100·0	..	..
(*c*) Mosquitoes kept on the floor.	0·0	0·0	12·0	24·0	58·0	74·0

SOLUBILITY OF DICHLOROVOS IN WATER KEPT IN THE ROOM

During the evaluation of dichlorovos against adult insects, it was observed that the mosquito larvae (*C. fatigans*) died when these were kept in water in bowls in the room which was fitted with the dispenser (Table III). The quantity of dichlorovos dissolved in water under varied conditions was determined by bioassay against *C. fatigans* larvae. For this, a standard curve of *lc-pr* regression line was first obtained, using the method recommended by the World Health Organization

TABLE III.

Kill in C. fatigans *larvae when exposed in the room fitted with 'dichlorovos' dispensers.*

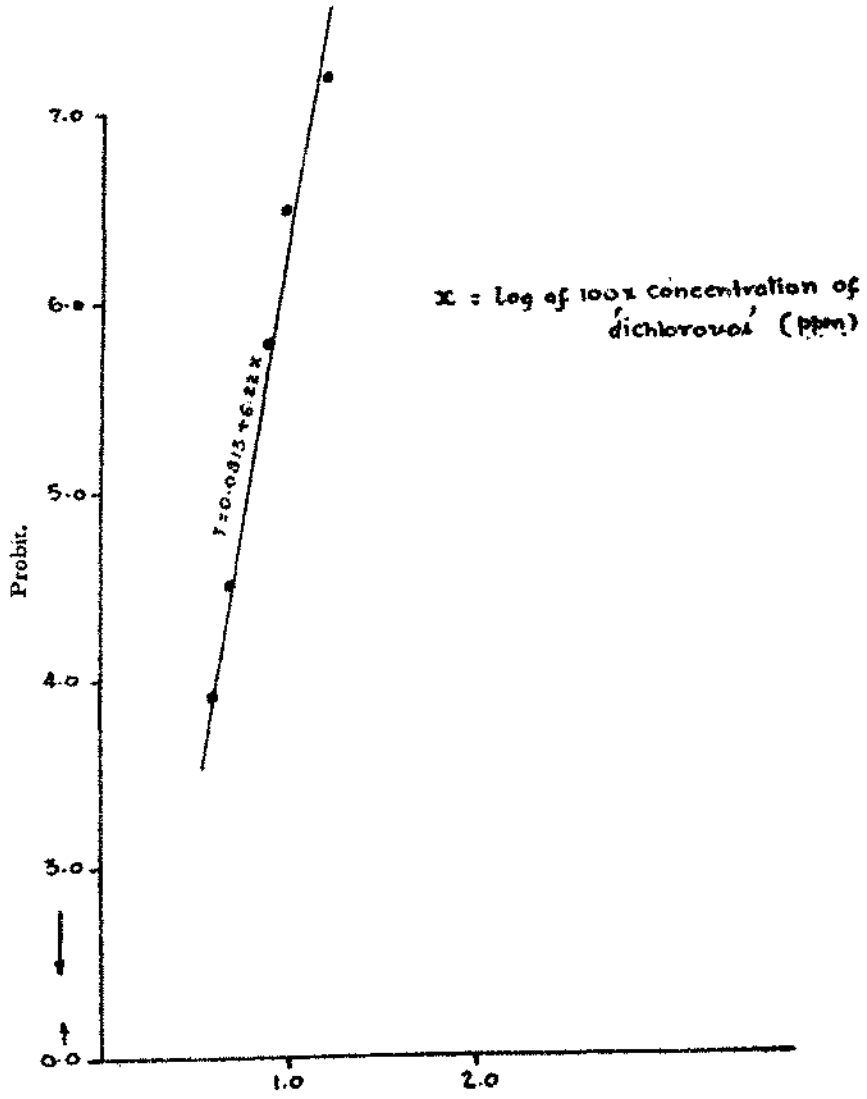
Ratio of dispenser to space.	Amount of water exposed.	PERCENTAGE MORTALITY IN FOURTH-INSTAR *C. fatigans* LARVAE AFTER :	
		24 hours.	48 hours.
1 dispenser per 6·12 cubic metres.	250 c.c. in enamel bowl (20·3 cm. diam.). Surface area of water 12·6 sq. cm.	100·0	..
1 dispenser per 6·12 cubic metres.	4,000 c.c. in enamel bowl (40·64 cm. diam.). Surface area of water 113·1 sq. cm.	100·0	..
1 dispenser per 33·0 cubic metres.	250 c.c. in bowl (20·3 cm. diam.).	100·0	..
1 dispenser per 33·0 cubic metres.	4,000 c.c. in enamel bowl (40·64 cm. diam.).	19·0	100·0

* The above room was ventilated by opening two windows (size 1·1 m. × 0·5 m.) completely, and the door (2·0 m. × 0·93 m.) was kept open about a quarter.

for assaying the susceptibility of mosquito larvae (World Health Organization, 1960). Late third-instar *C. fatigans* larvae were exposed at 23-25°C. to various concentrations of technical dichlorovos in water prepared by mixing one c.c. of solution of dichlorovos (freshly prepared in water) and 250 ml. of water. The mortality in *C. fatigans* larvae was recorded after an exposure period of 24 hours. The results thus obtained were analysed and the standard curve was drawn which is depicted in Graph 1.

GRAPH 1.

Showing the fitted pr-lc. regression line of technical dichlorovos against C. fatigans larvae.



Varying amount of water was kept for different periods of time in the Peet-Grady chamber and in the room where DDVP dispensers were suspended. Aliquot quantities of water, taken from the exposed water, were mixed with tap water to which mosquito larvae were exposed for 24 hours and then the mortality in them was recorded. Using the standard curve and the mortality, concentration of dichlorovos in the exposed water was determined. The dissolution of dichlorovos was determined by multiplying the concentration thus obtained by the dilution factor. The dissolution of dichlorovos in water under varied conditions, as estimated, is shown in Table IV.

TABLE IV.

Amount of 'dichlorovos' dissolved in water (p.p.m.) exposed to its vapours in the chamber.

Quantity of water.	Period of exposure (hours).	Ratio of 'dichlorovos' dispenser to space.	Concentration of 'dichlorovos' attained (in p.p.m.).
250 c.c. in small bowl.	24	One dispenser at the rate of 6·12 cubic metres in Peet-grady chamber.	0·5
4,000 c.c. in big bowl.	24	One dispenser at the rate of 6·12 cubic metres in Peet-grady chamber.	0·17
4,000 c.c. in big bowl.	120	One dispenser at the rate of 6·12 cubic metres in Peet-grady chamber.	0·4
250 c.c. in small bowl.	24	One dispenser at the rate of 33·0 cubic metres in the room.	0·25
4,000 c.c. in big bowl.	24	One dispenser at the rate of 33·0 cubic metres in the room.	0·059
4,000 c.c. in big bowl.	120	One dispenser at the rate of 33·0 cubic metres in the room.	0·17

The results indicated that the concentration of dichlorovos attained in water kept in rooms or premises where dichlorovos dispensers were used depended upon the quantity of water exposed, concentration of dichlorovos in air and the exposure period. However, the water in the enamel bowl (40·64 cm. diam., 4,000 c.c.) exposed for 24 hours in Peet-Grady chamber was found to cause 100 per cent kill for a period of six days subsequent to its removal from the chamber, in the larvae of *C. fatigans* when these were exposed to it daily for 24 hours.

CONCLUSIONS.

The investigations showed that dichlorovos has high contact toxicity both to *C. fatigans* and *Ae. aegypti*. Studies carried out on the residual fumigant action of dichlorovos dispenser (with Montan wax) received from the Communicable Diseases Centre, Savannah (Ga.), U.S.A., have shown its utility against mosquitoes*. The dispenser, at the rate of one dispenser per 33 cubic metres, was found to exert residual effect for more than 8 weeks. In the partially ventilated room the effect was, however, found to be much less.

* These findings are in conformity with the results obtained by other workers elsewhere.

The results obtained on the preliminary evaluation of dichlorovos dispenser in the well* have indicated the possibility of its use for the control of mosquitoes which breed in the wells as a single dispenser was found to give satisfactory kill in the mosquitoes for a period of about 8 weeks. This effect was, however, observed in the caged mosquitoes and it is to be evaluated under field conditions before definite conclusions could be drawn.

Present studies have yielded interesting information regarding absorption of dichlorovos vapours by the water kept in the testing chamber and its toxic effect on mosquito larvae. While this would be an additional advantage in the control of *Ae. aegypti* and *A. stephensi*, yet the possibility of ingestion of dichlorovos with the water by mammals, including human beings, exists. The possible hazards as a result of this cannot be completely ruled out even though the dosage was found to be very small. Therefore, this factor has to be given due consideration while assessing the utility of this insecticide for mosquito control.

SUMMARY.

The toxicity of dichlorovos as a contact insecticide and also as a persistent fumigant to mosquitoes, was determined. Dichlorovos vapours were found to be effective against mosquitoes where the ventilation is limited.

Dichlorovos dispensers received from the Communicable Diseases Centre, Savannah, U.S.A., were found to have persistent fumigant effect against mosquitoes. A single dispenser in a well was found to give satisfactory kill in the mosquitoes for a period of about 10 weeks. The effect in a closed room was similar.

The dichlorovos vapours were also found to produce toxic effects on mosquito larvae which were kept in the room fitted with the dispensers. The amount of the vapour of dichlorovos absorbed by the water kept in the chamber under different conditions was determined using a bio-assay method.

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* *A. stephensi*, an important vector of malaria in urban areas of India, was reported resistant to DDT in Madras State by Rajagopalan *et al.* (1956). Subsequently it has been reported resistant to DDT from Salem, Madras State, by Roy (1963) and from Andhra Pradesh by Sitaraman (1962). This species is mainly a well-breeder and during the National Malaria Control Eradication Programme the treatment of well-walls with DDT was undertaken. Obviously part of the spray also went into the well-water, thereby intensifying selection at both the larval and adult stages.

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EFFECTS OF DICHLORVOS (DDVP) ON THE BEHAVIOUR OF *A. GAMBIAE*.

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INTRODUCTION

DURING studies in experimental huts to assess the vulnerability of *A. gambiae* to the fumigant insecticide dichlorvos, it was found that lower mortalities occurred in huts with mud-lined roofs than in huts with grass-roofs. Furthermore, in huts with mud-lined roofs the greater proportions of the dead mosquitoes were found in the window-traps, whereas in huts with grass-roofs most of the dead mosquitoes were found inside the huts. The results of bio-assays indicated that the lower mortalities in huts with mud-lined roofs may have been due, in part, to some loss in toxicity of the insecticide by direct action of the mud surface. A higher proportion of dead mosquitoes in the window-traps of huts with mud-roofs was found to occur, however, even in instances where there were similar mortalities in the two types of hut. A possible explanation of this difference was that mosquito behaviour was affected by dichlorvos (Smith, Park and Hocking, 1963). A series of three studies was therefore made from 1961 to 1963 to determine the effect of dichlorvos on the behaviour of *A. gambiae*, and the results of the studies are presented in this paper.

METHODS.

The techniques employed in the three studies described below became progressively more elaborate as they were developed to study "behaviour" without interfering with routine assessments of toxicity.

Study I.—A detailed analysis was made of the results of routine assessments previously obtained (Smith, Park and Hocking, 1963) from four huts with grass roofs and four with mud-lined roofs. Each hut contained a single dichlorvos dispenser, except for the control huts, and results from each hut were obtained over a period of two months.

Study II.—Hand-collections of mosquitoes resting in experimental huts at 10.00 a.m. were added to the techniques of routine assessment (Hocking *et al.*, 1960) since in routine assessments there were no records of the number of mosquitoes alive in huts. With this additional information it was possible to obtain a strictly comparable distribution of mosquitoes between window-traps and indoors, for treated and untreated huts. The studies were made for two months in four huts, two of which contained single dispensers of the Ciba (C 3) type.

Study III.—Studies were made to determine the relative contributions of technical dichlorvos and plasticiser materials to the mortalities and changes in behaviour previously detected, since the vapour from the dispensers comprised not only dichlorvos but also a solvent dibutyl phthalate or dioctyl phthalate. The problem of how to assess "behaviour" simultaneously with "toxicity" was largely overcome by (1) Substituting a "Resting Count" for the earlier "Hand-catch"; (2) Combining counts of fed and gravid mosquitoes into one group—the "feeding section"; (3) Use of an Index of Repellency which indicates how the proportion of the total mosquitoes that are found in the window trap in a treated hut compares with that proportion in an untreated hut and so gives a more practical indication of the importance of changed behaviour in terms of mosquito control. Details of the Repellency Index are published elsewhere (Smith, 1963).

The studies were made in thirteen experimental huts with grass roofs, and were in duplicate with the exception of one control hut. Single dispensers, provided by Ciba Limited, were suspended in the huts as previously described (Smith, Park and Hocking, 1963). Types of dispensers used were Ciba—IX containing technical dichlorvos only, Ciba—X containing plasticiser only and Ciba—VIII containing dichlorvos and plasticiser. Studies were also made in two huts which contained a sheet of filter-paper treated daily with dibutyl phthalate, with the amount added based on daily weight loss from plasticiser dispensers. With the exception of three huts maintained for comparative purposes, the experimental huts were fitted with modified window-traps (Smith and Webley, 1963) to reduce the fumigant effect that occurred in the original type of window trap (Smith, Park and Hocking, 1963). It is noted that a somewhat similar type of modified window trap was successfully used to aid egress of *Anopheles culicifacies* into window-traps (Pal and Sharma, 1952).

RESULTS.

STUDY I.

(*a*) *Indoor and window-trap mortalities.*—The first indication that the insecticide affected the behaviour of *A. gambiae* was that in routine assessments of mortality, although there were wide differences between individual huts, there were on average, high floor counts in huts with grass roofs, whereas in huts with mud-roofs most mosquitoes did not die until after entering the window-trap even at similar overall mortalities. The example below gives the combined results of four treated huts with grass and four with mud-roofs.

The results (Table I) showed that the percentage of dead mosquitoes found on the floor of the grass-roofed huts was 62 per cent compared with 26 per cent for mud-roofed huts, whereas in the window-trap, mortalities were 42 per cent in the grass-roofed huts and 51 per cent in the mud-roofed huts.

(*b*) *Mortalities of unfed and fed mosquitoes.*—Less than half as many mosquitoes were caught in the grass than the mud-roofed huts (Table I) and 48 per cent of the

catch was unfed compared with 75 per cent in the mud-roofed huts. Of these unfed mosquitoes, the percentages that were dead were 56 per cent in the grass and 60 per cent in the mud-roofed huts. In the fed and gravid mosquitoes the percentages that were dead were 73 per cent in the grass-roofed huts as against 56 per cent in the mud-roofed huts. Also the percentages of the dead mosquitoes that were unfed were 42 per cent in the grass and 76 per cent in the mud-roofed huts (Table II).

TABLE I.

Distribution of mosquitoes indoors and in window-traps of huts treated with dichlorvos in plasticiser solution.

	Grass:				Mud:			
	Dichlorvos.		Control.		Dichlorvos.		Control.	
	Total caught.	Per cent distribution.	Total caught.	Per cent distribution.	Total caught.	Per cent distribution.	Total caught.	Per cent distribution.
Window-traps—Alive	1,339	35	2,087	78	3,085	41	2,882	74
Window-traps—Dead	965	25	604	22	4,227	44	1,025	26
Floor	1,551	40	0	0	1,449	15	0	0
Total	3,857	100	2,691	100	9,661	100	3,907	100
Per cent mortality		65		22		59		26

TABLE II.

Per cent mortalities of unfed and fed mosquitoes in experimental huts.

Abdominal condition.	Per cent.	Grass:				Mud:			
		Dichlorvos.		Control.		Dichlorvos.		Control.	
Unfed	Living	48	21	48	34	75	30	58	38
	Dead		27		14		45		20
Fed	Living	41	4	9	9	18	5	9	6
	Dead		37		0		13		3
Gravid	Living	11	10	43	35	7	6	33	30
	Dead		1		8		1		3

With regard to the distribution of the different abdominal stages, 6 per cent and 5 per cent were unfed and dead on the floor in the grass-roofed hut and mud-roofed hut respectively, compared with 34 per cent and 10 per cent for those fed and dead (Table III). Furthermore, of all unfed mosquitoes that died, 76 per cent reached the window-trap in the grass-roofed hut and 88 per cent in the mud-roofed hut. Of those fed or gravid that died, 12 per cent reached the window trap in the grass-roofed hut and 31 per cent in the mud-roofed hut.

TABLE III.

Per cent distribution of unfed and fed mosquitoes in experimental huts.

	GRASS :						MUD :					
	Dichlorvos.			Control.			Dichlorvos.			Control.		
	MT.	ET.	F.	MT.	ET.	F.	MT.	ET.	F.	MT.	ET.	F.
Unfed ...	38	4	6	33	15	0	67	3	5	48	10	0
Fed ...	6	1	34	7	2	0	7	1	10	4	5	0
Gravid ...	8	3	0	9	34	0	6	1	0	12	21	0

MT = Morning window trap. ET = Evening window trap. F = Floor.

STUDY II

(*a*) *Indoor and window-trap mortalities.*—The results (Table IV) show that the number of dead mosquitoes found on the floor on the grass-roofed hut was twice that of the mud-roofed hut, and when the mortality was considered in percentage of the total catch, this difference was more than three times. The number of dead mosquitoes in the window trap were on the other hand half as great again in the mud-roofed hut as in the grass-roofed one.

TABLE IV.

Mortalities in the hut and in the window-trap of experimental huts.

	GRASS :				MUD :			
	Dichlorvos.		Control.		Dichlorvos.		Control.	
	Total caught.	Per cent distribution.	Total caught.	Per cent distribution.	Total caught.	Per cent distribution.	Total caught.	Per cent distribution.
Window-trap—Alive	531	49	1,131	37	889	57	131	38
Window-trap—Dead	198	18	449	16	329	22	32	10
Floor—Dead	286	27	0	0	132	8	0	0
Hand catch—Alive	66	6	1,462	47	207	13	174	52
Total	1,081	100	3,092	100	1,557	100	337	100
Per cent mortality		45		16		30		9

(*b*) *Mortalities of unfed and fed mosquitoes.*—In both types of hut approximately half of the catch was unfed (Table V). In these unfed mosquitoes the percentage mortalities were virtually indentical (35 and 34 respectively). But in the fed and gravid mosquitoes the mortalities were 56 per cent in the grass-roofed hut as against 25 per cent in the mud-roofed hut. Of the unfed mosquitoes that died, 75 per cent reached the window-trap in the grass-roofed hut and 93 per cent in the mud-roofed hut. Of those fed and gravid that died, only 12 per cent succeeded in reaching the window-trap in the grass-roofed hut and 39 per cent in the mud-roofed hut.

TABLE V.

Per cent mortalities and distribution of mosquitoes, of different abdominal stages, in experimental huts.

Abdominal condition.	Per cent.	Grass: Dichlorvos. MT	ET	HUT	Grass: Control. MT	ET	HUT	Mud: Dichlorvos. MT	ET	HUT	Mud: Control. MT	ET	HUT
Unfed	Living	33	1	0	14	5	1	32	0	1	13	1	1
	Dead	10	3	5	3	5	0	16	1	1	5	2	0
Fed	Living	6	1	5	1	3	38	9	0	12	2	2	48
	Dead	3	0	22	1	1	0	5	0	7	1	0	0
Gravid	Living	5	3	1	3	11	8	14	2	0	10	10	3
	Dead	0	2	0	0	6	0	0	0	0	0	2	0

MT = Morning window trap. ; ET = Evening window trap.

TABLE VI.

Weekly number of surviving A. gambiae, *in huts as shown by hand-catches, and leaving the huts as shown by the window-traps.*

		Week: 1	2	3	4	5	6	7	8	Total.
Huts with dichlorvos (C 3) dispensers.										
Grass-roof	Hand catch.	64	2	0	0	0	0	0	0	66
	Window-trap catches.	93	104	122	74	10	17	25	86	531
	Total survivors.	157	106	122	74	10	17	25	86	597
	Percentage in window-traps.	59	98	100	100	100	100	100	100	89
Mud-roof	Hand catch.	88	23	41	4	7	14	17	13	207
	Window-trap catches.	95	195	284	70	23	73	95	54	889
	Total survivors.	183	218	325	74	30	87	112	67	1,096
	Percentage in window-traps.	52	89	87	95	77	84	85	81	81
Control huts.										
Grass-roof	Hand catch.	69	110	247	92	102	233	292	317	1,462
	Window-trap catch.	69	104	291	110	73	101	206	177	1,131
	Total survivors.	138	214	538	202	175	334	498	494	2,593
	Percentage in window-traps.	50	49	54	54	42	30	41	36	44
Mud-roof	Hand catch.	10	6	51	26	4	12	16	49	174
	Window-trap catches.	3	7	18	18	1	16	23	46	131
	Total survivors.	13	13	69	44	5	28	39	95	305
	Percentage in window-traps.	23	54	26	41	20	57	59	48	43

(c) *Location of the surviving mosquitoes.*—Of the surviving unfed mosquitoes, almost all were found in the window-trap (100 per cent in grass-roofed and 98 per cent in mud-roofed). Of the surviving fed mosquitoes 60 per cent were found in the trap of the grass-roofed hut and 43 per cent in the trap of the mud-roofed hut. Survival inside the grass-roofed hut was largely restricted to the first week (before the dispenser was fully active) (Table VI) and for the 3rd to 8th weeks, 100 per cent

of the fed and unfed survivors were found in the window-trap, whereas in the mud-roofed hut from the 3rd to 8th weeks only 86 per cent of all the surviving mosquitoes were found in the window-trap.

(*d*) *Repellency Index.*—The Index was 1·3 for the grass-roofed hut where the average mortality was 45 per cent., and 1·6 for the mud-roofed hut where the mortality was 30 per cent (Table IV).

STUDY III.

(*a*) *Indoor and window-trap mortalities.*—Dispensers, containing both dichlorvos and plasticiser, maintained mortalities of 93 to 97 per cent for three months; compared with one month for dispensers containing dichlorvos only. Dispensers containing only plasticiser were slightly but nevertheless distinctly toxic, confirmed by the small numbers of dead mosquitoes on the floors of the huts treated with plasticiser dispensers (Table VIII) and daily with dibutyl phthalate. Mortalities by dispensers with both dichlorvos and plasticiser were consistently higher in huts with window-traps with polythene funnels than in huts fitted with unmodified window-traps (Table VII). The mortalities were respectively greater by ; first month 14 per cent, second month 16 per cent, third month 8 per cent and fourth month 6 per cent.

TABLE VII.

Mortalities in A. gambiae *entering experimental huts with different types of dispensers (started December 5, 1962).*

Type of dispenser.	Experimental hut numbers.	PER CENT 24 HOUR MORTALITY WITH THE NUMBER OF *A. gambiae* IN PARENTHESIS : Age of dispensers in months.			
		0—1	1—2	2—3	3—4
Huts fitted with window-traps with plastic funnels.					
Technical dichlorvos (Ciba IX) ...	7 and 11	75 (24)	33 (158)	23 (344)	
Plasticiser only (Ciba X) ...	2 and 13	38 (16)	21 (370)	31 (296)	
Dichlorvos and plasticiser (Ciba VIII)	3 and 4	87 (8)	93 (156)	74 (105)	23 (82)
Dibutyl phthalate	12 and 14	19 (21)	18 (444)	23 (229)	
Control huts	15 and 18	4 (23)	13 (512)	21 (300)	13 (550)
Huts fitted with window-traps with funnels of cotton netting.					
Dichlorvos and plasticiser (Ciba VIII)	9 and 10	73 (33)	77 (280)	66 (128)	17 (127)
Control huts	1	33 (21)	10 (195)	15 (242)	8 (392)

Sixty-four per cent of the total catch was of dead mosquitoes on the floor of the huts with the Ciba VIII dispensers compared with 8 per cent for technical dichlorvos. The percentage of the total catch, dead in the window-trap of the hut with technical dichlorvos (Ciba IX) was on the other hand three times the percentage in the hut with the ordinary dispensers. The distribution of mosquitoes in huts with dispensers containing plasticiser only were similar to those in untreated huts.

TABLE VIII.

Mortalities in the hut and in the window trap of experimental huts.

	Technical dichlorvos (Ciba IX):		Dichlorvos and plasticiser (Ciba VIII):		Plasticiser (Ciba X):		Control.	
	Total caught.	Per cent distribution.	Total caught	Per cent distribution.	Total caught.	Per cent distribution.	Total caught.	Per cent distribution.
Window-trap—Alive	376	43	39	11	508	34	750	31
Window-trap—Dead	77	8	9	3	159	10	145	6
Floor—Dead	73	9	221	64	15	1	0	0
Resting count—Alive	339	39	75	22	826	55	1558	63
Total	865	100	344	100	1508	100	2453	100
Per cent mortality		29		86		26		16

(*b*) *Mortalities of unfed and fed mosquitoes.*—A third of the catch of mosquitoes was unfed in the hut with the technical dichlorvos compared with 18 per cent for the hut with dichlorvos and plasticiser, 14 per cent for plasticiser only, and 15 per cent for the control hut (Table IX). In these unfed mosquitoes. the percentage mortalities were 25 per cent in the huts with technical dichlorvos and 4(per cent, 20 per cent and 13 per cent in the other huts. But in the fed and gravid mosquitoes the mortalities were 16 per cent in the hut with technical dichlorvos and 71 per cent, 10 per cent and 5 per cent in the other huts. Of the unfed mosquitoes that died, 91 per cent reached the window-trap of the hut with technical dichlorvos and 30 per cent, 93 per cent and 100 per cent for the other huts. Of those fed and gravid that died, 22 per cent reached the window-trap of the hut with technical dichlorvos, less than one per cent in the hut with dichlorvos and plasticiser, and 91 per cent and 100 per cent in the other huts.

TABLE IX.

Per cent mortalities and distribution of mosquitoes, of different abdominal stages, in experimental huts.

		Technical dichlorvos (Ciba IX):		Dichlorvos and plasticiser (Ciba VIII):		Plasticiser (Ciba X):		Control:	
		Window-trap.	Hut.	Window-trap.	Hut.	Window-trap.	Hut.	Window-trap.	Hut.
Unfed	Living ...	22	4	6	4	9	3	11	2
	Dead ...	8	1	2	6	2	0	2	0
Fed	Living ...	4	26	1	14	4	31	3	35
	Dead ...	0	8	0	59	1	1	0	0
Gravid	Living ...	19	8	5	3	21	21	17	26
	Dead ...	3	0	0	0	7	0	4	0

TABLE X.

Repellency indices for A. gambiae entering experimental huts with different types of dispensers. (Started December 5, 1962).

	AGE OF DISPENSER IN MONTHS :			
	0 — 1	1 — 2	2 — 3	3 — 4
Huts fitted with window-traps with plastic funnels.				
Technical dichlorvos (IX)	0·5	1·6	1·3	
Plasticiser only (X)	1·2	1·2	1·2	
Dichlorvos and Plasticiser (VIII) ...	0·2	0·2	0·6	0·5
Dibutyl phthalate	0·8	1·1	1·9	
Control huts	1·0	1·0	1·0	1·0
Huts fitted with window-traps with funnels of cotton-netting.				
Dichlorvos and Plasticiser (VIII) ...	0·7	0·7	1·0	0·7
Control hut	1·0	1·0	1·0	1·0

(c) *Location of the surviving mosquitoes.*—Of the surviving unfed mosquitoes 86 per cent reached the window-trap of the hut with technical dichlorvos and 69 per cent, 76 per cent and 81 per cent in the other huts. Of those fed and gravid that survived, 38 per cent reached the window-trap of the hut with technical dichlorvos and 24 per cent, 33 per cent and 24 per cent in the other huts.

(d) *Repellency Index.*—Indices less than unity for dispensers containing both dichlorvos and plasticiser showed that the rate of kill was quicker than the rate of leaving the grass-roofed huts (Table X). With dispensers containing technical dichlorvos only, indices of 1·6 and 1·3 in the second and third months indicated that the rate of leaving was greater than normal at mortalities of 33 per cent and 23 per cent (Table VII).

DISCUSSIONS.

Study I.—Mortalities of 73 per cent in the feeding section, i.e. fed plus gravid, in grass-roofed compared with 56 per cent in mud-roofed huts, showed that the former huts were more toxic to the epidemiologically important section. The percentage mortalities among the unfed mosquitoes were, if anything, however the other way round being 56 per cent in the grass and 60 per cent in the mud-roofed hut. Thus the very large proportion of unfed mosquitoes in the mud-roofed huts reduced the difference between the mortalities, as a whole, between the two types of hut. The high proportion of dead mosquitoes in the window-traps of the mud-roofed huts was due to the overwhelmingly large numbers of dead unfed mosquitoes in the morning window-trap. It is thought that the mud-roofed huts were in some way more attractive than the grass-roofed huts to the recently emerged mosquitoes, which then nearly all entered the window-traps.

Study II.—The results confirmed those of the previous study by showing that the mortalities of the feeding section were higher in the grass-roofed huts, *viz*: 56 per cenet compared with 25 per cent in the mud-roofed huts. The percentage mortalities among the unfed mosquitoes were, however, similar in both types of hut (35 per cent and 34 per cent) and since the unfed mosquitoes were also present in similar proportions in both types of hut i.e. about half the hut-catch, they had less effect in reducing the difference in mortalities, as a whole, between the two types of hut (Table IV and and Table I), or increasing the proportion of the total catch in the window-trap of the mud-roofed hut.

The location of the greater proportion of the surviving mosquitoes in the window-trap of the grass-roofed hut, compared with the mud-roofed hut, at moderate mortalities, indicated that the insecticide was also possessed with irritant or repellent properties. Assessment of behaviour by studies on survivors alone was, however, made complex and difficult (*1*) because the unfed mosquitoes were less vulnerable than fed mosquitoes (*2*) because the unfed mosquitoes nearly all left the same night as they entered the hut whereas most fed mosquitoes remained indoors overnight (Smith, 1963). Thus, even in the absence of irritation or repellency an insecticide could by selectively killing the feeding section of the population resting indoors, thereby increase the relative proportions of surviving mosquitoes entering the window-trap in any night, and thereby give a spurious indication of repellency. A further complication with a quick-acting insecticide was that in the interval of three hours between window-trapping and making a resting count (or a hand-catch) some fed mosquitoes would die and so a count of survivors in the hut and in the window-trap would indicate an increased egress compared with a control hut.

These complexities were avoided by using the 'Repellency Index' which showed that there was, however, greater egress than normal from treated huts giving mortalities of 45 per cent and 30 per cent. The irritant or repellent effect of the insecticide was, however, offset by its concurrently rapid toxic action.

Study III.—Dispensers containing dichlorvos and plasticiser together, i.e. Ciba—VIII type gave high kills for a longer period than dispensers containing dichlorvos alone, i.e. Ciba—IX type. The higher kills in huts with window-traps with plastic funnels may have been due to higher concentrations of dichlorvos in the atmosphere of the huts due to restricted ventilation of the plastic funnels. The Repellency Indices in Table X show that with the Ciba—VIII dispensers any repellency was offset by rapid toxicity, but with the Ciba—IX dispensers repellency outweighed the rate of kill in the second month, in spite of a third of the mosquitoes dying that entered the huts (and three-quarters of that third died indoors). The average rate at which mosquitoes left was nevertheless 1·6 times that from the untreated huts. The plasticiser alone in Ciba—X dispensers had no overt effect on mosquito behaviour although it was slightly toxic (Table VIII).

The higher proportion (33 per cent) of unfed mosquitoes in huts treated with dichlorvos (Table X) than in the control huts (15 per cent) did not appear to be due

to the rapid toxicity of the insecticide because only one per cent of the unfed mosquitoes were found on the floor of the treated hut. A possible explanation was that there was some inhibition of feeding due to irritation by the dichlorvos, with consequent greater egress—again reflected in the high Repellency Index during the second month (Table X) and the higher proportion of survivors in the window-trap (Table IX). An earlier study also indicated that the behaviour of fed and gravid mosquitoes was also affected by dispensers containing dichlorvos in plasticiser solution (Smith, 1963).

In conclusion, it may be stated that there is some evidence that dichlorvos has irritant or repellent properties, as well as being rapidly toxic. It is not possible, with our present experimental techniques, to define the magnitude of the properties in more than the most approximate terms because we do not know (1) what proportions of the mosquitoes that enter treated huts leave through the open eaves, compared with control huts; (2) how important outdoor repellency is, i.e. repellency before entering the treated hut. The first point is at present under study, but the second point would probably be better resolved in a village trial where data could be obtained from many huts instead of a few experimental huts. Present evidence gives no indication that the irritant or repellent properties would be detrimental to mosquito control but assessment of kill might be more difficult under field conditions.

SUMMARY.

1. The effects of dichlorvos, from different types of dispenser, have been studied on the behaviour of *A. gambiae* in field trials with experimental huts.

2. The lower mortalities in mud-roofed than in grass-roofed huts, previously observed, were explained by the considerably lower kills inflicted on fed and gravid mosquitoes in the former type of hut. This difference was smothered to a greater or lesser degree by the proportion of unfed mosquitoes in the total catch.

3. The higher proportion of dead mosquitoes in the window-traps of huts with mud-roofs was largely due to greater numbers of unfed mosquitoes in the morning window-trap. Mud-roofed huts appeared to be more attractive than grass-roofed ones to unfed mosquitoes.

4. Technical dichlorvos had irritant or repellent properties which the present techniques indicated to be of no great practical importance to mosquito control because they were greatly offset by the concurrently rapid toxic action of the insecticide.

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A STUDY ON THE JOINT ACTION OF DIAMINO-DIPHENYL-SULPHONE (DDS) AND PYRIMETHAMINE IN THE SPOROGONY CYCLE OF *PLASMODIUM GALLINACEUM*: POTENTIATION OF THE SPORONTOCIDAL ACTIVITY OF PYRIMETHAMINE BY DDS.

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DDS alone appeared to possess low schizonticidal activity in *P. gallinaceum* (Ramakrishnan *et al.*, 1961). The potentiation of the schizonticidal activity of pyrimethamine by diamino-diphenyl-sulphone (DDS) in *P. gallinaceum* and *P. bastianelli* infections was described in a previous work (Basu *et al.*, 1962). In combination with pyrimethamine, the smallest dose required to be effective was found to be much lower than the equi-potent dose of any of the two drugs used singly to produce a set effect.

In the present investigation, the smallest schizonticidal dose combination of DDS and pyrimethamine, as stated above, was tried as a single administration to determine its sporontocidal activity in *P. gallinaceum*. The equi-potent as well as higher dosages of the drugs singly were also tried. The results of these studies are presented in this paper.

MATERIAL AND METHODS.

Fowls (Rhode Island red and white leghorn) weighing 0·7 to 2·7 kg. were inoculated intravenously with erythrocytic forms of *P. gallinaceum**, each with the standard inoculum of $0{\cdot}5 \times 10^6$ parasitized cells per kg. body weight.

Smears were examined from each bird daily to determine the extent of parasitaemia against 10^4 erythrocytes. Fresh batches of laboratory-bred *Aedes aegypti*

* The strain of *P. gallinaceum* was the same as used by Jaswant Singh, Basu and Ray (1962).

were fed daily, separately, on each of the experimental and control birds commencing on the day the infection involved 2 to 6 cells per 10^4 erythrocytes. One single dose of the drugs, either singly or in combination, was administered on D—day preceded by daily feeding of batches of mosquitoes for 3 days, namely, D—3, D—2 and D—1 day. Batches of mosquitoes were fed on D—day also and subsequently daily through D+10 days or a shorter period.

Parallel feeding of *Aedes aegypti* mosquitoes on control fowls continued during the corresponding D—2 through D+9 days. Fed mosquitoes were dissected and examined for sporozoites after the expiry of extrinsic incubation period. The temperature and humidity conditions remained the same for all batches of mosquitoes during the extrinsic incubation period.

DOSAGES OF THE DRUGS.

Previous work (Basu *et al.*, 1962 *loc. cit.*) on the potentiation of the schizonticidal effect of pyrimethamine by DDS had shown :—

(*i*) the equi-potent doses of DDS and pyrimethamine singly were 826 mg. and 0·18 mg./kg. respectively for schizonticidal effect in *P. gallinaceum* infection given as a single dose and (*ii*) the most effective dose combinations of the two drugs against *P. gallinaceum* evaluated at ED_{95}. which required the smallest dose of either drug, were DDS 1·4 mg. and pyrimethamine 0·00032 mg. per 50 g. administered twice daily for 3½ days. Converted to a single dose, the combination was DDS 196 mg./kg. plus pyrimethamine 0·045 mg./kg.

In the present investigation the following dosages have been used :—

(*a*) Combination :— DDS 210 mg./kg. plus pyrimethamine 0·028 mg./kg. single dose. Thus, the dose of pyrimethamine was almost half of that which had been the smallest effective component of the combination for schizonticidal activity.

(*b*) Pyrimethamine singly, three dosages, namely 0·028, 0.078 and 2·0 mg./kg. The latter two figures represented dose levels much above the equi-potent dose of the combination vide (*a*) above.

(*c*) DDS singly, two dosages, namely 210 and 300 mg./kg. The doses were close to the equi-potent dose of the combination vide (*a*) above.

RESULTS.

(*a*) SPOROZOITE RATES IN CONTROL FOWLS.

The results (Table I) showed that the sporozoite rate recorded as 60 per cent on D—2 day increased steadily to 86 per cent on D+1 day ; thereafter the rates declined daily till D+5 day. On D+6 day, there was slight increase of the rates followed by further decline on D+7 day and finally became nil on D+8 day. The parasite counts on the corresponding days recorded highest parasitaemia on D+3 day, followed by precipitous decline on D+5 day.

TABLE I.

Results in control fowls (sporozoite rate in Aedes aegypti, *average of 4 lots fed on 4 fowls).*

Day of feeding.	Number of mosquitoes dissected.	Number showing sporozoites.	Sporozoite rate (per cent).	Parasite count per 10^4 erythrocytes (average of 4 fowls).	Remarks.
D—2	5	3	60	2	D-day corresponded to the day of drug administration in the treated groups.
D—1	45	34	76	38	
D	36	30	83	688	
D+1	29	25	86	1923	
D+2	36	22	61	4250	
D+3	35	19	54	5275	
D+4	26	13	50	5150	
D+5	18	5	28	25	
D+6	11	4	36	2	
D+7	20	3	15	0	
D+8	16	*Nil*	*Nil*	0	
D+9	5	*Nil*	*Nil*	0	

(b) PYRIMETHAMINE (*singly*)

The results of single doses of 0·028, 0·078 and 2·0 mg./kg. of pyrimethamine on the sporogony cycle are contained in Tables II, III and IV.

TABLE II.

Investigations on sporontocidal activity of pyrimethamine 0·028 mg./kg. single dose in P. gallinaceum (*sporozoite rate in* Aedes aegypti, *average of 3 lots fed on 3 fowls).*

Day of feeding.	Number of mosquitoes dissected.	Number showing sporozoites.	Sporozoite rate (per cent).	Parasite count per 10^4 erythrocytes (average of 4 fowls).	Remarks.
D—3	16	0	0	2	Two fowls died by D+6 day. One which survived for the subsequent four days, did not produce gut or gland infection in the mosquitoes fed and the peripheral blood of this fowl became parasite-free from D+6 day onwards.
D—2	25	16	64	25	
D—1	39	26	67	100	
D	31	19	61	893	
D+1	30	21	70	1,770	
D+2	32	13	41	2,340	
D+3	27	7	26	906	
D+4	22	5	23	503	
D+5	25	*Nil*	*Nil*	403	

Two batches of three fowls each were treated with the above doses of 0·028 and 0·078 mg./kg. Batches of mosquitoes fed on each of these fowls were dissected and sporozoite rate determined. The sporozoite rates showed a gradual decline from D+2 day in the mosquitoes fed on fowls treated with a single dose of 0·028 mg./kg. of pyrimethamine. The rates in mosquitoes fed on fowls treated with a single dose of 0·078 mg./kg. of pyrimethamine showed a still slower progressive

decline beginning with D+4 day. Similar slow progressive decline of sporozoite rates was also observed in mosquitoes fed on control fowls (Table I) beginning from D+2 day. Thus no sporontocidal activity was manifest in the above doses of pyrimethamine.

TABLE III.

Investigations on sporontocidal activity of pyrimethamine 0·078 mg./kg single dose in P. gallinaceum (*sporozoite rate in* Aedes aegypti, *average of 3 lots fed on 3 fowls*).

Day of feeding.	Number of mosquitoes dissected.	Number showing sporozoites.	Sporozoite rate (per cent).	Parasite count per 10^4 erythrocytes (average of 3 fowls).
D−3	40	7	18	3
D−2	51	20	39	13
D−1	34	28	82	65
D	34	31	91	233
D+1	32	31	97	733
D+2	34	30	88	737
D+3	38	35	92	1,097
D+4	39	28	72	1,900
D+5	42	30	71	2,178
D+6	38	19	50	1,940
D+7	28	13	46	1,180
D+8	44	14	32	1,287
D+9	39	21	54	673
D+10	33	21	64	234

TABLE IV.

Investigations on sporontocidal activity of pyrimethamine 2 mg./kg. single dose in P. gallinaceum (*sporozoite rate in* Aedes aegypti, *average of 6 lots fed on 6 fowls*).

Day of feeding.	Number of mosquitoes dissected.	Number showing sporozoites.	Sporozoite rate, (per cent).	Parasite count per 10^4 erythrocytes (Average of 6 fowls).
D−2	21	12	57	30
D−1	84	59	70	86
D	73	65	89	321
D+1	118	0	0	933
D+2	123	0	0	333
D+3	106	0	0	69
D+4	89	0	0	8
D+5	126	15	12	2
D+6	123	12	10	1
D+7	98	10	10	0
D+8	77	0	0	0
D+9	69	0	0	0
D+10	58	0	0	0

Single dose of 2 mg./kg. of pyrimethamine, however, was outstandingly effective in that the sporozoite rate of 89 per cent on D−day was reduced to nil on D+1 day. Complete sporontocidal activity continued till D+4 day. During D+5 to D+7 day, the gametocytes recovered their infectivity and sporozoite rates of 10 to 12 per cent were recorded. Mosquito dissection results, thereafter, were negative.

(c) DDS (*singly*)

A single dose of DDS 210 mg./kg. was administered to 4 separate fowls (Table V). Batches of mosquitoes fed on these fowls were dissected after the expiry of the extrinsic incubation period and the sporozoite rates determined. The rate declined slowly from D+1 day but again increased from D+4 day for 3 consecutive days to become finally nil by D+8 day.

TABLE V.

Investigations on sporontocidal activity of DDS 210 mg./kg single dose in P. gallinaceum (*sporozoite rate in* Aedes aegypti, *average of 4 lots fed on 4 fowls*).

Day of feeding	Number of mosquitoes dissected.	Number showing sporozoites.	Sporozoite rate (per cent).	Parasite count per 10^4 erythrocytes (average of 4 fowls)
D−3	16	3	19	4
D−2	41	28	68	12
D−1	38	32	84	131
D	42	38	90	526
D+1	34	27	79	1575
D+2	53	17	32	1850
D+3	72	12	17	474
D+4	62	30	48	140
D+5	52	28	54	14
D+6	64	25	39	4
D+7	60	4	7	3
D+8	58	*Nil*	*Nil*	3
D+9	64	*Nil*	*Nil*	4
D+10	48	*Nil*	*Nil*	5

A single dose of DDS 300 mg./kg. was followed by slow decline of the sporozoite rate from D+1 day (Table VI). The rate increased on D+5 day to be

TABLE VI.

Investigations on sporontocidal activity of DDS 300 mg./kg. single dose in P. gallinaceum (*sporozoites in* Aedes aegypti, *average of six lots fed in six fowls*).

Day of feeding.	Number of mosquitoes dissected.	Number showing sporozoites.	Sporozoite rate (per cent.)	Parasite count per 10^4 erythrocytes (average of 6 fowls).
D−2	51	22	43	18
D−1	60	50	83	45
D	73	62	85	293
D+1	64	53	83	983
D+2	68	37	54	804
D+3	93	20	22	344
D+4	85	16	19	167
D+5	89	50	56	200
D+6	69	32	46	400
D+7	66	11	17	1
D+8	62	5	8	2
D+9	72	3	4	21
D+10	41	9	22	250

followed again by gradual decline. At no time during the period of observation, were the mosquitoes rendered non-infective. DDS alone, thus appeared to possess no sporontocidal activity.

(*d*) DDS AND PYRIMETHAMINE COMBINATION

A single dose of pyrimethamine 0·028 mg./kg. combined with DDS 210 mg./kg. was administered to 5 separate fowls (Table VII). Batches of mosquitoes fed on these fowls were dissected after the expiry of the extrinsic incubation period and the sporozoite rates determined. The rate was found to be 88 per cent for two consecutive days namely D—1 and D days. The rate declined abruptly to 17 per cent on D+1 day. From D+2 day onwards, the rates remained nil for the rest of the observation period. The sporontocidal activity of the combination, therefore, was striking.

TABLE VII.

Sporontocidal activity of a combination of pyrimethamine 0·028 and DDS 210 mg. per kg. single dose in P. gallinaceum *(sporozoite rate in* Aedes aegypti, *average of 5 lots fed on 5 fowls).*

Day of feeding.	Number of mosquitoes dissected.	Number showing sporozoites.	Sporozoite rate (per cent).	Parasite count per 10^4 erythrocytes (average of 5 fowls).
D−3	19	16	68	6
D−2	22	14	64	75
D−1	17	15	88	149
D	17	15	88	346
D+1	29	5	17	487
D+2	27	*Nil*	*Nil*	682
D+3	30	*Nil*	*Nil*	138
D+4	17	*Nil*	*Nil*	6
D+5	28	*Nil*	*Nil*	2
D+6	52	*Nil*	*Nil*	0
D+7	31	*Nil*	*Nil*	0
D+8	38	*Nil*	*Nil*	0
D+9	49	*Nil*	*Nil*	0
D+10	34	*Nil*	*Nil*	0

(*e*) STATISTICAL ANALYSIS

The effect on sporogony cycle manifested by different dosages of the two drugs, pyrimethamine and DDS, administered singly and in combination, was measured by comparing sporozoite rates (per cent) for groups of mosquitoes fed on fowls which were kept on these drugs and those kept under control. In attempting to determine the statistical significance of the changes in sporozoite rates, it was considered most suitable to compare separately the values obtained due to each drug with those of the control group. '*t*' tests were applied to pairs of series of such values (Table VIII).

TABLE VIII.

Statistical analysis of data.

Drug/dosage compared with control.	d. f.	't' value.	Remarks.
Pyrimethamine 0·028 mg./kg. ...	19	0·50	Not significant.
Pyrimethamine 0·078 mg./kg. ...	24	1·90	Not significant.
Pyrimethamine 2 mg./kg. ...	23	2·14	Significant at 5 per cent level.
DDS 210 mg./kg. ...	22	0·57	Not significant.
DDS 300 mg /kg. ...	22	0·52	Not significant.
Combination of 0·028 mg./kg. pyrimethamine and 210 mg./kg. DDS ...	24	2·01	Approaches significant at 5 per cent level.

The low value of '*t*' in dosages of pyrimethamine 0·028 and 0·078 mg./kg. showed that these dosages did not produce any sporontocidal activity. Pyrimethamine 2 mg./kg. showed significant variation of the sporozoite rate. Also the mosquitoes fed on fowls, which were kept on DDS 210 and 300 mg./kg., did not exhibit a variation, as could be seen from the value of '*t*' obtained in those dosages.

Combination of the two singly ineffective dosages, i.e., 0·028 mg./kg. pyrimethamine and 210 mg./kg. DDS, however, produced a marked drop in the sporozoite rate. The test showed that this variation approached statistical significance.

DISCUSSION.

Earlier investigation had shown that DDS did not possess any sporontocidal activity in *P. gallinaceum* (Ramakrishnan, Basu *et al.*, 1962). The present investigation with still higher doses of DDS, singly, confirmed it further.

A comparatively high dose of pyrimethamine alone, i.e., 0·7 mg./kg. (equivalent to adult human dose of 50 mg.) showed sporontocidal effect in *P. gallinaceum* for a short period only (Jaswant Singh *et al.*, 1953). In the present investigation smaller doses of pyrimethamine, namely, 0·028 and 0·078 mg./kg. understandably did not reveal any sporontocidal activity. A dose as high as 2 mg./kg., however, showed marked sporontocidal activity for a period of at least 4 days.

The combination of DDS and pyrimethamine, however, showed remarkable sporontocidal activity in that the mosquitoes, fed 48 hours after the drug administration in the host, failed to show any infection and continued to remain so for the subsequent days of observation. The sporontocidal effect was noticed even earlier as the sporozoite rate recorded a sharp decline in mosquitoes fed on the hosts, 24 hours after the birds received the drug dose. It would be worth noticing that the control fowls, although showing either low degree parasitaemia or complete latency, continued to infect mosquitoes from D+5 through D+7 days. In contrast, fowls, which received the single dose of the drug combination even in the presence of either high or low degree parasitaemia, failed to infect the mosquitoes from D+2 day onwards.

The dose of pyrimethamine (0·078 mg./kg.) alone, although greater than the equi-potent dose of the combination, failed to exhibit the sporontocidal activity. Similarly DDS alone, although close to the equi-potent dose of the combination, did not show any sporontocidal activity. That DDS potentiated the sporontocidal activity of pyrimethamine was thus clearly brought out. According to Basu *et al.* (1961) small doses of DDS, combined with a small dose of pyrimethamine, (100 and 50 mg. of the former and 12·5 mg. of the latter), proved adequate for treatment of both *P. falciparum* and *P. vivax* infections and indicated probable potentiation.

In view of the present finding that DDS potentiated the sporontocidal activity of pyrimethamine against *P. gallinaceum*, the combination appeared to offer the additional advantage of interception of transmission.

Further experimental evidence has indicated that the selection of resistant strain was difficult against the combination of DDS and pyrimethamine (unpublished).

SUMMARY.

A small dose of DDS, combined with a small dose of pyrimethamine, (210 mg./kg. of the former and 0·028 mg./kg. of the latter), showed marked potentiation of activity in the sporogony cycle of *P. gallinaceum* in *A. aegypti*.

Equi-potent or higher doses of the drugs used singly did not exhibit sporontocidal activity except with a very high dose of pyrimethamine.

Potentiation of the sporontocidal activity of pyrimethamine by DDS, in addition to the potentiation of its schizonticidal activity, appeared to widen the scope of use of the combination of these two drugs in the field of public health also.

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STUDIES ON INFESTATION OF *PISTIA STRATIOTES* LINN. BY THE CATERPILLAR OF *NAMANGANA PECTINICORNIS* HYMPS., A NOCTUID MOTH, AND ITS EFFECTS ON *MANSONIOIDES* BREEDING.

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INTRODUCTION.

MANSONIOIDES ANNULIFERA and *Mansonioides uniformis* are respectively the primary and secondary vectors of *Brugia malayi* in India. These species of mosquitoes are biologically dependant on certain aquatic plants, particularly *Pistia stratiotes*, during the aquatic phase of their life history. Their eggs are laid on the leaves of the aquatic weeds, and larvae and pupae remain attached to the roots of the host plant by means of specialised respiratory siphons, breathing the oxygen available in the spongy root tissue.

Another factor which is essential for the breeding of *Mansonioides* is the presence of organic pollution in the breeding places. In Kerala, this is essentially derived from the retting of coconut husks—a process which is indispensable for the coir industry. The presence of numerous water collections with *Pistia*, of which a large proportion are used for retting husks, provides the coastal tract of Kerala, with ideal conditions for the continual breeding of *Mansonioides* and for transmission of Malayan filariasis.

Any adverse change in the co-existance of these two essential ecological factors, which in effect mean air and food for the *Mansonioides* larvae, would bring about a reduction in their breeding, and help control of transmission of *Brugia malayi*. In this context any natural agent, which tends to destroy *Pistia stratiotes*, is of interest and worth investigation. The present paper deals with such an agent—a noctuid moth, *Namangana pectinicornis* Hymps., which has been observed to cause considerable destruction of *Pistia* in many parts of coastal Kerala. The present paper describes the life history of the moth and evaluates the effects of its infestation of *Pistia*, on the breeding of *Mansonioides*. The observations were carried out at Alleppey, in Kerala, during 1962-1963.

MATERIALS AND METHODS.

Observations in the field, as well as rearing experiments in the laboratory, were undertaken to study the various developmental stages of the moth. Adult moths were released for oviposition in 50 cm. cube mosquito-cages with small flowering

plants in pots, and bowls of water with *Pistia*. The egg masses were subsequently collected, hatched out and reared, in a round, porous, cement basin of 50 cm. radius. *Pistia*, with egg masses of the moth, were introduced amidst known numbers of healthy plants floated in the cement tank, and the various stages were observed as they developed. Also the average extent of destruction caused by a caterpillar per day, and the number of caterpillars required to destroy a unit area of *Pistia* surface, during the course of their larval period, were estimated. The preferences of the catepillar for the common aquatic weeds, and the rice plant, were studied.

In the field a large number of ponds were repeatedly observed every month to determine the seasonal prevalance of the moth. Two ponds, each about 10 metres in diameter, and with considerable moth infestation to start with, were selected at random and fortnightly observations carried out for more than a year to determine the extent of *Pistia* destruction caused by the caterpillar in nature. The effect of *Namangana* infestation of *Pistia*, on *Mansonioides* breeding, was studied by the examination of infested and uninfested *Pistia* ponds.

RESULTS.

LIFE HISTORY OF THE MOTH (PLATE I)

Egg.—The eggs are spherical, and roughly 200 microns in diameter. They are pearly white in colour with a greenish tint. They are laid in a compact mass cemented to the undersurface of the *Pistia* leaf near its edge, and covered by a silky material (Plate I, Fig. 1). Each egg mass contains from 70 to 120 eggs. Under a temperature range of 85 to 95 degrees Fahrenheit and an average relative humidity of 80 per cent, the eggs hatch out within a period of 48 to 60 hours.

Larva.—The tiny, slimy caterpillars that hatch out are pale-green in colour. The larvae are typically eruciform. They feed voraciously on the leaf (Plate I, Fig. 2), usually burrowing along in the direction of the longitudinal veins. The fully grown caterpillar is about 1·5 to 2 cm. long. The larval stages last from 15 to 20 days.

Pupa.—The obtect pupae are usually found in a tunnel burrowed in the leaf (Plate I, Fig. 3) or attached to the axils of the leaves. At first they are greenish but later change to a darkish brown colour with dull yellow bands. The adults hatch out within 48 to 60 hours.

Adult.—The adult (Plate I, Fig. 4) is a small and sluggish moth, measures only a centimetre in length. It has a silvery brown colour with dark and light speckles on the wings.

PREVALENCE OF MOTH INFESTATION.

The moth has a perennial prevalence ; all the different developmental stages occurring simultaneously in the same situation, throughout the year. However, the periods of peak occurrence coincide with each of the two monsoons of the year,

PLATE 1. Stages of *Namangana pectinicornis* Hymps.

FIG. 1. Egg mass.

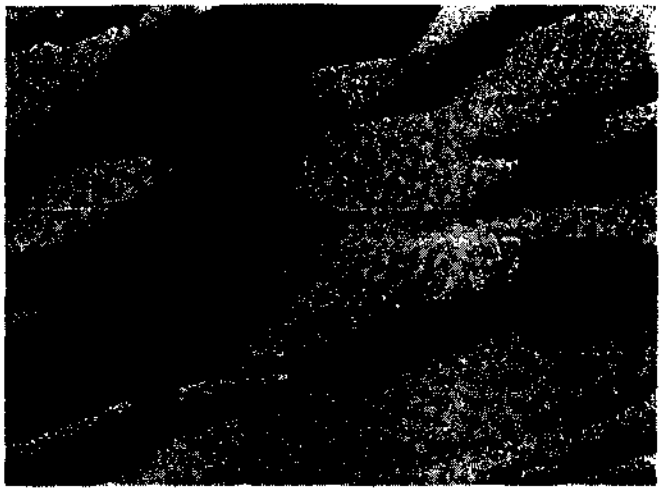

FIG. 2. Larva.

FIG. 3. Pupa.

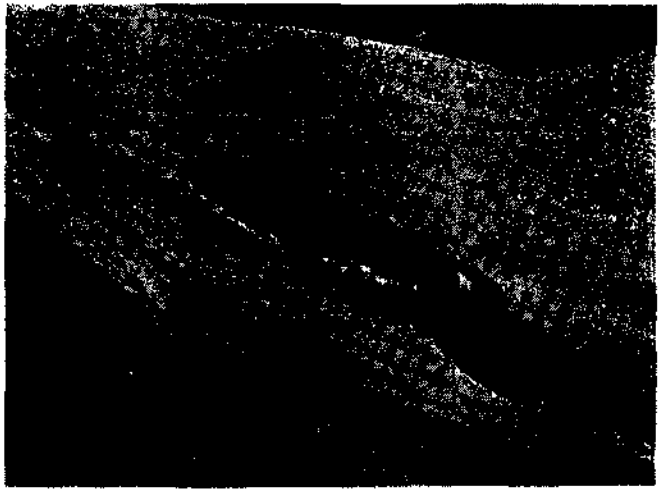

FIG. 4. Adult.

PLATE II.

Pistia plant before, and after, infestation of the caterpillar of the moth.

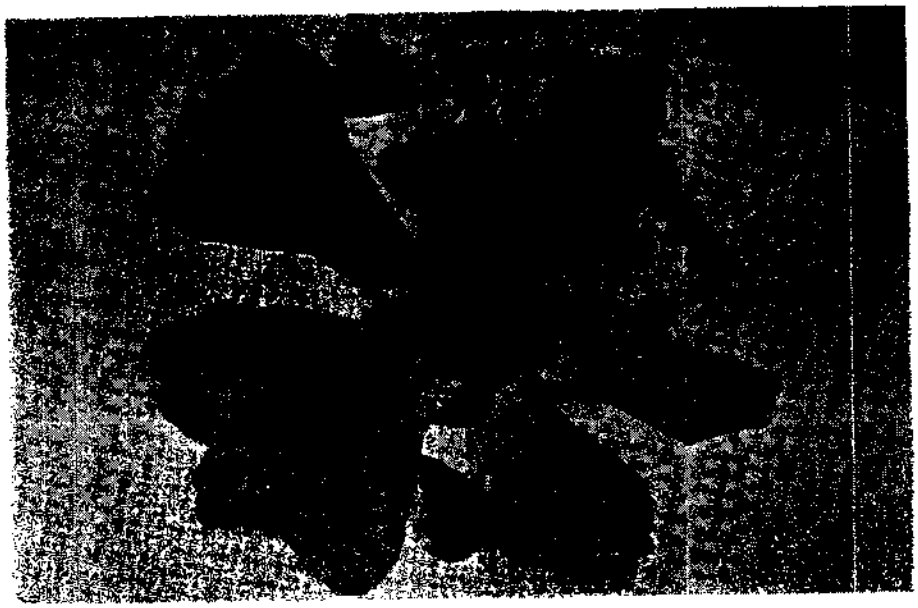

FIG. 1. Pistia plant before infestation of the caterpillar of the moth.

FIG. 2. The same pistia plant after infestation of the caterpillar of the moth.

The infestation becomes marked by June-July when there is a peak destruction which thereafter is maintained at a lower ebb ; and is followed by a second peak during October, which gradually wanes off. This corresponds almost with the flourishing period of *Pistia* growth during and after the rainy seasons. In Alleppey, during peak seasons, more than 75 per cent of the ponds are affected with 75 to less than 100 per cent infestation of the *Pistia*. During the dry months from February to April, 25 to 50 per cent of the ponds are infested with destruction of *Pistia* varying from 10 to 25 per cent. *Namangana* infestations have been observed also at Trivandrum, Quilon and Sherthallai areas of Kerala State.

DESTRUCTIVE CAPACITY OF THE CATERPILLAR.

It has been observed in the field as well as in the laboratory that, if present in good numbers, the caterpillars could bring about almost complete destruction of the leafy portions of *Pistia*. The nature and extent of destruction are illustrated in Plate II. It shows the same *Pistia* plant before and after infestation by the caterpillar, as observed in the laboratory. From laboratory observations it is estimated that about 100 caterpillars, developing from one average sized egg mass, could completely destroy the *Pistia* within an area of one sq. metre, during their larval life of 15 to 20 days. In the field, during the peak season, the number of caterpillars, per square metre *Pistia* surface, was always more than this estimated value, and naturally included caterpillars of different generations. On an average one growing caterpillar before pupation, eats up two sizeable heads of *Pistia*, which works out to be one average *Pistia* leaf per caterpillar per day.

SELECTIVE DESTRUCTION OF PISTIA.

In ponds with mixed vegetation, only *Pistia* was found infested. In the laboratory when the caterpillars were offered other plants, viz. *Echornia speciosa*, or *Salvinia auriculata* or rice plant they remained abstinent up to a maximum of five days, and eventually died. The predilection of the caterpillar for *Pistia stratiotes* is apparently specific.

EFFECTS OF MOTH INFESTATION ON *MANSONIOIDES* BREEDING.

Contrary to expectation it was observed that 50 per cent of the infested ponds showed *Mansonioides* breeding. It should be noted that in these ponds there was neither retting nor any other source of organic pollution. Apparently the source of the pollution which favoured breeding had been the decaying parts of the infested weed, and to a lesser extent, the excreta of the caterpillars. The total absence of breeding in *Pistia* ponds with neither husk steeping nor moth infestation is in support of the above fact.

DISCUSSION.

Pistia stratiotes Linn., so far as is known, is yet the preferred host plant for *Mansonioides* in Kerala (Iyengar, 1938 ; Burton, 1959 ; Joseph *et al.*, 1960). It was observed both in laboratory and field studies that *Namangana pectinicornis* Hymps. is capable for causing extensive destruction of *Pistia*. It was therefore expected that moth infestation would reduce *Mansonioides* breeding, and induced moth infestation could be used for the biological control of *Mansonioides*. But the study of the effect of moth infestation of *Pistia*, on *Mansonioides* breeding, has proved the contrary. In 50 per cent of the affected ponds, active breeding was present, both eggs and larvae of *Mansonioides* being found. This could be explained by the fact that (*i*) even in the most heavily infested ponds, the partially eaten leaves still afforded facilities for adult *Mansonioides* to oviposit ; (*ii*) the stems and roots being practically unaffected, the larvae and pupae were not deprived of attachment and could go through their normal course of development ; (*iii*) since these weeds can reproduce by stolons a fresh mat of *Pistia* often reappeared within a period of four weeks, (*iv*) the organic contamination, so essential for breeding, was obviously provided by the decay of the damaged or dead leaves of infested *Pistia* plants, and presumably by the excreta of the caterpillars as well.

Thus, despite its selective infestation, perennial prevalence, and high destructive potential, the caterpillar of *Namangana pectinicornis* could not possibly be used for practical *Pistia* control.

SUMMARY.

The life history of *Namangana pectinicornis* Hymps., a noctuid moth, the caterpillars of which were found infesting and causing damage to the *Pistia stratiotes* Linn., the host plant of *Mansonioides* mosquitoes in Kerala, is described. An account of the process of infestation is given on the basis of laboratory and field observations.

The possibility of using *Namangana pectinicornis*, for the biological control of *Pistia*, and thus of *Mansoniodes* is discussed in the light of the observations made. It is concluded that contrary to expectation, the presence of infestation would promote rather than inhibit *Mansonioides* breeding ; since, even in heavily infested situations, enough facilities remain for the oviposition of *Mansonioides* as well as for the completion of the aquatic phase of their life history. Further more, large scale decay of the affected *Pistia* leaves, together with excreta of the caterpillar, provide the factor of organic contamination of the water, so essential to *Mansonioides* breeding.

ACKNOWLEDGEMENT.

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HISTORY OF MALARIA AND ITS CONTROL IN DIFFERENT COALFIELDS OF INDIA.

A review of 15 years in retrospect

BY

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INTRODUCTION

In order to gain a proper perspective of malaria endemicity and to contemplate the prospects of eradication of the disease, a systematic review of the 15 years of intensive malaria control operations in the different coalfields of India, is presented.

Coal* is a chief fossil fuel obtained from the decomposition of plant material due to physico-chemical changes in geological times (over a stretch of 2½ billions of years in India). This fuel of supreme importance exists in horizontal and inclined seams in nature. These seam-bearing areas are called coalfields. The Indian coalfields are invariably associated with river basins. Their physiography, location in different States, and the division of the coalfields into larger and smaller groups, are described in detail in Table I.

THE SCHEME AND ITS PURVIEW.

By the proceeds of a cess, levied on despatch of coal by rail/road at the rate of 6 annas (37 Naye Paise) per ton, initiated in 1943-44, the Coal Mines Labour Welfare Fund was established in 1944, under the Ministry of Labour, Government of India. The Coal Mines Labour Welfare Organisation started functioning from 1944. The Antimalaria Section has since been a branch of the General Welfare Division of the Coal Mines Welfare Organisation. The cess was subsequently enhanced to 8 annas (50 Naye Paise) per ton from which a sum of Rupees one million per annum was earmarked for the antimalaria work. The per capita expenditure, year-wise, is set in Table I (*a*).

The Coalfields Malaria Control/Eradication Unit is an example of unity in diversity for it is dispersed so widely into 7 States of India. At the outset in 1945,

*Coal (1952) Bulletin No. 1—By Roy and Sharma—ISMAG Geological Society, Dhanbad (India).

there were only 4 large groups to commence with, *viz.*, Jharia, Raniganj, Pench Valley, Margherita, under the Assistant Directorate (Coalfields Branch). By gradual expansion of the activities, the other groups of coalfields came into the folds of this Unit, increasing the number to 10 by 1952 and are since maintained. While the 3 large coalfields in Damodar basin (Jharia, Raniganj and Hazaribagh) yield 70 per cent of coal, the total produce from the 10 fields together exceeds 85 per cent of the Indian coal output. The location of these 10 groups of coalfields has been marked in Map I.

TABLE I.

Physiography of the groups of coalfields of India under the Coalfields Malaria Control/Eradication Unit.

Serial number.	Name of the group of coalfields.	Location: District.	Location: State.	Associated with river valley.	Type of terrain.	Large/small group of coalfields.
1	Jharia	Dhanbad	Bihar	Damodar basin	Plain country.	Large
2	Raniganj	Burdwan	West Bengal	,, ,,	,, ,,	
3	Hazaribagh	Hazaribagh	Bihar	,, ,,	Undulating country.	
4	Margherita	Lakhimpur	Assam	Dehing basin	Hilly terrain.	Large
5	Pench valley	Chhindwara	Madhya Pradesh	Pench basin	Plain country.	Large
6	Korea	Sarguja	—do—	Hasdeo-Mand basin	Undulating country.	Small
7	Sambalpur	Sambalpur	Orissa	Mahanadi-Rampur basin	Plain country.	Both small
8	Talcher	Dhankanal	—do—	— do —	— do —	
9	Chanda	Chanda	Maharashtra	Wardha-Irai basin	,,	Small
10	Hyderabad	Khambum and Adilabad	Andhra Pradesh	Godavari-Pranhita basin	,,	Small

TABLE I (*a*).

Cost on malaria control measures, incurred by the Coalfields Malaria Control/Eradication Unit.

Years.	Per capita cost, in rupees, for the population protected.	Remarks.
1945-47	Data not available.	
1947-1952	Every year approximately Re. 1/-	(Chowdhury 1953)
1953	Re. 1·30	
1954	Re. 1·23	
1955	Re. 1·41	
1956	Re. 0·91	
1957	Re. 1·00	
1958	Re. 0·99	
1959	Re. 0·84	
1960	Re. 1·20	

Each coalfield has a number of collieries/coal mines. Quite a good number of the miners are housed in chowrahs* in the vicinity of mines. Since villages

**Terraced one-roomed tenements built in colliery colonies.*

MAP 1.

Location of 10 groups of coalfields in the respective States of Indian Dominion.

situated in the proximity of these coal mines supply a fair proportion of labour, such of the villages that lie within the radius of half a mile of any colliery, are also included in this scheme.

Labourers as coal miners are drawn from native as well as neighbouring districts/States, so that the population is not static and non-homogeneous. Also as their main profession is agriculture, they seldom remain on mining for they return home at least twice a year for transplanting/harvesting and such other private errands. Particulars in regard to the number of collieries and villages and their respective population protected, are furnished in Table II.

TABLE II.

Number of collieries and villages and their respective population, as in 1960, under the Coalfields Unit Malaria Control.

Serial Number.	Group of coalfields.	Collieries.		Villages.		Total population.
		Number.	Population.	Number.	Population.	
1	Jharia	427	8,13,528	180	68,041	3,81,569
2	Raniganj	220	2,26,690	134	1,10,201	3,36,891
3	Margherita	10	16,200	21	13,212	29,412
4	Pench Valley	30	62,892	51	35,551	98,443
5	Korea	10	43,690	3	2,360	46,050
6	Hazaribagh	64	1,76,203	63+ (46 hamlets)	34,593	2,10,796
7	Sambalpur	3	4,707	5	4,931	9,638
8	Talcher	3	4,456	15	6,942	11,398
9	Chanda	7	24 149	2	14,171	38,320
10	Hyderabad	3	1,31,000	Nil	...	1,31,000
Total (Ten)		777	10,03,515	475+ (46 hamlets)	2,90,002	12,93,517
				Total population about 13 lakhs (1·3 million).		

Out of the 13 lakhs (1·3 million) population under control, a little over ten lakhs (one million) is in the collieries, and a little under 3 lakhs in the villages included in the Coalfields Malaria Control Scheme. About 30 per cent of the total colliery labour population is permanent, 40 per cent is the non-static labour population of villages, and 30 per cent the static dwellers in the nearby villages.

HISTORY RECAPITULATED.

Rao (1944) reported malaria in near epidemic form in the Jharia coal mines in 1936. As a result of this high incidence of malaria, the Mines Board of Health, Jharia, started small scale antilarval work in this area in 193<. The Asansol Mines Board of Health, Raniganj, however, had been carrying out antilarval work since 1931.

Malaria was reported to have occurred in an epidemic form in the coalfields of Bihar and Bengal in 1943-44 (Jaswant Singh, 1948; Chowdhury, 1953). The high morbidity from malaria in miners at the crucial time of World War II (1939 to

1945) hampered the production of coal. This drew the attention of the Government of India and in 1944, Army Antimalaria Units were organised for the larger groups of coalfields, *viz.*, Jharia, Raniganj, Margherita and Pench Valley.

The civil anti-malaria organisation started functioning in the aforesaid 4 larger coalfields under the Director of the then Malaria Institute of India*, since December 1, 1945 (Afridi, 1961 ; Jaswant Singh, 1961 ; Covell, 1961)†. This organisation worked under the Ministry of Health with headquarters at Dhanbad. After December 1950, the administrative control of this organization was reoriented to the Ministry of Labour The Coalfields Branch came to be called the Coalfields Unit of Malaria Control (from 1951)/Eradication (from 1958) with the Chief Malaria Officer as the Unit Officer at headquarters under the Coal Mines Welfare Commissioner. The staff position is given in detail in Appendix I.

The technical control, however, continued to be of the Malaria Institute of India*.

MALARIOGENIC CONDITIONS.

The coal bearing belt exists mostly in the plain country in contiguity of river basins. The malaria endemicity varies from hypo-endemic to meso-endemic. Cyclical occurrences of high incidence, once in ten years as observed in Raniganj coalfields, have been a common feature. Rao (1944 *loc. cit.*) also recorded a period of 4 years of high incidence followed by three years of low incidence from Jharia mines. There is no canal irrigation in the vicinity of any coalfield. As such the problem is of seasonal malaria, associated with cultivation of rice (rain-fed) Of the ten groups of coalfields, seven are in plains, two in areas where land is undulating and one in hilly terrain. This last group of coalfields (Margherita) also differs from the rest of the coalfields in respect of vector, the predominance of the species of *Plasmodium* and season of transmission.

The malariogenic features that are specific to coalfields and deserve special mention (Chowdhury, 1953), are :—

(i) As a result of coal excavation breeding places are increased.

(ii) The tropical aggregation of labour affords easy chances for the spread of malaria, due to mixing of susceptible and parasite-harbouring people.

(iii) Probably new strains of plasmodia are introduced by the to and fro movement of the labour between their homes and the coal-mines.

(iv) Construction of large net-work of railway sidings, and rail roads for transportation of coal, block the natural gradient and cause stagnation of water, and

(v) Bunds are placed across natural water courses to collect water for domestic purposes, which result in formation of seepages and swamps.

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* Now known as the National Institute of Communicable Diseases, Delhi.

† *Official History of the Indian Armed Forces in the Second World War 1939-1945 : Medical Services : Preventive Medicine. Part II. Malaria Control*, pp. 374-375.

[Reprinted in *Ind. J. Mal.*, **16**, 4, (December 1962) pp. 393-574].

MALARIA CONTROL.

Brief history of control measures, coalfield-wise and chronologically arranged, is described in Appendix II. The local bodies were engaged in small scale anti-larval work from 1931 in Raniganj, from 1938 in Jharia and from 1941 in Margherita. Malaria control measures were organised and started by the army authorities by 1944 in the coalfields of Jharia, Raniganj, Pench Valley and Margherita. The civil organisations replaced these army units by the end of 1945. The control measures in force in the 4 large coalfields from 1944-1947 were mainly anti-larval, consisting of application of parisgreen, oiling, deweeding, canalisation, and minor engineering works. Spray killing (knockdown) of adult mosquitoes with pyrethrum solution was also done on limited scale. D.D.T. residual spraying was carried out in these coalfields from 1947 on limited scale.

In the meantime several other coalfields acceeded to this organisation as years rolled, e.g., the Hazaribagh coalfields in 1947, the Sambalpur, Talcher and Chanda coalfields in 1950, Korea in 1951—(However, the Korea coalfield was under the Malariologist, Bengal-Nagpur Railway from 1945 to 1951)—and the Hyderabad coalfield in 1952.

Indoor residual spraying with D.D.T. in all the coalfields was started in 1948 at a dosage of 50 mg./sq.foot. During the transmission season, three rounds of spray were carried out at intervals of 6 to 8 weeks. The anti-larval measures were carried out during the non-transmission season.

Malaria control measures on the pattern of the National Malaria Control Programme were started from 1954 in all the 10 coalfields with the standard scheduled dose of 100 mg. per sq.foot twice a year. The National Malaria Eradication Programme was launched in 1958-59 on the basis of 3 years of attack phase, with the formulation and dosage according to standard scheduled pattern, with total coverage as the key-note.

EPIDEMIOLOGICAL ASSESSMENT.

Malaria in the coalfields before the start of control measures was hypo- to meso-endemic with varying degree of seasonal exacerbations. The Margherita coalfield, however, was situated in the hyperendemic zone. According to Senior White (1943), a portion of the Korea coalfields was also hyperendemic (Chirimiri colliery situated in deep ravine bed, coal outcropping). It was here that the opening of the first two collieries and the construction of the East-Indian Railway was affected by fulminant malaria. Thus the area was dreadful even to the indigenous aboriginals, who used to shudder at inhabiting the area.

The coalfields are in general meso-endemic for malaria with the exception of a few collieries, *viz.*, Chirimiri (in the Korea group) and Margherita from the hilly terrain in Assam with high endemicity to hyperendemicity (Senior White, 1943; Jaswant Singh, 1948; Chowdhury, 1953). According to Chowdhury (1953 *loc. cit.*)

the vector in Margherita coalfield is *A. minimus* and the preponderance of species of malaria parasite is *P. falciparum.*

MALARIOMETRY.

The three malariometric indices, as described below, for the period 1945-1960, are presented in Table III and also in Charts 2 and 3, coalfield-wise and year-wise. Reduction in these indices was less pronounced in the early years (1945-1948), when only anti-larval measures were in force. After 1948, when the indoor residual insecticide application commenced, these indices showed progressive reduction, reaching the lowest level by 1959-60.

(*a*) *Malaria case rate per mille.*—The malaria morbidity rate was 549 and 600 respectively in the Raniganj and the Margherita coalfields in 1945. After 12 to 14 years of control measures, mainly D.D.T. indoor residual spraying, this index fell to 12 and 37 in the respective coalfields in the year 1960. The bars drawn on simple 3—scale log (Chart 2) show a gradual reduction in the incidence of malaria in all the coalfields, for the rate is below 20 by 1960. Margherita alone stands an exception, with 37 as its reduced rate of incidence in 1960.

(*b*) *Child spleen rate per cent.*—Only in the Margherita and the Pench Valley coalfields this index was as high as 23·0 and 19·9 per cent respectively to commence within 1946 and 1948, while in other coalfields the rate was below 13 per cent. But after years of malaria control operations, this rate was also considerably reduced in all the coalfields, reaching one and less than one per cent by 1959-60 (Chart 3).

(*c*) *Child parasite rate per cent.*—The data is available from 1948 onwards when this index in the beginning was 10·0 and 9·4 per cent from Hazaribagh and Jharia respectively. The fluctuations in the parasite index (25 per cent from Raniganj in 1949 and 22 per cent from Chanda in 1951) probably indicate post-epidemic curves. In general, this index has also depicted a down-ward trend, being in the vicinity of zero by 1959 (Chart 3).

PARASITOLOGY.

(*a*) *Parasite positives among fever cases.*—In Table IV are depicted the results of microscopic examination of blood smears from different coalfields, year-wise and species-wise. The result of examination of blood smears for fever cases reveals that parasite positives range between 0·5 and 4·0 per cent in the coalfields, except in Margherita where it is 20 per cent. This exceptionally high rate from Margherita may be due to introduction of parasite positives as a result of tropical aggregation of labour.

The maximum number of parasite positive cases occurs in the rainy season each year. The percentage of infection species-wise differs only in the Margherita coalfield where *P. falciparum* has been predominant (75 per cent), but in the rest of the coalfields the predominant species of *Plasmodium* has been *vivax* (70 to 99 per cent). This observation conforms with the record of Chowdhury (1953 *loc. cit.*).

CHART 2.

Malaria case rate per 100 of population.

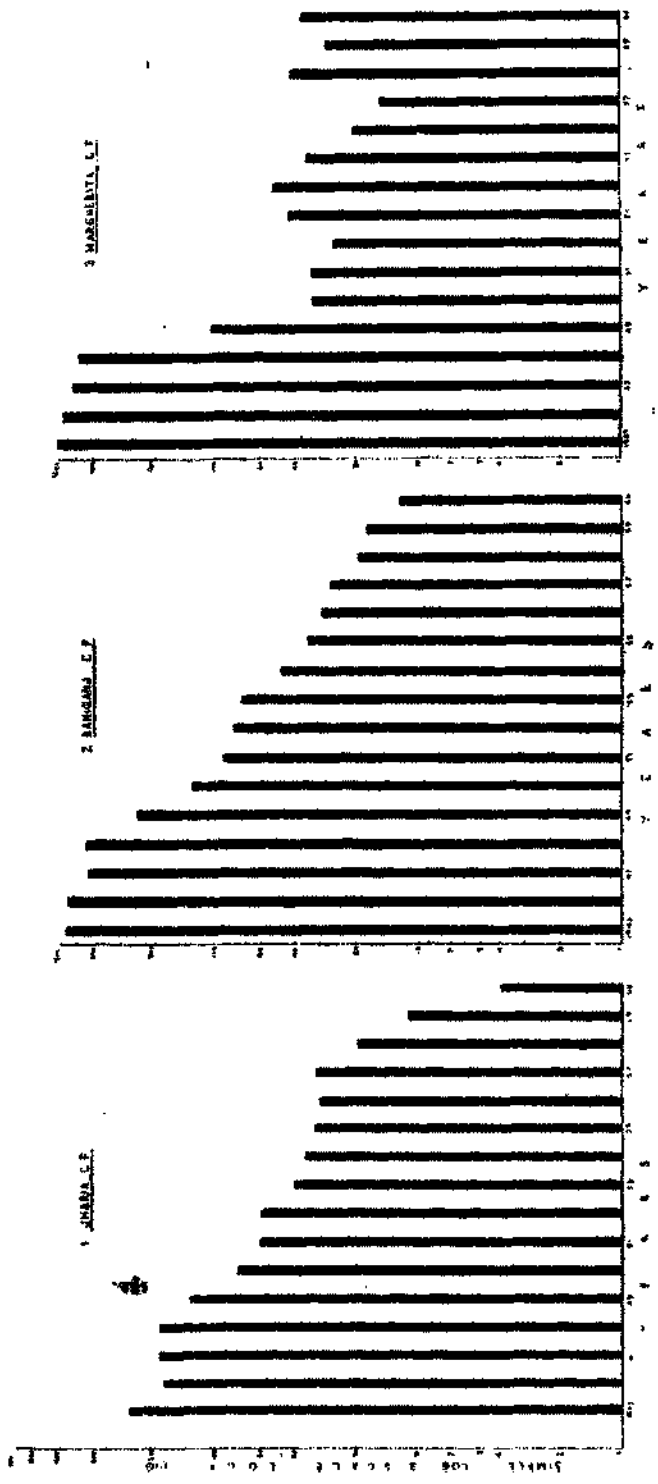

CHART 2. (*Contd.*)

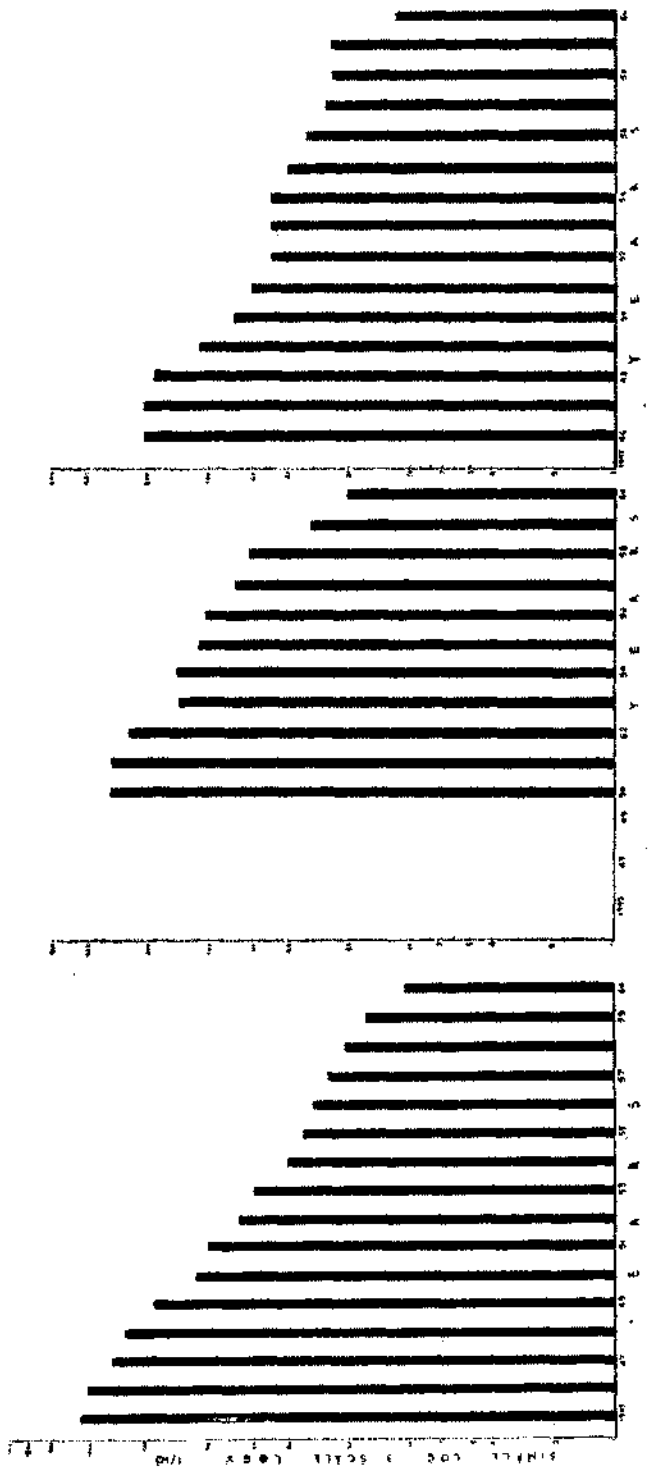

CHART 2 (*Concld.*)

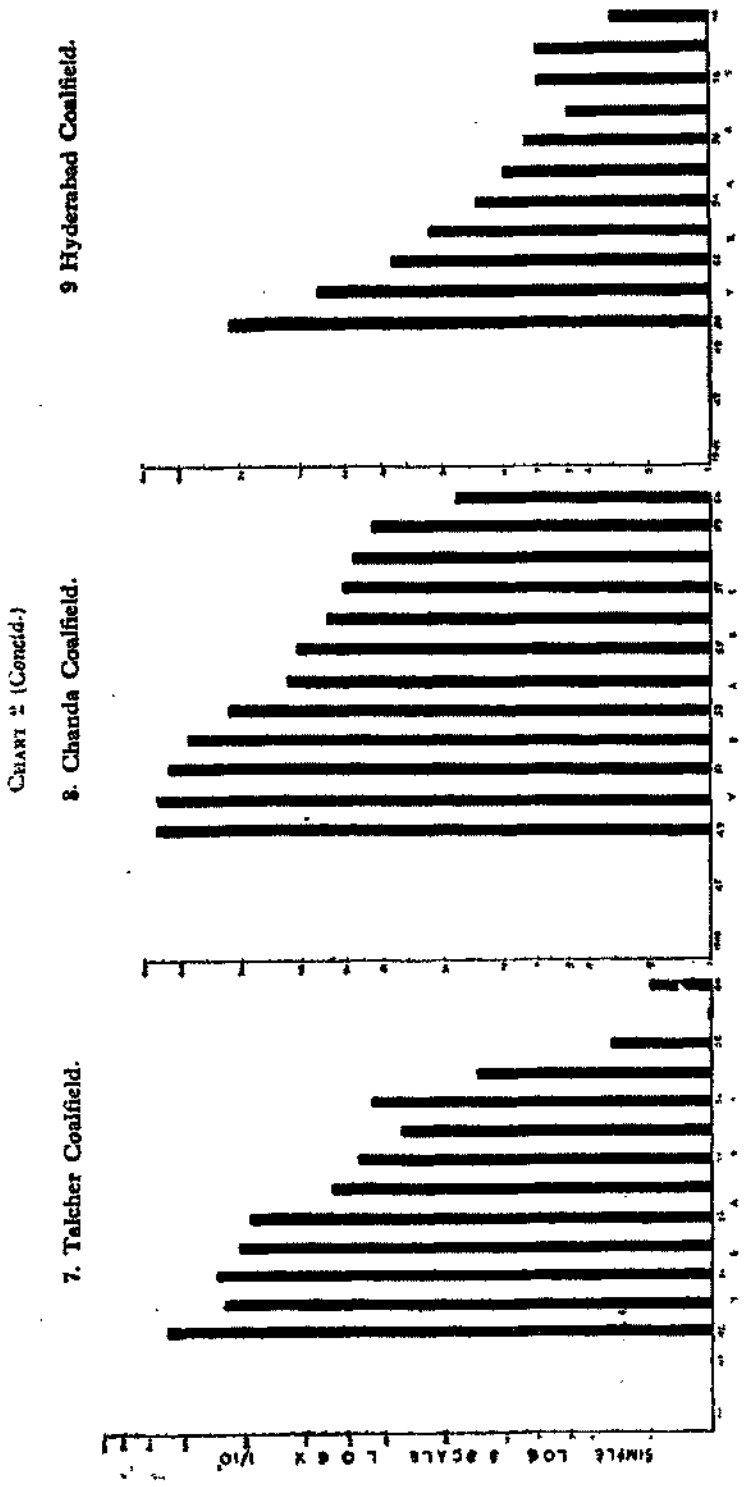

CHART 3.

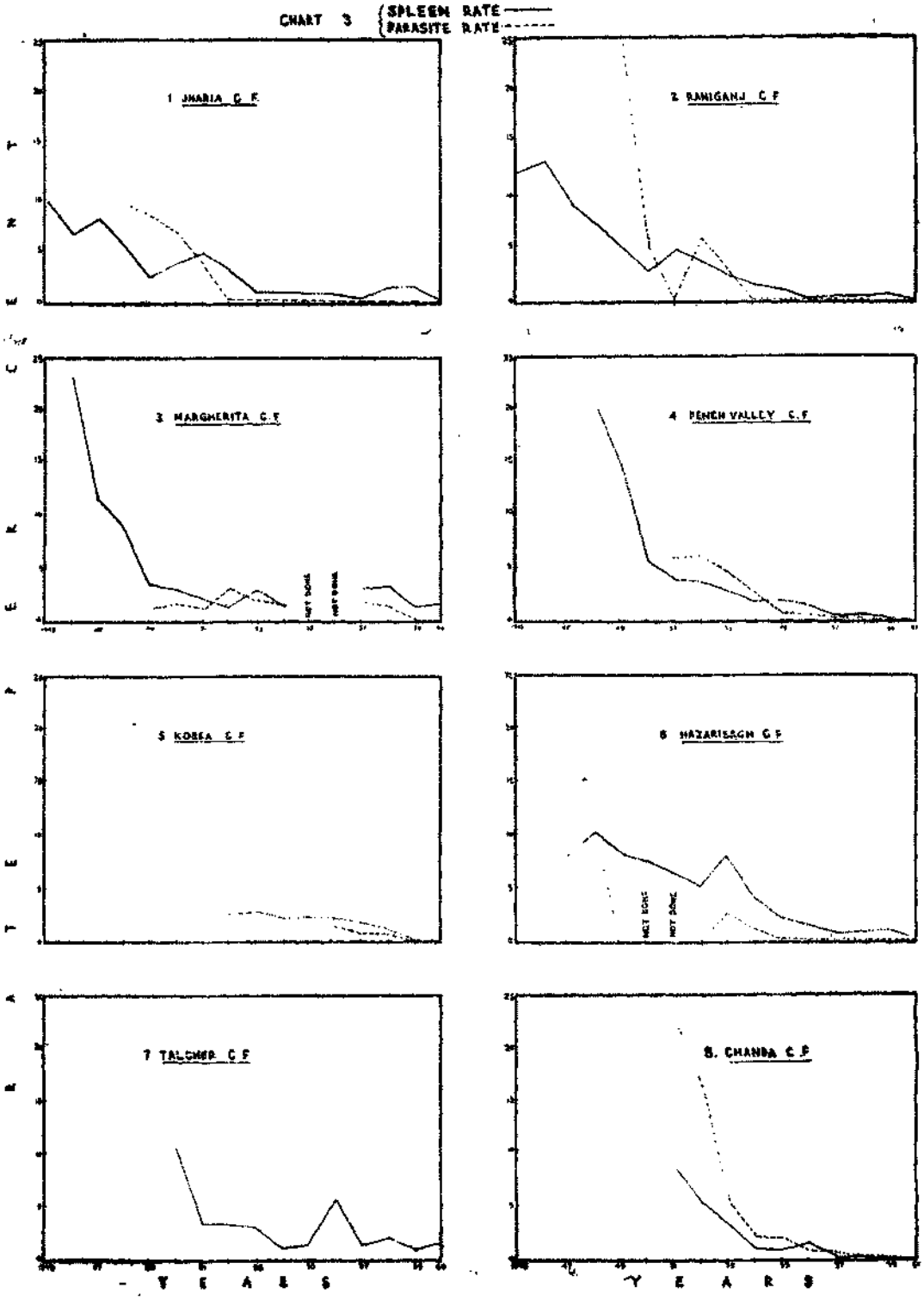
CHART 3
SPLEEN RATE
PARASITE RATE
1 JHARIA C F
2 RANIGANJ C F
3 MARGHERITA C F
4 PENCH VALLEY C F
5 KOREA C F
6 HAZARISAGH C F
7 TALCHER C F
8. CHANDA C F
HOT SONE
HOT SONE
HOT SONE
YEARS
YEARS

TABLE III.

Different malariometric indices from the different coalfields, year-wise, from 1945 to 1960.

Year.	1			2			3			4			5		
	Jharia.			Raniganj.			Margherita.			Pench Valley.			Korea.		
	(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)
1945	266	9·9	..	549	12·4	..	600	..	..	430	..	..	..	..	..
1946	180	6·3	..	540	13·3	..	560	23·0	..	395	..	..	..	..	..
1947	186	7·7	..	425	8·4	..	480	11·2	..	302	..	..	..	..	..
1948	187	5·2	9·4	430	7·4	..	463	7·6	..	262	19·9	..	..	..	..
1949	135	2·4	8·8	241	5·5	25·0	102	3·4	0·8	187	13·8	..	..	..	..
1950	78	3·7	6·9	130	2·8	5·5	32	2·8	1·4	116	5·3	..	310	..	..
1951	60	4·9	3·7	92	4·6	0·3	33	2·3	0·8	101	3·6	5·6	305	..	..
1952	60	3·1	0·4	83	3·7	6·2	26	1·7	2·7	71	3·3	5·4	252	2·6	..
1953	42	1·0	0·3	74	2·4	2·7	44	2·7	1·9	60	2·6	4·4	140	2·7	..
1954	37	1·0	0·1	48	1·5	0·1	50	1·3	1·4	42	1·9	2·1	144	2·3	..
1955	33	1·0	0·2	35	1·1	0·1	35	..	..	34	1·9	0·9	114	2·5	..
1956	31	0·4	0·2	30	0·4	0·2	20	..	..	31	1·3	0·5	104	2·3	1·4
1957	32	0·5	0·2	28	0·5	0·1	15	3·0	1·8	26	0·6	0·3	73	1·9	0·7
1958	20	1·5	0·1	20	0·5	0·0	42	3·8	1·3	21	0·5	0·3	64	1·3	0·7
1959	11	1·7	0·0	18	0·6	0·1	28	1·3	0·0	17	0·1	0·1	32	0·8	0·0
1960	4	0·1	0·0	12	0·1	0·0	37	1·4	0·0	11	0·1	0·1	21	0·3	0·0

Year.	6			7			8			9			10		
	Hazaribagh.			Sambalpur.			Talcher.			Chanda.			Hyderabad		
	(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)
1945	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
1946	203	..	..	..	..	..	..	..	..	..	..	..	..	..	..
1947	209	8·2	..	..	..	..	..	..	..	..	..	..	..	..	..
1948	183	10·4	10·0	..	..	..	478	..	..	..	..	..		..	..
1949	111	8·1	2·5	..	..	..	248	..	..	532	..	..	..	..	..
1950	76	7·6	..	..	..	..	269	10·7	..	524	..	..	219	..	..
1951	62	6·3	..	..	..	..	202	3·4	..	460	8·8	22·0	80	..	..
1952	50	5·1	0·0	..	..	..	187	3·3	..	365	5·3	16·0	36	..	..
1953	50	8·0	2·8	..	..	..	72	2·9	..	223	3·2	5·4	23	..	..
1954	50	4·2	1·2	..	..	..	56	1·0	..	121	1·2	2·2	14	..	..
1955	41	2·3	0·0	..	..	..	33	1·4	..	106	0·8	2·1	10	..	..
1956	32	1·5	0·1	..	..	..	48	5·8	..	74	1·3	1·0	8	..	..
1957	27	0·7	0·0	..	..	..	14	1·4	..	64	0·2	0·6	5	..	..
1958	25	1·0	0·0	..	..	..	3	2·0	..	60	0·1	0·2	7	..	..
1959	25	1·1	0·1	..	..	..	1	0·7	..	45	0·1	0·0	7	..	..
1960	12	0·3	0·0	..	..	..	2	1·1	..	17	0·0	0·0	3	..	..

(..)=Data not available. (a)=Malaria cases per mille. (b)=Child spleen rate (per cent) (c)=Child parasite rate per cent.

Stray instances have been recorded of *P. malariæ* infections only from 3 groups of coalfields. The feature in the Jharia area was apparently different prior to 1945, for Rao (1944) recorded 19 per cent positives in the mass blood survey during the period of high incidence in the Jharia Mining Settlement of which 54 per cent were *P. falciparum*, 35 per cent *P. vivax* and 5·8 per cent *P. malariæ*.

TABLE IV.

Parasite positives for malaria among fever cases from different coalfields, year-wise and specieswise.

Name of the group of coalfields.	Year.	Number of blood smears examined.	Number found positive microscopically.	Plasmodia: *P. vivax*	*P. falciparum.*	*P. malariæ.*	Mixed (*P. vivax* and *P. falciparum*).
1. Jharia	1955	2832	52	32	11	..	9
	1956	2365	56	42	10	2	2
	1957	2111	46	35	5	..	6
	1958	2377	34	30	4	..	..
	1959	3123	32	31	..	..	1
	1960	1940	15	14	1	..	..
Total for 6 years :—		14748	235	184	31	2	18
Percentage :—			2·0	79·0	13·0	1·0	7·0
2. Raniganj	1955	5010	44	39	4	..	1
	1956	4973	33	28	5	..	..
	1957	3499	22	13	8	1	..
	1958	2261	10	8	2	..	..
	1959	2639	21	17	4	..	..
	1960	2155	6	5	1	..	..
Total for 6 years :—		20557	136	110	24	1	1·
Percentage :—			0·5	80·0	18·0	1·0	10
3. Margherita	1958	42	4	2	2	..	..
	1959	114	24	3	21	..	..
	1960	288	60	17	43	..	..
Total for 3 years :—		444	88	22	66	..	..
Percentage :—			20·0	25·0	75·0	..	..
4. Pench valley	1955	2132	141	125	2	..	14
	1956	2441	148	135	..	2	11
	1957	2135	123	106	1	2	14
	1958	1358	14	13	...	..	1
	1959	1806	4	4	..	..	..
	1960	1982	2	2	..	..	..
Total for 6 years :—		11854	432	385	3	4	40
Percentage :—			4·0	88·0	1·0	1·0	10·0
5. Korea	1958	48	3	3	..	..	..
	1959	20	1	1	..	..	..
	1960	59	1	1	..	..	..
Total for 3 years :—		127	5	5	..	..	..
Percentage :—			4·0	100·0	..	..	..

TABLE IV (*Contd.*).

Name of the group of coalfield.	Year.	Number of blood smears examined.	Number found positive microscopically.	PLASMODIA : *P. vivax.*	*P. falciparum.*	*P. malariae.*	Mixed (*P. vivax* and *P. falciparum*)
6. Hazaribagh	1955	757	35	20	11	..	4
	1956	806	23	16	6	..	1
	1957	646	21	12	8	...	1
	1958	504	12	10	2	..	..
	1959	709	24	20	4	...	..
	1960	579	12	12	..	..	..
Total for 6 years :—		4001	127	90	31	..	6
Percentage :—			3·0	71·0	25·0	...	4·0
7. Talcher	1957	89	8	8	..	..	..
	1958	44	2	2	..	..	..
	1959	191	3	1	1	..	1
	1960	328	7	3	4	..	..
Total for 4 years :—		652	20	14	5	..	1
Percentage :—			3·0	70·0	25·0	..	5·0
8. Chanda	1957	256	15	14	1	..	..
	1958	412	14	14	...	..	..
	1959	353	5	5	..	..	..
	1960	225	1	1	..	..	..
Total for 4 years :—		1246	35	34	1	...	..
Percentage :—			3·0	99·0	1·0	..	..

(*b*) *Infant parasite index.*—Infant blood smears have been collected and examined since 1957 (Table V). Only 5,369 infant blood smears have been collected over a period of 4 years from 8 groups of coalfields. Positive infant bloods have been encountered only from the Pench Valley coalfields in 1957-58 while in the rest of the coalfields the results were negative. The numbers have been particularly insufficient from the Margherita coalfields. This index has not been followed systematically, as such no discussion is possible.

In the Sambalpur and the Hyderabad coalfields, no infant blood smears have been collected.

ENTOMOLOGY.

Anopheline factor.—The entomological investigations have not been of the required standard. Adult mosquito collections were made on a limited scale during the early period of this study in quite a few coalfields (Table VI). The record is based on sampling in close proximity of coalmines in a few localities.

TABLE V.

Infant blood smears examined for malaria parasites in the coalfields of India during the period 1957 to 1960.

(Done in between spraying intervals only, with the existing staff).

Name of the group of coalfields.	Years.	Number of infant blood smears examined.	Positives, if any.	Infant parasite rate per cent.	Remarks.
Jharia ...	1957—1960	1054	None	0·00	
Raniganj ...	—do—	1210	None	0·00	
Margherita ...	—do—	251	None	0·00	
Pench Valley	—do—	1487	5	0·34	
Korea ...	—do—	306	None	0·00	Positives detected in the years 1957-1958 only and parasite rate for 2 years only 0·53.
Hazaribagh ...	1958—1960	469	None	0·00	
Sambalpur ...		No data	No data		
Talcher ...	1957—1960	178	None	0·00	
Chanda ...	1957—1960	414	None	0·00	
Hyderabad ...		No data	No data		

TABLE VI.

Anopheline species recorded from the major groups of coalfields of India.

Serial number	Species of anopheline	Group of coalfields.				
		Jharia.	Raniganj.	Margherita.	Pench Valley.	Hazaribagh.
1	*A. aconitus*	—	+	—	+	—
2	*A. aitkeni*	—	—	+§	—	—
3	*A. annularis*	+	+	+	+	+
4	*A. barbirostris*	+	+	+	+	—
5	*A. culicifacies*	+	+	—	+	+
6	*A. fluviatilis*	+	+	—	+	+
7	*A. gigas*	—	—	+†	+	—
8	*A. hyrcanus*	+	+	+	+	+
9	*A. insulæflorum*	—	—	+§	—	—
10	*A. jamesi*	—	+	—	+	—
11	*A. jeyporiensis*	—	—	—	+	+
12	*A. karwari*	—	+	+	—	—
13	*A. kochi*	—	—	+	—	—
14	*A. leucosphyrus*	—	—	+†	—	—
15	*A. maculatus*	—	—	+	+	—
16	*A. moghulensis*	—	—	—	+	—
17	*A. minimus*	—	—	+	—	—
18	*A. pallidus*	+	+	+	+	+
19	*A. philippinensis*	+	+	+	—	+
20	*A. ramsayi*	—	+	—	—	+
21	*A. splendidus*	+	+	—	+	+
22	*A. stephensi*	+	—	—	+	+
23	*A. subpictus*	+	+	+	+	+
24	*A. tessellatus*	—	+	—	+	—
25	*A. theobaldi*	—	—	—	+	—
26	*A. turkhudi*	—	—	—	+	—
27	*A. umbrosus*	—	—	+	—	—
28	*A. vagus*	+	+	+	+	+
29	*A. varuna*	+	+	—	—	—

N.B. Records according to monthly reports for 1946-1948.

— = not collected. ; + = collected. ; † = recorded as larvæ only. ; § = recorded as larvæ also.

Altogether 29 species of anophelines have been recorded from the five larger coalfields in India. The 12 species of anophelines recorded from the Jharia coalfields are identical to those recorded by Sen *et al.* (1960) in the 5 years of study (1953-58) from the then unsprayed area near Dhanbad. Rao (1944 *loc. cit.*) in his study between 1938-1943, recorded 14 species of anophelines which include the present record of anopheline fauna of the Jharia Mining area, except *A. varuna*, but with the addition of *A. jamesi*, *jeyporiensis*, and *tessellatus*. The same author also collected larvae for 3½ years up to 1943, and recorded 14 species, including all those recorded as adults except *A. tessellatus*, but with the addition of *A. maculatus*.

Of the 29 species of anopheline mosquitoes, 19 are common while the remaining ten, viz., *aitkeni*, *gigas*, *insulaeflorum*, *kochi*, *leucosphyrus*, *moghulensis*, *minimus*, *ramsayi*, *theobaldi* and *umbrosus* are rare.

Very little is known of the bionomics of the anopheles of coalfields area. Rao (1944) recorded *A. fluviatilis* in maximum numbers from human dwellings in the Jharia Mining Settlement whereas Senior White (1943) recorded *A. fluviatilis* in high numbers from cattle-sheds in the Hazaribagh coalfields (Ramgarh). The observations of Sen *et al.* (1960) from near Dhanbad also conform to those of Senior White. In the experience of the present authors, the *A. fluviatilis* from the Dhanbad area is more zoophyllic as all the 97 blood meal samples tested were of bovine origin. Collections of this species were also maximal from cattle-sheds.

Vectors of malaria.—Senior White and Adhikari (1940) recorded infected *A. pallidus* in Chanda district.

Senior White (1943 *loc. cit.*) found *A. fluviatilis* positive in the Hazaribagh hilly range (a little away from the Hazaribagh coalfield area). Rao (1944) incriminated *A. fluviatilis* as primary vector and *A. stephensi* as secondary and described *A. annularis*, *culicifacies*, and *pallidus* as poor vectors, *i.e.*, possibly potential vectors in the Jharia Mining Settlement. Sen *et al.* (1960) could not incriminate any vector, obviously because of control measures in the vicinity over a prolonged period. The same authors also observed that *A. fluviatilis* was prevalent in high densities in Dhanbad plains, preferring cattle-sheds as day-time resting places. Dissections of over 2,200 specimens of *A. fluviatilis* between 1949-1958, at the Jharia Malaria Laboratory, were negative for malaria infection. They suggested that further study was needed before concluding whether the species has changed its habits in the course of a decade or that the species prevalent is of a different biotype.

Chowdhury (1953) observed that in coalfields of India the established vectors are *A. minimus* in the Margherita coalfields and *A. culicifacies* in Jharia. However in the coalfields of Raniganj, Pench Valley and Hazaribagh *A. culicifacies* was also presumed to be a vector.

DISCUSSION.

An analysis of the data of malaria endemicity, in the different coalfields of India, shows that in the major groups of coalfields that constitute over 85 per cent

of the entire coal mines of India (constituting 90 per cent of the population of the coalfields) all the three malario-metric indices were high in 1945. Slight reduction in the endemicity was, however, noticed as a result of larvicidal measures. Actual systematic control by spraying of residual insecticides commenced in 1947-48 and resulted in appreciable decrease in the three indices in three years.

In the smaller groups of coalfields, *viz*, Korea, Talcher, Chanda and Hyderabad, which acceeded to the scheme between 1950-52, it took two years of intensive control measures to have any appreciable reduction in the malaria indices.

Thus in all the coalfields, reduction in the three indices is evident from 1953-54, after 3 to 5 years of continuous indoor residual insecticidal spraying. The lowest levels in these indices were, however, achieved by 1959-60. Malaria rates in Margherita continued to fluctuate, adding support to the hyperendemic conditions that prevailed there earlier.

From the two smaller groups, *i.e.*, Sambalpur and Hyderabad, adequate or continuous data are not available.

SUMMARY AND CONCLUSIONS.

The malaria endemicity in the coalfields, the control measures adopted, and the success obtained in controlling it, are described in detail.

Except for the Margherita area, where the malaria morbidity was high due to the location of the coalfield in hyperendemic zone, in the rest of the coalfields, the indices progressively fell by 1954 and reached a very low level by 1959-60.

Malaria eradication programme was launched in the coalfields in 1958, basing the attack phase lasting for 3 years (1958-59, 1959-60 and 1960-61). Two rounds of DDT spraying at a dosage of 100 mg. sq.ft. per round and totality of coverage were the main features of the attack phase.

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APPENDIX I.

Full complement of Staff of the Coalfields Unit of Malaria Control/Eradication as it stood in the year 1960.

Government of India
(Ministry of Labour)
Coal Mines Welfare Commissioner

Chief Malaria Officer
(Coalfields),
Dhanbad.

- 1 Head Clerk
- 1 Senior Clerk
- 1 Stenographer
- 1 Storekeeper
- 1 Accountant
- 1 Clerk
- 1 Draftsman
- 2 Peons

- 1 Entomologist
- 1 Laboratory Assistant
- 1 Insect Collector

Raniganj (West Bengal)

- 1 Malaria Officer
- 1 Additional Malaria Officer

- Malaria
- tor
- ia Inspectors
- ia Field Workers

- countant
- orekeeper
- erk
- ter
- ivers
- ons
- owkidar

- 9 Malaria Inspectors
- 8 Malaria Sub-Inspectors
- 7 Insect Collectors
- 5 Laboratory Assistants
- 12 Senior Malaria Field Workers
- 82 Malaria Field Workers

Jharia (Bihar)

- 1 Malaria Officer
- 1 Additional Malaria Officer

- 1 Accountant
- 1 Storekeeper
- 1 Clerk
- 7 Drivers
- 1 Fitter
- 2 Peons
- 1 Chowkidar

- 1 Assistant Entomologist
- 8 Malaria Inspectors
- 12 Malaria Sub-Inspectors
- 5 Laboratory Assistants
- 5 Insect Collectors
- 9 Senior Malaria Field Workers
- 77 Malaria Field Workers

Hazaribagh (Bihar)

- 1 Malaria Officer

- 1 Accountant
- 1 Storekeeper
- 1 Clerk
- 4 Drivers
- 2 Fitters
- 1 Peon
- 1 Chowkidar

- 4 Malaria Inspectors
- 3 Malaria Sub-Inspectors
- 2 Laboratory Assistants
- 4 Insect Collectors
- 5 Senior Malaria Field Workers
- 42 Malaria Field Workers

Chanda (Maharashtra)

- 1 Assistant Entomologist
- 1 Malaria Inspector
- 1 Malaria Sub-Inspector
- 1 Insect Collector
- 11 Malaria Field Workers

Korea (Madhya Pradesh)

- 1 Senior Malaria Inspector

- 1 Clerk
- 1 Malaria Sub-Inspector
- 1 Driver

- 2 Malaria Inspectors
- 16 Malaria Field Workers

Pench Valley (Madhya Pradesh)

- 1 Malaria Officer

- 1 Storekeeper
- 1 Clerk
- 1 Driver
- 1 Peon
- 1 Fitter
- 1 Chowkidar

- 3 Malaria Inspectors
- 1 Laboratory Assistant
- 2 Insect Collectors
- 24 Malaria Field Workers

Sambalpur (Orissa)

- 1 Insect Collector
- 4 Malaria Field Workers

Talcher (Orissa)

- 1 Senior Malaria Inspector
- 1 Insect Collector
- 5 Malaria Field Workers

Note : Staff at Hyderabad is maintained by the respective collieries

APPENDIX II.

Malaria control operatians in the Coalfield Unit — Chronological record.

Serial number.	Name of the coalfields group.	State in which located.	CHRONICLE		Agency of malaria control.	Nature of malaria control.
			From.	To.		
1	Jharia	Bihar	1938	May, 1944	Jharia Mines Board of Health	Small scale anti-larval work.
			June, 1944	November, 1945	Army Malaria Control Unit	Intensive larval control.
			December, 1945	December, 1950	Asst. Directorate (Coalfields Branch) Malaria Institute of India	Intensive larval control up to 1947 and then onwards D.D.T. as adulticide (in all coalfields).
			January, 1951	1960 and to-date	Coalfields Malaria Control Eradication Unit under the Chief Malaria Officer (Coal Mines Welfare Organisation)	Mainly adulticides using D.D.T. and B.H.C., supplemented by minor larvicidal work.
2	Raniganj	West Bengal	1931	May, 1944	Asansol Mines Board of Health	Small scale antilarval work.
			June, 1944	Onwards to-date	Same as in the Jharia coalfields	As in the Jharia coalfields.
3	Margherita	Assam	1941	1942	Under Coal Co. and the Assam Medical Research Council.	Small scale anti-larval work.
			1943	November, 1945	Army Malaria Control Unit	Oiling, pyrethrum spraying and even D.D.T. at the fag end of World War II.
			December, 1945	To-date	Same as in the Jharia coalfields.	As in the Jharia coalfields.
4	Pench Valley	Madhya Pradesh	June, 1944	1960 and to-date		-do-

(Continued)

APPENDIX II (*Concld.*).

Serial number.	Name of the coalfields group.	State in which located.	CHRONICLE : From.	CHRONICLE : To.	Agency of malaria control.	Nature of malaria control.
5	Korea	Madhya Pradesh	1945	1951	Under the Malariologist, Bengal-Nagpur Railway.	Mainly anti-larval work, later supplemented by D.D.T. as adulticide.
			January, 1952	Onwards to-date	Same as in the Jharia coalfields.	As in the Jharia coalfields.
6	Hazaribagh	Bihar	1947	December, 1950	-do-	1947 onwards, D.D.T. as adulticide in all the coalfields, supplemented by minor larvicidal work.
			1951	1960 and to-date		
7 8	Sambalpur Talcher	Orissa	1950	1960 and to-date	do-	-do-
9	Chanda	Maharashtra	1950	1960 and to-date	do-	-do-
10	Hyderabad	Andhra Pradesh	1952	1960 and to-date	-do-	-do-

1947 :—D.D.T. was used as residual insecticide (adulticide) in limited areas.

1948-1953 :—In all the coalfields, D.D.T. was used as indoor residual spray @ 50 mg. per sq. foot, 3 rounds a year, during the presumed transmission season (July—November) at 6 to 8 weeks interval.

1954-1958 :—The Nation-wide Malaria Control Programme.
D.D.T. 100 mg. per sq. foot, 2 rounds a year, with totality of coverage.

1958-1959 :—Attack phase of National Malaria Eradication Programme launched.

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INVESTIGATIONS ON PLAGUE IN KOLAR DISTRICT (MYSORE STATE).

Part I.

Studies on some aspects of epidemiology.

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INTRODUCTION.

PLAGUE was a major pestilence, the ravages of which filled many dark pages in the history of communicable diseases. The total number of deaths recorded from this disease in India alone, from 1896 to 1918, was estimated to be more than ten millions (Seal, 1960).

A comprehensive review of the present knowledge of plague, with special reference to India, was published by Pandit and Krishnaswami (1963). A study of mortality due to plague in India during the last six decades revealed that there was steady and significant decline in the specific case mortality due to plague from 183·3 per 100,000 in the first decade (1898-1908) to 1·8 per 100,000 during the period 1949-1958 (Seal and Patnaik, 1963). Detailed analysis of deaths due to plague during the last ten years in the different States of India (Seal and Patnaik, 1963 *loc. cit.*) brings out that the disease has persisted in a few areas, particularly in the States of Madras and Mysore. These two States have not been free from human plague cases during any of these years. Of the three areas endemic for plague in India, *i.e.* (*i*) in North India along the foot of the Himalayas in the Punjab, Uttar Pradesh and Bihar, (*ii*) in Central India along the Central India hilly tracts, chiefly in Madhya Pradesh, and (*iii*) in the Deccan plateau of Peninsular India in Andhra Pradesh, Madras, Maharashtra and Mysore States (Sharif, 1951), the last has been having human cases continuously since 1930.

A team from the National Institute of Communicable Diseases was deputed in December, 1962, to help the Mysore State Government to investigate plague situation in Kolar District and plan control measures. The team worked in the area from December, 1962 to April, 1963, and studied the epidemiology of the

disease, the rodents (chiefly domestic) and the rat-flea fauna in the district*. The susceptibility status of the fleas to some of the chlorinated hydrocarbon insecticides, and the efficacy of different insecticides in flea control, were also studied. The observations on the epidemiology are reported in this paper.

TERRAIN AND TOPOGRAPHY OF KOLAR DISTRICT.

Kolar District in Mysore State (Map 1) is situated over an area of about 3,188 square miles on the Deccan plateau. Most of the area is hilly. The district is divided into eleven taluks (Map 2) and has a population of about 12 lakhs (1961 census). The rainfall is low, the average annual precipitation being about 28-29 inches. The monsoon usually starts from mid-July and lasts till October or November, with a peak in September-October.

The population is mostly rural, the urban areas being limited mainly to the taluk headquarters. The Kolar Gold Fields are located in Bangarpet, one of the southern taluks. The inhabitants are mostly indigenous, though the gold-mines area has a good percentage of workers from the adjoining States of Madras and Andhra Pradesh. The general living standard of the residents outside the mining area is poor. Most of the houses, particularly in the rural areas, have one or two multipurpose rooms, often with inadequate ventilation and poor lighting.

HISTORY OF PLAGUE IN MYSORE STATE.

Karve and Sundararajan (1935) recorded that plague first made its appearance in Mysore State in August, 1898. The infection was traced in a railway passenger coming from Bombay State where an outbreak was prevalent at the time. Plague appears to have persisted in Mysore State since then. Available records show that cases of plague were reported from all the districts of the State during the early fifties. Since 1952, however, the incidence recorded a gradual decline and plague disappeared except in Mysore and Kolar districts, and subsequently Kolar has remained the problem district.

A persisting focus of plague has continued to exist in Kolar District. The annual record of attacks and deaths from plague in the district during the last 34 years (1930 to 1963) is depicted in Table I and Graph 1. A cyclical periodicity is noticed in the occurrence of plague cases, a rise in the incidence being noticed almost with a rhythmic peak every 7-8 years. The monthly data regarding plague in the districts of Kolar (Mysore), Salem (Madras) and Chittoor (Andhra Pradesh), adjoining the inter-state borders, are incorporated in Table II.

Of the eleven taluks in the district, plague has been reported only from seven taluks since 1959, *i.e.*, Malur, Bangarpet, Kolar, Mulbagal, Srinivaspur, Chintamani and Sidlaghatta. Talukwise analysis is not available for earlier years.

The control measures till about a decade ago consisted mainly of the conventional procedures like cynogas fumigation of burrows, anti-plague inoculation in

Krishnaswami, A.K., Krishnamurthy, B.S., Ray, S.N., Singh, N.N. and Chandrahas, R.K. (1963) *Ind. Jour. Mal. 17*, 2-3, pp. 193-203.

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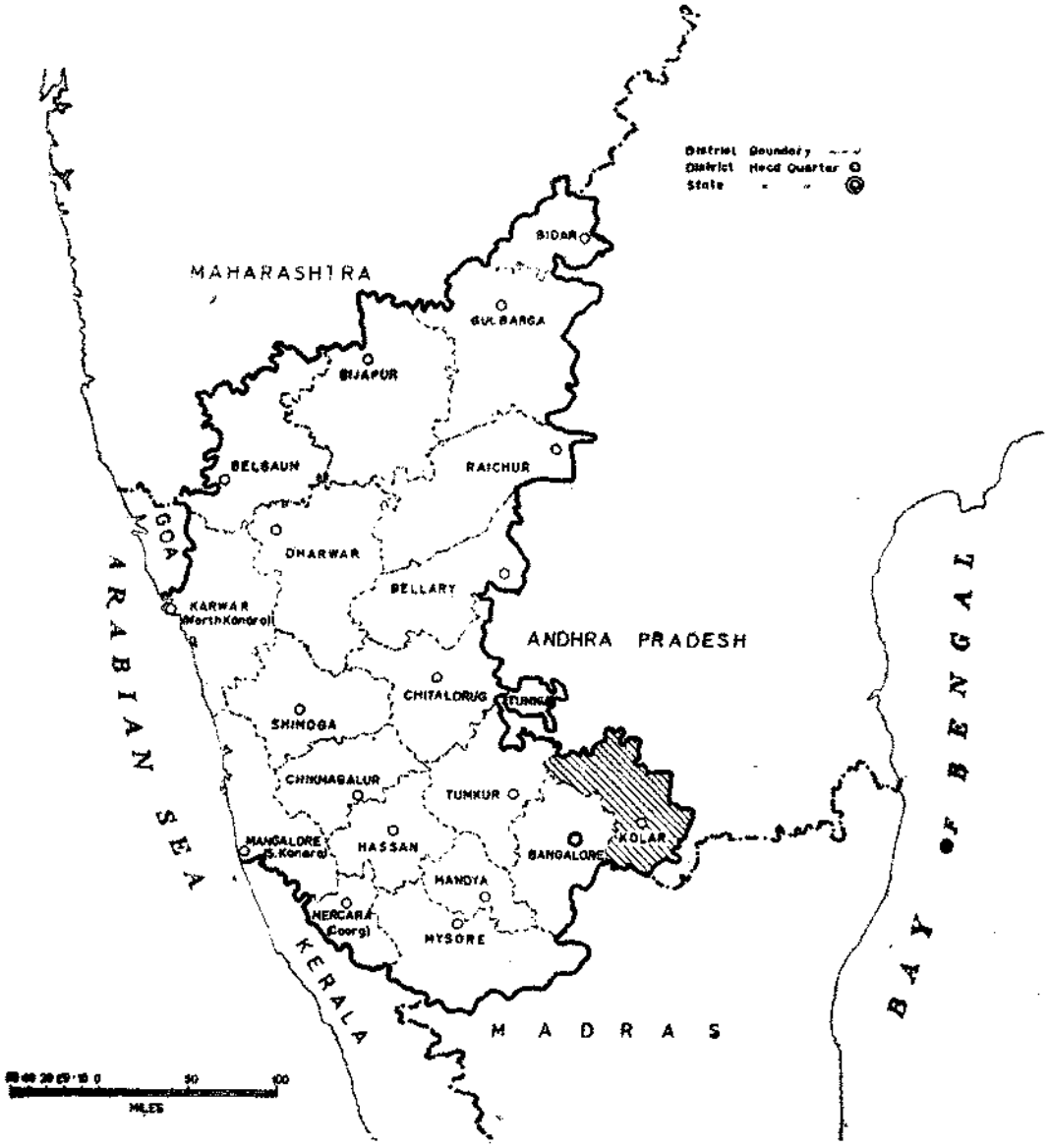
areas where rat-falls/plague cases were reported and treatment of cases with sulpha drugs. With the advent of the residual insecticides, spraying and insufflation of rat-burrows with residual insecticides was resorted to on a limited scale in localised areas, and treatment of cases with streptomycin in addition to the measures already in force.

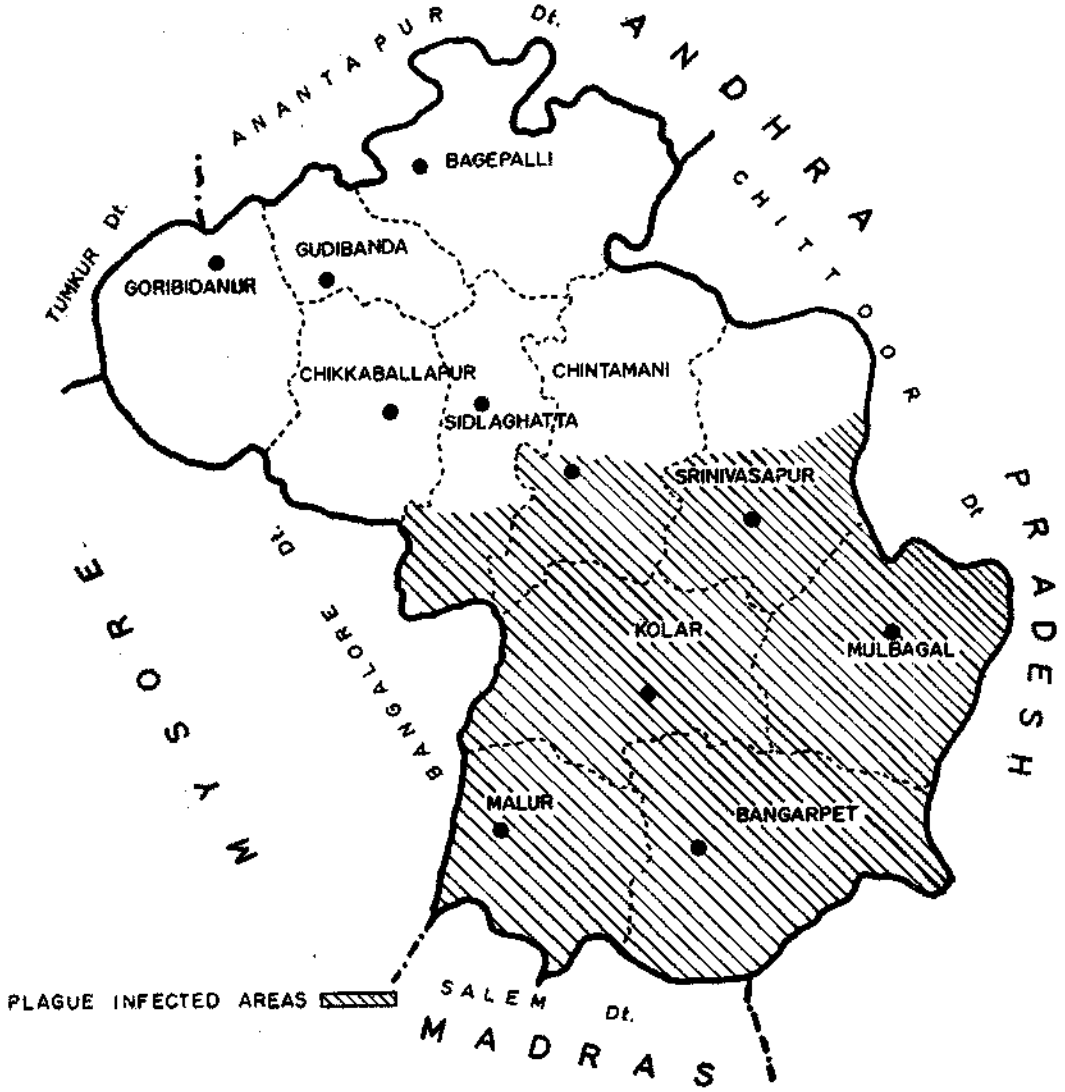
MAP 1.

MYSORE STATE—Showing the different Districts.



MAP 2.

Kolar District showing the different taluks and adjoining States.

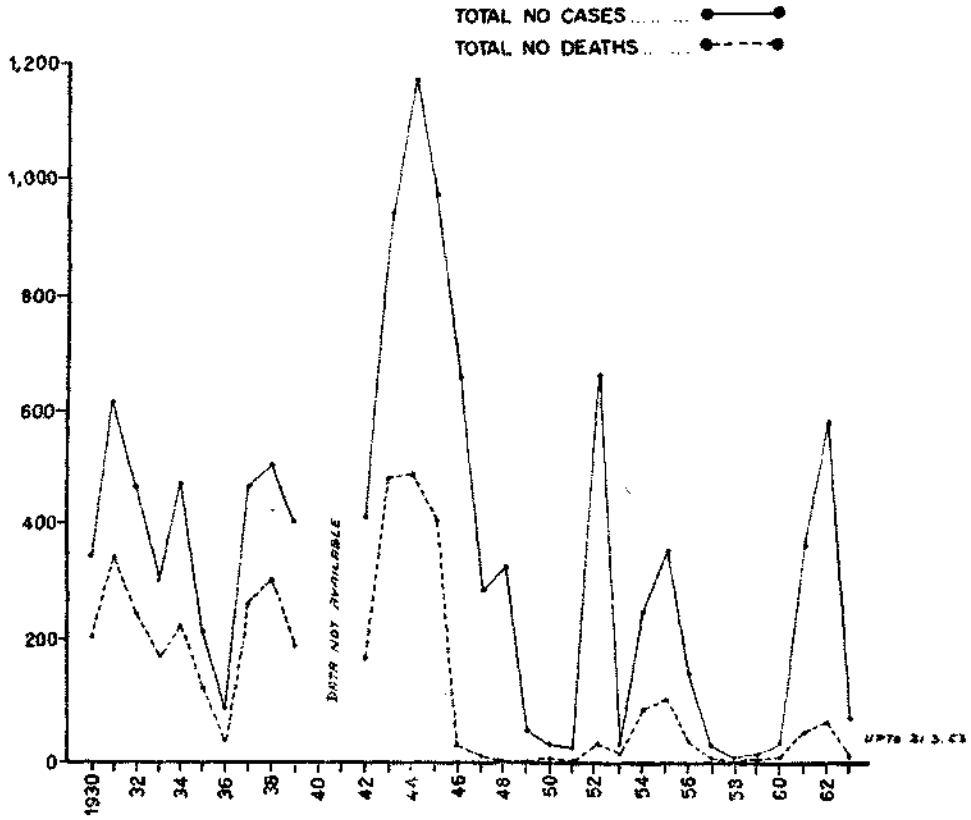


The implementation of the National Malaria Control Programme in 1953 and later of the Eradication Programme in 1958-59 brought the entire Kolar District under DDT house spray. Being an endemic area for malaria, two rounds of spray were applied annually—according to the logistics of the National Malaria Eradication Programme. The case incidence of plague in the district registered a sharp decline to 5 in 1958 and rose to 12 in the following year. This lull in the record

of human cases was, however, transient and was followed during the subsequent years by a steady rise to 30, 373 and 585 cases during 1960, 1961 and 1962. Whatever effect the district-wide DDT spray had on the rat-fleas and on plague transmission, appeared to have waned and the transmission again started as during the pre-National Malaria Eradication Programme years. The resurgence of plague occurred in spite of the DDT spraying under the National Malaria Eradication Programme which was discontinued only with effect from 1962.

GRAPH I.

Annual incidence of cases and deaths due to plague in Kolar District, Mysore State (1930—1963).



THE PRESENT PROBLEM.

It was obvious that plague was persisting in Kolar District, and the incidence of human cases had been progressively on the increase since 1960. During 1962, there were 585 cases with 67 deaths from 103 villages in seven of the eleven taluks in the district.

TABLE I.

Annual distribution of cases and deaths in Kolar District (1930-1963).*

Year	Total number of cases	Total number of deaths	Year	Total number of cases	Total number of deaths
1930	344	203	1947	290	6
1931	619	346	1948	329	Not available
1932	467	249	1949	50	-do-
1933	302	178	1950	26	3
1934	475	227	1951	21	Not available
1935	215	123	1952	664	31
1936	85	34	1953	31	18
1937	470	265	1954	253	90
1938	508	305	1955	360	105
1939	406	193	1956	149	38
1940	Not available		1957	25	7
1941	-do-		1958	5	1
1942	418	176	1959	12	4
1943	944	483	1960	30	7
1944	1179	192	1961	373	53
1945	976	408	1962	585	67
1946	661	m26	1963 (up to March 21, 1963)	77	10

* Data obtained from the District Health Officer, Kolar.

TABLE II.

Monthly incidence of plague in the district of Kolar (Mysore), Salem (Madras), and Chittoor (Andhra Pradesh), for the years 1962 and 1963.*

Month	1962										1963					
	Kolar		Bangalore		Salem		Coimbatore		Chittoor		Kolar		Salem		Chittoor	
	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D
January	34	8	..	..	..	..	..	..	..	..	24	2	..	..	10	2
February	40	9	..	..	..	..	..	..	4	1	58	7	14	3	7	..
March	79	6	..	..	..	..	..	..	3	1	18	1	6	1	12	2
April	26	6	..	..	..	..	..	..	..	..	4	..	..	..	..	..
May	4	..	..	..	5	1	.	..	..	..	..	..	3	1	..	..
June	39	4	..	..	17	3	..	..	..	..	..	..	5	1	..	..
July	64	4	4	1	6	4	..	..	..	..	12	..	..	..	..	..
August	77	9	..	..	28	5	..	..	7	..	5	..	..	..	..	..
September	67	7	..	..	18	4	..	..	4	..						
October	69	7	..	..	..	..	..	..	..	..						
November	50	5	..	..	6	1	3	..	1	1						
December	29	1	..	..	..	..	..	..	..	..						
Total	563	65	4	1	80	18	3	..	19	3						

* Data obtained from the Central Bureau of Health Intelligence

C = Number of cases.

D = Number of deaths.

Sporadic cases of plague continued to occur in the Hosur Taluk of Salem District (Madras State), and since 1959 the number of cases had steadily gone up in this area also. One hundred cases were recorded in 31 villages of the taluk in 1962, with 20 deaths. In recent months, cases of plague were reported from Palamaner and Punganur taluks of Chittoor District (Andhra Pradesh). Thus the three adjoining districts of the Mysore, Madras and Andhra Pradesh States have active foci of plague.

A peculiarity that struck the observers was that the cases were all mild and ambulent and did not look toxic at all. Acute illness and confinement to the bed was more an exception. This was the case irrespective of the vaccination status of the affected persons. The clinical picture of the cases made one even suspect whether they were real cases of plague till they were confirmed by bacteriological examination. Consistent history, however, was always available that prior to the human attacks, there were rat-falls in the house concerned or in the neighbourhood. There was no panic among the general public and notification of the rat-falls or the human cases was also not prompt.

THE PRESENT INVESTIGATIONS

The investigations planned for studying the causes for the persistence of plague in the area were planned in two phases. The first phase, which was a short term investigation, covered studies on the epidemiology of plague, including studies on the domestic rodent fauna and the rat-fleas. Studies also included observations on the susceptibility status of the rat-fleas and evaluation of the efficacy of some of the newer insecticides in plague control. The second phase of the investigations was planned on a long term basis, mainly to unravel the existence of the sylvatic rodents in the maintenance of the reservoir of the infection and their infecting the domestic rodents. The investigations under the latter phase are proposed to be taken up later*.

The studies detailed under the first phase were completed during a period less than four months (from 11th December, 1962 to 29th March, 1963). This was facilitated by the establishment of a number of field laboratories which reduced the time and distance for the transportation of material collected in the field. Bacteriological examination of the material obtained from human cases, rodents and fleas was carried out chiefly at the State Public Health Laboratory at Bangalore and partly at the field laboratory† established at Kolar.

The epidemiological investigations carried out covered the following aspects :

(i) Analysis of the available data regarding the occurrence of plague cases since its resurgence in 1959 ;

* Arrangements have been made (as this paper goes to the press) to commence these investigations from January, 1964.

† The Director, Indian Council of Medical Research, arranged with the Director, Haffkine Institute, Bombay, for the services of two Bacteriologists (Mr. Y.S. Nimbkar and Dr. M.K. Habbu) for establishing the temporary field laboratory at Kolar.

(*ii*) Studies on the domestic rodents ;

(*iii*) Studies on the rat-fleas ; and

(*iv*) Bacteriological studies.

(i) ANALYSIS OF AVAILABLE EPIDEMIOLOGICAL DATA.

Following a decline almost to the point of total disappearance during the year 1958, the number of cases started increasing during the following year. All the twelve cases in 1959 were confined to two villages in the taluk of Malur, where no cases had been reported during the previous year. It is interesting to note that starting from two villages in this southern-most district in 1959, there has been a progressive increase in the number of villages affected during the following years, located in the other taluks in the district. A talukwise analysis of the number of villages affected (Table III) reveals that a larger number of villages in the taluks, along the inter-state borders, got involved with centrifugal spread to the villages away from the State borders.

TABLE III.

Taluk-wise analysis of the number of plague cases in the villages of Kolar District (1959-1962).

Taluk.	NUMBER OF VILLAGES AFFECTED DURING THE YEAR :			
	1959.	1960.	1961.	1962.
Malur	2	7	22	21
Mulbagal	0	1	20	33
Bangarpet	0	1	7	22
Kolar	0	0	6	15
Srinivaspura	0	1	6	7
Sidlaghatta	0	0	2	2
Chintamani	0	0	0	3

Data were available from nineteen villages regarding the location of the houses where the human plague cases had occurred. In sixteen of the villages, it was observed that the first case had occurred in the houses in the periphery and immediately adjoining the fields. Subsequent cases were reported from the houses in the interior. While in three of these villages the case incidence did not proceed beyond the first house in the outlying area, no such correlation was noticed in the other three villages. It is not possible at this stage to say whether this observation is of any significance and whether the final transfer of infection was from the peri-domestic to the domestic rodents in houses situated at the periphery of the villages which subsequently led to the human infection.

SEASONAL INCIDENCE.

Monthly data on the morbidity and mortality due to plague are presented in Table IV. Transmission appears to be in progress almost during all the months

though the intensity shows wide variations. The main bulk of cases occurred during the months June to October, with the peak in August-September. During some years a rise in the number of cases was noticeable during March, indicating a spring transmission.

SEX AND AGE INCIDENCE.

Detailed information was collected regarding the sex and age of 322 of the human cases which occurred during 1962 and 1963 (Table V). More than 68 per cent (222 out of 322 cases were among the age-groups 6 to 30 years. One hundred and twenty-one (37·7 per cent) of the 322 cases were among males and the rest (62·7 per cent) were among females. The higher incidence of cases among females can probably be explained on the ground that they remain indoor for a greater time and thereby the risk of exposure is definitely enhanced. Seal and Pattnaik (1963 *loc. cit.*) observed that the incidence curve was similar in both sexes, the majority of cases being recorded between 6 and 30 years, with a peak incidence in the age-group 11 to 20 years.

CLINICAL FEATURES OF PLAGUE CASES IN KOLAR.

A number of plague cases were examined during the investigations. Usually the inguinal, axillary and cervical glands were affected.

BACTERIOLOGICAL INVESTIGATIONS.

Because of the mild and ambulent nature of the cases and the low case mortality it was possible for one even to suspect whether *P. pestis* was the aetiological agent. The history of rat-falls which always preceded the human cases, and the response of the cases to the usual chemotherapeutic agents, streptomycin and sulphadiazine, however, dispelled any such doubts. It was, however, considered essential to isolate the organisms and study their virulence. Material from human cases, rats and fleas were examined.

(*a*) *Bubo punctures.*—The Medical Officers in charge of the Primary Health Centres, Local Fund Dispensaries, were trained for obtaining bubo-puncture material from suitable cases whenever they were called upon to attend to a case, and forward the material for bacteriological examination. They were provided with Agar slants and broth tubes for this purpose. Material from buboes was obtained in 22 human cases during the period of examination. *P. pestis* was isolated from three of these cases.

(*b*) *Rat tissues.*—A large number of rats, collected during the investigations, were subjected to bacteriological examination. The strangulated rats, after removing the fleas, were subjected to detailed post-mortem examination. Over 2,000 rats of different species were subjected to such examination, but no macroscopic pathological changes were observed in any. Small portions of their viscera (spleen, liver and heart) were taken in sterile normal saline. Ninety-seven such pooled

TABLE IV.

Monthly data on morbidity and mortality due to plague in Kolar District (Mysore State).

Year.	JAN.		FEB.		MARCH		APRIL		MAY		JUNE		JULY		AUG.		SEP.		OCT.		NOV.		DEC.	
	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D	C	D
1954	2	2	5	3	3	2	8	4	*Nil*	*Nil*	33	11	35	9	32	6	60	22	46	20	12	4	17	7
1955	34	9	50	31	64	12	10	3	9	3	9	3	38	8	47	14	35	9	31	14	14	3	19	6
1956	29	5	78	25	20	2	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	..	*Nil*	17	4	*Nil*	*Nil*	1	0	4	2	*Nil*	*Nil*
1957	*Nil*	*Nil*	15	4	5	1	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	..	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	5	2
1958	5	1	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	..	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
1959	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	..	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	4	2	8	2
1960	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	1	1	*Nil*	*Nil*	11	2	*Nil*	*Nil*	*Nil*	*Nil*	15	3	*Nil*	*Nil*	3	1
1961	52	10	35	2	10	0	8	3	*Nil*	*Nil*	10	4	36	3	21	2	40	4	68	15	51	6	42	4
1962	36	7	43	10	62	6	28	5	5	0	40	4	60	4	112	13	60	5	61	7	54	5	24	1
1963 (Up to March 21, 1963.	25	5	44	5	8	0																		

C = Cases ; D = Deaths.

TABLE V.

Age and sex distribution of plague cases in Kolar District (Mysore State) during 1962-1963 (up to Feb. 21, 1963).

Age, in years.	Female.	Male.	Total	Remarks.
0— 5 years	14	12	26	
6—10 ,,	44	21	65	
11—15 ,,	26	23	49	
16—20 ,,	26	16	42	
21—25 ,,	23	13	36	
26—30 ,,	21	9	30	
31—35 ,,	13	7	20	
36—40 ,,	16	6	22	
41—45 ,,	5	9	14	
46—50 ,,	8	1	9	
51—55 ,,	2	2	4	
56—60 ,,	1	1	2	
61—65 ,,	*Nil*	*Nil*	*Nil*	
66 and above	2	1	3	
Total	201	121	322	

samples, from 572 rats, were examined with negative results. In addition, viscera of 5 dead rats, collected from among reported "rat-falls", were also examined but were negative.

(*c*) *Rat-fleas.*—Some of the fleas collected from the trapped rats were pooled and sent up for bacteriological examination. Twenty such flea pools, each containing from one to ninety fleas, were subjected to bacteriological examination. One of the collections (30 fleas) from Uddappanhalli (Kolar Taluk) yielded positive result, and *P. pestis* was isolated. The collection consisted of free fleas from an infected house.

P. pestis, isolated from the flea pools as well as from bubo-puncture material, was sent to the Haffkine Institute, Bombay, for typing and to test its virulence against standard strains maintained by them. They confirmed that the *P. pestis* received from us was as virulent as their strain.

DISCUSSION.

The reappearance of human plague in some parts of the country, after varying periods of absence, has indicated that the mere freedom from human cases of plague in any area is no matter for complacence. This also has shown that the fact that there are no cases of human plague, would not justify the conclusion that plague has disappeared from an area. Other factors concerned in the epidemiological chain, and other criteria adopted for pronouncing an area free from endemic plague, need to be studied carefully. The development of events in areas like Kolar have shown that plague can remain dormant over a number of years, only to flare up again and spread in the community with the return of favourable conditions. Nguyen-van-Ai (1959) reported the occurrence of 50 cases of plague in 1956 in two endemic areas of South Vietnam where no cases had occurred since the previous outbreak in 1948. It would appear essential to investigate the different links in the epidemiology of the disease to unravel the factors responsible for the maintenance of the reservoir of the infection (sylvatic) during the quiescent period.

The persistence of plague in the rural areas in certain locations in India still remains a problem. According to Pollitzer (1954) plague has been, as a rule, short-lived in the villages affected. Appearance of the diseases, early in the season, leads to a "complete outbreak" with numerous rat-falls and human victims. This is followed by a marked reduction in the susceptible rodent population, leading finally to a disappearance of the disease. Introduced late in the season limited epizootics, with limited or no human cases, results. With the decline of plague in an area, the infection probably recedes to its original hosts, the wild rodents. Baltazard *et al.* (1958), following their observations in Uttar Pradesh, concluded that plague was not static there but was constantly shifting from one place to another through contiguity of colony infection amongst wild rodents, which eventually transferred the infection to the commensal rats on their path. This resulted in epizootics amongst the commensal rats in the villages, which were often followed by human cases.

Pollitzer (1954 *loc. cit.*) concluded that local endemicity in rural areas was an exception. The general tendency of the infection to spread to contiguous localities created a condition characterised by marked changes in the localisation and intensity of the outbreaks from season to season.

The observations recorded during the present investigations showed some evidence regarding the possibility of the infection originating from the sylvatic reservoir(s).

Plague in Kolar District has so far remained chiefly a rural problem. None of the urban centres of the district (with the exception of Masti and Mulbagal towns) have been involved. The affected villages are rarely contiguous.

The total absence of infection among the 576 domestic rats examined also indicated that the reservoir had to be looked for elsewhere. Francis (1962) (*personal communication*) also observed that the examination of thousands of domestic rats yielded negative results. The reason for this may be that the infected and acutely ill rats do not leave their burrows and rarely come into the traps. It is probably hard to get a positive finding in the rat tissues from among the trapped animals except when an epidemic is raging. This is further substantiated by the negative finding in 17 flea pools from trapped rats. P. *pestis* was isolated from one out of three pools made from wandering fleas. This pool was made of collections from a house where human plague cases had been reported. It would be logical to conclude that the wandering fleas had abandoned the rat(s) which had died of plague infection.

The temporary lull in the incidence of plague in Kolar District can partly be attributed to the use of DDT over the entire district under the National Malaria Eradication Programme. It was observed that the fleas were resistant to DDT and the transmission which had re-established even when the DDT spraying was in progress, became more intense with the withdrawal of DDT since 1962.

Detailed epidemiological investigations were made in a number of cases occurring during the period when the investigations were in progress. Majority of the cases were of indigenous origin. A few cases gave history of movement and stay in places outside the State, but such history was often unreliable, as this was not confirmed by any other source, and in few instances even contradicted by other members of the household. A few of the cases investigated showed that the attacks occurred within the fifth day following antiplague inoculation. In a personal communication, Francis (1962) reported that there have been instances where plague attacks occurred about a month following plague vaccination. The need for an evaluation of the plague vaccine is obvious. The results of such evaluation will help to determine the vaccination policy in endemic plague.

The early success of DDT in keeping the transmission of plague under control till about 1959 led to the abandoning of any semblance of routine anti-rodent measures in Kolar as well as in most other parts of the country (excepting Madras

State) where such an organisation existed in the past. There seems to be little justification for such a step, even if the belief that the plague situation had been brought under control had been well founded. It is well known that rodents, apart from spreading human disease, cause considerable damage to property, food and buildings. Rodents are known to cause serious damage to a variety of food and vegetable crops, orchards, and agricultural produce in the fields and godowns.

Though India has been free from plague for a considerable period, a few foci continue to exist in areas like Madras, Mysore, Andhra Pradesh and Himachal Pradesh. Recently a case of rat-fall was reported from Bahraich District in Uttar Pradesh but no human cases followed. Cases have been reported after the lapse of several years from countries from which plague was believed to have disappeared. Such reports have been received from Palestine, Brazil, Burma, California in U.S.A., and Vietnam (Nguyen-van-Ai, 1959 *loc. cit.*). The history of plague in Mysore State itself shows that the incidence has a regular periodicity, with a rise every 7 — 8 years (Graph 1). It is, therefore, essential that persistent and sustained measures be started and maintained for the destruction of rats. The only hope lies in the implementation of rodent control measures on a community-wide scale.

The Government staff of the various departments working in the villages, especially those under Community Development Projects, as well as the members of the local institutions, should be trained in the theory and practice of rat control measures such as poison baiting, fumigation of rat-burrows, etc. The importance and urgency of reporting rat-falls and suspected plague cases should be impressed on the workers and the general public through various publicity procedures. Periodic fumigation of the food godowns, railway wagons and goods sheds will go a long way not only in eliminating the rodent pests, but also reducing the chances of passive transportation of infected rats and vector fleas. Such passive transportation of fleas have been reported chiefly through cotton and grain trade (King and Pandit, 1931).

The activities of the different departments such as Agriculture, Community Development, Public Health, etc., should be co-ordinated for organising large scale anti-rat compaigns. Above all, everything should be done to secure public participation in the anti-rat compaigns. The masses should be educated on the methods and benefits of rat destruction. False impressions regarding the causes and spread of plague should be dispelled through posters and film-shows. There is a common belief among the villagers of Kolar District that feeding on a rat dying due to plague, is lethal to a cat or dog so fed. The villagers usually resort to this "test" whenever there is a rat-fall in the locality and get into a complacent mood when the cat or dog survives the meal, and feel that the rat death is of no importance.

A great deal of organised and co-ordinated effort is necessary to enlist the co-operation of the masses in the rodent control operations which have to be maintained on a sustained basis. Spasmodic efforts are wasteful. Demonstration projects in the villages help in such projects.

SUMMARY.

Results of investigations on certain aspects of the epidemiology of plague in Kolar District (Mysore State) are reported. Plague, which has been endemic in the area, showed a decline towards the later half of last decade and once again re-established itself. The endemicity is limited to an area near the inter-state borders of Mysore, Madras and Andhra Pradesh and is confined to the districts of Kolar, Salem and Chittoor respectively in the three States. The endemicity is restricted to the rural areas only.

A large number of rodents and flea-pools were examined for *P. pestis*. The organisms were isolated from one of the flea-pools collected from a house where human plague case was also reported. All rats were negative. Three of the bubo-puncture material taken from human cases were also positive for *P. pestis*.

The need for further investigation, to determine the role of peri-domestic and sylvatic rodents as reservoirs of the infection and for the implementation of co-ordinated and sustained measures for mass extermination of rats, is stressed.

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INVESTIGATION ON PLAGUE IN KOLAR DISTRICT (MYSORE STATE).

Part II.

Rats and rat-fleas*

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(National Institute of Communicable Diseases, Delhi.)

[September 18, 1963.]

VARIOUS aspects of the epidemiology of plague in Kolar District (Mysore State) were studied by the National Institute of Communicable Diseases during 1962-63. Krishnaswami *et al.* (1963) have reported details of these investigations.

The study of the rodents and their ectoparasites is an integral part of any investigation on plague. Politzer (1954) cites from "*Bhagavat Purana*", a Sanskrit poetical work written centuries ago, wherein it is recognised that human plague is linked with the death of the domestic rodents. King and Pandit (1931), Sitarama Iyer (1933) carried out extensive surveys of rats and rat-ectoparasites in the erstwhile Madras Presidency (present Madras and part of Andhra Pradesh) and in a few districts of Mysore State. The National Institute of Communicable Diseases undertook the survey of rats and fleas of Kolar District (Map 1) as a part of the studies on the epidemiology of plague in that area. The results of the survey carried out during December, 1962 to March, 1963 are recorded in this communication.

(A) RATS.

To study the prevalence of rats, they were trapped alive using wire cage (wonder type) traps. Usually these traps were set in alternate houses and shops

* Part of these studies were carried out under the auspices of the Enquiry "Assessment of Susceptibility Status of Insects of Public Health Importance" of the Indian Council of Medical Research.

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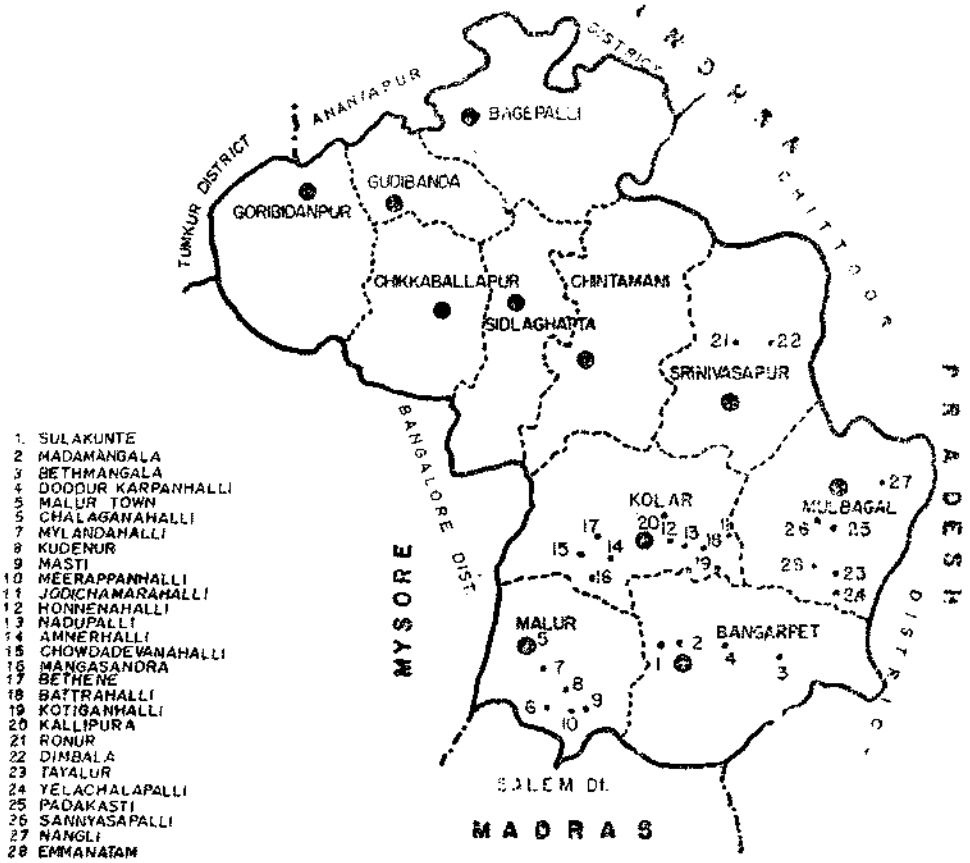
in the villages or towns late in the evening, with suitable baits. The traps, with the rats that came into them, were collected next morning and all the trapped rats were transferred into one or two cages which were covered with cloth bags. The traps were then cleaned and laid with fresh bait in those houses where they had not been set the previous day. Rats thus collected were directly transported to the field laboratory which was situated at a convenient distance. They were strangulated according to the World Health Organization standard technique* using sponge forceps.

MAP 1.

Kolar District showing the different villages where plague investigations were carried out.



The dead rats were de-fleased, identified and recorded species and sex-wise. The details of the villages investigated, number of traps set, rodents collected,

* Technique, using sponge forceps, was developed at the National Institute of Communicable Diseases, Delhi, and is now recommended by the World Health Organisation as one of the standard methods for immobilizing or strangulating rodents for collection of fleas from them. (*World Health Organization Technical Report Series 265*).

species and sex of rats, and rat index are recorded in Table I. A total of 2,846 rodents were trapped from 24 villages and four towns (Tg. HQ) during the period of investigations in all the taluks where epidemiological investigations were reported by Krishnaswami *et al.* (1963 *loc. cit.*). Of these 2,607 were *Rattus rattus* and the remaining 239 rodents consisted of *Mus musculus* (230), *Suncus murinus* (7), *Tatera indica* (2) in the order of their numerical prevalence. One each of *Rattus norvegicus* and *Bandicota indica* were collected from the field and a kitchen garden near Doddurkarpanhalli Village and Malur Town respectively.

TABLE I.

Details of domestic rodents captured indoors in the Kolar District of Mysore State.

Taluka	Villages	Number of traps set.	Species of rodents:								Total number of rats.	Rat Index*
			R. rattus		*M. musculus*		*S. murinus*		Others			
			Female	Male	Female	Male	Female	Male	Female	Male		
Bangarpet	Sulakunte	209	89	52	18	3	..	..	..	..	162	0·7
	Madamangala	88	41	13	..	..	..	..	..	..	54	
	Bethmangala	160	167	44	..	..	1	..	..	..	212	1·3
	Doddur Karpanahalli	35	18	8	3	1	..	..	..	..	30	1·8
Malur	Malur Town	76	77	21	31	..	..	1	..	..	130	1·7
	Challaganhalli	123	143	34	2	1	..	..	..	..	180	1·5
	Mylandahalli	113	105	30	..	..	..	..	..	..	135	1·2
	Kudieanur	100	109	45	3	3	..	..	..	..	159	1·6
	Masti	420	138	87	3	5	..	..	1†	..	235	0·6
	Meerappanhalli	90	82	20	..	..	..	..	..	..	102	1·1
Kolar	Joddichamarahalli	353	75	32	11	4	..	..	..	..	122	0·3
	Honnenhalli	105	79	23	4	4	..	..	..	..	110	1·0
	Nadupalli	70	32	8	1	..	..	..	..	..	41	0·6
	Ammerhalli	100	58	25	5	7	..	..	..	..	95	1·0
	Chowdadenahalli	70	6	2	11	3	..	..	..	..	22	0·3
	Mangasaundra	100	53	18	3	3	..	..	..	..	77	0·8
	Bethane	40	16	6	4	6	..	..	1†	..	33	1·0
	Kotiganhalli	234	33	12	2	..	1	..	..	..	48	0·2
	Bhattrahalli	116	48	27	6	2	..	..	..	..	113	1·0
	Kallipura	92	29	12	2	2	..	..	..	..	45	0·5
Srinivaspur	Ronur	87	71	17	29	5	..	..	..	..	122	1·4
	Dimbala	168	59	18	10	6	..	..	..	..	93	0·6
Mulbagal	Tayalur, Yelachalapalli and Padakasti.	174	85	20	5	3	..	2	..	..	115	0·7
	Sannysapalli	97	41	9	..	..	..	..	..	..	50	0·5
	Nangali	170	128	26	13	5	..	..	..	..	172	1·0
	Emmanathum	170	152	34	1	1	1	..	..	..	189	1·1
	Total	3554	1964	643	167	63	3	4	2	..	2846	

* Average number of rats per trap. † *Tatera indica* field-rat.

Attempts were also made to collect rodents from the field by opening rat-burrows. Altogether forty-seven rats were captured from fortynine rat-burrows. The species encountered were either *Tatera indica* or *Bandicoota bengalensis*.

Rattus rattus.—This was the most prevalent species of rat encountered in this area, constituting 91·6 per cent of the total rats trapped in human dwellings and shops during the period. It was observed that the prevalence of the brown or grey-bellied and the white-bellied varieties of this species was approximately same in all the villages surveyed. In one village, Dimbala (Srinivaspur Taluk) a single specimen of albino rat was collected from one of the traps along with grey ones.

Mus musculus.—From the daily collection of trapped rats it was noted that this species was next to *Rattus rattus* in its preponderance. It formed 8·1 per cent of the total rodents collected.

Tatera indica.—Specimens of *T. indica* were usually collected by opening the rat-burrows in the field. Professional rat catchers were employed to dig up rat-burrows. The technique used by them was to blow smoke into the rat tunnels and then to dig them up. Fresh mud blocking of the rat tunnels, as protection against natural enemies, was used by these people as to guide to denote the presence of rats in the burrows.

*Bandicoots**.—During the period of investigation, four specimens of this species were collected. Three specimens (*B. bengalensis*) were from five burrows opened in the field adjoining the villages and one (*B. indica*) from a burrow in the kitchen of a house.

Rattus norvegicus.—Only one specimen of *R. norvegicus* was collected in a trap when a number of these were laid in the field where the harvested crop had been stacked in ricks.

RAT INDEX AND THE SEX RATIO OF THE RODENTS TRAPPED.

The data obtained (Table I) indicated that the average number of rats per trap varied from 0·2 to 1·59 in the villages surveyed, with a gross value of 0·84. It was also observed that 75·1 per cent of the rodents trapped were females and the rest were males. The high proportion of females among the trapped rats seemed to show that more female rats went out of their burrows.

(B) RAT FLEAS.

(i) PREVALENCE.

During the period of investigation, altogether 9,380 fleas were obtained from the rodents collected. The technique recommended by the World Health Organization was employed for collecting fleas from trapped rats. Air was blown on the fur of the immobilized/strangulated rats, using a large rubber bulb (motor horn bulb with a small glass tube.) The fleas easily surfaced or dropped off into the enamel tray held below and were collected with a flea collecting aspirator. A number of fleas were also recovered from the cloth bags used for covering the rat traps which, in the day's collection of rats, had been put before transporting them to the field

* It was reported by the local people that *B. bengalensis* was the species which collected large stores of grains from the field before harvesting time and was the most destructive to crops.

laboratory from the village or town where they were trapped. Details of fleas collected from rats, from the cloth bags, flea index, and the ratio of *cheopis* and *astia* are summarised in Table II. The fleas thus collected mainly consisted of two species, namely, *X. cheopis* and *X. astia*. Only two specimens of *X. braziliencis* and of *C. canis* were also recorded.

(ii) FLEA INDEX.

Majority of the fleas (97 per cent) were collected from *R. rattus* and the density was found highest. *M. musculus* had fewer fleas. The flea density per mouse was less than one. The flea index in the villages investigated varied between 1·1 to 7·6.

In most of the cases no fleas were found on the peridomestic or sylvatic rodents collected from the field. However, two fleas were collected from two specimens of *T. indica* from village Uddappanohalli. One bandicoot which was caught from a burrow very near a house (in the kitchen garden of a house at Malur) had 158 fleas on it. The majority of these fleas were *X. astia*.

The association of fleas with different species, sex and size of rodents, were recorded in 20 villages of 5 taluks in the district. No correlation could be obtained in the flea densities with reference to the species, sex or size of the rodents.

Attempts were made to collect free wandering fleas from the houses by aspirators and by 'candle-dish' method cited by Politzer (1954 *loc. cit.*). Only forty-two fleas could be collected by the latter method. Sentinel method of collecting fleas, using a guinea-pig as the sentinel pig, was also tried ; no fleas or other ecto-parasites could be collected. The trials were inadequate to make any observations regarding its utility.

(iii) SUSCEPTIBILITY STATUS OF THE RAT-FLEAS TO CHLORINATED HYDROCARBON INSECTICIDES.

Kolar District has been treated with insecticides sprays (DDT) under the National Malaria Control/Eradication Programme ; one round in 1954 and two rounds during the malaria transmission season since 1955 till 1961 were given in this area. The last treatment under the programme was carried out in 1961. The area entered consolidation phase of the programme during the following year. BHC indoor residual spraying and dusting of rat-burrows have been carried out periodically as focal control measures in plague-infected and adjoining villages.

Susceptibility to DDT, BHC and dieldrin of the fleas collected was determined using the technique and kit recommended by the World Health Organization (*World Health Organization Tech. Rep. Series 191*).

Analysis of the results of the susceptibility tests are tabulated in Table III. The investigations revealed that resistance to DDT of both the species was

TABLE II.

Information on rat-fleas collected from the indoor trapped rodents of Kolar District, Mysore State.

Taluka.	Villages.	Number of fleas collected from rats.	Number of fleas collected from bags.	Total fleas.		Flea index.	Estimated relative prevalence (per cent) of :		Remarks
							X. cheopis	*X. astia*	
Bangarpet	Sulakunte Madamangala	In these villages, fleas were not recorded separately.		589	(589)	2·7	82·7	17·3	Estimated percentage of fleas found in bags, on the basis of total 7569 fleas, was 16·1 percent.
	Bethmangala			502	(403)	2·4	63·3	36·7	
	Doddur Karpanahalli			Nil	(Nil)	0·0	0·0	0·0	
Malur	Malur Town	253	107	360	(360)	2·8	84·7	15·3	
	Challaganahalli	472	182	654	(539)	3·6	88·1	11·9	
	Mylandahalli	192	83	275	(212)	2·0	85·4	14·6	
	Kudianur	388	150	538	(486)	3·4	83·1	16·9	
	Masti	212	194	406	(406)	1·7	97·5	2·5	
	Meerappanhalli	147	29	176	(185)	1·7	96·7	3·3	
Kolar	Joddichamarhalli	307	109	416	(416)	3·4	86·3	13·7	
	Honnenhalli	350	55	405	(405)	3·7	59·2	40·8	
	Nadupalli	263	50	313	(276)	7·6	64·8	35·2	
	Ammerhalli	419	62	481	(413)	5·1	36·6	63·4	
	Chowdadenahalli	31	4	35	(..)	1·6	..	..	
	Mangasaundra	402	55	457	(342)	5·9	99·1	0·9	
	Bethane	138	12	150	(70)	4·5	10·0	90·0	
	Bhattrahalli	505	43	548	(491)	4·8	62·5	37·5	
	Kotiganhalli			174	(70)	3·6	95·7	4·3	
	Kallipura			251	(227)	5·6	90·3	9·7	
Srinivaspur	Ronur	391	7	398	(398)	3·3	75·9	24·1	
	Dimbala	284	22	306	(280)	3·3	85·4	14·6	
Mulbagal	Taylur, Yelachalapalli and Padakasti.			54	(53)	0·5	98·1	1·9	
	Sannysapalli			241	(241)	4·8	99·6	0·4	
	Nangali	1108	37	1145	(914)	6·7	83·8	16·2	
	Emmanathum	485	21	506	(152)	2·7	95·4	4·6	
	Total	6347	1222	9380					

N.B.—Figures in parenthesis are the numbers of fleas identified and used for estimation of relative prevalence of different species.

TABLE III.

Summary of results of susceptibility status of X. cheopis *and* X. astia *to D.D.T., B.H.C. (gamma) and Dieldrin.*

Taluk.	Village.	Date of last spray of DDT under the National Malaria Eradication Programme.	Date of spray with B.H.C. under the spot control programme for plague.	Flea index.	LC50 OF FEMALE FLEAS (WITH ONE HOUR EXPOSURE TO THE INSECTICIDE PAPERS) :					
					D.D.T.		B.H.C. (gamma).		Dieldrin.	
					X. cheopis.	*X. astia.*	*X. cheopis.*	*X. astia.*	*X. cheopis.*	*X. astia.*
Bangarpet	Sulakunte	Nov. 25, 1961	Jan. 29, 1962	2·8	>4·0	>4·0	>0·1	0·060	0·45	0·1
	Madamangala	Nov. 25, 1961	Oct. 18, 1962	2·0	4·0	..	0·1			
	Bethamangala	Nov. 4, 1961		2·4	4·0	>4·0	0·06	0·071	0·2	0·1
	Doddur Karpanhalli	June 12, 1961	Jan. 17, 1963	0·0*	..	..	..	..	..	..
Malur	Malur Town	Dec. 29, 1961		3·0	>4·0	<4·0	H	0·051	..	..
	Chalaganhalli	Dec. 20, 1961		3·6	>4·0	>4·0	0·09	0·05	H	S
	Mylandahalli	Dec. 27, 1961	July 28, 1962	2·0	>4·0	4·0	0·09	S		
	Kudianur	Dec. 27, 1961	Nov. 2, 1961	3·4	>4·0	<4·0	0·03	>0·1		
	Masti	Dec. 18, 1961	Nov. 25, 1962	1·1	>4·0	>4·0	0·05	0·071		
	Meerappanhalli	Dec. 14, 1961	Aug. 7, 1961	1·7	..	..	H	S		
Kolar	Jodichamarahalli	Jan. 7, 1962		4·3	>4·0	>4·0	>0·1	S	0·32	
	Honnenahalli	Dec. 20, 1961		3·5	>4·0	>4·0	H	0·060		
	Nadupalli	Dec. 20, 1961		7·6	>4·0	>4·0	H	0·10		
	Ammarhalli	Jan. 16, 1962		5·1	>4·0	>4·0	0·18	0·076		
	Chowdadenahalli	Jan. 16, 1962		1·5	..	..	..	..		
	Mangasandra	Jan. 18, 1962		5·6	>4·0	4·0	H	0·032		
	Bethene	Dec. 20, 1961		4·5	†	..	†	0·038		
	Battranalli	Dec. 20, 1961		4·8	>4·0	>4·0	0·06	0·045		
	Kotiganhalli	Jan. 13, 1962	Aug. 15, 1962	3·8	>4·0	4·0	>0·1	‡		
	Kallipura	Jan. 12, 1962		5·8	>4·0	>4·0	>0·1	..		
Srinivaspur	Ronur	Oct. 30, 1961		3·2	4·0	>4·0	H	H		
	Dimbala	Oct. 30, 1961	Aug. 5, 1962	3·1	4·0	<4·0	H	S		
Mulbagal	Taylur	Nov. 29, 1961	Dec. 17, 1962							
	Yelachalapalli	Dec. 8, 1961	Nov. 21, 1962	0·5	>4·0	§	§	..		
	Padakasti	Nov. 29, 1961	June 26, 1962							
	Sannyasapalli	Nov. 28, 1961		4·8	>4·0	S	..	..	0·24	
	Nangli	Dec. 14, 1961		6·7	>4·0	<4·0	H	0·05		
								0·1		
	Emmanatam	Dec. 29, 1961	June 27, 1962	1·7	<2·0	†	>0·025	‡		

* The village had been sprayed and dusted with B.H.C. only a day before the R.E.P. survey.

† The flea population was mainly composed of *X. astia*. Only 2 *X. cheopis* were found.

‡ Very few *X. astia* were collected in these villages.

§ Flea density was low in these villages.

H=LC50 values could not be estimated due to high heterogeneity in response.

S=Sample tested was inadequate to draw valid conclusions.

widespread in the area. The LC50 (for female fleas) was found to be more than 4 per cent DDT* with one hour exposure in most of the villages tested. Mohan (1960) estimated the susceptibility of fleas in two villages (Challanganahalli and Masti in Malur Taluk) and found that both *Xenopsylla cheopis* and *X. astia* were resistant to DDT. This indicated that the fleas in Kolar District continued to be resistant to DDT despite the withdrawal of DDT spray under the National Malaria Eradication Programme and the absence of selection pressure for over a year.

The susceptibility of *X. cheopis* to gamma BHC was estimated in 19 villages. The response of the fleas to BHC in many of the villages indicated that the flea population was highly heterogeneous. This heterogeneity was probably due to the presence of resistant fleas in high frequency. The LC50 value of *X. cheopis* to BHC, wherever determined, ranged from 0·03 to 0·1 per cent gamma BHC. The concentration of 0·01 per cent of gamma BHC, reported to cause 100 per cent mortality in the susceptible strain of fleas (Sharma and Joshi, 1960), yielded significantly high percentage of survivors in the fleas of Kolar District. The tolerance of the local fleas to BHC thus ranged from three to ten times that of susceptible ones.

The susceptibility status of *X. cheopis* female against dieldrin was also found to be slightly higher (LC50 varied between 0·2 to 0·3) than that of susceptible ones (LC50=0·035) as estimated by Sharma and Joshi (1961 *loc. cit.*).

DISCUSSION.

King and Pandit (*1931 loc. cit.*) who surveyed the adjoining area of the Andhra and Madras States for rats and rat-fleas and Seetharam Iyer (*1963 loc. cit.*) who similarly investigated different areas in Mysore State, including Kolar Gold Fields, showed that the majority of the rodents collected by them were *Rattus rattus*. Data recorded in the present investigations confirms these findings. *Rattus rattus* comprised 91·6 per cent of the total rats collected in dwelling houses. The average rat index of 0·8 per trap indicates a high rat population. The other species of rodents, collected by trapping in dwellings and shops of the villages and towns investigated, revealed that *Mus musculus* was second to *Rattus rattus* in prevalence. Very rarely other rodents were trapped in the cages. The occurrence of two specimens of *T. indica* in traps, even though not important in itself, is of interest ; it denotes the possibility of contact between domestic and wild rodents and transfer of ectoparasites.

There were reports in the villages that bandicoots were entering domestic premises, though none was caught in the traps used†.

Following factors, possibly, have contributed to the apparent increase in the rat population : (*i*) increased activity in food production, (*ii*) increased food storage without proper storage facilities, and (*iii*) paucity of predators of the rodents, especially cats.

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* LC50 of *X. cheopis* (female) in village Emmanatam (Mulbagal Taluk) was recorded to be less than 2 per cent DDT.

† Wonder type wire cage traps were not considered suitable for trapping bandicoots by Dr. Deoras, Entomologist, Haffkine Institute, Bombay.

It was observed that the rats trapped in the houses or dug out of burrows in the field were all apparently healthy and active, and the proportion of females trapped was more as compared to the males. On no occasion were two different species of rodents found in the same burrow. None of the rodents collected, both from rat traps or from burrows, were sick or dead.

Of the two species of rat-fleas, *X. cheopis* and *X. astia*, encountered in the locality, the former predominated. Both species were recorded from all the localities investigated in different proportions. No fleas could be collected from the field, either from the animals caught or from their nests. Neither larval nor pupal stages were encountered at any time. Large number of fleas were collected from the cloth-bags used for covering the cages in which the day's collection of rodents were transported (Table II). Sometimes the number of fleas recovered from these bags were fifty per cent of those collected from the rats. Stark and Kinney (1962) opine that fleas abandon their hosts when they are disturbed. This perhaps may be the reason for such large number of fleas getting into the bags.

The susceptibility tests showed that both the species of the rat-fleas continued to be resistant to DDT and the tolerance to BHC of *X. cheopis* females ranged from 3 to 10 times that of normal. The fleas, however, were found to be susceptible to dieldrin. The spraying history of the area indicates that ample opportunity for selection to DDT was provided by the DDT spraying carried out under the National Malaria Control/Eradication Programmes. Besides, under the local plague control measures, BHC water dispersible powder was either sprayed on the walls as a residual treatment or BHC dust insufflation of rat-burrows, in the plague-affected and surrounding villages, was carried out. This seems to have resulted in the selection of resistant strains amongst the fleas. The build-up of large population of resistant individuals in the flea population seems to have resulted by constant in-breeding of the fleas confined to rat-burrows which are almost isolates. Thus intensive selection has brought down the susceptible population of the fleas, both in *X. cheopis* and *X. astia*, to a very low level.

With the withdrawal of DDT spray under the National Malaria Eradication Programme in the Kolar Unit in 1962, as the Unit entered the consolidation phase, it was expected that the proportion of susceptible fleas in the flea population would increase. However, the observations revealed that this had not happened and in widely separated areas the fleas still continued to be highly resistant to DDT. The resistance to BHC, however, was not of a high degree and did not interfere with the existing control measures of plague with BHC.

No difference in the resistant status to BHC was observed among the flea population of female *X. cheopis* collected from villages treated once, twice or thrice with BHC under the plague control measures due to repeated infection of these villages.

The gross flea index ranged from 1·1 to 7·6 with an average of 3·3 in the area. *X. cheopis* index (Table II) seems to be above the critical level of 1·0 per rat which

is said to be sufficient to cause plague epidemics.* Seetharama Iyer (1953 *loc. cit.*) similarly found high *cheopis* index from areas where plague had been reported. He recorded 0·27 as *X. brasiliensis* index in Kolar Gold fields. This species was conspicuous by its absence during the present investigation except in one village where two specimens were collected.

By and large, the flea density in the area is considerably high and has a low susceptibility to DDT and BHC. The use of BHC under the Spot Control Programme of the State wherein the plague-affected villages and the neighbouring villages are subjected to cyanofumigation, insufflation of rat-burrows with BHC and spraying of BHC water dispersible powder of the dwelling-houses would increase the possibility of further selection of resistance to BHC which may affect its use for the control of fleas in the coming years.

The above studies revealed that *X. cheopis* is considerably more tolerant than *X. astia* to both the insecticides tested and the males in both the species are more susceptible than the females.

CONCLUSIONS.

The rat and the rat-flea population in the investigational area is considerably high. Conditions for fulminating epidemics and continuation of endemic conditions exist due to the high rate of rat and rat-fleas. The resistance in fleas to DDT and their tolerance to BHC have been well established in this State. The factors responsible for increase in the rat population and continuance of high density also were revealed from the observations. No correlation could be established between the size or species of rat to the infestation of fleas with them. The field rodents surprisingly showed no fleas either on them or in their nests. This was possibly due to smoke that was used in catching them.

SUMMARY.

Studies in respect of the rat and rat-fleas, their prevalence and the susceptibility status of rat-fleas were carried out. The rat and flea index in the investigational areas were seen to be very high. The fleas, *X. cheopis* and *X. astia*, were observed to be highly resistant to DDT and their tolerance was continuously increasing due to the increased exposure to BHC. However, the fleas were found to be susceptible to dieldrin.

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* Roy, D.N. and Brown, A.W.A. (1954) *Entomology—Medical and Veterinary.* Excelsior Press, Calcutta.

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INVESTIGATION ON PLAGUE IN KOLAR DISTRICT (MYSORE STATE).

Part III.

Comparative evaluation of D.D.T., B.H.C. and Diazinon water dispersible powder and dust formulations against rat-fleas in villages near Kolar Town (Mysore State)*

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[November 30, 1963.]

MOHAN (1962) reported resistance of rat-fleas, *X. cheopis and X. astia* in villages Masti and Challeganahalli of Kolar District. Krishnaswami *et al.* (1963) confirmed high physiological resistance in both these species of fleas to D.D.T. in these and 24 other villages as well as 4 towns in the seven plague-affected taluks of Kolar District (Map 1). They also established the existence of tolerance to gamma B.H.C. in the fleas in widely separated areas of the district. In view of the resistant status of fleas, it was considered desirable to determine the comparative effectiveness of different insecticidal treatments for flea control in the area. Studies were carried out on the effect of residual treatment of all dwelling houses as against dust insufflation of rat-runs and rat-burrows only with chlorinated as well as organophosphorus compounds. Results of these trials, which were carried out in villages around Kolar Town, are presented in this communication.

MATERIAL AND METHODS

Nine villages around Kolar were selected for these trials (Sketch 1). The villages were small in size and were easily approachable by road from Kolar Town. Villages, where silk-worm rearing was in vogue, were carefully avoided as this in-

* These studies were partly financed by the Indian Council of Medical Research through the enquiry, "Assessment of Susceptibility Status of Insects of Public Health Importance."

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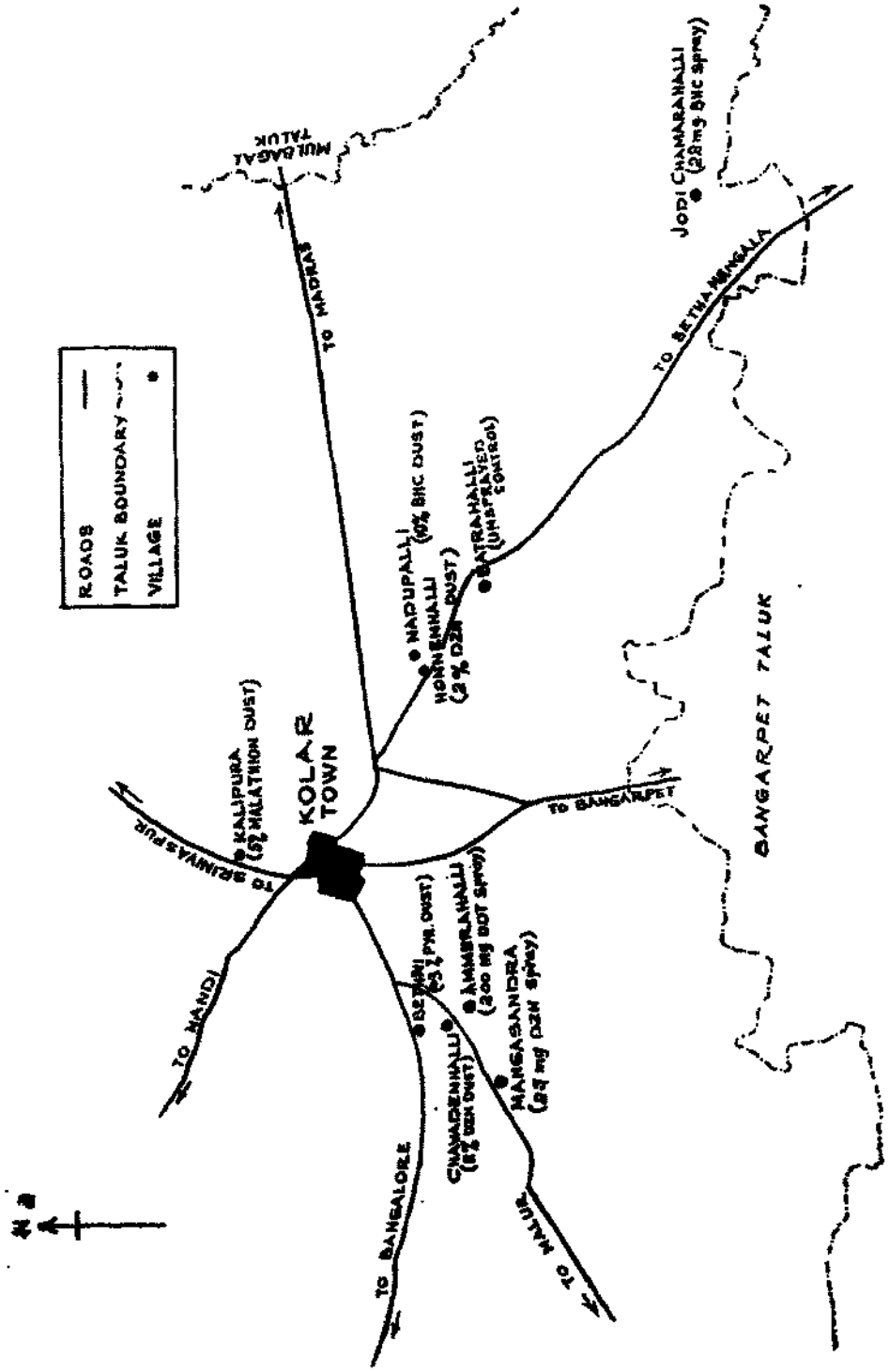
MAP I.

Kolar District showing the different villages where plague investigations were carried out.

terfered with the insecticidal treatment of the whole village. Each of the trial villages was treated separately with either residual spray, using water dispersible powders of D.D.T., B.H.C. or Diazinon, or insufflated with dust formulation of B.H.C., Diazinon or Malathion. Details of villages, insecticidal formulation used, the dosage and particulars of treatment, are summarised in Table I.

Indoor residual treatments were carried out using water dispersible formulations from which sprays of requisite concentrations were prepared. Stirrup pumps were used to apply the insecticide. The walls and the ceilings were thoroughly sprayed, with special attention to the areas where the floor and the wall as well as the wall and the ceiling met. Rat-runs under the eaves, outside the houses, were also sprayed. Special attention was paid to ensure the correct dosage and complete coverage.

Dust insufflation of rat-burrows and rat-runs was carried out using blowers employed for cyanofumigation. Efforts were made to locate, as far as practicable, and insufflate all the rat-holes inside and outside the houses.



Sketch 1.

Location of the trial villages selected for insecticidal treatments near Kolar Town, (Mysore State.)

TABLE I.

Details of villages selected for insecticidal treatment against rat-fleas near Kolar Town, Mysore State.

Serial number.	Name of village.	INSECTICIDAL FORMULATION AND TYPE OF TREATMENT :			Date of treatment.	HOUSE TREATED :		
		Insecticidal formulation.	Type of treatment.	Dosage per house or per square metre.		Total number of houses.	Number of houses treated.	Untreated houses, Refused/ Locked.
1	Bhattrahalli	(Untreated)	(Comparison Village)	(Comparison Village)	(Comparison Village)	63	Untreated	..
2	Jodichamarahalli	50 per cent B.H.C. water dispersible powder (6·5 per cent gamma isomer)	Indoor residual spray	0·2 gm. per sq. metre	Feb. 6, 1963	97	78	19
3	Nadupalli	10 per cent B.H.C. dust	Insufflation of rat-burrows and dusting rat-runs	0·5 kg. per house	Feb. 7, 1963	53	49	4
4	Ammerahalli	75 per cent D.D.T. water dispersible powder	Indoor residual spray	2·0 gm. per sq. metre	Feb. 8, 1963	90	76	14
5	Mangasandra	40 per cent Diazinon water dispersible spray	—do—	0·25 gm. per sq. metre	Feb. 9, 1963	88	75	13
6	Chowdadenahalli	2 per cent Diazinon dust	Dusting of rat-runs and insufflation of rat-burrows	0·5 kg. per house	March 7, 1963	43	39	4
7	Honnenahalli	—do—	—do—	—do—	April 10, 1963	56	55	1
8	Kallipura	5 per cent Malathion dust	—do—	—do—	July 12, 1963	23	20	3

TABLE II.

Details of the pre- and post-insecticidal treatment flea-index with the number of rats trapped and fleas collected.

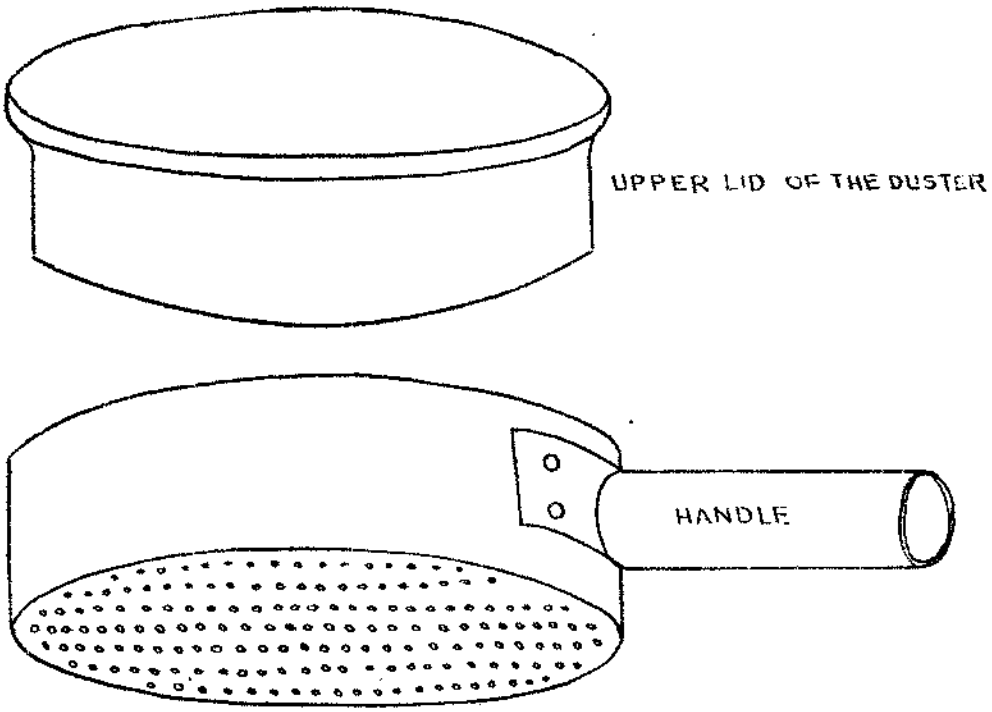
	Insecticide and the type of treatment.	PRE TREATMENT FLEA-INDEX :			POST-TREATMENT FLEA-INDEX :																						
					March			April			May			June			July			August			September			October		
		Number of rats trapped.	Number of fleas collected.	Flea-index.	Number of rats trapped.	Number of fleas collected.	Flea-index.	Number of rats trapped.	Number of fleas collected.	Flea-index.	Number of rats trapped.	Number of fleas collected.	Flea-index.	Number of rats trapped.	Number of fleas collected.	Flea-index.	Number of rats trapped.	Number of fleas collected.	Flea-index.	Number of rats trapped.	Number of fleas collected.	Flea-index.	Number of rats trapped.	Number of fleas collected.	Flea-index.	Number of rats trapped.	Number of fleas collected.	Flea-index.
	Comparison village (Untreated)	113	548	4·8	32	142	4·4	26	64	2·46	25	63	2·52	40	105	2·62	29	95	3·28	9	30	3·33	11	62	5·64	9	47	5·2
1963	50 per cent B.H.C. water dispersible powder (6·5 per cent gamma isomer)	49	212	4·3	43	36	0·8	14	8	0·57	10	6	0·6	26	42	1·6	15	55	3·66	14	43	3·0	15	49	3·27	..	..	..
1963	B.H.C. dust 10 per cent.	41	313	7·6	25	36	1·4	37	27	1·37	36	111	3·08	24	38	1·6	18	60	3·3	5	24	4·8	8	39	4·87	..	..	..
1963	D D.T. spray (75 per cent D.D.T. water dispersible powder).	95	481	5·1	45	53	1·1	52	69	1·33	16	21	1·32	18	45	2·5	71	289	4·0	43	196	4·56	30	108	3·6	..	..	..
1963	Diazinon 40 per cent water dispersible powder.	77	428	5·6	19	83	4·8	46	78	1·69	21	51	2·43	31	24	0·8	30	91	3·0	31	114	3·7	20	46	2·3	..	..	..
63	Diazinon dust (2·0 per cent)	22	34	1·5	..	..	..	5	5	1	6	8	1·33	8	0	0·0	14	32	2·3	16	46	2·9	6	24	4·0	..	..	..
33	Diazinon dust (2·0 per cent)	110	387	3·5 (Feb. 10, 1963) 2·09 (April 10, 1963)	..	..	..	54	113	2·09	40	45	1·12	27	47	1·74	2	15	7·5	22	100	4·54	15	96	6·4	..	..	..
3	Malathion dust (5·0 per cent)	10	39	3·9 (June 13, 1963)	..	..	..	..	..	..	..	..	..	..	..	..	2	9	4·5	..	..	..	5	2	0·4	7	42	6·0

* Observations were continued only in these villages after September, 1963.

Rat-holes in the thatched ceiling were also treated. Treatment of the rat-runs with the dust formulations was achieved by using a special type of container with holes on one side simulating a sieve (Fig. 1). A long wooden handle was attached to the container filled with the dust formulation, so that tapping on this handle would release small quantities of the dust along the rat-runs. The insecticidal treatment of all the villages was carried out under the direct supervision of one of the authors.

FIG. 1.

Sketch of metal duster used for dusting rat-runs.



COLLECTION OF DATA

Rats were trapped alive in wire cages [wonder type (Roy and Brown, 1954)]. They were either strangulated or immobilised using a pair of sponge forceps and were defleased by blowing air on the fur. The fleas were picked up, using a small aspirator equipped with a rubber bulb. The number of fleas thus obtained was recorded. These fleas were processed with the potash method and identified under a stereo-microscope. Monthwise details of observations, such as the number of rats trapped and the number and species of fleas obtained, are mentioned in Table II. The flea index was determined at intervals of 30 days after the treatment of the villages.* The pre and post-insecticidal treatment flea-indices in the trial villages near Kolar Town are summarised in Table III.

* Flea index, immediately after treatment, was not determined due to shortage of staff and time.

TABLE III.

Details of flea-index (pre-and post-insecticidal treatment) in trial villages near Kolar Town where evaluation of different insecticidal treatments against the oriental rat-fleas was carried out.

Serial number.	Name of the village.	Insecticidal treatment, with date.	Monthly rat-flea index (*X. cheopis and X. astia*):								
			Pre-treatment.	Post-insecticidal treatment.							
			February, 1963.	March, 1963.	April, 1963.	May, 1963	June, 1963.	July, 1963.	August, 1963.	September, 1963.	October, 1963.
1	Bhattrahalli	Comparison village (untreated).	4·8	4·4	2·46	2·52	2·62	3·28	3·33	5·64	
2	Jodichamarahalli	B.H.C. spray (0·2 gm. B.H.C. per sq. metre) Feb. 6, 1963.	4·3	0·8	0·57	0·6	1·6	3·66	3·0	3·27	
3	Nadupalli	B.H.C. dust (10 per cent B.H.C. dust) Feb. 7 1963.	7·6	1·4	1·37	3·08	1·6	3·3	4·8	4·87	
4	Ammerahalli	DDT. spray (75 per cent DDT. water dispersible powder). Feb. 8, 1963.	5·1	1·1	1·33	1·32	2·5	4·0	4·56	3·6	Observations discontinued.
5	Mangasandra	Diazinon spray (0·25 gm. per sq. metre) Feb. 9, 1963.	5·6	4·3	1·69	2·43	2·8	3·0	3·7	2·3	
6	Chavadadenahalli	Diazinon dust (2·0 per cent diazinon) March 7, 1963.	1·5 (March 7, 1963)*	..	1·0	1·33	1·6	2·3	2·9	4·0	
7	Honnenahalli	Diazinon dust (2·0 per cent Diazinon) April 10, 1963.	2·09 (April 10, 1963)*	..	..	1·12	1·74	7·5	4·54	6·4	
8	Kallipura	Malathion dust (5·0 per cent Malathion) July 12, 1963.	3·9 (July 13, 1963)*					Treated with malathion dust	No rats were caught.	0·4	6·0

* Dates of pre-treatment collections where the insecticidal treatments were carried out later than February, 1963.

RESULTS AND DISCUSSION

It would be observed from the data summarised in Table III that B.H.C. residual spray at 0·22 gm. per sq. metre was effective in keeping the flea index low for a period of approximately 12 weeks.† D.D.T. at a dosage of 2 gm. per sq. meter was nearly as effective as the B.H.C. spray. Diazinon residual spray did not result in any reduction in the flea population as seen from the first post-treatment index.

Amongst the dust insufflation treatments B.H.C. dust gave a residual effect of approximately 8 weeks, whereas Diazinon dust (2 replicate treatments of separate villages with 2 per cent dust) produced no significant reduction in the flea density. The results with malathion dust have not been conclusive as no rats could be trapped during the first post-treatment rat-ectoparasite survey of that village.

The post-treatment flea survey of the trial villages carried out in March, 1963, showed that flea index in villages sprayed with B.H.C. or D.D.T. formulation as well as the village insufflated with B.H.C. dust showed a heavy reduction as compared to the pre-spray levels. There was practically no reduction in the village sprayed with diazinon formulation, indicating that this insecticide spray had no effect against rat-fleas. The flea index in the untreated comparison village, Bhatterahalli, in March, 1963, was nearly the same as that of February, 1963. However, there was a fifty per cent reduction in the flea index of this village recorded in April, 1963. Reduction of flea population was restricted only to the untreated village as in the insecticide-treated villages the flea index in April was nearly the same as that of March, 1963. However, the flea index in the untreated village, in spite of the decline, was still higher than that of the treated villages. The data also indicate steady increase in flea index after the slight decline in April and by July, 1963, the flea index was more than half of the pre-treatment index. The loss of effectiveness of both Diazinon spray as well as dust insufflation treatment was obvious ; the effect did not last beyond four weeks in both the cases.

CONCLUSIONS

Results of these investigation indicated that residual spray of dwellings with B.H.C. water dispersible powders was most effective in reducing the flea density to the minimum and maintained at the same level for almost 12 weeks following treatment.‡ It was also observed that though the susceptibility of rat-fleas was very low to D.D.T., an enhanced dosage of the same insecticide at 2·0 gm. per sq. metre (double the dosage applied by the National Malaria Eradication Programme) signi-

† Francis (1963) reported six months residual effectiveness of B.H.C. treatment against rat-fleas.

‡ Based on the results of these trials, recommendations were made to the Mysore State Government to undertake B.H.C. indoor residual spray in the seven plague affected taluks of Kolar District. Following completion of the first round of spray in August 1963, total interruption of transmission to man occurred and the incidence of human plague in Kolar District has been brought down to zero since then. Although D.D.T. residual spray at double the dosage (2 gm. per sq. metre) had also been effective against fleas, it could not be employed for interruption of transmission of plague as the flea population already had a high proportion of resistant individuals and this would have resulted in intensification of the selection.

ficantly reduced the flea index for 12 weeks. Diazinon, applied either as a residual spray or as dust insufflation, was seen to be ineffective in bringing down flea population in the treated villages. B.H.C. dust was most effective amongst the dust insufflation treatments. Trials with malathion dust treatment were inconclusive.

SUMMARY

Field trials with different insecticidal formulations (used both as residual spray and dust insufflation) against rat-fleas in villages near Kolar Town are described. The results have shown that residual treatment of dwelling houses had the maximum effectiveness against fleas. It also showed that the organophosphorus compound, Diazinon, was ineffective both as residual spray and insufflation treatment.

ACKNOWLEDGEMENT

The authors gratefully thank Dr. S.D. Narayan Gowda, Deputy Director of Public Health, Bureau of Epidemiology, Bangalore, and Dr. B. Raghvan, District Health Officer, Kolar, Mysore State, for their help and active co-operation.

The assistance rendered during the insecticidal treatment by Shri Shamanna, Senior Malaria Inspector, National Malaria Eradication Programme Unit, Kolar, and Sarwasri Bhagat Ram and Nasib Singh is duly accredited.

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Indian Journal of Malariology, 17, 2-3, June-September 1963.

Review

PRACTICAL MALARIOLOGY (2nd edition) by PAUL F. RUSSELL, LUTHER WEST, REGINALD D. MAXWELL and GEORGE MACDONALD. pp. i-xiv, 1-750, with numerous illustrations. Oxford University Press, New York, Toronto and London. (1963).

THE first edition of this book, published in 1946, was received well by the malariologists throughout the world. Since then rapid advances have been made in the various fields of malariology ; notably in chemotherapy, vector control and in the concepts and methods of malaria control. The exo-erythrocytic phase in the life-cycle has been demonstrated in all the four species of human malaria The policy of limited malaria control has been displaced in many countries by that of total eradication.

The above factors have necessitated not only a thorough revision of the text but in some cases the rewriting of whole chapters. The principal objective, however, remains unchanged, i.e. to give clinical, laboratory and field information about malaria, capable of being put to use in daily practice. An important addition is the chapter on malaria eradication. This includes an account of principles, processes and criteria employed and the various phases of the programme, the organisation and legislation etc.

Other subjects discussed which merit special mention include the evolution of the malaria parasite, the use of phase-contrast and electron microscopy, serodiagnosis, use of infected material for experimental purposes and testing for susceptibility to insecticides. The physiology of the parasite, the latest antimalarials and insecticides with the problems of resistance posed by them, laboratory and field techniques, and mosquito bionomics are explained in great detail. A new chapter on vector eradication has been added. The keys to the *Anopheles* of the world have been revised with the help of various workers who have been duly acknowledged. The omission of Authors' Index in the previous edition has been rectified in the present edition.

The authors have very rightly indicated that the book is dedicated to "All who in one way or another are engaged in eradicating malaria from the world", as the most important aspect of malariology today is eradication rather than control of the disease. Emphasis has therefore been given to the principles, and administration of malaria eradication, chiefly through imagocidal measures.

Notwithstanding the achievements in the field of malariology, it has been mentioned in the preface to the book that certain lacunae in our knowledge remain still to be solved. For instance, the biochemistry and physiology of *plasmodium* are relatively obscure and no satisfactory method of *in vitro* culture has been devised. The relation between simian and human malaria requires clarification.

Parasite-resistance to drugs and vector-resistance to insecticides are both important problems.

The book is an outstanding contribution to the field of malariology. The get-up of this monumental work is excellent. It is pleasant to read, stimulating to workers both in research and field and an ideal text book for students of malariology. The collaboration of Professor G. Macdonald, Director of the Ross Institute, London, is a welcome feature of the revised edition.

There are a few omissions here and there. In the historical introduction under residual insecticides (pp. 20-21), the role of the organic phosphorus compounds has not been mentioned. Comparative characters of *Plasmodia* of man in stained thin smears are described in Table I (pp. 66-67). It would have been useful if a similar table for stained thick smears was also given along with it for comparison purposes. As regards Manson's theory (p. 9, para 2) it is stated that, "Manson succeeded in tracing the filaria through the stomach wall of the mosquitoes into the abdominal cavity and then into thoracic muscles. During this passage the parasite increased in size, developing a mouth, an alimentary canal and other organs". It should have been mentioned here that the parasite does not increase in size during its passage through stomach wall on to the thoracic muscles but all the development of the parasite takes place in the thoracic muscles of the mosquito.

To sum up, the book is an outstanding contribution to malariology and masterly in presentation. There is no doubt that the authors have fully and admirably achieved the objective laid down in the preface. The book is bound to be welcomed by all those engaged in the study of malaria and its control.

Sep. 1, 1963. D.S. and S.P.R.

Indian Journal of Malariology, **17**, 4, *December 1963*.

ANNOUNCEMENT

INDIAN JOURNAL OF MALARIOLOGY

THE Indian Council of Medical Research announces the retirement of the *Indian Journal of Malariology* with the publication of the December 1963 issue (Vol. XVII, No. 4) after a distinguished record of service to all malaria workers of the world for 34 years.

Many of the malaria workers in India as well as in the world are busy in the execution of the respective National Malaria Eradication Programmes. Research on the problems of this disease is now restricted to a few workers. There has, therefore, of late been a great paucity of papers to be printed in this Journal. Further, the research in malariology has become refined and multi-disciplined like all other medical research. For a wider dissemination of scientific knowledge, specialised work today has to be published in a variety of specialised journals in order to reach the workers of the respective disciplines. Research papers can be published in any one of the several journals that are now available in India.

The Journal has achieved its original objective. It was started when there were not many suitable periodicals for publishing results of innumerable investigations on malaria conducted in various parts of the country. The Malaria Institute of India (now known as National Institute of Communicable Diseases) has continuously disseminated knowledge, propagated ideas and contributions of the workers through the pages of this Journal. This contributed to the undertaking of the eradication of malaria from the country.

The Journal was started in 1929 by the Indian Research Fund Association (now the Indian Council of Medical Research) as *Records of the Malaria Survey of India* about the same time as the Malaria Survey of India was formed. The main objective of the Journal was to disseminate knowledge and thus get an awakening among the public in general, and the public health workers and the administrators in particular; with special reference to its prevalence, suffering of humanity and its effect on the economy of the country.

There were a number of valuable reports and other articles relating to malaria in India which had either not been published or published in the local provincial publications that were not easily accessible to all the malaria workers in India. A systematic search for these reports, journals or records was made by the then Director of the Malaria Survey of India, Major J.A. Sinton, and a bibliography of malaria in India was compiled. This was published as the first issue of the Journal. Besides, a number of valuable memoranda, which were not readily available for general use, were reprinted in the second issue.

The name of the Journal was changed to that of *Journal of the Malaria Institute of India* in 1938 in conformity with the change in the name of the Institute. Later, the scope of the Journal was widened and articles from not only workers of

the Institute and from other parts of the country, but also from eminent scientists from abroad, appeared in the Journal. It was, therefore, considered once again appropriate to change the name to *Indian Journal of Malariology* in 1947.

The *Indian Journal of Malariology* has been a quarterly periodical published in March, June, September and December. It has in all completed thirty volumes containing 1,112 articles (122 from foreign contributors), comprising 14,935 pages by about 475 workers. Besides covering all aspects of malaria (human, avian, simian and rodents—physiology of malaria parasite, pathology, chemotherapy, immunology, epidemiology, control and eradication) and studies on mosquitoes both *Culex* and *Anopheles* (bionomics, distribution, vectorial capacity, relation to malaria destruction by use of insecticides, resistance of insecticides), the Journal has covered valuable knowledge on rat-fleas, house-flies, bed-bugs, trypanosomes, blackwater fever, kala azar, filariasis, plague, etc.

The Editor records his gratitude for the cooperation received from the contributors, publishers and the printers.

—EDITOR.

Indian Journal of Malariology, **17**, 4, December 1963.

A CHART ON THE CHAIN OF FILARIASIS TRANSMISSION.

BY

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[January 2, 1963†.]

THIS Chart is meant to illustrate the chain of transmission of human filariae which are carried by mosquitoes. There are two species of such filariae, namely, *Wuchereria bancrofti* and *Brugia malayi*. Both these are characterized by nocturnal periodicity. The Pacific strain of *W. bancrofti*, on the other hand, is characteristically non-periodic. Despite their morphological and physiological differences, the life histories of these filariae within their respective vectors as well as within the human host are essentially the same, and can be represented by a common chart.

Charts showing the life cycle of *W. bancrofti* have been published by various authors of text books in tropical medicine and parasitology (e.g., Napier, 1946; Chatterjee, 1957; Lapage, 1958 and Faust, 1959). But none of these charts represents the peculiar epidemiological features that characterize the process of transmission of moquito-borne human filariases. These features are :—

(1) The symptomless carriers who constitute the main reservoir of infection.

(2) The need for repeated infective bites by infected vectors for the establishment of the symptomless carrier state.

(3) The need for further repeated inoculations (hyperfilariation) for the manifestation of filarial disease.

(4) The filarial patient seldom playing any role in transmission since, in the majority of cases, the peripheral blood becomes free from microfilariae when disease sets in.

The present Chart seeks to emphasize the above points besides illustrating the life cycle of the parasite in man (the definitive host) and in the mosquito (the intermediate host).

It is realized that this Chart is still far from being comprehensive because there are yet some important lacunae in our knowlege of the life history of mosquito-borne human filariae. For instance, hardly any thing is known regarding the life of these parasites from the time of their entry into the human system, as infective larvae, up to their appearance as adult filariae in the lymphatics. Again,

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† The Chart, originally received with the manuscript of this paper, was 'multicoloured'. Some technical difficulty having been experienced in reproducing the 'multicoloured' chart, the author was requested to forward 'single-coloured' chart. Hence publication of this paper has been delayed. —*EDITOR.*

recent work in Malaya (Edeson *et al.*, 1955) has indicated the possible and even probable role of animal reservoirs in human filariasis. As these mysterious aspects of the lives of these parasites come to light, suitable modifications will have to be made in the Chart.

ACKNOWLEDGMENTS

The author is indebted to Dr. S.P. Ramakrishnan, Director, National Institute of Communicable, Diseases, Delhi, for critical comments, especially for pointing out the necessity of incorporating in the Chart, the second and third epidemiological points mentioned above. Thanks are also due to Dr. N.G.S. Raghavan, Deputy Director, National Institute of Communicable Diseases, for the suggestion that the Chart be published.

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Indian Journal of Malariology, **17**, 4, December 1963.

NATURAL MORTALITY IN MOSQUITOES OF THE *CULEX VISHNUI* GROUP IN SOUTH INDIA

BY

R. REUBEN

(*Virus Research Centre*

[December 1,

INTRODUCTION

NATURAL mortality of vectors has long been recognised as an important factor in the spread of mosquito-borne diseases. Estimates of this factor in the field are, however, few. Previous reports on mosquito longevity in South India are those of Russell and Rao (1942), and Laurence (1963).

Macdonald (1957) showed how mortality could be calculated from the ratio of the number of mosquitoes at the infective stage to the total number of mosquitoes infected with any stage of the parasite. The method becomes impracticable when one tries to apply it to virus carriers. For example, members of the *Culex vishnui* group have been incriminated as vectors of Japanese encephalitis in the North Arcot District of Madras State (*unpublished data, Virus Research Centre, Poona*). Our knowledge of this particular vector-virus relationship is still inadequate, and in any case infection rates are liable to be too low for reliable ratios to be obtained.

Davidson (1955) showed that the proportion of parous mosquitoes in a population is related to their survival. All the parous mosquitoes in a population must have lived at least as long as it takes to complete one gonotrophic cycle. If n be the number of days between the blood-meal and oviposition, and p is the probability of survival through one day, then p is given by $\sqrt[n]{\text{the proportion of parous mosquitoes in the sample of mosquitoes examined}}$. Percentage daily mortality is then $(1 - p) \times 100$. This relationship holds only if the population is being renewed at a steady rate over the period for which the estimate is being made.

Laurence (1963) has discussed the objections to the use of the parous rate for calculating p. He compared mortalities of two filarial vectors obtained from parasite rates and parous rates of the same mosquitoes. There was no significant discrepancy between the pairs of estimates so obtained. Thus the method is valid for the mosquito species he worked with in the Vellore area. Samarawickrema (1962), working on *Culex fatigans* in Ceylon, showed that successive gonotrophic cycles were synchronous with stages of development of microfilariae, thus showing that no delay occurred between oviposition and the blood-meal which marks the beginning of a new cycle. The available evidence, therefore, goes to show that the parous

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

rate can be used for species which breed continuously throughout the year and have at all times easy access to a source of blood, and to breeding places. These conditions are fulfilled by *Culex "vishnui"*. Only the parous rate has been used in the present observations.

METHODS

Mosquitoes for this study were collected from three villages around Vellore, in the North Arcot District of Madras State. Collections were made every morning from Magoon-type traps baited with bullocks. Most of the mosquitoes were freshly-fed, with ovaries at Stage II or II-III. They were dissected shortly after being brought to the laboratory. Very occasionally, and in negligible numbers, the catch included mosquitoes with ovaries at Stage III-IV or later stages. These were ignored. The presence or absence of tracheolar coiling in the ovary was used to determine whether or not a mosquito was parous, as described by Detinova (1962). Mosquitoes were dissected in distilled water, and the tracheoles examined after the ovaries had dried. Plate I [Fig. 1(*a*) and 1(*b*)] show the appearance of parous and nulliparous ovaries respectively after drying. The method was first tried on mosquitoes of known parity status, and was found to hold. No attempt was made to look for follicular relics.

Not all the mosquitoes collected in a day were dissected. Fifty mosquitoes from each village were selected at random from the day's catch. During the summer months, when only a few mosquitoes entered the traps, the entire catch was dissected. Parous rates were calculated on the results of each month's dissections. It was assumed that over the short period of a month the population of *Culex "vishnui"* remained fairly steady.

The gonotrophic cycle was determined every month in the laboratory. Since *Culex "vishnui"* oviposits with difficulty in the laboratory, batches of mosquitoes were dissected at intervals after the blood-meal to find out when the eggs were fully developed.

RESULTS

Natural mortality was determined every month from November, 1960 to May, 1962. During the summer months the gonotrophic cycle of *Culex "vishnui"* was found to take two days, while in cool wet weather it varied between three and four days. On one occasion mosquitoes took 5 days to complete the gonotrophic cycle. Parous rates also varied from season to season. Nulliparous mosquitoes were found throughout the year, and there was no indication in the course of this study that large emergences of young mosquitoes were taking place periodically. The relative proportions of parous and nulliparous mosquitoes remained reasonably constant from day to day within the period of a month.

Table I presents the data from mosquitoes trapped in three villages, and the estimates of natural mortality based on them. There were some months in which

PLATE I.

FIG. 1. (*a*) Ovary of a parous female of *Culex "vishnui"*. × 50

FIG. 1. (*b*) Ovary of a nulliparous female *Culex "vishnui"* showing tracheal coiling. × 50

TABLE I.

Monthwise analysis of parity data on Culex "vishnui" *from three villages in North Arcot District.*

Month and year.	Length of gonotrophic cycle in days (n).	Villages														
		Venkatapuram					Sathuperi					Kammavanpeth				
		Number nulliparous.	Number parous.	Proportion of parous.	p.*	Per cent mortality.†	Number nulliparous.	Number parous.	Proportion of parous.	p.*	Per cent mortality.†	Number nulliparous.	Number parous.	Proportion of parous.	p.*	Per cent mortality†.
November, 1960	3—4	106	138	0·599	0·84—0·88	16—12	63	171	0·731	0·90—0·93	10— 7	67	250	0·789	0·92—0·94	8—6
December, 1960	4	71	115	0·618	0·89	11	63	81	0·563	0·87	13	148	318	0·682	0·91	9
January, 1961	4	117	206	0·638	0·89	11	37	75	0·670	0·90	10	140	247	0·638	0·89	11
February, 1961	3	70	123	0·637	0·86	14	37	46	0·554	0·82	18	156	230	0·596	0·84	16
March, 1961	2—3	35	66	0·654	0·81—0·87	19—13	113	130	0·535	0·73—0·81	27—19	229	204	0·471	0·69—0·78	31—22
April, 1961	2	81	89	0·524	0·72	28	32	63	0·663	0·81	19	22	39	0·639	0·80	20
May, 1961	2	10	13	0·565	0·75	25	52	40	0·435	0·66	34	69	58	0·457	0·68	32
June, 1961	2	81	180	0·690	0·83	17	30	46	0·605	0·78	22	260	276	0·515	0·72	28
July, 1961	2	141	456	0·764	0·87	13	41	94	0·696	0·83	17	152	153	0·502	0·71	29
August, 1961	2	85	387	0·820	0·91	9	12	21	0·636	0·80	20	73	173	0·703	0·84	16
September, 1961	2—3	145	519	0·782	0·88—0·93	12— 7	8	20	0·714	0·85—0·89	15—11	53	186	0·778	0·88—0·92	12—8
October, 1961	3	179	433	0·708	0·89	11	32	123	0·794	0·93	7	144	319	0·689	0·88	12
November, 1961	3—4	91	252	0·735	0·90—0·93	10— 7	121	387	0·762	0·91—0·93	9—7	131	498	0·792	0·93—0·94	7—6
December, 1961	4	75	220	0·746	0·93	7	118	286	0·708	0·92	8	99	321	0·764	0·93	7
January, 1962	4—5	81	122	0·601	0·88—0·90	12—10	41	41	0·500	0·84—0·87	16—13	221	335	0·603	0·88—0·90	12—10
February, 1962	3—4	87	92	0·514	0·80—0·85	20—15	16	10	0·385	0·73—0·79	27—21	371	448	0·547	0·82—0·86	18—14
March, 1962	3	87	90	0·509	0·80	20	9	8	0·471	0·78	22	208	267	0·562	0·83	17
April, 1962	2	21	32	0·604	0·78	22	1	0	..	..	..	36	48	0·571	0·76	24
May, 1962	2	15	40	0·727	0·85	15	0	5	..	..	..	48	75	0·610	0·78	22

* p = Chances of survival through a single day.

† Per cent mortality = $(1-p) \times 100$.

some mosquitoes took longer than others to complete the gonotrophic cycle. For example, in November 1960, $n = 3$ or 4. The parous rate in that month at Venkatapuram, one of the villages, was 0·599. p was therefore either $\sqrt[3]{0{\cdot}599}$ or $\sqrt[4]{0{\cdot}599}$, giving values of 0·84 or 0·88. Daily mortality therefore lay between 16 per cent and 12 per cent for this village in November 1960.

In all the three villages, mortality was higher in summer than in winter. Since the results from all the three were fairly similar, it was decided to combine the data, since the numbers of mosquitoes caught at each village individually were so low in some months as to make results based on them unreliable.

The pooled results are shown in Table II. The total numbers of mosquitoes on which parity was determined each month now rises, and the parous rates which are based on them become proportionately more reliable. Ninty-five per cent confidence limits for parous rates are included in the Table. It will be seen that only in April and May of each year does the variation exceed $\pm$ 0·04. The values of p corresponding to the lowest and highest values of the parous ratios, are given in the next column.

It may be argued that combining the data from three different places is unjustified. It can, however, be seen by comparison of Tables I and II that the mortality pattern obtained from the combined data is very similar to that obtained from individual villages, but has the advantage of being based on larger numbers of dissections.

From Table II it would seem that the mortality rate from August to January is between 6 per cent and 14 per cent. After February there is a rise in mortality, and highest mortalities occur in March to May when the values obtained vary from 15 per cent to 38 per cent. After June, mortality begins to fall again. This sequence of events was found to occur in all the three places studied and in both years of the study.

Table II also includes rainfall data and temperatures taken at one of the villages. North Arcot District receives some rainfall in June from the south-west monsoon, but most of its rainfall comes from the north-east monsoon in September-November. High mosquito mortality corresponds with hot dry months of the year, and low mortality with cool wet weather.

The average number of mosquitoes taken in ten trap nights in different months is included in Table II for comparison. In general it would appear that catches are highest in October to December. The greater longevity of mosquitoes during the rains, together with the increase in available breeding places, could well be contributory factors to the increase in the total population which occurs at this time. Graph 1 illustrates the seasonal variation in mosquito mortality, mosquito population (as shown by trap catches), and climatic factors.

TABLE II.

Natural mortalities calculated monthly on the basis of the combined data from three villages in North Arcot District, together with the average number of Culex "vishnui" *caught per ten trap-nights and meteorological data from one of the villages.*

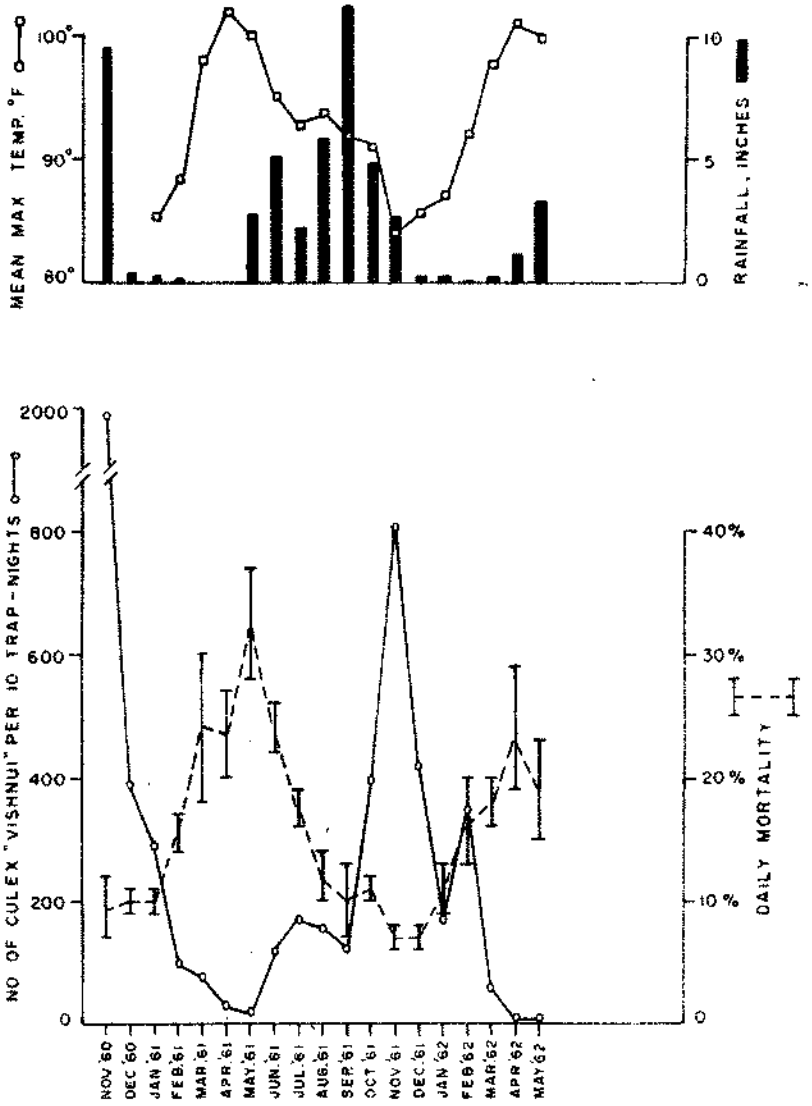
Month.	Length of gonotropic cycle in days (n).	Number with known parity.	Number parous.	Proportion parous ± 95 per cent confidence limits.	p.*	Per cent mortality.†	Number of *Culex "vishnui"* caught per 10 trap nights.	Temperature F°: Mean maximum.	Actual maximum.	Mean minimum.	Actual minimum.	Rainfall inches.
November, 1960	3—4	815	579	0·710 ± 0·031	0·88—0·93	12— 7	1978·6	..	..	..	..	2·50
December, 1960	4	796	514	0·646 ± 0·033	0·89—0·91	11— 9	399·4	..	..	..	..	0·45
January, 1961	4	822	528	0·642 ± 0·032	0·89—0·91	11— 9	289·0	85·4	95	67·7	58	0·27
February, 1961	3	662	399	0·603 ± 0·037	0·83—0·86	17—14	104·0	88·4	95	66·8	59	0·15
March, 1961	2—3	771	400	0·519 ± 0·035	0·70—0·82	30—18	78·6	98·1	105	74·1	66	0·00
April, 1961	2	326	191	0·586 ± 0·054	0·73—0·80	27—20	27·4	101·9	109	76·4	71	0·00
May, 1961	2	242	111	0·459 ± 0·063	0·63—0·72	37—28	18·8	100·2	109	78·8	74	2·88
June, 1961	2	873	502	0·575 ± 0·033	0·74—0·78	26—22	111·5	95·2	101	78·4	72	5·03
July, 1961	2	1,037	703	0·678 ± 0·028	0·81—0·84	19—16	170·2	92·6	97	77·2	68	2·28
August, 1961	2	751	581	0·774 ± 0·030	0·86—0·90	14—10	161·0	93·6	97	77·0	72	5·93
September, 1961	2—3	931	725	0·779 ± 0·027	0·87—0·93	13— 7	124·1	92·1	97	75·8	71	11·24
October, 1961	3	1,230	875	0·711 ± 0·025	0·88—0·90	12—10	400·5	91·0	96	76·3	72	4·98†
November, 1961	3—4	1,480	1,137	0·768 ± 0·021	0·92—0·94	8— 6	813·4	84·2	89	70·0	63	2·60
December, 1961	4	1,119	827	0·739 ± 0·026	0·92—0·94	8— 6	421·2	85·6	90	68·1	63	0·26
January, 1962	4—5	841	498	0·592 ± 0·033	0·87—0·91	13— 9	172·8	87·2	94	66·1	60	0·30
February, 1962	3—4	1,024	550	0·537 ± 0·031	0·80—0·87	20—13	348·8	92·3	98	69·0	59	0·09
March, 1962	3	669	365	0·546 ± 0·038	0·80—0·84	20—16	63·1	97·7	101	73·1	62	0·34
April, 1962	2	138	80	0·580 ± 0·082	0·71—0·81	29—19	13·1	101·2	110	78·7	75	1·10
May, 1962	2	183	120	0·656 ± 0·069	0·77—0·85	23—15	13·4	99·8	111	82·8	76	3·44

* p = Chances of survival through a single day.

† Per cent mortality = $(1-p) \times 100$.

GRAPH 1.

Seasonal variation in natural daily mortality of Culex "vishnui" *together with variation in catches and meteorological conditions.*



DISCUSSION

The *Culex vishnui* group consists of at least three species in the Vellore area. At the time this study was begun it was not possible to distinguish between adults of these species, so they were considered together. It is possible that these species may differ from each other as regards mortality. This is a possibility which will

have to be tested before the epidemiological significance of the mortality rate can be assessed. It may perhaps be permissible to make some comments at this stage. In an area where there is seasonal variation in mosquito numbers and mosquito mortality, one would expect that successful virus transmission would be most probable in the season when the population is high and the mortality is low. These conditions exist during the north-east monsoon, between September and November. There is some evidence that there is increased virus activity at this time.

Estimates of the natural mortality of *Culex fatigans* and *Anopheles peditaeniatus* are available from the Vellore area for the period October to December 1961 (Laurence, 1963). For *Culex fatigans*, values ranged from 14 per cent to 24 per cent and for *Anopheles peditaeniatus* from 15 per cent to 24 per cent. These values were obtained by using both parasite rates and parous rates. For the same period, estimates of mortality for *Culex "vishnui"* are lower, ranging from 6 per cent to 12 per cent.

Russell and Rao (1942), using very different methods, estimated a mortality of 50 per cent every two days in *Anopheles culicifacies*, in another part of South India. Their experiments were conducted between May and October. Macdonald (1952) showed that Russell and Rao's data could be recalculated to give a daily mortality of 22·5 per cent.

SUMMARY

Estimates of natural mortality of *Culex "vishnui"* in South India, based on parous rates, have been made over a period of nineteen months. This group of mosquitoes has been incriminated as the vector of Japanese encephalitis in this area. The mortality in August to January was between 6 per cent and 14 per cent, but was higher during the rest of the year. Highest mortalities occurred during the hot dry months from March to May. The known season of virus transmission in this area corresponds with the season when mosquito mortality is low.

ACKNOWLEDGMENTS

The author is grateful to Professor D. S. Bertram of the London School of Hygiene and Tropical Medicine for his advice, and for permission to use his data for November, 1960. Professor P. Armitage, also of the London School, kindly commented on the reliability of parous rates. The interpretation put upon the data presented is, however, the author's responsibility.

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A NEW DESIGN OF INSECTICIDE TESTING CHAMBER.

BY

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[December 5, 1963.]

INTRODUCTION

THE usual methods for screening potential contact insecticides are those in which either the insecticide is applied in scaler concentrations and the percentage mortalities assessed at the end of an arbitrary period of 24 or 48 hours, or a population exposed to a single concentration for a fixed period of time and the percentage mortality compared at the end of 24 hours. In cases where space sprays are to be screened, Peet Grady's (1928) method is quite satisfactory.

Evaluation of contact insecticides by topical application with the micrometer syringe is no doubt very precise for determining LD_{50}'s accurately but needs good laboratory facilities and well-trained technicians. One of the many earlier methods, cited by Busvine (1957) for screening potential insecticidal compound, is by treating the interior of a glass container with an insecticide solution and exposing the insects to this surface after the insecticide film is completely dry.

Fay (1953) improved the method by treating strips of filter papers, rolling them into cylinders and covering the two open ends by wire-netting collars. The method has been further improved by Busvine and Nash (1953) by limiting the area of the untreated surface to the base of the specimen-tube. The present communication describes a new design of apparatus for bio-assay.

MATERIALS AND METHOD

The apparatus essentially consists of the following parts : (*i*) a glass tube, 7 inches long and one inch in diameter, the lower one inch of this tube is drawn into a cone and is fused with another glass tube one inch long and 0·5 inch in diameter (Fig. 1·A) ; (*ii*) a glass rod 9 inches long and 1/8th inch in diameter, the lower end of this rod being moulded into a knob wide enough to close the opening between the glass tube and the cone (Fig. 1-B) ; (*iii*) a cork 0·5 inch in length and large enough to fit into the upper end of the tube with a hole in its middle to allow the free movement of the rod (Fig. 1-C); (*iv*) a disc of filter-paper, one inch in diameter and with a hole corresponding to the one in the cork (Fig. 1-D) ; (*v*) an ordinary pin, the upper 3/4th of which is bent horizontally into horse-shoe shape (*Fig. 1-E*) ; (*vi*) a strip of filter-paper, 6×4 inches, is sprayed with the insecticide and rolled into the tube while the disc of paper is fixed to the base of the cork with the help of the horse-shoe shaped pin and treated with insecticide. Fig. 2 shows the arrangement

Fig. 1-A.

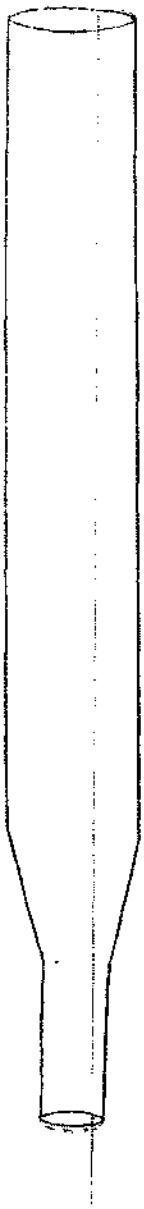


Fig. 1-B.

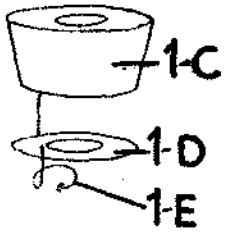


Fig. 2.

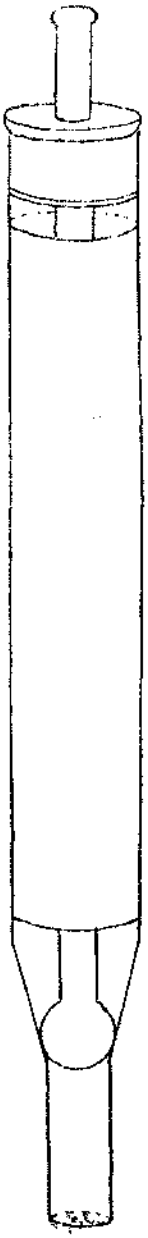


Fig. 1-A.

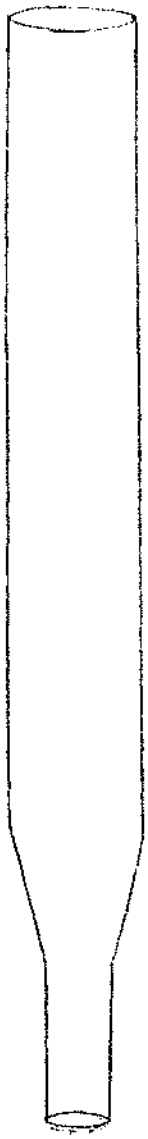
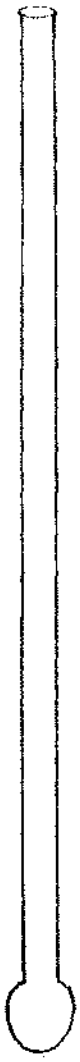


Fig. 1-B.



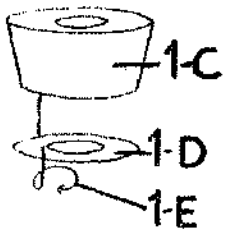
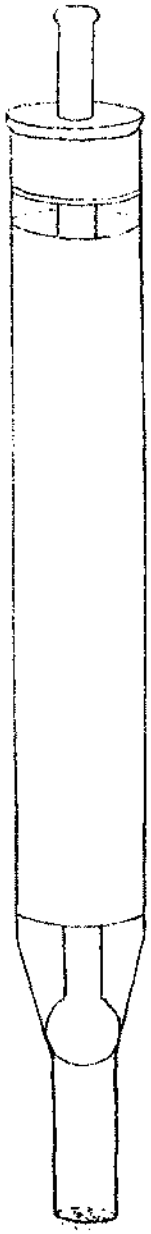


Fig. 2.



of the various components in position. The test insects are anaesthetised with carbon dioxide, counted, sexed and introduced into the tube by slightly lifting the cork. A minute or so later, the insects recover from the effect of carbon dioxide and settle on the treated filter-paper. The number of insects that are knocked down can be recorded at regular intervals of time. The selection of the concentration of the insecticide and the interval of time can be standardised by doing a few preliminary experiments.

DISCUSSION

The method described here facilitates observation of two essential facts about the flying insects, *viz.*, (*a*) different stages of knocked down, (*b*) mortality, when an insect population is exposed to a surface treated with an insecticide.

For reasons of uncertainty about the pathological conditions of insects in toxicological work, this aspect has not been given much importance. The new design of the apparatus helps in observing the initial and advanced paralysis and to record the percentage mortality amongst the paralysed insects. Essential prerequisites of an ideal insecticide, quick knock-down and irreversible paralysis can be determined by this method in a single operation.

The advantage of this method is that the paralysed insects can be removed at any time during the experiment without disturbing the rest of the insects under test. On account of the preferential behaviour of flying insects to rest on rough surfaces available, the untreated resting site becomes almost negligible so that the insects are in contact with the insecticide almost all the time during the experiment. This method is simple and precise. It needs no elaborate equipment and can be used both in the laboratory and the field.

LIMITATIONS

1. Since the method is based on the assumption that flying insects prefer rough surface over a glazed one, any change in this behaviour of insects would render the method ineffective.

2. Fabrication of either the tubes or the glass rods, though simple, would need the assistance of a glass blower.

SUMMARY

A design of an apparatus for the assessment of insecticide toxicity to flying insects is described. It takes advantage of the preferential behaviour of these insects to rest on the rough surface instead of glazed ones. It provides for removing the paralysed insects at any time during the experiment without disturbing the other insects under test, and helps the recording of observations while the experiment is in progress. It is simple and precise.

ACKNOWLEDGMENT

The author would like to express his sincere thanks to Dr. B. Mukerji, Retired Director, Central Drug Research Institute, for his keen interest in the project, and to the glass-blowing section for fabrication of the apparatus.

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EXPERIMENTAL SUSCEPTIBILITY OF *ANOPHELINE* MOSQUITOES TO SIMIAN MALARIA IN THE NILGIRIS, MADRAS STATE, SOUTH INDIA*

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[December 10, 1963.]

INTRODUCTION

ENZOOTIC simian malaria in *Macaca radiata radiata* in the Nilgiris of the Madras State was described by Ramakrishnan and Mohan (1962). It was possible by the authors to infect laboratory-reared *A. stephensi* and *A. fluviatilis* with simian malaria parasites by feeding them on infected monkeys. It was also possible to infect clean *Macaca radiata radiata* monkeys with *P. cynomolgi* and *P. inui* parasites by using *A. stephensi* as an experimental vector in the laboratory.

During the course of one-and-half years of investigation (March, 1962 to August, 1963) on epizootology of simian malaria, a total of eighty-nine *Macaca radiata radiata* monkeys were captured from different localities of the Nilgiris. Thirty-five of these were found to be naturally infected. Satya Prakash and Chakrabarti (1962) described the morphology and periodicity of these parasites and concluded that the parasites were either *P. inui* Halberstadter and Prowazek or *P. cynomolgi* Mayer. This paper records the work and results of attempts at experimental infection of *Anopheline* mosquitoes and clean *Macaca radiata radiata* monkeys to these parasites.

MATERIAL AND METHOD

The mosquitoes utilized for these studies were mainly obtained from the colonies maintained at the insectary. A small number of wild-caught *Anopheline* mosquitoes collected from Kallar, which is situated at the foot-hills of the Nilgiris, were also used.

* This work was mainly financed from the regular budget of the National Institute of Communicable Diseases and partly from the grants of the Indian Council of Medical Research and the World Health Organization.

Macaca radiata radiata monkeys were captured from different parts of the Nilgiris and were utilized for the present investigation. Some of them were found to be infected either with *P. inui* or with a mixed infection of *P. cynomolgi* and *P. inui*, on capture. The rest of the monkeys were not showing any parasites immediately after capture. These monkeys were used in the present investigation either to infect different batches of *Anopheline* mosquitoes or to complete the transmission of simian malaria from one monkey to the other through the experimentally infected *Anopheline* mosquitoes.

After the suitability of the donor monkeys was determined (all these monkeys had mixed infection of *P. cynomolgi* and *P. inui*), different species of *Anopheline* mosquitoes were fed simultaneously on the infected monkey. In each feeding, two known susceptible species of mosquitoes, *A. stephensi* (Type) and *A. fluviatilis*, were included as control. Sample dissections of the midguts and glands were made at regular intervals in order to evaluate the rate of infection and susceptibility of different species.

Prior to transmission of the malaria through infective mosquitoes, the recipient monkeys were usually splenectomised in order to prove that they were free from any previous infection. Out of the eight monkeys utilized in this investigation, four were splenectomised and were observed for periods varying from twenty-two to one-hundred-and-fifteen days prior to the infective bites by the mosquitoes. Three others were baby monkeys which were captured along with the mothers. When repeated blood examinations, varying from one to five months, did not show any parasites in their blood, they were treated as clean animals. Only one monkey (M-78) was used which was weighing 5 lbs. and was not splenectomised. The blood smears were examined and found negative for a period of seven days after capture and the monkey was then utilized for experimental feeding due to shortage of other clean animals. The prepatent period in this monkey was seventeen days and the nature of parasitaemia was similar to that of a fresh infection.

RESULTS

The temperature during different periods of the investigation varied between 75°F. and 90°F. The relative humidity was between 51 and 86 per cent. Among the different species of *Anopheline* mosquitoes used for these studies, *A. stephensi*, *A. fluviatilis*, *A. tessellatus* and *A. elegans* showed gut and gland infections when fed on monkeys infected with mixed infection of *P. cynomolgi* and *P. inui*. The results of experimental feeding of these mosquitoes are shown in Table I. The analysis of the proportion of positives, month-wise and species-wise, is depicted in Table II.

It would be seen from Tables I and II that all the four species of mosquitoes were susceptible to the simian infection under laboratory conditions. All of them showed high oöcyst counts under suitable identical conditions.

It was possible to infect clean *Macaca radiata radiata* monkeys with mixed infection of *P. cynomolgi* and *P. inui* by using the three species, *A.*

TABLE I.

Results of dissection of laboratory-bred Anopheline *mosquitoes, fed on monkeys showing mixed infection of* P. cynomolgi *and* P. inui.

Serial number of experimental feeding on monkeys.	Date of feeding.	*A. stephensi*.					*A. fluviatilis*.					*A. tessellatus*.					*A. elegans*.				
		Number of gut dissected.	Number positive.	Number of gland dissected.	Number positive.	Percentage of gland infection.	Number of gut dissected.	Number positive.	Number of gland dissected.	Number positive.	Percentage of gland infection.	Number of gut dissected.	Number positive.	Number of gland dissected.	Number positive.	Percentage of gland infection.	Number of gut dissected.	Number positive.	Number of gland dissected.	Number positive.	Percentage of gland infection.
1	March 6, 1962	9	9	31	20	64	4	4	6	5	32	*									
2	July 6, 1962	10	10	17	16	94	21	21	19	10	52	*		*			*		*		
3	July 10 and 11, 1962	6	6	110	82	74	4	4	106	101	94										
4	August 30, 1962	0	0	124	89	71	0	0	126	101	80										
5	September 27, 1962	0	0	23	8	36	0	0	48	17	35										
6	October 3, 1962	0	0	56	36	64	0	0	28	15	53										
7	November 13, 1962	4	4	31	16	51	5	5	68	31	44	7	7	3	2	66					
8	December 3, 1962	3	3	15	13	86	6	6	7	6	85	2	1	2	1	50					
9	December 21 and 24, 1962	0	0	46	38	71	0	0	137	112	81	9	9	9	8	88					
10	January 9, 1963	0	0	16	9	56	0	0	15	3	20	*	*	*	*	*	*	*	*	*	*
11	February 4, 1963	8	4	8	2	25	8	6	8	2	25	*	0	*2	0	*	2	2	4	4	100
12	August 13, 1963	1	0	6	0	0	4	2	10	0	0	*	*	*	*	*	*	*	*	*	*

* No feeding was attempted.

TABLE II.

Percentage of experimentally fed Anopheline *mosquitoes which showed sporozoites in their glands during different months of the investigation.*

Species of *Anopheline* mosquitoes fed on infected monkeys.	March, 1962	April, 1962	May, 1962	June, 1962	July, 1962	August, 1962	September, 1962	October, 1962	November, 1962	December, 1962	January, 1963	February, 1963	March, 1963	April, 1963	May, 1963	June, 1963	July, 1963	August, 1963
A. stephensi	64	*	*	*	77·1	71·1	36·0	64·0	51·0	75·4	56·0	25·0	*	*	*	*	*	0·0
A. fluviatilis	82	*	*	*	88·8	86·0	35·0	53·0	44·0	81·9	20·0	25·0	*	*	*	*	*	0·0
A. tessellatus	*	*	*	*	*	*	*	*	66·0	81·8	*	0·0	*	*	*	*	*	*

* No feeding was attempted.

stephensi, *A. fluviatilis* and *A. tessellatus*, as experimental vectors. Experimental transmission with *A. elegans* was not attempted due to non-availability of sufficient number of these mosquitoes. *A. elegans* has been recently incriminated as a natural vector of simian malaria in the Nilgiris (Choudhury *et al.*, 1963). The results of the experimental transmission in the monkeys are shown in Table III. It would be seen therefrom that out of eight recipient monkeys, which were bitten by infected mosquitoes, only one remained negative without showing any parasitaemia. All the seven monkeys showed mixed infection of *P. cynomolgi* and *P. inui* after prepatent periods varying from eleven to forty-seven days. On each occasion, *P. cynomolgi* appeared first, followed by *P. inui* within three to five days.

DISCUSSION

A. maculatus, *A. culicifacies*, *A. splendidus* and *A. annularis* have been described by Mulligan (1935) as experimental vectors of *P. cynomolgi*. It was observed by him that *P. cynomolgi* infection could easily be transmitted to healthy monkeys by bites of these mosquitoes. Green (1932) was able to infect *A. maculatus*, *A. kochi* and *A. vagus* with *P. cynomolgi* but failed to transmit the infection into clean monkeys by inoculation of sporozoites.

The experimental insect vector of *P. inui* was, however, unknown for a long time as mentioned by Russell (1946). From available literature it appears that the first reference of successful mosquito transmission of *P. inui* was made by Garnham (1951). Working on pre-erythrocytic cycle of *P. inui*, he found that *A. maculipennis atroparvus* could be successfully infected under laboratory conditions, the sporozoites appearing three weeks after the infective feed. In *A. stephensi* and *A. gambiae*, he observed that the development was stopped in the oocyst stage. All the transmission studies in the present investigation were carried out with a mixed infection of *P. cynomolgi* and *P. inui*, as pure infection was not available in nature in the captured monkeys. It was observed that *A. fluviatilis*, *A. tessellatus* and *A. stephensi* were infected with sporozoites of *P. cynomolgi* and *P. inui* after an extrinsic incubation period of eleven, twenty and twenty-three days respectively. The sporozoites were sometimes found to appear earlier than mentioned above in case of *A. stephensi* and *A. tessellatus* but the correct identification of the species was not possible.

Ramakrishnan and Mohan (1962 *loc. cit.*) observed that *A. stephensi* could be infected with a mixed infection of *P. cynomolgi* and *P. inui*, the sporozoites appearing thirteen days after the infective feed. The results of the present studies confirm the above finding. Wide variation in the prepatent period was observed in the recipient monkeys during the present investigation. It took forty-seven days to show patent parasitaemia in case of one monkey which had received the bites of two *A. stephensi* mosquitoes. This happened to be the lowest dose that was attempted. The difference in the dose of the sporozoites must be one of the main factors for the wide variations observed in the prepatent period.

TABLE III.

Experimental transmission of mixed infection of P. cynomolgi *and* P. inui *by* A. stephensi, A. fluviatilis *and* A.tessellatus.

Serial number of experimental feeding.	Species of mosquitoes employed.	Species of parasites and the serial number of the monkey on which the mosquitoes were fed for infecting them.	Species and the total number of infected mosquitoes fed on clean monkey for transmission (confirmed by post-parandial dissection of the glands).	Serial number of the recipient monkey on which mosquitoes were fed for transmission and the period of observation prior to the infective bite in each animal.	Results.	Prepatent period for *P. cynomolgi*.	Prepatent period for *P. inui*.
1.	*A. fluviatilis*	M. 75	*A. stephensi* : 15	M. 73 (Baby monkey) 23 days	Remained negative.	..	..
	A. stephensi	(*P. cynomolgi* and *P. inui*)	*A. fluviatilis* : 15	M. 78 7 days	Became positive with *P. cynomolgi* and *P. inui* parasites.	17 days	20 days
2.	*A. fluviatilis*	M. 74	*A. fluviatilis* : 18	M. 93			
	A. stephensi	(*P. cynomolgi* and *P. inui*)	*A. stephensi* : 16	(Baby monkey) 155 days		14 days	19 days
3.	*A. stephensi*	M. 92 (*P. cynomolgi* and *P. inui*)	*A. stephensi* : 2	M. 76 (Baby monkey) 116 days		47 days	50 days
4.	*A. fluviatilis*	M. 76	*A. fluviatilis* : 10	M. 106			
	A. stephensi	(*P. cynomolgi* and *P. inui*)	*A. stephensi* : 2	(After splenectomy) 22 days		26 days	31 days
	A. tessellatus		*A. tessellatus* (infected glands) : 3	M. 77* (After splenectomy) 115 days		25 days	30 days
5.	*A. fluviatilis*	M. 95	*A. fluviatilis* : 4	M. 98			
	A. stephensi	(*P. cynomolgi* and *P. inui*)	*A. stephensi* : 12	(After splenectomy) 68 days		11 days	14 days
6.	*A. fluviatilis*	M. 77	*A. fluviatilis* : 14	M. 99			
	A. stephensi	(*P. cynomolgi* and *P. inui*)	*A. stephensi* : 7	(After splenectomy) 107 days		14 days	18 days

* This monkey received inoculation of 3 infected glands of *A. tessellatus* in serum saline, i/v.

SUMMARY

A. stephensi, A. fluviatilis, A. tessellatus and *A. elegans* were found to be susceptible under laboratory conditions to a mixed infection of *P. cynomolgi* and *P. inui* obtained from *Macaca radiata radiata* monkeys captured from different localities of the Nilgiris. The successful transmission of the simian malaria parasites into clean monkeys, with the help of *A. stephensi, A. fluviatilis* and *A. tessellatus* as the experimental vectors, is recorded.

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INCRIMINATION OF *ANOPHELES ELEGANS* JAMES (1903) AS A NATURAL VECTOR OF SIMIAN MALARIA IN THE NILGIRIS, MADRAS STATE, INDIA*.

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[December 10, 1963.]

INVESTIGATIONS were commenced in February, 1962, to determine the natural vector (s) responsible for the enzootic simian malaria described by Ramakrishnan and Mohan (1962). A small hamlet, situated on the foot-hills of the eastern side of the Nilgiris, was selected for the studies, as ideal conditions for the transmission of simian malaria were prevalent in the area. The hamlet is 457 metres above the sea-level and is situated on the Coonoor-Mettupalaiyam Road, about 15 miles from Coonoor. It is covered with dense tropical forest in which arecanut gardens and a few mango groves are scattered. These arecanut gardens and the mango groves, with some jack-fruit trees, are the favourite haunts of monkeys. It was observed that the monkeys rested during the night in these locations, especially during the mango and jack-fruit seasons, extending from May to August. The temperature and humidity during this period remain optimum for the transmission of malaria.

OBSERVATIONS

During the observation period, extending over a little more than a year, approximately 6,000 *Anopheline* and *Culicine* mosquitoes were collected and dissected for determining the presence of natural infections in them. Thirty-one wild-caught mosquitoes, which included *Culex vishnui*, *Culex bitaeniorhynchus*, *Aedes (aedes) pseudomediofasciatus*, *Armigeres obturbans* and *A. elegans*† were found infected in nature. In some instances, the sporozoites detected in these mosquitoes were inoculated into uninfected *Macaca radiata radiata* monkeys, whenever they were available, to establish their simian origin or otherwise.

* This work was mainly financed from the regular budget of the National Institute of Communicable Diseases and partly from the grants of the Indian Council of Medical Research and the World Health Organization.

† Christophers (1933) recognized only *A. leucosphyrus* Donitz, 1901 as a valid species with *elegans* James, 1903 as a synonym. Colless (1956 and 1957) has split *A. leucosphyrus* group into 13 species. This group is represented in India by *A. elegans* James and *A. balbacensis balbacensis* Baisas. The latter has not as yet been found in the investigation area.

In the months of June and July, 1963, 84 female specimens of *A. elegans* were collected along with a large number of specimens of *A. tessellatus* and *A. maculatus* from one of the arecanut gardens during the day. *A. elegans* were collected from dark, damp and shady places of the gardens and also from shrubs near the base of the arecanut trees. The availability of sufficient males of these species in the same place showed that the breeding was going on in the vicinity of the arecanut gardens. In these gardens, water from the mountain streams is allowed to stagnate around the base of the arecanut trees for about a week and the plots are then drained. This intermittent flooding of alternate areas in the gardens leaves enough swamps for the breeding of the mosquitoes. *Anopheline* and *Culicine* larvae of different species were collected from these places.

Out of 84 *A. elegans* females captured and dissected, 10 showed sporozoites in the glands which were confirmed by staining. The sporozoites were slender and their length varied from 8 to 14 microns. Eight other mosquitoes showed oocysts in their mid-guts. Four uninfected* *Macaca radiata radiata* monkeys were inoculated with sporozoites obtained from four of these infected mosquitoes. All the four monkeys developed mixed infection of *P. cynomolgi* and *P. inui* after a prepatent period of 8, 8, 11 and 13 days respectively. One monkey showed *P. inui* first which was followed by *P. cynomolgi*. The reverse happened in the other three. The results of the experimental inoculation of sporozoites are mentioned in Table I.

The sporozoites detected in the wild-caught *Culicine* mosquitoes failed to produce infection when inoculated into other clean monkeys.

In the monkeys inoculated with sporozoites from *A. elegans*, *P. inui* parasites were detected after a prepatent period of 10, 11, 13 and 20 days respectively. Wharton *et al.* (1962) observed the appearance of *P. inui* after a prepatent period of 17 days in a rhesus monkey which was inoculated with the sporozoites obtained from one wild-caught *A. leucosphyrus* in Malaya. The prepatent period in the experimental animals in case of *P. cynomolgi* was observed to be between 8 and 12 days. Eyles *et al.* (1963) inoculated a rhesus monkey with sporozoites obtained from one wild-caught *A. balbacensis introlatus* in Malaya. The monkey showed *P. cynomolgi* parasites on the eleventh day. In the laboratory *Macaca radiata radiata* monkeys, on whom experimentally infected *A. stephensi*, *A. fluviatilis* and *A. tessellatus* were fed, showed a prepatent period varying from 11 to 26 days with *P. cynomolgi* parasites (Choudhury *et al.*, 1963).

Laboratory-reared *A. elegans*, *A. stephensi* and *A. fluviatilis* mosquitoes were fed during the month of August, 1963, on an infected *Macaca radiata radiata* monkey (M-126) showing a mixed infection of *P. cynomolgi* and *P. inui*. The

* The fact that the recipient monkeys were free from malarial infection was proved by removing the spleen in case of three monkeys. They remained negative during an observation period of 60 to 114 days. The fourth recipient monkey was born in the laboratory and was observed and found negative for three months prior to experimental inoculation.

TABLE I.

Results of inoculation of sporozoites from wild-caught A. elegans *into uninfected* Macaca radiata radiata *monkeys.*

Serial number.	Number of the recipient monkey.	Observation period before infection.	Dose of inoculation.	Result of the inoculation.	Prepatent period in the recipient monkey.	Remarks.
1	*M-119 (splenectomised)	100 days after splenectomy	Sporozoites from one mosquito in monkey serum saline.	Mixed infection of *P. cynomolgi* and *P. inui*.	11 days	*The monkey died of high parasitaemia and the resultant anaemia after 20 days of patent parasitaemia
2	M-126 (splenectomised)	114 days after splenectomy	Sporozoites from one mosquito in monkey serum saline.	Mixed infection of *P. cynomolgi* and *P. inui*.	8 days	
3	M-8 (splenectomised)	60 days after splenectomy	Sporozoites from one mosquito in monkey serum saline.	Mixed infection of *P. cynomolgi* and *P. inui*.	13 days	
4	Baby of M-96 (Born in Coonoor laboratory) (spleen intact)	102 days	Sporozoites from one mosquito in monkey serum saline.	Mixed infection of *P. cynomolgi* and *P. inui*.	8 days	

average temperature during this period was 82·4°F and the average relative humidity was 55 per cent. Among five *A. elegans* mosquitoes fed on the monkey, one showed heavy infection of the midgut when dissected on the 8th day after feeding. Another moribund mosquito in this group was dissected on the 13th day and showed infection of the mid-gut and the glands. The remaining three mosquitoes were dissected on the 14th day and showed heavy infection of the glands. Contrary to this, very poor infection was obtained in the other two species of mosquitoes, *A. stephensi* and *A. fluviatilis*, which were fed simultaneously as control on the same infected monkey and dissected after the same interval. These two species have been found to be otherwise quite efficient vectors of simian malaria under laboratory conditions (Choudhury *et al.*, 1963). The results of the experimental infection are shown in Table II.

From Table II it is seen that *A. elegans* is an efficient vector under laboratory conditions. The observation is of course based on only a very small number of these mosquitoes, which were available for the experimental feeding. Efforts, to colonise this species in the laboratory, have not yet been successful.

TABLE II.

Results of dissection of the laboratory-reared mosquitoes fed on Monkey Number 126, showing infection of P. cynomolgi *and* P. inui.

Species of mosquitoes fed.	Number of mid-gut dissected.	Number found with oocysts.	Number of mosquitoes dissected for glands.	Number showing sporozoites.	Remarks.
A. elegans	2	2	4	4	One *A. elegans* mosquito was dissected for both gut and glands.
A. fluviatilis	4	2	10	*Nil*	
A. stephensi	1	*Nil*	6	*Nil*	

Wharton and Eyles (1961) incriminated *A. hackeri* as a vector of simian malaria in Malaya. Since then two other species of the *leucosphyrus* group, namely *A. leucosphyrus* and *A. balbacensis introlatus*, have been incriminated as vectors of simian malaria in Malaya (Wharton *et al.*, 1962 and Eyles *et al.*, 1963). The present findings have established one more species of the *leucosphyrus* group as a natural vector of simian malaria. *A. elegans* thus becomes the fourth of this group to be incriminated as a vector of simian malaria. Contrary to the findings in Malaya by Macdonald and Traub (1960) of *A. leucosphyrus* attacking man at ground-level inside the forest, *A. elegans* was never found on human baits during the present investigation. Not a single specimen of this mosquito was collected from a monkey bait kept 20 feet above the ground level in a mango grove situated very close to the arecanut garden. *A. tessellatus*, *Culex* and *Aedes* group of mosquitoes were regularly collected from this bait. It was also not possible to collect *A. elegans* mosquitoes from a monkey bait kept on the ground in the same arecanut garden. Freshly-fed *A. elegans* mosquitoes were, however, collected from the shrubs near the monkey bait. The result of the precipitin test, carried out at the National Institute of Communicable Diseases, Delhi, with the blood-meals of these mosquitoes, showed the presence of monkey-blood in three out of 11 specimens tested. The results of the tests are shown in Table III.

TABLE III.

Results of the precipitin test carried out from the blood-meals of A. elegans *captured in July and August, 1963, from an arecanut garden in the investigation area.*

Month of capture of the mosquitoes.	Number of blood-meals examined.	Number showing positive reaction to :					Remarks.
		Human.	Bovine.	Dog.	Pig.	Monkey.	
July	8	..	..	..	..	2	*The rest did not show any reaction against any of the antiseras tested.
August	3	..	..	..	..	1	
Total	11*	..	..	..	..	3	

The present observations have not revealed evidence of any other vector (s) of simian malaria in the Nilgiris. Because of the low density of *A. elegans* (one per

two man-hours) and absence of their association with man, this species does not appear likely to transmit the simian malaria infection to man.

SUMMARY

A. elegans James, one of the species of the *Leucosphyrus* group, has been shown to be infected in nature with *P. cynomolgi* and *P. inui* in a small hamlet of the Nilgiris, Madras State, India.

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INVESTIGATION ON THE REAPPEARANCE OF SUSPECTED HUMAN PLAGUE IN GOBICHETTI-PALAIYAM TALUK OF COIMBATORE DISTRICT, MADRAS STATE, INDIA*

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INTRODUCTION

PLAGUE broke out in epidemic form in India in 1895-1896 and took toll of a large number of human lives for about two decades. Since then there has been a gradual decline in the plague death rates. Punjab, Uttar Pradesh and Bombay got badly involved during this period. The other States which were affected to a lesser degree were Bihar, Orissa, Central Provinces, Hyderabad and Mysore of the British period. During the last decade, plague started disappearing from most of the States in India, with the exception of Mysore and Madras which have never been completely free ever since 1898 (Seal, 1960a). The plague situation in these States has recently been reviewed by Seal and Patnaik (1963). Of the twelve districts of the Madras State, Kanyakumari, Tirunelveli and Ramanathapuram have been free from plague for about the last two decades. Other districts have also become free since 1953, except some portions of Salem District where plague still continues smouldering.

The Coimbatore District, which is adjoining Salem, has been free from any attack since 1950. The reported attacks and deaths from plague in the nine districts of Madras, between 1940 and 1960, are mentioned in Table I. It is observed from Table I that during the five years from 1943 to 1947, Coimbatore District had the major brunt of onslaught as compared to other districts. The incidence of plague declined gradually during the following three years till it disappeared completely. The endemicity in the Madras State, thereafter, remained confined to Salem District only. After a lapse of eleven years, plague is once again threatening to reappear in Coimbatore District.

THE PRESENT EPISODE

Three instances of rat-fall occurred on October 9, 1962, in Kalmandipuram Village of Talavady Panchayat Union under the Gobichettipalaiyam Taluk of

* This investigation was carried out under the auspices of the *"Assessment of Susceptibility Status o Insects of Public Health Importance"* enquiry, financed by the Indian Council of Medical Research.

TABLE I.

Reported attacks and deaths from plague in different districts in Madras State between 1940—1960

Year.	SALEM :		NILGIRIS :		COIMBATORE :		NORTH ARCOT :		SOUTH ARCOT :		MADURAI :		TANJORE :		TRICHIRAPALLI :		CHINGLEPUT :		TOTAL :	
	A*.	D†.	A.	D.	A.	D.	A.	D.	A.	D.	A.	D.	A.	D.	A.	D.	A.	D.	A.	D.
1940	386	184	15	11	219	76	..	..	..	..	6	4	..	..	..	..	...	..	626	275
1941	205	106	88	11	148	50	13	11	..	..	..	..	..	..	..	..	..	..	384	178
1942	254	125	111	51	160	74	4	3	..	..	3	1	..	1	..	..	..	..	532	255
1943	463	239	92	45	6,876	4,796	18	15	..	..	15	14	..	..	74	..	..	..	7,464	5,109
1944	341	163	61	34	1,673	1,184	32	22	..	..	20	18	..	..	..	..	2	1	2,129	1,422
1945	529	303	370	163	1,294	1,076	40	35	58	22	17	10	..	..	..	..	..	..	2,308	1,609
1946	397	187	153	60	3,577	1,996	57	30	57	22	15	7	..	..	..	..	..	..	4,256	2,311
1947	376	179	117	18	2,901	1,495	675	454	250	120	579	206	12	8	22	9	1	1	4 933	2,510
1948	285	132	66	13	401	294	40	19	67	25	217	93	17	9	..	..	..	..	1,093	585
1949	166	68	20	8	39	7	28	18	...	..	6	2	15	8	..	..	..	..	281	111
1950	3	1	7	4	2	..	..	..	..	..	..	..	..	..	..	..	..	..	12	5
1951	6	5	..	..	..	..	..	..	3	2	..	..	..	..	7	6	..	..	16	13
1952	18	10	3	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	23	12
1953	9	6	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	9	6
1954	8	6	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	8	6
1955	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
1956	2	1	..	..	..	..	..	..	..	..	..	..	..	..	...	..	..	..	2	1
1957	4	1	...	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	4	1
1958	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..
1959	17	7	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	17	7
1960	29	14	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	29	14

* A = Attacks.

† D = Deaths.

The districts of Ramanathapuram, Tirunelveli and Kanyakumari had no attacks and deaths due to plague.

Coimbatore District. Rat-falls occurred regularly in this village from the beginning of November, 1962, but failed to attract the attention as the death among the rodents was thought to be due to the rat poison used by the villagers. On November 14, 1962, five smears collected from dead rats were sent to King Institute, Madras, for bacteriological examination. The finding of plague bacilli in these smears left no doubt that the rat-falls were due to plague.

Two girls, aged six and twelve years, living in adjoining houses in this village showed a high rise of temperature and cervical adenitis. Dead rats, whose smears showed *Pasteurella pestis* on examination, had been collected from these houses, only a few days prior to the illness of these girls. The physician of the Primary Health Centre at Talavady made a provisional diagnosis of plague in these two cases. The girls were treated with chloramphenicol and sulphadiazine and made an uneventful recovery. The material taken from the infected lymph glands, after the administration of the drugs, did not show any plague bacilli. A boy, aged fourteen years, of the same village showed inflammation of the axillary lymph glands with a high rise of temperature. He was also similarly treated with the above drugs and was cured. No further attack was reported from this or any of the surrounding villages and the rat-falls also ceased to occur due to the very active antiplague measures that were undertaken from the middle of November. The present investigation on the occurrence of human plague was undertaken from January 18 to 22, 1963.

Gobichettipalaiyam Taluk is situated in the north-west of Coimbatore District bordering Mysore State. It is divided into five Panchayat Unions, of which Talavady is in the extreme north-west. It consists of twenty villages and seventy-two hamlets with a population of 24,500. The villages are all scattered in the forest belt and the communication between individual villages is very poor. The panchayat headquarter, Talavady, is situated on a plateau along the road from Satyamangalam to Chamarajanagar (Mysore State), about 42 miles from Satyamangalam.

Talavady is situated at an altitude of 2,400 feet and the average rainfall is about 30 inches, occurring mainly from October to February. There is an acute shortage of water in this Union as the subsoil water table is low and the supply from the springs and mountain streams is extremely inadequate.

The socio-economic condition of the people is comparatively low. There is no electricity in the villages and no regular means of communication with the surrounding areas. The average housing condition of the villagers is poor. Most of the houses are constructed with mud and stones. The walls of the houses are not even plastered well with mud, due to scarcity of water. Plenty of cracks were found in the walls of the houses which were plastered with earth. These cracks were mainly due to the rocky and sandy nature of the soil available over there. During the investigation, many rat-holes and rat-burrows were seen in the walls of almost

every house. There is only one winter crop consisting of *ragi** and dry millets during the course of the whole year. This Panchayat Union was previously endemic for plague in the past. Table II shows the number of reported attacks and deaths due to plague in this Union during 1940-1962. It is observed from this table that there was a gradual decline in the attack-rate from 1940 to 1950, followed by a lull of eleven years without any attack.

TABLE II.

Reported attacks and deaths from plague in the Talavady Panchayat Union from 1940 to 1962.

Year.	TALAVADY PANCHAYAT UNION :	
	Attacks.	Deaths.
1940	108	37
1941	14	3
1942	7	2
1943	73	33
1944	23	10
1945	28	11
1946	35	11
1947	19	5
1948	4	2
1949	0	0
1950	1	0
1951-61	0	0
1962	3 (suspected)	0

Kalmandipuram Hamlet, where the rat-falls and the plague cases occurred, is situated nine miles north of Talavady and is about five miles from the Chamarajanagar District border of Mysore State. It is a small hamlet consisting of about one hundred houses and is surrounded by shrubs and fields of *ragi*, the main winter crop. This hamlet is fairly isolated from the surrounding localities.

ANTIPLAGUE MEASURES CARRIED OUT BEFORE THE INCIDENT†.

There is one permanent team consisting of one Plague Health Inspector, three mistrys‡ and six labourers looking after the antiplague work of this Union. They are engaged in the insufflation of rat-burrows and dusting of the rat-runs with 10 per cent D.D.T., rat trapping and periodical flea survey. It takes about nine months for the team to complete one round of the whole panchayat. Just before the out-break, a flea survey was conducted by this team and a flea index of four was determined in Kalmandipuram and the surrounding villages.

SUSCEPTIBILITY TEST ON RAT-FLEAS COLLECTED FROM RODENTS OF THE INFECTED AND SURROUNDING VILLAGES

The panchayat had also been receiving two rounds of D.D.T. spraying each year since 1955, carried out by the National Malaria Control/Eradication Programme.

* *Eleusine coracana*.

† Antiplague measures are carried out as a routine by the State Public Health authorities.

‡ Overseers.

Two hundred fourteen fleas were collected from 97 rats captured during the three days of investigation. The flea index was determined to be 2·2. *X. astia* was the only species found in the collections. The results of the tests are depicted in Table III. It is observed from this Table that the fleas were resistant to D.D.T. When exposed to 4 per cent concentration of this insecticide for one hour, there was no mortality in the females and a small percentage of mortality in the males. The fleas were exposed to 4 per cent D.D.T. for 24 hours and this gave a mortality rate between 50 and 60 per cent in both sexes combined.

TABLE III.

Results of susceptibility test with X. astia *collected from Talavadi Panchayat Union, Gobichettipalaiyam Taluk, Coimbatore District, Madras State.*

Species.	Insecticides.	Concentration (Per cent).	Number of fleas exposed :			Percentage of fleas dead :			LC (Per cent).
			Total.	Male.	Female.	Total.	Male.	Female.	
X. astia	DDT (One hour's exposure)	1·0	8	6	2	0·0	0·0	0·0	>4·0
		2·0	9	5	4	*0·0	*1·2	0·0	
		4·0	9	3	6	*0·0	*16·1	0·0	
			7	..	7	0·0	..	0·0	
	DDT (24 hours' exposure)	4·0	8	3	5	*53·0	*100·0	*50·0	
			8	2	6	50·0	100·0	33·3	
			20	6	14	60·0	80·0	37·5	
	Dieldrin (One hour's exposure)	0·05	18	7	11	*0·0	*0·0	0·0	Females =0·12
		0·1	17	3	14	*33·7	*100·0	*19·6	
		0·2	13	5	8	*71·1	*50·0	*84·3	
		0·4	11	3	8	100·0	100·0	100·0	
		0·8	5	1	4	100·0	100·0	100·0	
	Gamma BHC (One hour's exposure)	0·005	9	3	6	0·0	0·0	0·0	
		0·01	14	1	13	*1·6	0·0	H*10·4	
		0·02	8	6	2	*21·7	*37·0	0·0	
		0·04	6	2	4	*16·6	*37·0	*6·2	

* Percentage mortality corrected by Abbott's formula.
Control mortality in all these series = 20·0 per cent.
Control mortality in the rest of the series = 0·0
H = Heterogeneous response.

The LC_{50} value for *X. astia* females against Dieldrin was estimated to be 0·12 per cent. The same value for a susceptible strain from Delhi has been reported by Sharma and Joshi (1961) to be 0·01 per cent.

The tests against gamma BHC were performed with the laboratory impregnated filter papers. The LC_{50} value for *X. astia* could not be determined as enough fleas were not available during the period of investigation to run through all the concentrations. When exposed to 0·04 per cent of gamma BHC for an hour, the mortality rate in *X. astia* females was found to be 6·2 per cent only. The LC_{50} values for *X. astia* females of a susceptible Delhi strain was determined to be 0·001 per cent. This showed an indication of increased tolerance of the fleas to gamma BHC.

MEASURES TAKEN AFTER THE OUTBREAK

(*i*) All the houses in Kalmandipuram and the surrounding villages, within five miles radius, were sprayed with gamma BHC.

(*ii*) The rat-burrows in these houses were fumigated with cyanide gas.

(*iii*) In addition, rat-burrows were insufflated and rat-runs dusted with gamma BHC.

(*iv*) Mass inoculation of the population was carried out with antiplague vaccine (Haffkin's).

With the institution of these measures in November, 1962, there was no further report of any rat-fall or human case.

PROBABLE CAUSE OF THE OUTBREAK

There was a gradual rise in the density of the fleas as D.D.T. failed to control them due to the emergence of a resistant strain. This increase in the density of the fleas helped in the overflow of infection from wild rodents to the domestic ones either directly or through the peridomestic ones. The factors responsible for keeping the infection limited to the wild rodents and its occasional overflow into the domestic ones are not quite clear.

Though a fairly good number of rat-falls occurred in Kalmandipuram during October and November, 1962, the report of suspected human cases was surprisingly low in spite of the fact that the housing conditions showed a close association between villagers and the rodent population. The absence of *X. cheopis*, not a single specimen of which was found during the course of the investigation, might be the limiting factor in the spread of the disease in the human and rodent population.

Seal (1960*b*) and Deoras and Gokhale (1958) observed the changing pattern in the distribution of fleas in Calcutta and Bombay respectively. It was found that there was relative increase of *X. astia* in Calcutta and *X. cheopis* in Bombay. Whether such a thing happened in Talavady, could not be confirmed due to non-availability of the previous data about the distribution of fleas in this place. King and Pandit (1931) and King and Iyer (1933) reported the presence of *X. astia*, *X. cheopis* and *X. braziliensis* in Coimbatore Town and surrounding areas. But no authentic data about the previous flea distribution in Talavady could be obtained.

SUMMARY AND CONCLUSION

Rat-falls, which were found to be due to plague bacilli, occurred during October and November, 1962, in Kalmandipuram Village of Talavady Panchayat Union under the Gobichettipalaiyam Taluk of Coimbatore District. The district had been free from any attack of plague since 1950. This Panchayat Union was endemic for plague, with the exception of the last eleven years.

Three suspected human cases, which could not be confirmed by laboratory tests, occurred in the village during November, 1962. On investigation, the housing condition of the villagers revealed close association between them and the rodent population. The survey of the fleas showed *X. astia* as the prevalent species and it was found to be resistant to D.D.T. This insecticide was being used to control the fleas for a number of years in this locality. After the incident, D.D.T. has been replaced by gamma BHC but susceptibility tests, carried out early in 1963, showed incipient resistance of the fleas to this insecticide. Hence, frequent checks should be made to determine the level of tolerance of the fleas if this insecticide is going to be continually used.

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SUSCEPTIBILITY STATUS OF RAT-FLEAS, *X. CHEOPIS* AND *X. ASTIA*, TO CHLORINATED HYDROCARBON INSECTICIDES IN THE SALEM, COIMBATORE AND MADURAI DISTRICTS OF MADRAS STATE*.

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HUMAN plague almost completely disappeared from India, except in certain parts of the Madras and Mysore States, during the later part of the last decade. In 1958, only four deaths from plague were recorded throughout India which is the lowest number ever recorded since 1898. During the last decade, widespread application of D.D.T. and other residual insecticides by the National Malaria Control/Eradication Programme Organization was, to a great extent, responsible for the spectacular decline in the incidence of plague cases and in the densities of rat-fleas in most of the places throughout India. The role of insecticides used in agriculture and preservation of grains cannot be assessed. With the report of emergence of different insecticide-resistant rat-fleas in many parts of India during the last three years, the revival of plague transmission to man appears imminent in some of the previous plague-endemic areas. The appearance of resistance in rat-fleas against different insecticides in many parts of India has previously been reported by Patel *et al.* (1960), Mohan (1960, 1962), Sharma and Joshi (1961*a*), Ramakrishnan *et al.* (1961) and Krishnamurthy and Joshi (1962).

Susceptibility tests of rat-fleas against different chlorinated hydrocarbon insecticides were undertaken in different parts of Madras State such as Salem Town, Hosur and Denkanikota Panchayat Unions of Hosur Taluk (Salem District), Talavady Panchayat Union of Gobichettipalaiyam Taluk (Coimbatore District) and in Madurai Town.

Out of the twelve districts of Madras State, only Salem District (Hosur Taluk) has continued to be persistently endemic for plague. Hosur Taluk has an area of 11,166 sq. miles and is contiguous to the plague-endemic Malur Taluk of Kolar District of Mysore State. The Taluk is divided into five circles or panchayat unions consisting of Hosur, Denkanikota, Berigai, Thally and Kalamangalam. Among these, Denkanikota and Berigai are more persistently endemic than others. The results of the flea susceptibility tests, carried out in the above mentioned places, are presented in this paper.

* These studies were carried out under the auspices of the "*Assessment of Susceptibility Status of Insects of Public Health Importance*" enquiry, financed by the Indian Council of Medical Research.

MATERIAL AND METHODS

The fleas were collected from rodents obtained from the different investigation areas. The rats and mice were killed by strangulation and the fleas were collected with the help of an aspirator as they tried to escape from their dead hosts. They were gently transferred into a clean glass jar and kept there for two to three hours for observation. The dead and injured fleas were discarded before the actual tests were carried out.

The susceptibility status of the fleas was determined by the World Health Organization provisional technique (*W.H.O. Tech. Rep. Series No. 191*, 1960). The D.D.T. and Dieldrin-impregnated filter papers, supplied by the World Health Organization, were used while testing the fleas against these insecticides. Whatman filter papers No. 1 were impregnated with different concentrations of gamma B.H.C. by the Busvine-Nash (1954) method and utilized for tests against this insecticide.

Strip of the treated paper, measuring 5 cm. × 2·5 cm. each, was folded longitudinally in the form of "Z" and was used for the tests. The fleas were exposed to the insecticidal paper for one hour in a clean glass tube 15 cm. long and 1·5 cm. in diameter. After the exposure period, the fleas were transferred into clean holding tubes containing a similar strip of untreated filter paper and the mortality counts were made after twenty-four hours. Appropriate control was kept for each test. In certain instances, where the mortality even in the highest concentration of the insecticide was very low, the fleas were exposed for twenty-four hours in that concentration and the mortality was recorded without further holding period. The fleas were mounted suitably and identified species and sex-wise. The data were analysed by the probit analysis method (Finney, 1952) and LC_{50} values were obtained from the graph by the eye estimation method.

RESULTS

The history of insecticidal treatment by the National Malaria Control/Eradication Programme, and by the Public Health Department, as an antiplague measure in the different investigation areas is depicted in Table I. The results of the susceptibility test against D.D.T., Dieldrin and gamma B.H.C. are shown in Table II.

(i) *Denkanikota and Hosur Panchayat Unions.*—From the different villages of these unions, two-hundred-ten and nine-hundred-twenty fleas were collected for tests, giving a flea index of 0·82 and 3·0 respectively. *X. cheopis* was found to be the predominant species in both the places. The ratios between *X. astia* and *X. cheopis* were 1 : 6·5 and 1 : 5 respectively. The fleas in both these places were found to be highly resistant to D.D.T. When exposed to four per cent concentration of this insecticide for one hour, there was no mortality. The fleas were, however, found to be quite susceptible to Dieldrin and gamma B.H.C. in

TABLE I.

History of insecticidal treatments and the flea indices of the different investigation areas where fleas susceptibility tests were carried out.

Name of the place where the susceptibility tests were carried out.	Date of testing.	Flea index.	History of insecticidal application.	Remarks.
Denkanikota Panchayat Union (Hosur Taluk)	November 30, 1962 to December 15, 1962	*0.82	(i) Three rounds of D.D.T. at the dosage of 100 mg. per sq. ft. from 1959 to 1961 by the National Malaria Eradication Programme Organization. (ii) Spraying of the roof-wall and floor-wall angles of houses with 5 per cent D.D.T. from 1951-55. Dusting of the rat-runs and rat-burrows with 10 per cent D.D.T. from 1951 onwards. Since 1962, D.D.T. has been replaced by gamma B.H.C.	*Intensive antiplague measures were being carried out when the tests were conducted.
Hosur Panchayat Union (Hosur Taluk)	November 10, 1962 to December 15, 1962	3.9	(i) Three rounds of D.D.T. at the dosage of 100 mg. per sq. ft. from 1959 to 1961 by the National Malaria Eradication Programme Organization. (ii) Spraying of the roof-wall and floor-wall angles of houses with 5 per cent D.D.T. from 1951-55. Dusting of the rat-runs and rat-burrows with 10 per cent D.D.T. from 1951 onwards. Since 1962, D.D.T. has been replaced by gamma B.H.C.	..
Madurai Town	September 16, 1963 to September 22, 1963	1.1	Three rounds of D.D.T. at the dosage of 100 mg. per sq. ft. from 1959 to 1961 by the National Malaria Eradication Programme Organization.	..
Salem Town	November 9, 1962 to December 15, 1962	2.97	Twelve rounds of D.D.T. at the dosage of 100 mg. per sq. ft. from 1955 to 1962 by the National Malaria Control/Eradication Organization.	..
*Talavady Panchayat Union (Gobichetti-palaiyam Taluk)	January 18, 1963 to January 22, 1963	2.2	(i) Two rounds of D.D.T. at the dosage of 100 mg. per sq. ft. each year since 1955 by the National Malaria Control/Eradication Organization. (ii) Insufflation of rat-burrows and dusting of the rat-runs with 10 per cent D.D.T. since the last ten years. Since 1963, D.D.T. has been replaced by gamma B H.C.	*Three suspected human plague cases occurred in this Union during November, 1962, after a lapse of 11 years.

TABLE II.

LC_{50} values of the fleas tested in the different investigation areas against D.D.T., Dieldrin and gamma B.H.C. and the same value of a susceptible Delhi strain for comparative studies.

Names of the different places where the flea susceptibility tests were carried out.	LC_{50} VALUES OF D.D.T. :						LC_{50} VALUES OF DIELDRIN :						LC_{50} VALUES OF GAMMA B.H.C. :					
	X. cheopis :			*X. astia* :			*X. cheopis* :			*X. astia* :			*X. cheopis* :			*X. astia* :		
	Male.	Female.	Combined.	Male.	Female.	Combined.	Male.	Female.	Combined.	Male.	Female.	Combined.	Male.	Female.	Combined.	Male.	Female.	Combined.
Delhi susceptible strain*	0·25	0·31	0·4	0·07	0·1	0·1	0·02	0·04	0·035	..	0·01	..	0·005	0·013	0·01	..	0·001	..
Denkanikota Panchayat Union	> 4·0	> 4·0	> 4·0	> 4·0	> 4·0	> 4·0	Between 0·1 and 0·2	0·2	Between 0·1 and 0·2	..	..	..	> 0·04	> 0·04	> 0·04	..	..	..
Hosur Panchayat Union	> 4·0	> 4·0	> 4·0	> 4·0	> 4·0	> 4·0	0·11	0·12	0·12	†H	†H	†H	0·02	0·03	0·025	0·02	0·03	0·025
Madurai Town	..	0·45	0·25	..	0·2	0·2	‡	‡	‡	‡	‡	‡	0·0025	0·02	..	†H	0·003	0·003
Salem Town	1·2	1·4	1·4	1·2	..	1·2	0·075	0·09	0·09	0·04	0·04	0·04	0·023	0·024	0·024	..	0·03	0·03
Talavady Panchayat Union	..	..	..	> 4·0	> 4·0	> 4·0	..	..	..	†H	0·12	0·12	..	..	..	..	..	> 0·04

* Sharma and Joshi (1961*b*.)

† H = Heterogeneous response.

‡ LC_{50} value could not be determined as all the fleas died in the lowest concentration (0·05).

Hosur and to Dieldrin alone in Denkanikota. From Table II it is seen that *X. cheopis*, tested at Denkanikota, was not susceptible to gamma B.H.C. When exposed to 0·04 per cent of this insecticide for an hour, these fleas did not show any mortality. Unfortunately they could not be tested against any further higher concentration due to nonavailability of sufficient number of fleas at the time of the tests. The LC_{50} value of a strain of susceptible *X. cheopis* from Delhi was determined to be 0·01 per cent (Sharma and Joshi, 1961*b*). From this it can be presumed that *X. cheopis*, collected at Denkanikota, was showing definite tolerance to gamma B.H.C.

(*ii*) *Madurai Town.*—Four-hundred-twenty eight fleas, which included both *X. cheopis* and *X. astia*, were collected from different wards of the town, giving a flea index of 1·1. The ratio between *X. cheopis* and *X. astia* was 1 : 2. The fleas were found to be completely susceptible to all the three insecticides. The LC_{50} values of the fleas against Dieldrin could not be determined as there was cent per cent mortality of the fleas in the lowest concentration of the test (0·05 per cent).

(*iii*) *Salem Town.*—One-thousand-thirty four fleas were collected from different wards of the town, giving a flea index of 2·97. *X. cheopis* was found to be the predominant species and the ratio between *X. astia* and *X. cheopis* was 1 : 4·6. The LC_{50} values for *X. cheopis* and *X. astia* against D.D.T. were determined to be 1·4 and 1·2 per cent respectively. The corresponding LC_{50} values of a susceptible Delhi strain were found to be 0·04 and 0·01 per cent respectively (Sharma and Joshi, 1961*b*). This shows that the fleas were showing certain amount of tolerance to D.D.T. The fleas were, however, completely susceptible to Dieldrin and gamma B.H.C.

(*iv*) *Talavady.*—Two-hundred-fourteen *X. astia* were collected from different villages of this union, giving a flea index of 2·2. The fleas were found to be highly resistant to D.D.T. When exposed to four per cent concentration of this insecticide for one hour, there was no mortality in the females and a small percentage of mortality in the males. The fleas were exposed to this concentration for twenty-four hours and this gave a mortality of 57·1 per cent. The fleas were found to be susceptible to Dieldrin. There was evidence of increased tolerance of the fleas to gamma B.H.C. The LC_{50} value against this insecticide could not be determined as enough fleas were not available during the period of investigation to run through all the concentrations. When exposed to 0·04 per cent gamma B.H.C. for an hour, the mortality rate in *X. astia* female was found to be 6·2 per cent only. The corresponding LC_{50} value of a susceptible Delhi strain was determined to be 0·001 per cent (Sharma and Joshi, 1961*b*).

DISCUSSION

From Table I, it is seen that Salem had received twelve rounds of D.D.T. spraying by the National Malaria Control/Eradication Programme. Madurai had received only three rounds by the National Malaria Eradication Programme. None

of them had received any insecticide as an antiplague measure. Denkanikota and Hosur Panchayat Unions had received three rounds of D.D.T. by the National Malaria Eradication Programme but they had received D.D.T. as an antiplague measure since 1951. Three rounds of D.D.T. by the National Malaria Eradication Programme did not produce any change in the susceptibility of the fleas tested at Madurai. Twelve rounds of D.D.T. spraying by the National Malaria Control/ Eradication Programme at Salem has, however, brought about some tolerance of the fleas to this insecticide. Spraying of three rounds of D.D.T. by the National Malaria Eradication Programme, coupled with antiplague D.D.T., has produced a very high degree of resistance of the fleas to this insecticide in Hosur and Denkanikota Panchayat Unions. Talavady has been exposed to eighteen rounds of D.D.T. spraying by the National Malaria Control/Eradication Programme and the antiplague D.D.T. since the last ten years. The fleas have been found to be highly resistant to D.D.T. From these, it appears that antiplague D.D.T. in the form of insufflation and dusting of rat-burrows and rat-runs has possibly contributed more towards the selection of resistant strain of fleas than the spraying of this insecticide by the National Malaria Control/Eradication Programme. In Denkanikota and Talavady, D.D.T. was replaced by gamma B.H.C. as an antiplague measure in 1962 and early 1963 respectively as the former did not have any effect on the flea density. The fleas in both the places have started showing a definite tolerance to the latter insecticide within a very short period. It is doubtful whether gamma B.H.C. will remain effective in controlling the density of fleas in these places after the end of another two or three years. Since human plague cases have been reported from both the places in the recent past, efforts should be made to control the fleas by active rat killing measures such as poisoning and destruction by trapping.

SUMMARY AND CONCLUSIONS

Susceptibility tests, carried out on rat-fleas collected from Denkanikota, Hosur, Madurai, Salem and Talavady, showed high degree of resistance to D.D.T. in Denkanikota, Hosur and Talavady and only slight tolerance in the Salem fleas. The fleas from all these places were found to be susceptible to Dieldrin and gamma B.H.C., except the Denkanikota and Talavady fleas, which showed definite tolerance to gamma B.H.C.

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REARING OF *IXODES PETAURISTAE* WARBURTON, 1933, IN LABORATORY.

BY

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[December 11, 1963.]

THOUGH several species of ticks of the genus *Haemaphysalis* have been reared in the laboratory successfully, difficulties had been experienced in regard to *Ixodes petauristae*†, a species of tick occurring commonly in the evergreen forests of Sagar and Sorab talukas of Shimoga District, Mysore State. In view of the infestation of small mammals in the forests by this species of tick, and the recent experimental evidence suggesting its potentiality as a vector of Kyasanur Forest Disease, it was necessary to study the biology of this species in great detail. Further taxonomic studies of the developmental stages, most essential for the investigations on the epidemiology of the disease, were not possible without successful laboratory rearing of this species.

The successful rearing of *Ixodes petauristae* was finally achieved by careful manipulation of the environment in which they were kept, and the selection of appropriate hosts for the purpose of feeding.

Two species of rats, *Rattus rattus wroughtoni* and *Rattus blanfordi*, were trapped alive in the forests and were kept in wire-cages placed over trays containing water. Over a hundred fully engorged nymphs of *Ixodes* dropped into these trays during the next few days. These nymphs were picked, dried on a filter-paper and were transferred individually to shell vials (2 inch × 1 inch), the open ends of which were covered with bolting silk and placed in desiccators provided with a 20 per cent solution of KOH. The engorged nymphs were observed to be crawling for about six to nine days after which they became quiescent. A waxy secretion, which appeared to facilitate the nymphs to adhere to the glass surface of the tube, was found on their ventral surfaces. In this quiescent stage, they were found for periods ranging from 31 to 62 days, after which they started moulting into adults which were identified as *Ixodes petauristae*.

The moulted adults were held in shell vials, covered with bolting silk, in the same desiccators provided with a freshly prepared solution of 20 per cent KOH. The moulted adults, though very inactive in the beginning, were found crawling

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

† *Ixodes kerri* Rao, 1955 [*Ixodes kerri*, a new species of tick from a flying squirrel from Southern India (Acarina: Culicidae)—*Jour. Bombay Nat. Hist. Soc.*, **52**, pp. 860-864] is a synonym of *Ixodes petauristae*.

inside the tubes and appeared to thrive very well at room temperature (the rainfall and temperature data are summarized in Table I).

TABLE I.

Monthly rainfall, mean monthly maximum and minimum temperatures at Sagar (Shimoga District).

	Rainfall, in inches.	Maximum temperature (degrees Fahrenheit).	Minimum temperature (degrees Fahrenheit).
Jul. 1962	39·19	78°	70°
Aug. 1962	28·47	78°	69°
Sep. 1962	7·32	82°	69°
Oct. 1962	9·03	85°	70°
Nov. 1962	2·27	85°	61°
Dec. 1962	1·70	85°	64°
Jan. 1963	0·00	85°	57°
Feb. 1963	0·58	90°	57°
Mar. 1963	3·50	93°	66°
Apr. 1963	0·06	92°	70°
May. 1963	5·82	92°	72°
Jun. 1963	7·59	82°	70°

The actively moving adults were transferred, one male and one female, to a petri-dish (60 mm. wide). The male tick was observed, moving actively and within a few minutes it was seen attaching itself to the female, with their ventral surfaces against each other. The male was seen slowly piercing the female genital aperture with its hypostome. The male palps were slowly pushed on either side of the hypostome, which was now deeply embedded in the female genital aperture. In this position, the pairs were seen coupled for five to seven hours. (It appears that fertilization does take place during the 'coupling process' inside the petri-dish, because these females laid fertile eggs after feeding and without coming into contact with any other males later).

These females were fed on a young Malabar giant squirrel (*Ratufa indica*), which in nature is a common host for the adults of this tick. (Baby monkeys of the species *Macaca radiata* and the common three striped palm squirrel, *Funambulus tristriatus tristriatus*, were also successfully used to feed adults). The female ticks were released in a metallic pill box cemented on to the back of the host and were seen to attach to the body of the host after about 24 to 36 hours. Only in two instances were males also released in the pill box along with the females. The females appeared to feed slowly for the first two or three days, after which they engorged very rapidly, swelling to about ten times the size of an unfed adult. The fed females detached nine to 13 days after they were released into the pill box, and were picked up, transferred individually to shell vials (2 inch × 1 inch) which were provided with a piece of filter paper and were placed in desiccators with 20 per cent KOH. The oviposition took place after about ten days and lasted several days.

Each female laid about 2,500 eggs (one egg mass was counted and numbered 2,686 eggs). The eggs were transferred with a brush to sterile baked clay pots,

about 2 inch in diameter and 3 inch high, filled to about half height with sterilized forest soil. The clay-pots were then placed in a tray (12 × 12 × 2 inch) containing water. By absorbing water through the porous clay walls, the soil in the pots attained almost the same consistency as the forest soil during the monsoon season. The object was to simulate as much as possible the natural conditions since *Ixodes* larvae were obtained from the forest floor in large numbers during the monsoon months.

The larvae started hatching after about 80 days, some after as long as 93 days, and started crawling out of the clay-pots after another week. The active larvae were transferred to 16 × 100 mm. tubes, closed at one end with plaster of paris and the other with bolting silk. The plaster of paris was kept wet always, and the larvae appeared to thrive well in such tubes.

Forest shrews and rats were employed as hosts to feed the larvae. The hosts were kept in wire-cages (9 × 9 × 9 inch) over trays containing water and the 'questing' larvae were transferred, with a brush, to the back of the host. The larvae, which dropped from the animals into the trays with water before attaching, were collected with a brush, dried on a filter-paper for a few seconds and replaced on to the back of the host. Most of the larvæ were found crawling into the fur of the host, and many could be seen after a few hours attached to the ears and head of the host animal.

The engorged larvæ, which detached after 4 to 5 days, were collected with a brush from the water and transferred to a sterile 50 ml. beaker, containing a circular piece of filter-paper and covered with bolting silk. The beakers, containing the engorged larvae, were placed in the centre of a white enamel tray. A wire-frame was provided in the tray to support lint pieces, which were kept constantly moist, so that the beakers containing the ticks were in a moist environment. The mortality among the engorged larvae was negligible.

Nymphs were found emerging after 25 to 30 days, and were seen actively crawling inside the beaker after two to three days. After a further 11 to 12 days, the nymphs were transferred with a brush on to rats and shrews. The animals were restrained inside a tight-fitting wire-gauze cover for a few hours to restrict their movement and to allow the nymphs to attach, after which the host animals were held in individual cages, over trays containing water. The engorged nymphs dropped after 4 to 5 days, and were picked up with a brush, dried on a filter paper, and transferred individually to shell vials (2 × 1 inch) covered with bolting silk. The shell vials, containing the engorged nymphs, were placed in a desiccator with 20 per cent KOH for moulting.

Following are the details of the time taken during the different phases of rearing:

LIFE CYCLE OF *IXODES PETAURISTAE* REARED IN THE LABORATORY (FROM NYMPH TO NYMPH).

	Duration in days	
Engorged nymphs dropped	0	
Engorged nymphs quiescent after	6 — 9	
Adults emerged after	31 — 62	
Adults started feeding after	30	
Adult fed for	9 — 13	(on *Ratufa indica*)
Females oviposited after	9 — 15	
Females continued laying eggs for	8 — 37	
Larvae hatched after	80 — 93	
Larvae started feeding after	15 —	(on rats and shrews)
Larvae fed for	4 — 5	
Engorged larvae quiescent after	6 — 7	
Nymphs emerged after	25 — 30	
Nymphs started feeding after	11 — 12	
Nymphs fed for	4 — 5	(on rats and shrews)
Duration of life cycle	238 — 288	days

The taxonomical studies on the larval and nymphal stages and a redescription of the adult, *Ixodes petauristae*, are being presented separately.

SUMMARY

Details of a successful method of rearing all the stages of *Ixodes petauristae* in the laboratory are given. Essentially the technique consisted of keeping the ticks in a very moist environment and the use of appropriate natural hosts for feeding. Eggs and larvae were held on wet mud, and the nymphs in the beaker surrounded by wet lint. The larvae and nymphs were fed on rats and shrews, and the adults on the giant squirrel. The complete life cycle took 238 to 288 days.

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FURTHER STUDIES ON INFESTATION OF *PISTIA STRATIOTES* LINN., BY THE CATERPILLAR OF THE MOTH, *NAMANGANA PECTINICORNIS* HYMPS., AND OTHER FACTORS THAT AFFECT *MANSONIOIDES* BREEDING IN THE PONDS IN KERALA.

BY

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[December 12, 1963.]

INTRODUCTION

EARLIER studies (George, 1963) on the effect of natural destruction of *Pistia* by the caterpillar of the moth, *Namangana pectinicornis* Hymps., have established the fact that, more than destroying *Pistia*, the moth infestation promotes *Mansonioides* breeding by adding pollution to the pond. In the present studies the prevalance of *Mansonioides* breeding in ponds is estimated with respect to factors such as the presence of *Pistia*, retting of coconut husks and leaves, moth infestation or other sources of organic pollution.

MATERIALS AND METHODS

The survey for the *Mansonioides* breeding was carried out during the pre-monsoon months of May and June, 1963. At this time of the year the number of water collections were minimum due to drought; there was considerable *Mansoniodes* breeding due to the concentration of organic pollution in water, and the number of ponds with *Pistia* have stabilised due to annual upkeep of the ponds. In all, 400 ponds were examined. These were classified according to the type of vegetation, the presence or absence of retting, moth infestation and *Mansonioides* breeding. The test for breeding was the presence of any of the developing stages of *Mansonioides*, i.e., eggs, larvae or pupae.

RESULTS

The studies have revealed that the predominant vegetation in 69 per cent of all the ponds was *Pistia*; in 15 per cent *Eichornia* and in 3 per cent *Lemna, Azolla* or *Salvinia*; the rest 13 per cent being free from any noteworthy vegetations, other than diatoms, other algae or some varieties of grasses.

Table I shows the distribution of retting among the different kinds of ponds. It was noted that even though only 21·7 per cent of the ponds with *Pistia* were used for retting, 50 per cent of the total retting took place in them. It was also noted

that the *Eichorina* ponds were preferentially used for retting ; 80 per cent of them being so used. Out of a total of 400 ponds of all sorts examined, 120 had retting in them, showing that retting was prevalent to the extent of 30 per cent.

TABLE I.

Distribution of retting in ponds with different kinds of vegetation.

Kinds of vegetation.	Number of ponds.	Percentage.	Number of ponds with retting.	Percentage of ponds with retting.	Percentage break-up of ponds with retting.
Pistia ...	276	69	60	21·7	50·0
Eichornia ...	60	15	48	80·0	40·0
Other vegetation ...	12	3	..	..	..
No vegetation ...	52	13	12	23·1	10·0
Total ...	400	100	120	30	100

TABLE II.

Analysis of the 276 Pistia *ponds showing the prevalence of* Mansonioides *with respect to that of retting and the moth.*

Ponds with	MOTH PRESENT :		MOTH ABSENT :		Total
	Mansonioides present.	*Mansonioides* absent.	*Mansonioides* present.	*Mansonioides* absent.	
Retting Present	0	0	36	24	60
Retting Absent	60	60	0	96	216
	60	60	36	120	
Total ...	120		156		276

Table II shows the status of the 276 *Pistia* ponds regarding the presence of *Mansonioides*, as also of the moth and of retting. Sixty out of the 276 *Pistia* ponds showed retting, i.e., 21·7 per cent of them. In 36 out of these 60 retting ponds and 60 out of the 216 *Pistia* ponds without retting, there was *Mansonioides* breeding. That means 60 per cent of the retting ponds, as against only 27·7 per cent of the non-retting ponds, show *Mansonioides*.

One hundred and twenty out of the 276 *Pistia* ponds, i.e., 43·5 per cent of the *Pistia* ponds showed moth infestation. In 60 out of these 120, as against only 36 out of the 156 moth-free ponds, there was *Mansonioides* breeding. That means 50 per cent of the moth-infested ponds as against only 23·1 per cent of the moth-free ponds showed evidence of *Mansonioides*.

On the whole, 96 out of the 276 *Pistia* ponds showed *Mansonioides*, i.e., 34·7 per cent. Of these 96 breeding ponds, 36, i.e., 37·5 per cent were retting ponds and 60, i.e., 72·5 per cent were moth-infested ponds.

An interesting finding was that not a single moth was found in any of the ponds with retting. Considering pollution of both sorts as due to retting as well as moth infestation, 53·3 per cent of the polluted ponds as against nil per cent of the otherwise clean ponds showed *Mansonioides* breeding.

DISCUSSION

According to earlier estimates (Iyengar, 1938) of the incidence of breeding in ponds at Pattanakaud, a few miles north of Alleppey where the present survey was done, 44·1 per cent of the retting ponds, as against 4·5 per cent of the non-retting ponds, showed *Mansonioides*. In the present studies, however, the corresponding figures are 60·0 per cent and 27·7 per cent respectively. Apart from differences in place and time, the higher rates for breeding in the latter studies are probably due to the fact that the test for assessing breeding in this case, included presence in the *Pistia* ponds of even the egg clusters in addition to larvae or pupae.

It was interesting to note that *Mansonioides* breeding in the non-retting *Pistia* ponds was entirely due to the pond-polluting part played by the caterpillar of *Namangana pectinicornis*, as evidenced by their invariable presence in these ponds and in view of the incomplete but massive *Pistia* destruction they generally bring about. Actually it has been found that 50 per cent of the ponds with the moth infestation breed *Mansonioides* (George 1963, *loc. cit.*) and that these ponds were conspicuous by the absence of any potent source of organic pollution other than the caterpillar infestation. Thus it has been established that moth infestation of *Pistia*, as it generally exists in nature, is a potent source of organic pollution to the pond, which is conducive to *Mansonioides* breeding.

Also it is inferred that the main sources of organic pollution, as are usually present in the ponds at Alleppey, are those associated with retting and moth infestation ; since none of the ponds without either of these kinds of pollutions showed *Mansonioides*. This compares with a meagre 1·4 per cent breeding in apparently non-polluted ponds reported in a different context by Iyengar (1934).

It is interesting to note the relative importance of retting and moth infestation in the production of organic pollution, conducive to *Mansonioides* breeding. Even though breeding is observed in 60 per cent of the retting *Pistia* ponds, as against only 50 per cent of the moth infested ponds, the difference is not statistically significant ($X^2 = 1{\cdot}23$ p 0·25). Hence it appears that moth infestation and retting may equally predispose to *Mansonioides* breeding. However, a wider prevalence of moth infestation at certain seasons of the year could make it a greater hazard. Also since moth infestation and retting are mutually exclusive, any programme aimed at the control of *Mansonioides* by control of organic pollution in *Pistia* ponds, will have to consider each of these factors independantly. Keeping the ponds free of *Pistia* is always a more feasible proposition than keeping them free of organic pollution, for the control of *Mansonioides* breeding.

SUMMARY AND CONCLUSIONS

This paper is based on the survey and study conducted in Alleppey Town, where the presence of various kinds of vegetations and sources of organic pollution in the ponds were studied to find out the relative effects of these factors on *Mansonioides* breeding. The following are the more important findings :—

1. Using of the ponds for retting is prevalent to a great extent, and by contributing the essential organic pollution it helps *Mansonioides* breeding in the *Pistia* ponds.

2. *Namangana pectinicornis* infestation practically enhances *Mansonioides* breeding, and because of its wider prevalence as compared to retting, is responsible for *Mansonioides* breeding in a larger number of ponds.

3. The moth infestation exists independently of retting in *Pistia* ponds, and therefore even if the habit of retting is discouraged, *Mansonioides* breeding could persist as long as *Pistia* and the moth co-exist.

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FILARIASIS IN KERALA STATE: FILARIA SURVEY OF KOTTAYAM MUNICIPALITY.

BY

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AND

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[February 27, 1963.]

DIFFERENT parts of Kerala State are being surveyed from time to time by the Filariasis Training Centre, Ernakulam*, since 1956. The results of these surveys bring out the current position of filariasis in the State. Many areas, which were believed to be free from filariasis, revealed varying degrees of filarial endemicity in the course of these surveys. The present paper sets out the results of a filaria survey undertaken in Kottayam Municipality, an area which had not been surveyed before and was believed to be free from filariasis.

Kottayam, a commercial town, known for forest products, is located on the bank of a big navigable stream. The town extends over an area of about 6·25 square miles, and is divided into 27 wards with a population of 44,204 (1951 census), comprising 49·8 per cent Christians, 45·5 per cent Hindus and 4·7 per cent Muslims.

The terrain is uneven with steep rises and falls. The soil is loamy but very fertile. The subsoil water level is very low. The source of water is from wells and ponds. There is no proper drainage system, most of the drains being kutcha but at places are lined by stones. There is not much stagnation of water, except in the low-lying areas, due to the hilly nature of the place. The disposal of night-soil in the municipal area is by hand removal and in the outskirts people resort to open air defaecation or use pit latrines.

The majority (64 per cent) of the people are agriculturists. The principal crops are rice, cocoanut, arecanut, plantains, sugarcane, tapioca, pepper, ginger and rubber. There are four small rubber factories, four plywood factories for making tea chests and one cement factory.

Cotton weaving, coir making, oil crushing, jaggery making and pottery are some of the important cottage industries.

Majority of the people are non-vegetarians. Rice is the staple food although the low-income groups occasionally resort to tapioca in place of rice.

* This has been shifted to Kozhikode from April 1, 1962.

The average annual rainfall in Kottayam is 115·1 inches. Both the monsoons contribute to this, the main precipitation occurring in June and July. Throughout the year, the relative humidity is fairly high.

The Municipality is carrying out antilarval operation on a small scale for the last five years by use of mosquito larvicidal oil.

METHODS AND MATERIAL

The present survey was carried out in all the wards of the municipality during February and May, 1961 and February, 1962, between 8 P.M. and 1 A.M. House-to-house visits were made in the areas selected at random, examining all the inmates of the houses, including infants. As far as possible, 20 cmm. of blood was collected from each individual and made into a thick smear on a clean glass slide. In addition, details with regard to the presence or otherwise of disease manifestations were recorded. Particulars in regard to duration of stay in Kottayam Town, permanent residence and the duration of swelling etc. were also noted.

The air-dried smears were dehaemoglobinised, using tap water, and stained with J.S.B. I stain for about five minutes and then examined for microfilariae. In positive smears, after identification of the species the number of microfilariae was counted and recorded. Mosquitoes were also collected from the different wards between 6 A.M. and 8 A.M. After identification, the female specimens were dissected and examined for filarial infection. The number and stage of developmental forms, and the site where they were found, were recorded. The types of breeding places were also recorded.

RESULTS

A total of 6,242 persons were examined, comprising 14·1 per cent of the population. Only 30 persons were found positive (8 *B. malayi* and 22 *W. bancrofti*) for infection and 16 had one or the other disease manifestation. An infection rate of 0·48 per cent and disease rate of 0·25 per cent and the average infestation of 4·7 microfilariae per 20 cmm. of blood were recorded.

Particulars in regard to infection, disease and endemicity rates and the average infestation for the different wards surveyed are shown in Table I. The same data for different age-groups are set out in Table II.

From the histories recorded, it was noted that, except three cases, none of the microfilaria carriers had any reasonably long stay in endemic areas away from Kottayam. At least four persons with disease stated that they had never gone out of Kottayam since birth and the duration of disease manifestation in them varied between 4 and 6 years. Thus the infections in them would appear to have been locally contracted. In those who had contracted the infection, before settling down in the Kottayam Municipality area, the duration of disease manifestation varied between 10 to 30 years.

TABLE I.

Infection, disease and endemicity rates and average infestation per 20 cmm. blood in Kottayam Municipality.

Ward number	Number of persons examined.	Number with infection.	Infection rate (per cent).	Number with disease.	Disease rate (per cent).	Endemicity rate (per cent).	Average infestation.
1	80	..	..	..	..	..	..
2	91	..	..	..	..	..	..
3	109	2	1·84	..	..	1·84	14·50
4	102	..	..	..	..	..	..
5	113	..	..	..	..	..	..
6	106	..	..	..	..	..	..
7	84	..	..	..	..	..	..
8	102	2	1·96	1	0·98	2·94	1·50
9	924	5	0·5	2	0·2	0·70	7·00
10	546	6	1·1	3	0·5	1·60	6·80
11	514	2	0·6	2	0·6	1·20	2·50
12	84	2	2·38	..	..	2·38	2·50
13	112	2	1·78	1	0·89	2·67	6·50
14	112	..	..	..	..	..	..
15	59	1	1·69	..	..	1·69	38·0
16	60	..	..	..	..	..	..
17	72	1	1·38	..	..	1·38	1·00
18	108	..	..	..	..	..	..
19	101	..	..	..	..	..	..
20	72	1	1·38	..	..	1·38	2·00
21 & 22	94	..	..	..	..	..	..
23	72	4	5·55	..	..	5·55	6·00
24	59	..	..	..	..	..	..
25	812	1	0·1	1	0·1	0·20	1·00
26	570	..	..	..	..	..	..
27	1,304	1	0·1	6	0·5	0·60	15·60
Total	6,242	30	0·5	16	0·3	0·80	4·77

TABLE II.

Infection, disease and endemicity rates in different age-groups of persons examined during the survey of Kottayam Municipality.

Age group, in years.	Number examined.	Number with disease.	Disease rate (per cent).	Number with infection.	Infection rate (per cent).	Average infestation.	Endemicity rate (per cent).
0—2	95	..	..	..	..	..	..
3—5	286	1	0·34	..	..	..	0·34
6—10	731	..	..	1*	0·13	12·00	0·13
11—20	1,596	1	0·06	9* 2†	0·68	7·06	0·74
21—30	1,275	3	0·23	6* 2†	0·62	10·60	0·85
31—40	976	4	0·40	1†	0·10	1·00	0·50
41—50	620	3	0·49	4* 2†	0·96	4·42	1·44
Above 50	650	4	0·61	2* 1†	0·45	3·75	1·06
Total	6,242	16	0·3	22* 8	0·5	4·77	0·8

* *W. bancrofti.* † *B. malayi.*

The disease manifestations noted were (*i*) swellings of the legs (12 cases), (*ii*) swellings of the arm (one case), (*iii*) swelling of one arm and one leg (one case), and (*iv*) glandular enlargement with fever (2 cases).

ENTOMOLOGICAL FINDINGS

The following species of mosquitoes were recorded :

C. fatigans
C. gelidus
C. bitaeniorhynchus
A. subpictus
A. vagus
A. hyrcanus
Mansonioides annulifera
Mansonioides uniformis
Lutzia sp.
Armigeres sp.
A. jamesi

The average per man-hour density of *C. fatigans* was 12·8. Female mosquitoes of all the species were dissected but only *C. fatigans* showed developing stages of filarial infection. The number of *Culex fatigans* dissected during the three visits to Kottayam Municipality and the infection recorded in them are shown in Table III.

TABLE III.

Results of dissection of C. fatigans *in Kottayam Municipality.*

Serial number of visit.	Serial number of the wards surveyed.	Period of survey.	Number dissected.	Number positive.	Per cent positive.	Number with infective larvae.	Per cent with infective larvae.
First	25, 26, 27.	Feb. 1961	142	*Nil*	*Nil*	*Nil*	*Nil*
Second	9, 10, 11.	May, 1961	151	2	1·3	*Nil*	*Nil*
Third	1 to 8 and 12 to 24.	Dec. 1961 to Feb. 1962	319	4	1·3	1	0·3

Culicine breeding was noted in kutcha drains, cess-pools in the backyard of houses and hotels, flushout tanks and stagnant water collections, while *Anopheline* breeding was confined to paddy fields, tanks and fresh water collections. There were plenty of tanks in the rural area of the municipality with much vegetation of *Pistia*, water hyacinth and lemna, and *Mansonioides* sp. were found breeding in them.

CONCLUSIONS

From the results of the filaria survey, it may be observed that transmission of filariasis has been going on in Kottayam Municipality for some time. The filarial infection as well as the disease rate is low. The entire Kottayam area was considered to be free from filaria infection but no surveys had been carried out in

this area in the past. One of the neighbouring taluks, Pathanamthitta, was surveyed by Iyengar (1938) and it was shown as free from filariasis. Another area, Vaikom Taluk, situated in the north, was surveyed in 1957 (*Malaria Institute of India Reports*, 1957) and *B. malayi* infection was recorded.

The infection and disease rates in Kottayam are 8·6 and 10·3 per cent respectively. It is probable that the period of transmission of the infection in Kottayam is short. From the mosquito dissections, it will be noted that only a few mosquitoes had shown filarial infection. The survey was carried out at random during the months of December, January, February and May. During these months, there were very few water collections. The density of the vectors was quite low. It might be possible that majority of transmission takes place in the post-monsoon period; as during monsoon, due to the heavy rain and hilly nature of the area, there will be considerable flushing of water which is unfavourable for breeding of mosquitoes.

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A NOTE ON THE OBSERVATION ON THE REAPPEARANCE OF *MICROFILARIA BANCROFTI* IN CASES TREATED WITH DIETHYL-CARBAMAZINE IN DOSES SCHEDULED UNDER THE NATIONAL FILARIA CONTROL PROGRAMME.

BY

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[December 15, 1963.]

INTRODUCTION

DIETHYLCARBAMAZINE, a synthetic drug, has been tried against most of the filarial infections, including those of the human beings. In India, under the National Filaria Control Programme (Raghavan, 1955), the dose schedule is 4 mg. per kg. boby-weight in a single dose treatment for a period of five days or 2 tablets (100 mg. each) for an adult above 18 years of age, 1½ tablets for 12 to 18 years, one tablet for 6 to 12 years and half tablet for 2 to 6 years of age for five days. The drug is used as a microfilaricide for sterilizing the blood of microfilaria and thus interrupting the transmission of filariasis.

Singh, Rastogi and Srivastava (1962) made observations on the day-to-day effect, and also during two days following the full course of treatment with Diethylcarbamazine, on the circulating *W. bancrofti* in doses scheduled under the National Filaria Control Programme, on 44 *W. bancrofti* positive cases at Bahraich (Uttar Pradesh). Observations on these cases were made, before the start of the therapy and also 24, 48, 72, 96 and 120 hours after the start of the therapy. The observations were concluded 48 hours after completion of therapy in these cases. The study revealed that in 43·1 per cent of the cases taken in the series, the microfilaria did not disappear completely from the peripheral circulation, although the infestation was brought to a lower level. Further they noted a sudden reduction in the number of circulating microfilariae within 24 hours of the start of the therapy. They also discussed the possibility of resistance of microfilaria to Diethylcarbamazine in some cases.

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The present note deals with observations made on 39 out of 44 cases taken by them in the initial trial, by following them monthwise for three months, and also in the sixth month after completion of the therapy. The observations thus recorded are set out below.

PROCEDURE

Thirty-nine out of 44 cases, referred to above, were followed. The blood smears were collected between 9·30 and 11·30 p.m. A measured quantity (20 c.mm.) of blood was collected with a calibrated pipette on slide for preparation of smear. These slides were later stained with JSB I stain (Jaswant Singh and Bhattacharji, 1944). The number of microfilariae were counted in each smear.

RESULTS

Details of the blood examination results for each of the 39 cases, before treatment, and at start of follow-up (48 hours after the completion of the therapy) as well as subsequent monthly and six-monthly follow-ups are given in Table I. The infection rate per cent and average infestation per 20 c.mm. of blood at the start of the follow-up as well as of the subsequent monthly and six-monthly examinations are set out in Table II. It is evident from Table II that the blood examination report at the start of follow-up reveals an infection rate of 41·02 per cent and average infestation as 2·3 per 20 c.mm. of blood. The report of the first monthly follow-up showed the infection rate as 56·4 per cent and average infestation as 3·8 per 20 cmm. of blood. Similarly the report of second monthly follow-up gave the infection rate as 58·9 per cent and average infestation as 7·6 per 20 c.mm. of blood. The third monthly follow-up infection rate was 53·8 per cent and average infestation 7·8 per 20 c.mm. blood. The blood examination report, after six-monthly follow-up, showed the infection rate of 43·5 per cent and average infestation as 6·8 per 20 c.mm. of blood.

It is further evident that there was an increase in the infection rate and average infestation in the first and second monthly follow-ups. In the third monthly follow-up, the infection rate started declining and in the sixth monthly follow-up the infection rate was considerably reduced, about the same as that at the start of the follow-up, although the average infestation remained a little high. Tables III-A and III-B set out the percentage increase in the infection rate and average infestation at the various follow-ups.

DISCUSSION

A close study of Table I shows that the behaviour of every case is different following the course of treatment and hence every individual case requires analysis for itself. From the observations recorded, it is evident that in more and more cases the microfilaria starts reappearing in the peripheral circulation as the time lapses and also that the average infestation is increased, although after six months

about the same number of persons showed microfilariae in the peripheral circulation though their average infestation remained high.

TABLE I.

Details of follow-up of blood examinations.

Case number.	Age, in years.	Number of microfilariae (per 20 c.mm. blood) before treatment.	Number of microfilariae (per 20 c.mm. blood) 48 hours after completion of treatment.	NUMBER OF MICROFILARIAE (PER 20 c.mm. BLOOD) AFTER :			
				1 month.	2 months.	3 months.	6 months.
1	10	66	6	1	6	3	5
2	19	12	*Nil*	3	3	*Nil*	6
3	32	36	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
4	24	9	*Nil*	*Nil*	1	*Nil*	*Nil*
5	19	86	*Nil*	6	16	17	16
6	25	10	1	2	2	2	2
7	28	62	1	3	2	14	13
8	37	6	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
9	11	19	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
10	13	10	1	2	6	6	*Nil*
11	28	1	*Nil*	1	1	2	2
12	14	136	1	5	28	25	*Nil*
13	34	1	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
14	6	4	1	*Nil*	*Nil*	4	*Nil*
15	12	1	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
16	25	47	*Nil*	3	6	7	*Nil*
17	17	59	2	11	14	16	21
18	18	382	6	2	7	11	8
19	21	48	1	3	8	4	*Nil*
20	17	61	*Nil*	7	9	6	4
21	14	178	1	*Nil*	9	8	4
22	41	35	1	5	7	6	4
23	16	42	*Nil*	5	7	1	*Nil*
24	30	1	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
25	33	36	1	2	2	2	2
26	25	3	*Nil*	1	1	*Nil*	*Nil*
27	28	17	1	*Nil*	*Nil*	*Nil*	*Nil*
28	30	14	1	5	6	2	*Nil*
29	9	7	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
30	32	20	6	3	12	14	10
31	35	9	*Nil*	3	*Nil*	*Nil*	4
32	13	3	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
33	20	3	*Nil*	*Nil*	*Nil*	*Nil*	3
34	14	8	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
35	28	4	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
36	19	55	6	8	11	10	12
37	24	12	*Nil*	2	3	4	1
38	62	2	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
39	41	9	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
Total		1514/39	37/16	83/22	177/23	164/21	117/17

TABLE II.

Infection rate and average infestation at times of various follow-ups.

BLOOD EXAMINATION REPORT AT THE START OF FOLLOW-UP :		FIRST MONTH'S FOLLOW-UP BLOOD EXAMINATION REPORT :		SECOND MONTH'S FOLLOW-UP BLOOD EXAMINATION REPORT :		THIRD MONTH'S FOLLOW-UP BLOOD EXAMINATION REPORT :		SIXTH MONTH'S FOLLOW-UP BLOOD EXAMINATION REPORT :	
Infection rate, per cent.	Average infestation, per 20 cmm. of blood.	Infection rate, per cent.	Average infestation, per 20 cmm. of blood.	Infection rate, per cent.	Average infestation, per 20 cmm. of blood.	Infection rate, per cent.	Average infestation, per 20 cmm. of blood.	Infection rate, per cent.	Average infestation, per 20 cmm. of blood.
41·02	2·3	56·4	3·8	58·9	7·6	53·8	7·8	43·5	6·8

TABLE III-A.

Initial and the follow-up infection rates with their per cent increase.

Initial infection rate at the start of follow-up.	FOLLOW-UP INFECTION RATES AND THEIR PER CENT INCREASE :							
	On the first monthly follow-up.		On the second monthly follow-up.		On the third monthly follow-up.		On the sixth monthly follow-up.	
	Infection rate.	Percentage increase.	Infection rate.	Percentage increase.	Infection rate.	Percentage increase.	Infection rate.	Percentage increase.
41·02	56·4	15·38	58·9	17·88	53·8	12·78	43·5	2·48

TABLE III-B.

Initial and the follow-up average infestations per 20 c.mm. of blood, with their per cent increase.

Initial average infestation per 20 c.mm. of blood at the start of follow-up.	FOLLOW-UP AVERAGE INFESTATIONS, PER 20 C.MM. OF BLOOD, WITH THEIR PER CENT INCREASE :							
	On the first monthly follow-up.		On the second monthly follow-up.		On the third monthly follow-up.		On the sixth monthly follow-up.	
	Average infestation.	Percentage increase.	Average infestation.	Percentage increase.	Average infestation.	Percentage increase.	Average infestation.	Percentage increase.
2·3	3·8	65·2	7·6	230·1	7·8	239·1	6·7	191·3

Out of the 39 cases followed, 12 (30·7 per cent) did not show the reappearance of microfilaria in the peripheral blood at any stage, while 9 (23·07 per cent) cases persistently showed the presence of microfilariae in their peripheral blood throughout the course of follow-up. In 11 (28·2 per cent) cases, microfilariae reappeared at some stage or the other while in 6 (15·3 per cent) cases microfilariae disappeared after 6 months, although they were present at the time of the immediate follow-up. The microfilaria load was maximum in the second month of the follow-up.

As the behaviour of every individual case is different, it is difficult to explain this reappearance and disappearance of microfilaria in the peripheral circulation of these cases. However, the possibilities are discussed below :

1. Possibility of some resistance against Diethylcarbamazine.

2. The possibility of Diethylcarbamazine having no effect on the productivity of the adult female worm of this strain. Rama Krishnan *et al.* (1963) have shown that Diethylcarbamazine had no sterilising effect on the adult female worms of *L. carinii* in white rat.

3. Possibility of Diethylcarbamazine in only suppressing and not sterilising completely the productivity of the adult female worm which later on regains its power and starts producing microfilaria.

4. Natural death of the infection in the body of the individual.

5. Presence of more than one strain of microfilaria and hence the difference in the behaviour.

As the observations recorded are quite different in every case and in view of the plausible possibilities discussed above, it is evident that the problem requires a further detailed study.

SUMMARY

Thirty-nine cases were followed-up at intervals of one month, two months, three months, and six months, after administering the full course of Diethylcarbamazine in dosage schedules adopted under the National Filaria Control Programme.

The results were tabulated which showed that there was an increase in the infection rate per cent as well as the average infestation per 20 cmm. blood in the first, second and third month of the follow-up. After six months, the infection rate was almost the same as at the start of follow-up, although the average infestation remained high.

Different behaviour of every individual case has been discussed and the possibilities for the same also explained.

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STUDIES ON THE EPIDEMIOLOGY OF *B. MALAYI* FILARIASIS IN KERALA STATE.

Periodicity of the microfilariae.

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[December 15, 1963.]

DURING recent years, much advance has been made on studies on the *B. malayi* infection. Two forms of *B. malayi* infection have been reported from Malaya. Turner and Edeson (1957) recorded a difference in periodicity between the *B. malayi* microfilaria carrier of Kuantan and that of Penang in Malaya. In the former group, there was less remarkable nocturnal rise and microfilariae were found in the peripheral blood during day-time whereas in the latter group the periodicity was strictly nocturnal. Based on the findings about the behaviour and appearances, Wilson *et al.* (1958) named the two types as semi-periodic and periodic forms of *B. malayi*, the former being prevalent in swamp forest areas and the latter in plain areas of north-west Malaya. Difference in the susceptibilities of the vectors, to the two forms of *B. malayi* infection, has also been recorded by them.

Great interest has been created in the studies on the semiperiodic form of *B. malayi* infection for its adaptibility to good number of vertebrate host other than man. Finding of *B. malayi*-like microfilariae in monkeys (*M. irus*) by Poynton and Hodgkin (1939) indicated that vertebrates, other than man, could be definite hosts of *B. malayi* infection. Subsequently, adult specimens of *Brugia malayi* have been recovered from animals (Buckly and Edeson, 1956). The adaptibility of semi-periodic form has been further confirmed by successful experimental inoculation to animals (Edeson and Wharton, 1957 ; 1958).

In this country, the largest single tract of *B. malayi* filariasis is confined to the central part of Kerala Stata (Raghavan, 1957) and it is estimated that there are about 1·5 million people living in this *B. malayi* endemic zone (Joseph *et al.*, 1962). Though the majority of this endemic zone is confined to plain areas of the central tract of Kerala State, patches of *B. malayi* infection have been reported in the hilly regions of Kerala State also (Iyer, 1901 ; Iyengar, 1938 ; Raghavan *et al.*, 1958 and Annual Reports of the Malaria Institute of India for the years 1960, 1961, 1962).

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In view of the findings of two forms of *B. malayi* infection from abroad as well as from the reports of *B. malayi*-like microfilariae in animals from this country (Pattanayak and Raghavan, 1956 ; Pattanayak, 1956), attempts were made to determine the periodicity of the *B. malayi* microfilariae in different parts of the Kerala State, including the hilly region, to observe if semi-periodic form of *B. malayi* exists in this State.

PROCEDURE

Microfilaria carriers from the plain areas were selected from the village of Thuravur in Shertallai Taluk, Palluruthy Panchayat, and from Kalathara which is an island located in the backwaters of Arabian Sea near Ernakulm. These areas are endemic for *B. malayi* filariasis (Iyengar, 1938 ; Annual Reports of the Malaria Institute of India for 1958, 1959, 1961).

Microfilaria carriers from Kottai and hill-tribe settlements in Quilon and Trivandrum districts were selected for periodicity studies for the hilly areas. These areas are endemic for *B. malayi* filariasis (Iyer, 1901 ; Iyengar, 1938 ; Raghavan *et al.*, 1958 ; Annual Report of the Malaria Institute of India, 1958) and are located on the ranges of western ghats at a height of about 1,000 feet above sea-level.

Microfilaria carriers, numbering 27 from the plain and 20 from hilly areas, were kept under observation in the field laboratory where facilities for sleeping were provided. 20 cmm. of blood was collected by finger-prick at the end of every two hours, commencing from 6 a.m., which was continued till 4 a.m. the following day. In one batch, this process was commenced at 7 a.m. and continued till 5 a.m. the next day. The blood collected was drawn into thick smears. These were air-dried and dehaemoglobinised with tap-water and subsequently stained with J.S.B. stain 1 (Jaswant Singh and Bhattacharji, 1944) for examination and enumeration of microfilariae present in the smears collected. From each individual, three smears were collected at each time and the average of microfilariae was calculated, taking into consideration the number of microfilariae present in each smear. All the microfilaria carriers were males ; females were not included as it was difficult to provide all facilities in the field-laboratory.

RESULTS

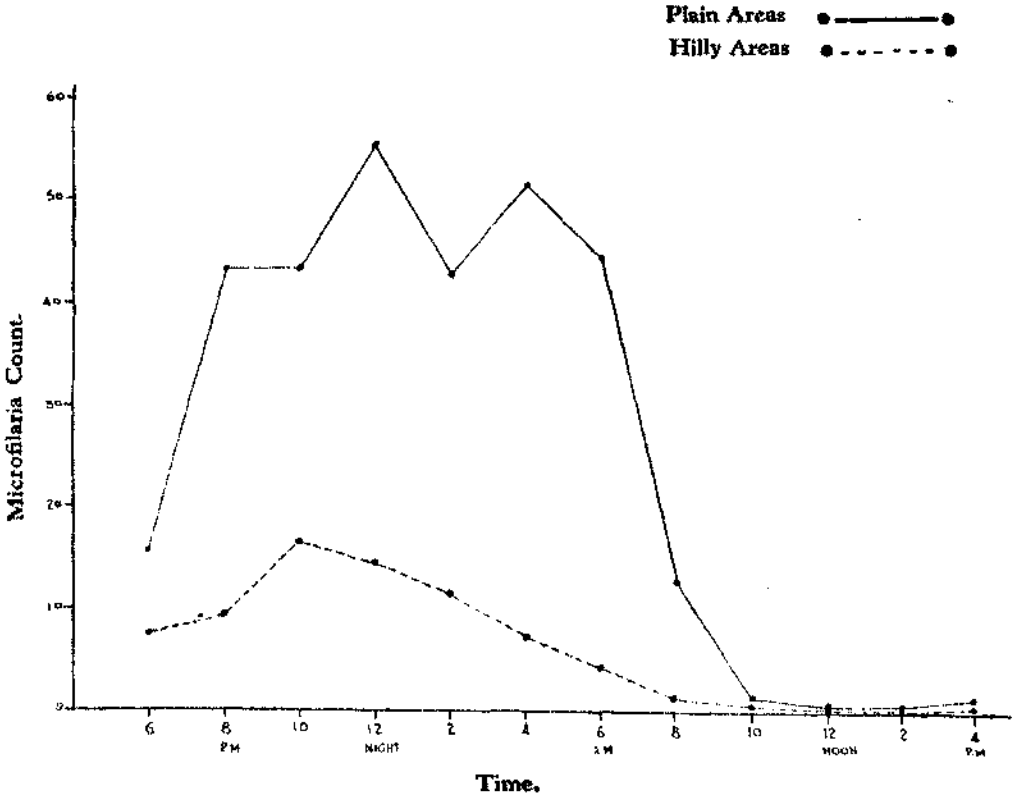
Results of the periodicity of each microfilaria carrier, both from plain and hilly areas, are shown in Tables I and II. The averages of the periodicity studies of microfilaria carriers for plain and hilly areas are depicted in Table III and Graph 1.

From the results contained in these Tables, it would be seen that microfilariae in every one of the individual carriers, both from the plains as well as from hills, exhibit nocturnal periodicity. Only in some cases, few microfilariae have been noted during day time, but the peak density has been reached only during night time. Two peak densities have been observed in the case of microfilaria carriers

from plain areas, *i.e.*, one peak is towards the midnight and the other in the early hours of the morning. But in the case of microfilariae carriers from hilly areas, the double peak is conspicuously absent. The peak density has been reached during early part of the night and thereafter it gradually declines. The double peak density observed in microfilaria carriers from plain areas, and single peak density in microfilaria carriers from hilly areas, can be observed from individual studies from Tables I and II as well as from the average for both the areas depicted in Table III and Graph I.

GRAPH I.

Comparison of periodicity of B. malayi *in plain and hilly Areas, according to the results shown in Table III.*



DISCUSSION

Periodicity of filarioidea within a genus or species is not a rigid or permanent phenomenon (Kessel, 1960). Apart from the semi-periodic and periodic *B. malayi* infection, variations have been noted in the case of *B. patei* (Heish *et al.*, 1959) and in the case of loiasis in monkeys (Duke, 1955). Thus the probability of encountering variations in the strictly periodic type of infection cannot be eliminated.

TABLE I.

Results of B. malayi *periodicity studies conducted at different places in the plain areas of Kerala State.*

Average number of microfilariae per 20 c.m.m. of blood.

Locality.	Case number.	TIME: 6 a.m.	8 a.m.	10 a.m.	12 noon.	2 p.m.	4 p.m.	6 p.m.	8 p.m.	10 p.m.	12 mid-night.	2 a.m.	4 a.m.
Thuravur	13316	7·3	0·3	Neg.	1·3	0·3	0·3	1·3	14·6	8·0	12·6	8·6	13·6
	13634	67·0	38·3	2·0	Neg.	1·3	1·3	11·4	33·6	20·3	43·5	44·3	92·6
	13024	44·3	12·3	1·0	2·6	1·6	4·6	23·0	37·0	41·0	32·6	27·0	29·3
	12331	2·6	0·6	Neg.	Neg.	Neg.	Neg.	2·0	5·0	6·0	5·6	3·6	2·3
	9702	10·0	7·3	Neg.	Neg.	Neg.	1·7	3·3	8·0	13·3	14·3	15·7	8·0
Average periodicity for the locality :—		26·2	11·7	0·6	0·8	0·7	1·6	8·2	29·5	17·9	21·3	19·8	29·2
Islands round about Ernakulam (Kalathara)	1	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·5	0·0	0·5	0·0	0·0
	2	4·0	0·5	0·0	0·0	0·0	0·0	3·5	6·5	8·0	10·0	5·5	6·0
	3	33·5	4·0	0·0	0·0	0·5	1·0	18·5	33·0	30·0	20·0	36·5	29·0
	4	44·0	22·0	2·0	1·5	1·0	8·5	3·0	38·5	26·5	28·0	31·0	42·0
	5	20·5	0·5	1·0	0·0	1·0	3·5	20·0	23·0	28·5	34·5	20·5	35·0
	6	11·0	2·6	0·5	0·5	0·0	0·0	30·0	8·0	17·0	17·0	15·5	13·0
	7	5·5	0·5	0·0	0·0	0·0	0·0	9·0	8·5	12·0	14·0	13·5	13·5
	8	76·0	19·6	0·5	0·0	0·0	0·0	26·6	50·5	80·5	96·5	91·5	90·5
	9	1·0	1·0	0·0	0·0	0·0	1·0	2·5	5·0	10·5	18·0	5·5	1·0
	10	10·2	0·5	0·0	0·0	0·0	1·5	6·5	10·5	11·0	13·5	8·0	13·0
Average periodicity for the locality :—		20·6	5·1	0·4	0·2	0·3	0·8	9·2	18·4	22·0	25·2	22·8	24·4
Palluruthy	1	40·3	6·0	1·0	0·7	1·0	0·0	14·3	31·3	26·3	24·7	42·7	47·3
	2	7·3	0·0	0·0	0·0	0·0	0·0	1·3	8·0	7·3	9·0	13·3	11·7
	3	216·0	58·8	7·0	0·6	1·3	7·0	60·0	205·0	233·0	322·0	201·0	339·0
Average periodicity for the locality :—		87·9	21·3	2·7	0·6	1·8	2·3	28·0	81·4	88·9	118·6	85·3	99·3
Thuravur*	5694	0	0	0	0	0	0	0	0	0·3	0	0	0
	5871	0	0	0	0	0	0	0·43	2	2·7	2·7	2·7	2
	6191	9·3	1·7	0·7	0	0	0·3	2·5	7·7	11·7	13·5	8	10·7
	6214	1·6	0	0	0	0·3	1·6	5·6	13	11·3	1	11·3	20·7
	6210	2·5	0	0	0	7	4·5	10	4	0	17	22·5	10
	6233	8	2	0	0·5	0	2	3	32	3	30·5	33·5	24
	6225	8	0·5	0·3	0	0·3	1·3	14·7	29	17	23	52	21·3
	6209	31·5	0·5	1·5	0·5	1·5	2	22·5	60·5	86	116	33	99·5
	6192	26·7	6·7	1·0	0·0	0·7	1·3	20·5	27·3	33·7	45·0	40·0	40·0
Average periodicity for the locality :—		9·7	1·2	0·4	0·1	1·1	1·4	8·7	18·4	18·3	27·6	28·1	25·4

* Study was commenced at 7 a.m. and continued up to 5 a.m. (24 hours) at two-hourly intervals.

TABLE II.

Results of B. malayi *periodicity studies conducted at different places in the hilly areas of Kerala State.*

Average number of microfilariae per 20 c.mm. of blood.

Locality.	Case number.	Time: 6 a.m.	8 a.m.	10 a.m.	12 noon.	2 p.m.	4 p.m.	6 p.m.	8 p.m.	10 p.m.	12 mid-night	2 a.m.	4 a.m.
Kottai (Hilly area)	1	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	1·0	9·0	9·0	6·0	1·0
	2	1·0	Neg.	Neg.	Neg.	Neg.	Neg.	3·0	1·0	3·0	1·0	3·0	1·0
Average periodicity for the locality :—		5·0	0·0	0·0	0·0	0·0	0·0	1·5	1·0	6·0	5·0	4·5	1·0
Kani Settlements (Hilly area)	1	21·0	13	6	0	0	0	3	11	14	13	10	6
	2	1	0	0	0	0	0	0	3	1	2	4	1
	3	3	0	0	0	0	0	0	2	4	6	2	2
	4	3	0	1	0	0	1	0	7	8	7	4	3
	5	0	0	0	0	0	0	0	2	2	2	1	1
	6	0	0	0	0	0	0	0	0	2	4	1	2
	7	0	0	0	0	0	0	0	3	3	1	0	0
	8	4	0	0	0	0	0	0	4	2	2	3	1
	9	0	0	0	0	0	0	0	2	1	1	1	0
	10	2	0	0	0	0	0	2	5	6	5	6	5
	11	40	11	0	0	0	0	42	64	88	99	81	46
	12	14	6	1	0	0	0	1	2	24	26	16	18
	13	4	0	0	0	1	9	34	52	47	32	36	31
	14	35	2	1	0	0	9	93	79	144	132	112	97
	15	1	0	0	0	0	0	1	2	1	1	1	0
	16	1	0	0	0	0	0	4	4	5	6	1	1
	17	38	13	0	0	0	2	67	78	121	86	66	22
	18	1	0	0	0	0	0	7	5	16	11	4	8
Average periodicity for the locality :—		9·3	2·7	0·5	0·0	0·1	1·2	14·1	18·1	27·2	24·2	19·4	13·6

TABLE III*.

Results of periodicity studies on B. malayi *in plain and hilly areas.*

Time.	Average periodicity of microfilaria in the plain areas (Kerala State).	Average periodicity of microfilaria in the hilly areas (Kerala State).
6 p.m.	15·2	7·8
8 p.m.	43·1	9·6
10 p.m.	43·0	16·6
12 midnight	55·0	14·0
2 a.m.	42·6	11·9
4 a.m.	51·0	7·3
6 a.m.	44·0	4·9
8 a.m.	12·7	1·4
10 a.m.	1·2	0·3
12 noon	0·5	0·0
2 p.m.	0·6	0·05
4 p.m.	1·6	0·6

* Results of the batch from a plain area, where studies were commenced at 7 a.m , have been excluded for calculation of the average.

It is with this in view that the present investigations were undertaken. From the results, it is evident that the *B. malayi* microfilariae exhibited nocturnal periodicity only.

In some cases, microfilariae were present in small number during day-time in the peripheral blood although the peak density was during night. Similar findings have also been observed by Low, Manson-Bahr and Walter as quoted by Manson-Bahr (1957), where the periodicity curve was absolutely nocturnal.

From Table III, it will be observed that there is a difference in the peak density of the microfilariae. In the plain areas group, there are two peaks whereas in the hilly areas it is single. In the case of *W. bancrofti* infection also, two peaks have been observed in the microfilaria density but "it is not clear whether a depression is an artefact (due to simple variation) or a genuine phenomenon (as is more probable)" (Hawking, 1960). Although in the hilly areas, the peak is single, unlike in the plain areas, it appears that it does not affect the transmission of the disease in view of the high blood infection and filarial endemicity of the community as observed by Raghavan *et al.* (1958). The peak density of microfilaria in hilly areas has been during early hours of the night. This might be to suit the vector, *M. uniformis*, which is more prevalent during early part of the night (Raghavan *et al.*, 1958).

Nocturnal periodicity of *B. malayi* infection in Kerala State has been observed in limited scale in certain areas by Iyengar (1938) and Raghavan *et al.* (1958) also. From the present results, which are based on a larger scale from all the *B. malayi* areas, the absence of semi-periodic form has been confirmed. No difference in the periodicity pattern of *B. malayi* microfilaria, prevalent in hilly areas and plain areas, could be demonstrated

From the village of Thuravur and islands round about Ernakulum, *B. malayi*-like infection in dogs was recorded (Pattanayak, 1956 ; Annual Reports of the Malaria Institute of India for 1960, 1961, 1962). The adult worms are yet to be identified. Since semi-periodic *B. malayi*, which has adaptibility to vertebrate hosts other than man, is absent it will be of interest to know if these animal infections are of *B. malayi*.

SUMMARY

Periodicity of microfilaria of *B. malayi* in the single largest tract (Kerala State) of India has been studied.

Nocturnal periodicity has been recorded both in plain areas and hilly areas. Absence of semi-periodic form has been demonstrated.

In view of the absence of semi-periodic form of *B. malayi*, the identification of the adult worms from dogs and cats, showing *B. malayi*-like infection, will be of interest.

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A NOTE ON NATURAL SIMIAN MALARIA INFECTION IN KERALA STATE, INDIA.

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[December 15, 1963.]

A SURVEY conducted in the Nilgiri Hills, Madras State, revealed an enzootic focus of simian malaria (Ramakrishnan and Mohan, 1962). A similar survey was made for simian malaria in Kerala State. The results of the same are set out in the present note.

The monkey population in Kerala State is very sparse. The greater concentration is on the slopes of the western ghats. In the plane areas, it is extremely scanty. Shooting of monkeys is prohibited in the entire State. They were collected by trapping which was a time consuming method, involving a patient waiting for long hours which quite often ended in failure. Similar experiences had been encountered during the survey in the Nilgiris referred to above.

Two places in the Kerala State were surveyed for simian malaria during 1962, namely Nilambur and Alleppey. Nilambur is located on the western slope of the western ghats, from the eastern slope whereof Ramakrishnan and Mohan (1962) reported enzootic focus of simian malaria. Nilambur was classified as hyperendemic in the National Malaria Eradication Programme of Kerala State. Alleppey is located in the central plain area of Kerala State and is hypo-endemic as classified in the National Malaria Eradication Programme of Kerala State.

During May and December, 1962, 11 monkeys (*Macaca radiata radiata*) were captured from Nilabur, out of which 7 (63·6 per cent) were found positive for malaria infection. The infection was mixed *P. cynomolgi* and *P. inui* and was later confirmed by inoculation of blood from infected monkeys from Nilambur into clean *M. rhesus* monkeys at Delhi. Blood for parasites was taken only from monkeys with intact spleen. Pure *P. cynomolgi* infection was not encountered during this survey. The parasites, in general, were scanty in positive smears.

A survey of natural malaria infections in the local mosquitoes was carried out. Morning and night catches of mosquitoes were made in huts near the areas from where positive monkeys were collected. The results of the dissection of gut and glands of the 118 female mosquitoes collected are shown in Table I. All the dissected mosquitoes were negative.

During February, 1962, survey was undertaken in Alleppey. The monkey population was very scanty. Only three brown (*M. radiata radiata*) monkeys were collected. None of them showed any malaria parasite even after splenectomy.

TABLE I.

Results of dissection of gut and gland of the 118 female mosquitoes.

Mosquito.	Number (males).	Number (females).	Number dissected.	Results.
A. fluviatilis	10	3	3	Negative
A. pallidus	..	1	1	Negative
A. jeyporiensis	3	5	5	Negative
A. tessellatus	3	..	..	Negative
A. jamesi	3	4	4	Negative
C. vishnui	7	15	15	Negative
C. gelidus	0	2	2	Negative
C. fatigans	0	2	2	Negative
C. bitaeniorhyncus	0	1	1	Negative
A. albopictus	3	7	7	Negative
Armigeres	14	78	78	Negative

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NOTE ON THE FLIGHT RANGE OF *CULEX FATIGANS*.

BY

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[December 22, 1963]

INTRODUCTION

THE flight range of the mosquito denotes the maximum distance up to which it can fly. The dispersal of mosquitoes depends on its flight range. *C. fatigans* is the vector of *W. bancroftian* filariasis and determination of its flight range at a particular place may be of importance for delimiting areas for anti-larval operations. The National Filaria Control Programme is based mainly on anti-larval measures and delimiting of areas for such measures may be necessary for its effective implementation.

Senior White (1934) and Afridi and Majid (1938) have also determined the ranges in the past. The present field study was undertaken by the National Filaria Control Unit, Basti (Uttar Pradesh) under natural climatic conditions, to determine the actual flight range of female *C. fatigans* in a place where antilarval measures are being undertaken.

METHOD

Majid (1937) has discussed the technique of marking and catching the mosquitoes, while the technique employed in this study for the determination of flight range of *C. fatigans* consisted of general collection of mosquitoes, sorting out of the female *C. fatigans* and dusting them with golden aluminium-powder by an atomiser. The dusting was thoroughly done so that all the mosquitoes could take a thin but a distinct coat of the powder. The dusting was done in order to identify the mosquitoes under experiment from the other mosquitoes. The golden-coloured mosquitoes were released outside the laboratory early in the morning and the recollection was made after twenty-four hours of their release. The search for the coloured mosquitoes was conducted in all the directions centripetally from the laboratory up to a distance of five miles.

The experiment was carried out for 13 days under natural climatic conditions and simultaneous collections were made. The various distances up to which the

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golden-coloured mosquitoes were recovered on the different days have been recorded in Table I. The distances were measured from the place of their release.

TABLE I.

Number of mosquitoes released after having been marked with golden aluminium powder and the number recovered from various distances.

Days of experiment.	Number of mosquitoes marked and released.	Number of marked mosquitoes recovered.	MAXIMUM DISTANCE AT WHICH RECOVERED :	
			(In Miles and Furlongs).	(In Kilometers).
1st day	83	*Nil*	..	..
2nd day	176	3	0—4	0·80
3rd day	292	7	1—2	2·01
4th day	233	5	1—0	1·60
5th day	303	6	2—0	3·21
6th day	182	2	2—0	3·21
7th day	245	6	2—4	4·02
8th day	265	4	2—4	4·02
9th day	316	7	2—2	3·62
10th day	318	9	2—5	4·22
11th day	326	6	1—7	3·01
12th day	219	5	2—6	4·42
13th day	*Nil*	7	2—4	4·02

The equipment employed during the experiment consisted of an atomiser, golden aluminium-powder, sucking tubes, flash lights, Barraud's cages and test-tubes. The same persons were engaged during the whole period of the experiment.

OBSERVATIONS AND DISCUSSIONS

For re-collection of the mosquitoes, searches were made from all types of dwellings, bushes, under culverts, treeholes and also from the aquatic vegetations. It was, however, found during these catches that the marked *C. fatigans* were recovered only from the indoor stations and not from the outdoor collections. It was further observed (Table I) that the maximum flight range of female *C. fatigans* was 2 miles and 6 furlongs (4·42 kilometre). On comparing the present results with those of Senior White (1934) as 2,310 yards (2·11 km.) for males and of Afridi and Majid (1938) as 5,500 yards (5·02 km.) for females and 2,860 yards (2·61 km.) for males, it is evident that the flight range varies for *C. fatigans* from place to place and that also of males and females. Moreover, it can now be put on record that from the experiments carried out for the determination of the flight range of female *C. fatigans* at the National Filaria Control Programme Unit, Basti (Uttar Pradesh), the maximum distance found was 2 miles and six furlongs (4·42 km.) under natural climatic conditions.

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HOUSE FREQUENTING BEHAVIOUR OF *C. FATIGANS*.

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VARIOUS epidemiological aspects of *W. bancroftian* filariasis have been studied by many workers in India (Iyengar, 1938 ; Raghavan and Krishnan, 1945 ; Raghavan, 1951, 1955 ; Ray, 1957 ; Bhatia and Wattal, 1958, and Ramakrishnan 1961). The present study was undertaken at the National Filaria Control Unit, Barabanki (Uttar Pradesh), in order to supplement the knowledge of epidemiology of *W. bancroftian* filariasis in a particular area.

METHOD

A village known as Beri in District Barabanki (Uttar Pradesh) which has no other village within a radius of one mile, was selected for the present study. All the 70 houses in the village were enumerated for regular observations. Before commencing the study, every house was visited daily by a team of five insect collectors for 5 to 7 days and the mosquitoes were collected for twenty minutes in each house, and then the per man-hour density was worked out with a view to have the average density of *C. fatigans* of the village/house (Table I). This also helped in fixing houses as the fixed catching stations for further study. The collections were made in the month of November during the morning hours from 7 a.m. to 11 a.m.

Six catching stations, having house numbers 2, 5, 10, 45, 56 and 62 from the representative areas of the village, were selected for further observations and hereafter known as fixed stations. Total mosquito catches were conducted in all the six fixed stations in the morning. The *C. fatigans* were sorted out separately from the total catch of each fixed station. They were kept in Barraud's cages and fed on glucose solution. In the evening, all actively flying *C. fatigans* were counted, properly dusted with golden powder (Majid, 1937) and were released in their respective fixed stations. The number of mosquitoes released in the six fixed stations was 492, out of which 209 were male and 283 female.

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TABLE I.

Density of C. fatigans *during one week from all the houses of Village Beri, District Barabanki.*

Houses :		Time spent :	Total number of *C. fatigans* collected :			Per man-hour density.
Serial number.	Type.	Hours—Minutes.	Male.	Female.	Total.	
1	O.S.	2—20	37	34	71	30
2	F.S.	2—20	52	40	92	40
3	O.S.	2—20	19	37	45	23
4	O.S.	2—20	41	70	111	49
5	F.S.	2—20	52	68	120	51
6	O.S.	1—40	5	9	14	8
7	O.S.	2—20	39	22	61	26
8	O.S.	2—20	20	22	42	18
9	O.S.	2—20	36	44	80	34
10	F.S.	2—20	53	50	103	44
11	O.S.	2—20	6	6	12	5
12	O.S.	2—00	6	11	17	9
13	O.S.	2—00	5	9	14	7
14	O.S.	2—20	17	21	38	16
15	O.S.	2—20	12	18	30	13
16	O.S.	2—20	18	19	37	16
17	O.S.	2—20	18	14	32	14
18	O.S.	2—20	11	26	37	16
19	O.S.	2—20	20	24	44	19
20	O.S.	2—20	21	22	43	18
21	O.S.	2—20	15	19	34	15
22	O.S.	2—20	13	21	34	15
23	O.S.	2—20	6	23	29	12
24	O.S.	2—20	15	23	38	16
25	O.S.	1—40	11	16	27	16
26	O.S.	2—20	12	22	34	15
27	O.S.	2—20	15	24	39	17
28	O.S.	2—20	11	17	28	12
29	O.S.	2—00	10	12	22	11
30	O.S.	1—20	6	11	17	13
31	O.S.	2—20	11	23	34	15
32	O.S.	2—20	7	21	28	12
33	O.S.	1—20	7	10	17	13
34	O.S.	2—20	4	30	34	15
35	O.S.	2—20	7	27	34	15
36	O.S.	2—20	8	30	38	16
37	O.S.	2—20	6	47	53	23
38	O.S.	1—40	4	24	28	17
39	O.S.	2—20	12	21	33	14
40	O.S.	2—20	11	19	30	13
41	O.S.	2—20	17	14	31	13
42	O.S.	2—20	20	19	39	17
43	O.S.	1—40	7	14	21	13
44	O.S.	2—20	17	10	27	12
45	F.S.	2—20	22	15	37	16
46	O.S.	2—00	10	17	27	14
47	O.S.	2—20	11	11	22	9
48	O.S.	1—00	6	6	12	12
49	O.S.	2—00	8	18	26	13
50	O.S.	2—20	9	20	29	12
51	O.S.	2—20	19	16	35	15
52	O.S.	2—00	9	11	20	10
53	O.S.	1—40	7	13	20	12

(Continued)

TABLE I. (*Concld.*)

Houses :		Time spent :	Total number of *C. fatigans* collected :			Man-hour density.
Serial number.	Type.	Hours—Minutes.	Male.	Female.	Total.	
54	O.S.	2—20	13	19	32	14
55	O.S.	2—00	12	15	27	14
56	F.S.	2—00	13	21	34	17
57	O.S.	2—00	12	23	35	18
58	O.S.	2—00	15	27	42	21
59	O.S.	2—00	10	31	41	21
60	O.S.	2—20	11	37	48	21
61	O.S.	2—20	15	33	48	21
62	F.S.	2—00	19	33	52	26
63	O.S.	2—20	16	27	43	18
64	O.S.	2—20	15	19	34	15
65	O.S.	2—20	11	33	44	19
66	O.S.	2—20	21	21	42	18
67	O.S.	2—20	14	24	38	16
68	O.S.	2—20	14	23	37	16
69	O.S.	2—20	20	36	56	24
70	O.S.	2—20	25	29	54	27

Note :—O.S — Other catching stations,
F.S — Fixed catching stations.

The re-collection of the coloured *C. fatigans* was started on the third day of their release, and continued on alternate days. Mosquitoes were searched for thirty minutes in each of the fixed stations, while in each of the other 64 stations search for only 15 minutes was done. The search was conducted till the 25th day of their release.

The equipment employed consisted of flash light, Barraud's cages, sucking tubes, test tubes and magnifying lenses. The same Insect Collectors were employed throughout the period of study.

OBSERVATIONS

During the period of observations it was observed that the houses with comparatively higher density of *C. fatigans* were ill-ventilated, dark and had plenty of hangings inside. In these houses the kitchen was separate from the living rooms and the breeding places were noticed adjacent to it. While on the other hand the houses with low density were exposed to light, cooking was done inside the living rooms, less of hangings inside the rooms and there were no breeding places nearby.

The average density of *C. fatigans* recorded from the fixed stations was 32·33 per man-hour, the highest being 51 and the lowest 16. The lowest density was recorded to be 5 from one of the other catching stations (Serial number 11) and the highest of 49 from catching station Number 4 as shown in Table I.

The total number of coloured *C. fatigans* recaptured, on different days after their release from fixed as well as from other catching stations, have been shown in Table II. The maximum number of coloured *C. fatigans* recaptured were recorded

on the 5th day of their release, the number being 58 and 29 from the fixed and other cathing stations respectively. The coloured mosquitoes could be recovered up to 13th day of their release after which none was found from any of the catching stations. The catching continued till 25th day.

TABLE II.

Number of coloured C. fatigans *recaptured on different days after release.*

Day after release.	NUMBER OF *C. fatigans* (COLOURED) RECAPTURED :				TOTAL :	
	From fixed stations.		From other stations.			
	Male.	Female.	Male.	Female.	Male.	Female.
2rd ...	9	13	*Nil*	*Nil*	9	13
5th ...	30	28	12	17	42	45
7th ...	12	10	2	4	14	14
9th ...	5	7	1	2	6	9
11th ...	9	14	2	*Nil*	11	14
13th ...	*Nil*	2	*Nil*	*Nil*	*Nil*	2
Total	65	74	17	23	82	97

Percentage of coloured *C. fatigans* recaptured from the fixed stations = 77·78.
Percentage of coloured *C. fatigans* recaptured from other stations = 22·22.

Out of the 492 *C. fatigans* released, the total number of coloured *C. fatigans* recaptured during the period of observations, from all the catching stations, was 179, out of which 12 were male and 97 were female. Thus 36·6 per cent of the coloured *C. fatigans* were recollected from the village. Of all the coloured *C. fatigans* recovered 77·78 per cent were from the fixed catching stations, while 22·22 per cent were caught from other stations. The other catching stations from where the coloured *C. fatigans* were collected, were situated either adjacent to the fixed stations or were only a few metres away from them.

CONCLUSION

(1) From the observations it was recorded that a high percentage, i.e. 77·78 per cent of coloured *C. fatigans* recaptured were from the fixed stations, while only 22·22 per cent from the other catching stations.

(2) The other catching stations, from which the coloured *C. fatigans* were recovered, were either adjacent or only a few metres away from the fixed catching stations.

(3) It is evident that *C. fatigans*, in general, does not change its dwelling place once it occupies, provided of course there is plenty of food for it and the breeding places are available in the vicinity. This behaviour of *C. fatigans* to occupy generally the same dwelling during its life time may, therefore, be one of the causes for the slow transmission of filariasis in a locality.

SUMMARY

The present studies were undertaken at the National Filaria Control Programme Unit, Barabanki, to study the 'House frequenting behaviour of *C. fatigans*'. On the basis of the observations it was recorded that a high percentage, *i.e.*, 77·78 per cent of *C. fatigans* stayed in the same dwelling once they had occupied while only 22·22 per cent of *C. fatigans* were found to change their original dwelling and were noticed to shift to the adjacent or very near houses from their original dwellings.

Hence it is evident that *C. fatigans* mostly remains in the same house during its life time and may change its original dwelling place probably due to the struggle for existence.

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OBSERVATIONS ON RURAL FILARIASIS IN SITAPUR DISTRICT (UTTAR PRADESH)

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INTRODUCTION

The district gazetteers of the districts of Gorakhpur and Varanasi of the United Provinces of Agra and Oudh (now known as Uttar Pradesh) have described about the prevalence of filariasis in these districts as early as 1909. It has been further indicated in the filaria map of India, presented by Magaw and Gupta (1927), that the disease was common in the eastern districts of Uttar Pradesh. Recently Raghavan (1955) also published a map of India showing the prevalence of the disease in almost all the eastern districts of this State. Further, with the launching of the National Filaria Control Programme during the year 1955-56, the Filaria Survey Units have delimited the problem of filariasis in the districts of Ballia (Rahman *et al.*, 1957), Basti (Rahman, *et al.*, 1957), Bahraich (Diwan Chand *et al.*, 1959), Gonda (Diwan Chand *et al.*, 1961), Deoria (Diwan Chand *et al.*, 1961), and Ghazipur (Diwan Chand *et al.*, 1961), all situated in the eastern zone of the State. But with the results obtained by the survey carried out during 1959 in Hardoi Town, which is situated in the central zone of the State, the filaria problem appears to have extended further and is present in the central zone of Uttar Pradesh also. As such, a survey was undertaken in the districts of Lucknow, Sitapur, and Hardoi. Details of the conditions prevailing in the rural areas of Sitapur are presented in this paper.

TOPOGRAPHY AND CLIMATIC CONDITIONS

The district lies in the north central plains of Uttar Pradesh between 27°6′ and 27°54′ north latitude, 80°18′ and 81°24′ east longitude, having an area of 2,208 sq. miles. The terrain comprises a larger portion of an upland plain with a

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smaller portion of low land or *Ganjar* area on the eastern side. It is bounded on the west and the south-east by the River Gomti, while towards the east flows the famous River Ghagra, giving a roughly rectangular shape to the tract. The whole district is of purely alluvial formation and the river deposits, after the floods, are usually fertile. The *Ganjar* tract is liable to annual inundations during rains, and the villages are situated on the highest available spots. In addition to a large number of streams and streamlets, the district contains a large number of lakes and tanks. Apart from these, there is a net work of irrigation channels of the Sarda Canal system traversing the plain area of the district.

The climatic conditions are generally healthy excepting in the low-lying areas. The mean temperature ranges between 45°F in the cold season and 95°F in the hot weather. The prevailing winds are generally from the east during monsoon and from the west during the rest of the year. The rainy season extends from the middle of June to end of September, the average rainfall being about 54 inches. Most of the transmission takes place during the rainy season.

PEOPLE AND THEIR SOCIO-ECONOMIC CONDITIONS

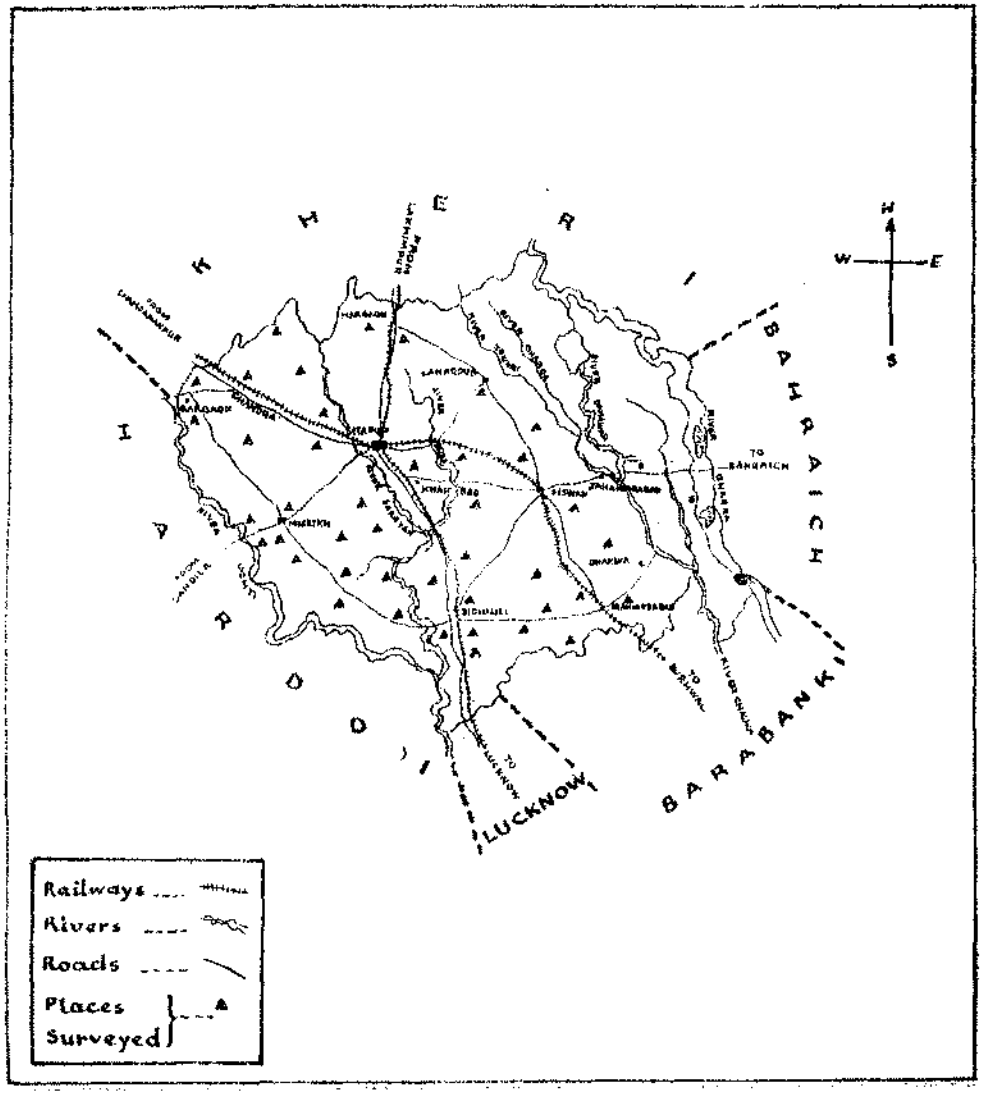
The population of the district is 16,15,928 (1961 census). As about 76 per cent of the area is agricultural, majority of the people depend on cultivation for their means of livelihood. Rice and wheat are the main crops while sugarcane is an important cash crop. The increase in the population has caused a decline in the land area per capita, which has resulted in a great strain on the economy of the people. The district is also inhabited by the landlords belonging to a great variety of castes and clans. The district headquarters town Sitapur is well connected by rail and roads with its neighbouring districts. A famous fair is also celebrated at Neem Sharanya and Misrikh every year. The chief trades of the district are sugar, plywood, groundnuts, *darris*, leather goods, tobacco, ropes, *gur* and *khandsari*.

MATERIAL AND METHODS

A random and unbiased sample of the population in villages was taken for survey as indicated in Map I, and in order to obtain the maximum number of blood slides wide publicity was done in the villages and hamlets by distributing printed pamphlets and contacting people personally during day-time. Attempts were made to collect maximum number of blood smears, consisting of a thick film prepared with three drops of blood (20 c.mm.) by visiting door-to-door in the villages at night between 8 p. m. and midnight from persons of all age-groups, both sexes, and both Hindus and Muslims. Collections of adult mosquitoes were made by trained insect-collectors with the help of sucking tubes. Night catches were also made for mosquitoes in these villages and the dissections were made for searching and counting the parasites the next day. The blood films were stained the following day with J. S. B. Stain I (Jaswant Singh and Bhattacharji, 1944) in the field and were examined later for counting the microfilariae in the positive

MAP 1.

Map of Sitapur District.



blood slides. While preparing the blood smears, people were examined for disease manifestations, *viz.*, hydrocele, elephantiasis, lymphadenitis and history of chyluria. The surveys of the rural areas of Sitapur were carried out during the year 1961-62.

OBSERVATIONS

The survey results, *viz.*, infection rate, average infestation, disease and endemicity rates, along with the entomological data, are detailed below :

INFECTION RATE

The blood films obtained from 5,883 persons of all age-groups, during the present observations, revealed the following results (Table I) :—

TABLE I.

Infection rate and average infestation according to age-groups.

Age-groups (Years).	Number of persons examined.	Number found positive for micro-filariae.	Infection rate, per cent.	Average infestation per 20 c.mm. of blood.
0 — 1	124	*Nil*	0·00	*Nil*
2 — 5	526	4	0·76	2·26
6 — 10	775	18	2·32	4·66
11 — 20	1,246	47	3·77	4·93
21 — 30	1,784	72	4·03	5·26
31 — 40	710	45	6·33	5·02
41 — 50	399	22	5·51	7·31
Above 50	319	25	7·83	4·04
Total	5,883	233	3·96	5·09

As is evident from the above data, the average infection rate for all ages is 3·96 per cent, which is comparatively lower than in the adjoining districts of Hardoi and Lucknow as reported by Singh (1960). The infection was found in both male and female children at the minimum age of 5 years in a village, Khagasia Mau. The infection rate showed an increase with the age, being maximum in the age-group of "above 50 years". The type of infection detected was *Wucheraria bancrofti*. The average infestation was observed to be 5·09 microfilariae per 20 c. mm. of blood while the maximum number of the parasites found in a single slide was 52.

DISEASE RATE

While collecting the blood-films during the survey the same individuals were examined for disease manifestation and out of the 5,883 persons only 98 showed to have different disease processes, in the form of hydrocele, elephantiasis of the limbs and chyluria. The disease rate was comparatively lower, being 1·66 per cent, as compared to that in the neighbouring districts of Hardoi and Lucknow. The youngest age at which the disease manifestations were noted, was in a girl of

ten years, with elephantiasis of the left lower limb. The incidence of the disease, according to age-groups, is shown in Table II.

TABLE II.

Disease processes according to various age-groups.

Age-groups (Years).	Number of persons examined.	Number of persons showing disease manifestation.	Disease rate, per cent.
0 — 1	124	*Nil*	0·0
2 — 5	626	*Nil*	0·0
6 — 10	775	1	0·13
11 — 20	1,246	8	0·64
21 — 30	1,784	42	2·35
31 — 40	710	27	3·80
41 — 50	399	16	4·01
Above 50	319	4	1·25
Total	5,883	98	1·66

The observations reveal that the disease showed appearance at the age of 10 years and increased to the maximum in the age-groups of above 40 years.

The incidence of various disease manifestations, observed in the various age-groups and both sexes, are tabulated in Table III which shows that the hydrocele and other genital lesions were commonest, followed by chyluria, in this area.

TABLE III.

Number of persons showing disease manifestations by age-groups and sex.

Age-groups (Years).	Disease manifestations:												
	Hydrocele.		Elephantiasis.								Chyluria.		Total.
			Right lower limb.		Left lower limb.		Right upper limb.		Left upper limb.				
	Male.	Female.	Male.	Female.	Male.	Female.	Male.	Female.	Male.	Female.	Male.	Female.	
0 — 1	..	..	..	..	..	..	..	..	..	..	..	..	..
2 — 5	..	..	..	..	..	..	..	..	..	..	..	..	..
6 — 10	..	..	1	..	..	..	..	..	..	..	..	..	1
11 — 20	8	..	..	..	1	2	..	..	..	..	1	..	12
21 — 30	36	..	..	1	..	2	..	..	1	..	3	..	43
31 — 40	18	..	2	..	2	..	..	..	2	..	7	..	31
41 — 50	12	..	..	1	..	1	..	..	..	..	2	..	16
Above 50	3	..	1	..	..	..	..	..	..	..	2	..	6
Total	77	..	4	2	3	5	..	..	3	..	15	..	109*

* Different disease manifestations in the same individual have been counted separately, *i.e.*, 98 persons have shown 109 disease manifestations. Some persons had more than one disease manifestation.

ENDEMICITY RATE

Out of 5,883 persons examined, 227 were found positive for microfilariae while 98 showed disease manifestations. There were only two persons showing both disease manifestations as well as infection in their blood. The endemicity rate of the rural areas of Sitapur District has been found to be 5·59 per cent which is low. The endemicity, according to the age-groups, is presented in Table IV.

TABLE IV.

Endemicity rates according to various age-groups.

Age-groups (Years).	Infection rate, per cent.	Disease rate, per cent.	Both disease and infection rate, per cent.	Endemicity rate, per cent.
0— 1	0·00	0·00	0·00	0·00
2— 5	0·75	0·00	0·00	0·75
6—10	2·32	0·13	0·00	2·45
11—20	3·77	0·64	0·00	4·41
21—30	4·03	2·35	0·11	6·27
31—40	6·33	3·80	0·00	10·14
41—50	7·83	1·25	0·00	9·09
Total ...	3·96	1·66	0·03	5·59

The data obtained during the survey have been further analysed in order to study their relation with the sex and religion. Tables V and VI show the infection, disease and endemicity rates amongst males and females, Hindus and Muslims, of rural areas.

TABLE V.

Infection, disease, mixed and endemicity rate according to sex.

Sex.	Number examined.	INFECTION RATE :		DISEASE RATE :		BOTH DISEASE AND INFECTION :		Endemicity rate, per cent.
		Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	
Male	3,924	167	4·25	93	2·39	2	0·25	6·57
Female	959	66	3·36	5	0·25	*Nil*	0·00	3·62
Total ...	5,883	233	3·96	98	1·66	2	0·03	5·59

TABLE VI.

Infection, disease, mixed and endemicity rates according to religion.

Religion.	Number examined.	INFECTION RATE:		DISEASE RATE :		BOTH DISEASE AND INFECTION :		Endemicity rate, per cent.
		Number.	Percentage.	Number.	Percentage.	Number.	Percentage.	
Hindus	5,388	221	4·10	91	1·68	2	0·03	5·75
Muslims	496	12	2·42	7	1·41	*Nil*	0·00	3·84

The statistical analysis of data showed that the disease and endemicity rates amongst the male members are higher than the females. There is, however, no difference in infection rate in the two sexes.

The data were analysed statistically with regard to incidence of infection, disease and endemicidity in the two groups. No positive difference was found with respect to these characteristics in the Hindus and Muslims.

ENTOMOLOGICAL OBSERVATIONS

Mosquito collections were made from various parts of the district from human and mixed dwellings as well as from the cattlesheds and a total of about 4,385 were captured. Out of these, 2,674 were *Culex fatigans*, the rest being *Anopheles*. Twelve *Culex fatigans* were found positive with developing microfilaria, giving the infection rate of 1·19 per cent. The infectivity rate worked out to be 0·89 per cent. None of the *Anophelines* were found positive. The results of dissection are tabulated in Table VII.

TABLE VII.

Results of mosquito dissections.

Mosquitoes.	Number captured.	Number dissected.	Number found positive.	Stage of development :			
				I	II	III	IV
C. fatigans	2,674	1,005	12	*Nil*	5	5	2
Anophelines	1,711	960	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*

It is confirmed that *Culex fatigans* is the vector of *W. bancrofti* infection found in the rural areas of Sitapur District. It was further observed that it breeds profusely in stagnant water with plenty of organic pollution, *viz.*, stagnant pools, cess-pits, ponds, stagnant drains, small nullahs, etc.

SUMMARY

Filaria survey of the rural areas of the Sitapur District (Uttar Pradesh) was conducted during 1961-62 and 5,883 persons were examined for the presence of infection and disease.

The microfilariae rate was found to be 3·96 per cent while the disease rate worked out to be 1·66 per cent, and the endemicity rate was noticed to be 5·59 per cent. The average infestation per 20 c.mm. of blood was 5·09 and the maximum number of microfilariae enumerated in one blood smear was 52. The type of infection detected was *W. bancrofti*.

The main disease manifestations were hydrocele, elephantiasis of the lower limbs and chyluria.

Culex fatigans has been incriminated as the vector which showed various development stages of the *W. bancrofti*.

Stagnant pools, drains, ponds, and pits for collecting kitchen sullage, have been found to be the potential breeding grounds for the vectors.

As also worked out by Singh (1960) in the epidemiological surveys carried out in the State during the last ten years, the endemicity of the *W. bancrofti* infection, which was believed to be originally restricted to eastern districts of the State, has now been found to be more widely distributed in the districts of central Uttar Pradesh also.

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STUDIES ON THE COMPARATIVE HOSPITABILITY OF *SALVINIA AURICULATA* AUBET AND *PISTIA STRATIOTES* LINN., TO *MANSONIOIDES ANNULIFERA* (THEOBALD) IN KERALA.

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INTRODUCTION

Salvinia auriculata is a water fern belonging to the family Salviniaceae. It is characterised like other members of the family by the presence of sessile fronds on the upper side of the floating stem and long root-like leaves hanging into the water. It is a Brazilian species, first introduced into India in 1933 (Biswas and Calder, 1937). The species of *Salvinia*, originally indigenous to India, are *S. natans* and *S. cucullata* (Biswas and Calder, 1937).

Until about three years ago, *S. auriculata* was not in evidence in Kerala. Iyengar (1938) mentions only one species of *Salvinia*, namely, *natans*, as commonly occurring in the Travancore area of Kerala. Burton (1960) found *S. auriculata* growing in a tank in Ernakulam. It is obvious that the weed got introduced into this State rather recently. How this happened, is not definitely known. However the weed has now invaded extensive sheets of water and has become a dominant aquatic vegetation practically all along the southern half of the coastal tract of Kerala. In many places it has crowded out *Pistia stratiotes* which has all along been the characteristic floating vegetation of the ponds, tanks, channels and canals in this area.

During the monsoons, when the salinity of the back waters and lagoons and other water courses connected with them is very low, the fern can be seen drifting in them in large matty patches. Along with the ebb and flow of the tides, these patches fluctuate between the back waters and the net work of inland channels and canals. During summer, when the salinity in the back waters is high, the fern withers

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and apparently dies away ; but enough of it remains in the channels and ponds so that with the onset of the rains, it resumes its luxuriant growth with redoubled vigour and spreads all over.

Salvinia auriculata, among other aquatic flora, has been reported to be an efficient natural host plant for *Mansonioides uniformis* in Ceylon (Antonipullai *et al.*, 1958), and it is considered to be of importance in the epidemiology of Brugian filariasis in that country. According to Burton (1960) this weed is said to have been introduced into Ceylon during World War II in order to camouflage water ways and thus to confuse enemy pilots.

Mansonioides annulifera is the primary vector of *Brugia malayi* in the coastal tracts of Kerala. *Pistia stratiotes* has been proved to be the normal host plant of *M. annulifera* not only in Kerala but in most parts of India. It has seldom been found to breed on other aquatic plants. But Burton (1960) demonstrated by laboratory experiments that gravid females could be made to oviposit on the leaves of *Salvinia auriculata* and the larvae to attach themselves to the root-like processes of the fern. However, he does not mention having come across natural breeding of *M. annulifera* on *Salvinia*.

The secondary vector of *Brugia malayi* in Kerala is *M. uniformis*. This mosquito is the primary vector of this infection in certain isolated foci in the hilly regions of Kerala, where *M. annulifera* is extremely rare or absent (Iyengar, 1938 ; Raghavan *et al.*, 1958). In the coastal areas, however, *M. uniformis* plays only a secondary role because of its comparatively low incidence and zoophilic habits. *M. uniformis*, unlike *M. annulifera*, is a highly adaptable species and has been reported to breed on a wide variety of aquatic plants including *Salvinia* (Antonipullai *et al.*, 1958). In the coastal tracts of Kerala, however, its host plant is usually *Pistia*. Burton (1960) has shown that it can thrive well on *Eichornia crassipes*, another common aquatic weed.

The present investigation was mainly concerned with the possible effects of the influx of *S. auriculata* on the breeding of *M. annulifera*, the primary vector of *B. malayi*, and to assess its natural potential as a host plant for the vector, in comparison with *P. stratiotes*, its natural host plant.

PROCEDURE OF STUDY

Field as well as laboratory experiments were conducted to determine the comparative suitability of *S. auriculata* and *P. stratiotes* for oviposition of *M. annulifera* and for the attachment of its larvae. The rate of output of adult *annulifera* from experimental ponds, stocked with the two weeds, was also studied. Finally a general reconnaissance of a typical locality, where *S. auriculata* had become the dominant aquatic vegetation, was made to assess the extent of *Mansonioides* breeding on this fern. The entire investigation was carried out during the dry months of February through July which represent the most favourable season for *Mansonioides* breeding.

(i) FIELD EXPERIMENTS

The venue of the field experiments was Ariparambu, a locality about three miles south-west of the township of Sherthallai.

(*a*) A preliminary random survey of the locality showed considerable incidence of *M. annulifera*. Fifteen ponds (diameter range about 6 to 10 metres), situated within an area of approximately 4 hectares, were selected for the experiment. They were divided at random into three comparable ecological groups as shown below and maintained as such for the duration of the experiment, *i.e.*, five months, February through June, 1963.

Group	Ponds numbered	Vegetation
A	1 to 5	*Pistia* only.
B	6 to 10	*Pistia* and *Salvinia* (equal proportions).
C	11 to 15	*Salvinia* only.

All the 15 ponds had been cleared of all vegetation before they were stocked with the respective weed. The ponds in Group B were stocked with equal quantities of *Pistia* and *Salvinia* (Plate I, Fig. 1). Cocoanut husk packing was then introduced into each experimental pond in the same manner as is done locally for retting husk for coir (Plate II). The retting husks would keep the water in ponds in a state of pollution, a condition so essential for the breeding of *Mansonioides* (Iyengar, 1938).

The preparatory part of the experiment was done during the middle of January. Actual observations began during the first week of February, after allowing a fortnight's time for the ecological factors to get stabilized in all the ponds. During this period the ponds were daily examined for egg masses and larvae of *Mansonioides*. Thereafter, *i.e.*, from February onwards, triweekly examinations were done. Every time at least 100 heads each of *Pistia* and *Salvinia*, taken from around the husk pack, were examined from the respective ponds. The number of egg masses and larvae per 100 heads of weed from each pond was recorded.

It must be mentioned here that no attempt was made to differentiate the egg masses specifically even though, as pointed out by Burton (1960), mature eggs containing the fully formed first-instar larva can be identified specifically under the microscope by siphonal characters. This is obviously not practicable in field studies like the present one in which the egg masses, being freshly laid, would be mostly immature, and therefore would not show up the differential characteristics unless they are kept in the laboratory for maturation and subsequent examination—a rather laborious process. It is possible that quite a few of the egg masses encountered might have been those of *M. uniformis* ; but examination of the larvae in the experimental ponds, which was done along with the search for the egg clusters, revealed that the predominating species was *M. annulifera* and the breeding of *M. uniformis*, if present at all, was too low to vitiate the main results. Differentiation

of the larvae of the two species with the naked eye is not difficult for an experienced person and is practicable. The larvae of *M. annulifera* can be distinguished by their longer siphon and the absence of a dark clypeal area which is characteristic of *M. uniformis* (Menon, 1940).

(*b*) Comparative observations on the output of adult *M. annulifera* from the experimental groups of ponds were also made by trapping the newly emerged adults in a floating trap.

The trap has a bottomless dealwood base 30 cm. × 25 cm., and 10 cm. high. This is surmounted by a four sided transparent pyramid made of polythene sheet stretched over a wire frame. The pyramid has an aperture 2·5 cm. in diameter at the apex. Over this pyramid, is placed a mosquito-cage having its base stitched in the manner of a four-sided funnel corresponding to the sides of the polythene pyramid so that the latter fits into the former, the sides and apertures of the two coinciding. The cage is provided with a wide sleeve on one side through which the trapped mosquitoes can be collected. To set the trap, the cage is detatched and the dealwood base, with the surmounting cone, is lowered into the breeding place in such a way as to enclose within it a rectangular section of the matty growth of the weed, *Pistia* or *Salvinia* as the case may be. The cage is then placed in position and the trap set afloat. The adult mosquitoes, which emerge from pupae attached to the roots of the plants imprisoned within the base of the trap, are caught in the cage and can later be collected.

For the present study, the trap was kept afloat day and night, consecutively for a number of days in one representative pond of each experimental group of ponds, 10 days for Group A, 9 days for Group B and 8 days for Group C. The collection of the trapped adults was done daily at 7 a.m.

(*ii*) LABORATORY EXPERIMENTS.

(*a*) *Oviposition.*—To study the oviposition preferences of *M. annulifera*, the following experiment was conducted :

Within a large mosquito cage (45×45×45 cm.), two identical earthenware bowls 15 cm. in diameter, containing pond water, were placed. Into one of the vessels a healthy *Pistia* plant was introduced and into the other a *Salvinia* plant about the same size as the *Pistia*. Every morning some adult females of *M. annulifera*, reared and fed on human blood in the laboratory, were let into the cage. The *Pistia* and *Salvinia* were examined each morning for egg masses and the number of egg masses found on each recorded. The egg masses, together with the leaf on which they were laid, were removed every morning so that they might not get mixed up with those laid on the following day.

The experimental cage was kept in a well ventilated room receivinig good diffused day-light. No artificial light was used. Plenty of moisture was provided for the mosquitoes by hanging wet towels over the cage. The mosquitoes were fed on raisins. Those that had laid were not removed but retained in the cage until the close of the experiments.

PLATE I.

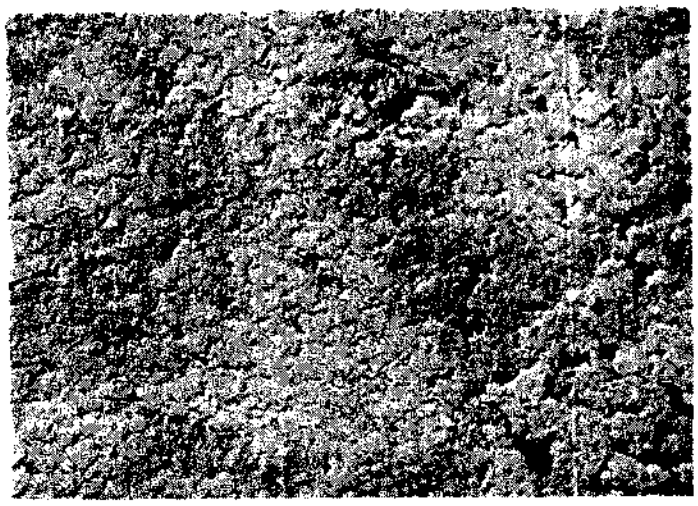


FIG. 1.– Mixed pond (*Pistia* and *Salvinia*). (Experimental Group B).

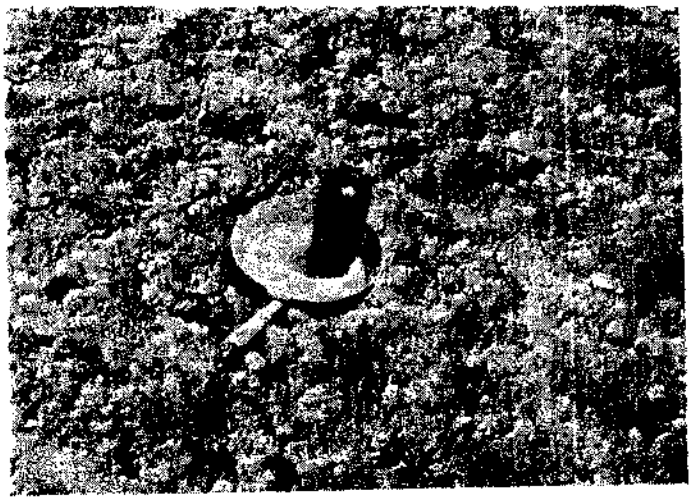
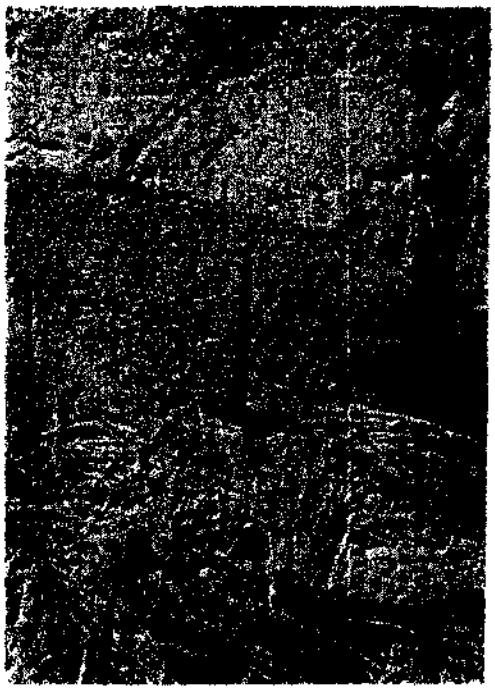


FIG. 2.– Pure *Salvinia* pond showing thick growth of the weed, forming an impervious mat. (Experimental Group C).

PLATE II.



Examination of an experimental pond for eggs and larvae of *Mansonioides annulifera*. Note coconut husk pack in the fore-ground.

A slight variation of the above experiment was also tried by offering *Pistia* and *Salvinia* in the same vessel for oviposition.

(b) *Attachment preferences of larvae.*—To observe larval attachment on *Salvinia* and *Pistia* the two plants were placed together in a large dish containing pond water so that the roots of *Pistia* and the root-like processes of *Salvinia* intertwined. A known number of full-grown larvae, collected afresh from a *Pistia* pond, were then introduced into the dish. After 24 hours the number of larvae found attached to each plant was recorded.

(iii) RECONNAISSANCE OF *MANSONIOIDES* BREEDING ON *SALVINIA*

In addition to the experimental observations, a general reconnaissance of *Mansonioides* breeding was also made during July, 1963, in two localities near Sherthallai Town, namely Thannirmukkam and Thiruvazha. Thannirmukkam borders on the backwaters and is crisscrossed by several channels, drains and canals communicating with the backwaters. Most of these had heavy growths of *Salvinia*. The isolated ponds and tanks in the area were also over-grown with the fern. *Pistia* had given place to *Salvinia* in most of the area and nowhere was it seen unless in association with *Salvinia*. In Thiruvazha, which is situated some 2 miles west of the backwaters, the water collections consisted mainly of isolated ponds and tanks. *Salvinia* was present in some of the ponds together with *Pistia*. The reconnaissance was mainly of water collections in which *Salvinia*, alone, or together with *Pistia*, grew in the presence of retting cocoanut husks, since a preliminary examination of several *Salvinia*-infested situations, without retting cocoanut husks, did not show any breeding.

RESULTS

(i) OVIPOSITION PREFERENCES OF *M. ANNULIFERA*

(a) *Field observations.*—Table I shows the monthly frequency of finding egg masses in the three groups of experimental ponds, respectively. The total number of observations in respect of a group of ponds is the sum total of the number of days on which the respective group of ponds in that series was examined. Table I also shows the total number of egg masses found during the observations, during each month. For Group B ponds (*Pistia* + *Salvinia*) separate data regarding the finding of eggs on the two plants were not recorded during the first two months, February and March.

It is interesting to note that during the first two months the number of positive observations in respect of A and C groups of ponds was quite low. By April, the number had considerably increased and then remained high for the rest of the observation period. This finding is explained by the fact that, as the process of retting of the cocoanut husks progresses, the organic pollution of the water in the ponds also increases, making them more and more attractive to the gravid females for oviposition.

TABLE I.

Comparative frequency of oviposition of M. annulifera *on* Pistia *and* Salvinia (*Field experiments*).

Month.	GROUP A (*Pistia* ONLY) :			GROUP B (*Pistia* AND *Salvinia*) :					GROUP C (*Salvinia* ONLY) :		
				Pistia.		Salvinia.					
	Total number of observations made.	Number of positive observations.	Total number of egg masses seen.	Total number of observations made.	Number of positive observations.	Total number of egg masses seen.	Number of positive observations.	Total number of egg masses seen.	Total number of observations made.	Number of positive observations.	Total number of egg masses seen.
February ...	75	5 (6·7%)	8	Separate data for *Pistia* and *Salvinia* not available.					65	4 (6·2%)	14
March ...	63	11 (17·5%)	24	—do—					59	13 (22·0%)	21
April ...	58	51 (87·9%)	102	60	35 (58·3%)	41	19 (31·7%)	26	60	17 (28·3%)	26
May ...	45	41 (91·1%)	116	45	34 (75·6%)	64	21 (46·7%)	32	45	19 (42·2%)	24
June ...	55	46 (83·6%)	103	55	35 (63·6%)	55	14 (25·5%)	21	55	12 (21·8%)	14
Total ...	**296**	**154 (52·0%)**	**353**	**160**	**104 (65·0%)**	**160**	**54 (33·8%)**	**79**	**284**	**65 (22·9%)**	**99**

To make a correct quantitative assessment of the oviposition preference of *M. annulifera* for one or the other of the two host plants, it is necessary to take into consideration both the positive observations and the number of egg masses found during the observations in respect of each. The preference can be estimated as the ratio of the total number of egg clusters found on *Pistia* to that found on *Salvinia*. The ratio for the individual months as well as the gross ratio for the entire experimental period is presented in Table II. Since the total number of observations has not been always the same for *Pistia* and *Salvinia*, the number of observations in regard to *Salvinia* was proportionately adjusted for that of *Pistia* and the adjusted number of egg masses for *Salvinia* (shown in Column 6 of Table II) has been used for calculating the ratio.

TABLE II.

Oviposition preference of M. annulifera *between* Pistia *and* Salvinia. *Comparison of Group A (pure* Pistia) *and Group B (pure* Salvinia) *ponds.*

Month	*Pistia*.		*Salvinia*.			Preference Ratio. (3) : (6)
	Total number of observations.	Total number of egg masses.	Total number of observations.	Total number of egg masses.	Adjusted number of egg masses.	
1	2	3	4	5	6	7
February ...	75	8	65	14	16·2	0·50
March ...	63	24	59	21	22·4	1·07
April ...	58	102	60	26	25·1	4·07
May ...	45	116	45	24	24·0	4·83
June ...	55	103	55	14	14·0	7·36
Total ...	296	353	284	99	101·7	3·47

The overall preference ratio is 3·47 : 1 in favour of *Pistia*, which fairly tallies with the preference ratio obtained in the field for April and May (4·07 : 1 and 4·83 : 1) as well as that obtained in the laboratory, namely 4·2 : 1 (*see* Table V). The values for February and March which are very low, and that for June which is very high, may therefore be considered abnormal, and the normal preference ratio, for practical purposes, reckoned to be around 4 : 1. While the low values for February and March are unaccountable, the reason for the abnormally high value for June is explained under the heading 'Discussion'.

Perhaps, the data obtained from ponds in Group B reflect the degree of preference more accurately, since *Pistia* and *Salvinia* were allowed to grow side by side in the same ponds, thus ensuring identity of environmental or other factors which might possibly influence the choice of situations for oviposition by the gravid *annulifera*. The preference ratios for the last three months, for which alone data were recorded, are stated in Table III.

TABLE III.

Oviposition preference of M. annulifera *in Group B ponds.*
(Pistia *and* Salvinia *mixed*).

Month.	*Pistia* :		*Salvinia* :		Preference Ratio (3) ÷ (5)
	Total number of observations.	Number of egg masses.	Total number of observations.	Number of egg masses.	
1	2	3	4	5	6
April ...	60	41	60	26	1·58
May ...	45	04	45	32	2·00
June ...	55	55	55	21	2·62
Total ...	160	160	160	79	2·03

It would appear from Table III that the preference ratio is about 2 : 1 in favour of *Pistia*. The variation in the ratio through the different months is not statistically significant :

$$X^2 = 2{\cdot}01. \quad D.F. = 3. \quad P > 0{\cdot}05.$$

Comparing the preference ratios given in Table III with the corresponding ratios in Table II, it is seen that the degree of preference in favour of *Pistia* is significantly lower when it is associated with *Salvinia* than when the two plants grow separately. Further, to test the correctness of this premise, a statistical comparison of the average numbers of egg masses found per positive observation in respect of the two situations was made. The results are furnished in Table IV.

It can be seen that there is no significant difference in the average number of egg masses per positive observation, between *Pistia* and *Salvinia* when they exist together (compare Columns 10 and 13). On the other hand when they are separate, the average number of egg masses on *Pistia* is about twice that on *Salvinia* (compare Columns 4 and 7). This explains the doubling of the preference ratio from 2 : 1 when the two weeds grow together (Table III), to 4 : 1 when they are separate (Table II).

(b) *Laboratory findings.*—Out of 128 gravid females introduced into the oviposition cage for egg laying, 94 laid. Of the 94 egg masses thus obtained, 76 were found on *Pistia* and 18 on *Salvinia*. As stated earlier, in one series of experiments *Pistia* and *Salvinia* were offered in separate containers, and in another in the same container. In the first series, 16 (80·0 per cent) were on *Pistia* and 4 (20 per cent) on *Salvinia*. In the other series, altogether 74 egg masses were laid of which 60 (81·1 per cent) were on *Pistia* and 14 (18·9 per cent) on *Salvinia*. These data are contained in Table V. The laboratory findings corroborate the preference ratio as obtained in the field observations.

TABLE IV.

Average number of egg masses per positive observation, on Pistia *and* Salvinia.

(Group 'B' Ponds)

Months.	Ponds with *Pistia*.			Ponds with *Salvinia*.			Ponds with *Pistia* + *Salvinia*.					
							Pistia.			Salvinia.		
	Positive observations.	Egg masses.	Average number of egg masses.	Positive observations.	Egg masses.	Average number of egg masses.	Positive observations.	Egg masses.	Average number of egg masses.	Positive observations.	Egg masses	Average number of egg masses.
1	2	3	4	5	6	7	8	9	10	11	12	13
April ...	51	102	2·00	17	26	1·53	35	41	1·17	19	26	1·37
May ...	41	116	2·83	18	24	1·26	34	64	1·88	21	32	1·52
June ...	46	103	2·24	12	14	1·17	35	55	1·57	14	21	1·50

TABLE V.

Oviposition of M. annulifera *on* Pistia *and* Salvinia *in the laboratory.*

Series.	Total number of egg masses obtained. (1)	Number of egg masses laid on:		Preference ratio (2) ÷ (3)
		Pistia. (2)	*Salvinia*. (3)	
(1) *Pistia* and *Salvinia* in separate vessels.	20	16	4	4 : 1
(2) *Pistia* and *Salvinia* in the same vessel.	74	60	14	4·3 : 1
Total ...	94	76	18	4·2 : 1

(ii) THE OCCURRENCE OF LARVAE OF *M. ANNULIFERA* ON *PISTIA* AND *SALVINIA*.

The results of examination of the experimental ponds for larvae are presented in Table VI. Group A (*Pistia* only) invariably showed the highest incidence followed by the Group B (mixed) and Group C ponds (*Salvinia* only), in that order. In Group B, the larvae were seen more frequently on *Pistia* than on *Salvinia*.

Attachment of the larvae of M. annulifera *to* Pistia *and* Salvinia.—In a series of 4 replicate laboratory experiments, with a known number of fourth-instar larvae placed in a white tray containing water and both the plants with their roots intertwined, it was observed after 24 hours that the number of larvae that were attached to *Pistia* was always greater than those attached to *Salvinia* (Table VII). However,

TABLE VI.

Comparative incidence of larvae of M. annulifera *on* Pistia *and* Salvinia (*Field experiment*).

Month.	GROUP A (*Pistia* ONLY) :			GROUP B (*Pistia* + *Salvinia*) :					GROUP C (*Salvinia* ONLY) :		
				Pistia.			Salvinia.				
	Total number of observations made.	Number of positive observations.	Total number of larvae seen.	Total number of observations made.	Number of positive observations.	Total number of larvae seen.	Number of positive observations.	Total number of larvae seen.	Total number of observations made.	Number of positive observations.	Total number of larvae seen.
February ...	75	73 (97·3%)	1,299	Separate data for *Pistia* and *Salvinia* not available.					65	39 (60·0%)	203
March ...	63	60 (95·2%)	1,826	—do—					60	43 (71·7%)	315
April ...	58	58 (100·0%)	3,670	60	51 (85·0%)	621	43 (71·7%)	303	60	37 (61·7%)	348
May ...	45	45 (100·0%)	2,439	45	44 (97·8%)	1,567	43 (95·6%)	779	45	36 (80·0%)	307
June ...	55	53 (96·4%)	3,671	55	47 (85·5%)	1,328	38 (69·1%)	532	55	14 (25·5%)	93
Total ...	296	289 (97·6%)	12,905	160	142 (88·8%)	3,516	124 (77·5%)	1,614	285	169 (59·3%)	1,266

the preference ratio was found to be highly variable. The gross ratio worked out to 3 : 1 in favour of *Pistia.**

TABLE VII.

Attachment of M. annulifera *larvae to* Pistia *and* Salvinia
(*Laboratory observations*).

Replicate serial number.	Number of larvae introduced for attachment.	Number of larvae attached: After one hour. To *Pistia.*	After one hour. To *Salvinia.*	After 24 hours. To *Pistia.*	After 24 hours. To *Salvinia.*
1	48	10 (20·8%)	1 (2·1%)	17 (35·4%)	1 (2·1%)
2	48	13 (27·1%)	Nil (0·0%)	20 (41·7%)	1 (2·1%)
3	50	Not observed.		18 (36·0%)	14 (28·0%)
4	70	Not observed.		41 (58·6%)	16 (22·9%)
Total ...	216			96 (44·4%)	32 (14·8%)

In each experiment, all but one of the several roots of the *Pistia* and one root-like process of the *Salvinia* had been clipped off prior to the introduction of the larvae. There were thus only two strands about 15 cm. long available for attachment for the 24 to 35 larvae used at a time. The fact that a large proportion of the larvae did not attach themselves to either of the two plants at all, was probably because the accommodation available for attachment was insufficient. This possibly accounts for the wide variation in the attachment preference.

(iii) OUTPUT OF *M. ANNULIFERA* ADULTS FROM *PISTIA* AND *SALVINIA.*

Table VIII furnishes the number of newly emerged adults obtained in the floating trap from representative ponds belonging to each of the three experimental groups of ponds.

From ponds in Group A (*Pistia* only) 43 adults were obtained in ten days; from Group B (*Pistia* and *Salvinia*) 25 adults in nine days; and from Group C *Salvinia* only) none at all in eight days. The average output per day per trap works out to 4·2 for Group A, 2·7 for Group B and nil for Group C.

It must be mentioned here that since only one floating trap was available, the collections from the three groups of ponds were not simultaneously made. The trap was used successively, first in Group A, then in Group C and lastly in Group B. The data, therefore, may not be strictly comparable; but seeing that the collections were made during April-May, when breeding was at its peak in all the three groups of ponds, the data are considered significant.

* The possibility of the larvae being preconditioned to *Pistia* cannot be ruled out, since they were obtained from *Pistia* ponds. The purpose of intertwining the roots of *Pistia* and the root-like processes of *Salvinia*, in observing the attachment preferences in the laboratory, was to minimise the possible effect of this factor, by rendering the sensory differentiation of the two weeds more difficult for the larvae. It cannot, however, be averred that any element of bias, that might possibly result from pre-conditioning of the larvae to *Pistia*, has been totally eliminated from the experiment.

TABLE VIII.

Output of M. annulifera *adults from* Pistia *and* Salvinia *(Trap collections).*

GROUP A. *Pistia* only :				GROUP B. *Pistia* and *Salvinia* :				GROUP C. *Salvinia* only :			
Days of collection.	Number of adults collected.			Days of collection.	Number of adults collected.			Days of collection.	Number of adults collected.		
	Male.	Female.	Total.		Male.	Female.	Total.		Male.	Female.	Total.
10	26	17	43	9	15	10	25	8	*Nil.*	*Nil.*	*Nil.*

(*iv*) GENERAL RECONNAISSANCE OF BREEDING OF *M. ANNULIFERA* ON *SALVINIA AURICULATA.*

The results are presented in Table IX. As already stated, it were the situations in which retting cocoanut husks were present that were particularly examined for larvae and eggs, since a careful preliminary examination of a very large number of water collections with *Salvinia* alone did not show any evidence of breeding. Out of 31 water collections with *Salvinia* and retting husks, including several with associated growths of *Pistia* and *Eichornia*, 7 showed presence of egg masses and/or larvae. In all these seven breeding places, *Salvinia* was associated with *Pistia* and retting husks ; and two had *Eichornia* as well as *Pistia*.

TABLE IX.

Mansonioides annulifera *breeding on* Salvinia-*infested water collections. (Results of a general reconnaissance).*

Associated vegetation.	Retting cocoanut husks.	LOCALITY : Thannirmukkom. Ponds. Number examined.	Thannirmukkom. Ponds. Number positive.	Thannirmukkom. Channels. Number examined.	Thannirmukkom. Channels. Number positive.	Thiruvazha. Ponds. Number examined.	Thiruvazha. Ponds. Number positive.	Remarks.
None ...	Present	1	..	6	..	..	.	
Pistia ...	Absent	3	..	1	..	4	..	
Pistia ...	Present	1	1	..	..	5	4	Egg masses found on *Salvinia* in one Pond.
Eichornia ...	Absent	1	..	2	..	..	..	
Eichornia ...	Present	1	..	1	..	..	..	
Eichornia, Pistia ...	Present	2	..	3	2	..	..	Several egg masses on *Pistia* ; larvae on *Pistia, Eichornia.*

DISCUSSION

The present studies, while confirming Burton's (1960) laboratory observation that *M. annulifera* would lay eggs on the leaves of *Salvinia auriculata* and that its larvae can use the root-like processes of the fern for attachment and respiration, demonstrate, on the basis of both laboratory and field observations, that *Salvinia* is not on the whole so favourable to the breeding of *M. annulifera* as *Pistia stratiotes*.

When *Pistia* and *Salvinia* were offered in the same cage for oviposition in the laboratory, the mosquito exhibited a marked preference (4 : 1) for *Pistia*. The field data gave more or less the same preference ratio as was obtained in the laboratory. However, in the field, when the two weeds were mixed, the preference ratio was 2 : 1, as against 4 : 1 when they were in separate ponds. All the same, it is clear that the gravid *M. annulifera* has an instinctive predilection for *Pistia* as shown by the oviposition frequencies for the three groups of experimental ponds. The pure *Pistia* ponds showed the highest frequencies, the mixed ponds came next and the pure *Salvinia* ponds last. But this predilection was more patent when the two weeds grew separately than when they grew together. Perhaps, when they co-exist in large masses as in the ponds of Group B, the mosquitoes are to some extent confused and unable to differentiate them. This possibly accounts for the lower preference ratio for the mixed ponds.

It would appear that the gravid female in her hurry to unburden herself would instinctively do so on the first suitable leaf surface that offers the essential physical conditions required for her to assume the characteristic posture for oviposition (Iyengar, 1938) and for the firm attachment of the eggs. These conditions are (*a*) that a surface (preferably the lower surface) of the leaf must graze the water, and (*b*) this surface must be sufficiently hairy or rough. If the first suitable leaf that she chances upon is that of *Salvinia*, she would oviposit on that. However, the two conditions mentioned above are far more readily available with *Pistia* than with *Salvinia*, and hence the larger proportion of egg masses found on *Pistia*. It is noteworthy that the frequency of oviposition in Group C (*Salvinia*) ponds during June was particularly low. This accounted for the exceptionally high preference ratio (7·36 : 1) in favour of Group A (*Pistia*) ponds, in which the frequency of oviposition continued to be at the same high level during April, May and June. The paucity of egg masses in the *Salvinia* ponds during June should be ascribed to some inhibitory factor which came in the way of oviposition. By June, the growth of *Salvinia* in the ponds had become so thick that it had formed an almost impervious mat over the water surface (Plate I, Fig. 2). This circumstance must have naturally rendered it difficult or impossible for many ovigerous females to gain access to the leaves touching the water, for successful oviposition.

The preference of the larvae for the roots of *Pistia*, rather than to the root-like processes of *Salvinia*, is well reflected by the laboratory findings as well as by

the data obtained from the field experiments in which the two weeds remained mixed (Group B). Burton (1960) found in his laboratory studies that the larvae of *M. annulifera* and *M. uniformis* often had difficulty in getting a firm hold on the root-like processes of *Salvinia*, because the peripheral tissue of these processes, which consists only of one layer of cells, does not have enough bulk into which the terminal hooks of the larval siphon could be sunk deeply. It is, therefore, quite probable that a considerable proportion of larvae would die for want of attachment facilities in ponds with *Salvinia* alone. That this does happen is indicated by the comparatively low larval densities in the pure *Salvinia* ponds (Table VI). If the chances of survival of the larvae on *Pistia* and *Salvinia* were equal, the ratio of larval densities should be about the same as the oviposition ratio in respect of the two weeds, namely 4 : 1 in favour of *Pistia* — assuming that the average number of eggs in a cluster is not affected by the species of host plant on which it is laid and is about the same whether it be *Pistia* or *Salvinia*. Any significant divergence of the larval density ratio from the oviposition ratio, in favour of one of the two weeds, should therefore be attributed to larval mortality in respect of the other. On the basis of this premise, the data presented in Table VI were statistically analysed to determine the relative chances of larval survival in the three situations represented by the three groups of experimental ponds. Excepting the difference in the type of vegetation present in them, the conditions influencing the breeding of *M. annulifera* in the different ponds were about the same. This being so, the essential factors which would determine the larval population are :—(*i*) the continuous addition of newly hatched larvae, (*ii*) the continuous decrease of larvae on account of the population of mature larvae, and (*iii*) the mortality of larvae. Taking these factors into consideration it can be proved that the ratio of the numbers of larvae found on the *Pistia* and *Salvinia* ponds respectively is equal to the product of the ratio of the withdrawal rate in *Salvinia* to the withdrawal rate in *Pistia* and the oviposition preference ratio. The theoretical aspects of this formula are explained by one of the present authors (S. Raman) in Appendix I. The formula can be expressed symbolically as follows :—

$$\frac{N}{N'} = \frac{p'}{p}\,\lambda$$

where N = Total number of larvae seen in Group A (*Pistia*) ponds.

N' = Total number of larvae seen in Group C (*Salvinia*) ponds.

p = Rate of withdrawal (probability of withdrawal) from Group A (*Pistia*) ponds.

p' = Rate of withdrawal (probability of withdrawal) from Group C (*Salvinia*) ponds.

λ = Oviposition preference ratio.

The calculation of the value of p'/p for the two groups of ponds is shown in Table X. Since the number of observations was different in some months the

values for the number of larvae have been proportionately adjusted in Column 6. The value of λ is 4 (*vide supra*)*.

A similar calculation for Group B ponds, in which both *Pistia* and *Salvinia* were present, is shown in Table XI. Here the value of λ is 2 (*vide supra*).

The gross ratio of the withdrawal rates is found to be 2·5 : 1 when the two weeds grow separately (Table IX).

The value for the month of June in Table X is exceptionally high (9·9), which indicates a great drop in the larval population in *Salvinia* ponds during that month. This finding is, however, quite compatible with the observation that the number of egg masses recorded for the *Salvinia* ponds was also very low for June. The fall in the frequency of oviposition, the reason for which has already been explained (*vide supra*), must naturally bring about a proportionate reduction in the larval population. On the other hand, since the frequency of oviposition continued to be, more or less, the same in the *Pistia* ponds during the latter months, there was no drop in the larval population in them on this account. Omitting the data for June, the ratio of withdrawal rates works out to 1·9 : 1. The withdrawal rate in *Salvinia* may thus be taken to be twice that in *Pistia*.

When *Pistia* and *Salvinia* grew together, the withdrawal rates of larval populations from the two weeds were almost equal, the gross ratio being about 1·1 : 1. The ratios for the individual months were not significanly different (Table X).

TABLE X.

Larval withdrawal ratio (*pure* Pistia *and pure* Salvinia *ponds*).

Month.	*Pistia* :		*Salvinia* :		Adjusted number of larvae.	*p'/p.
	Number of observations.	Number of larvae.	Number of observations.	Number of larvae.		
1	2	3	4	5	6	7
February ...	75	1,299	65	203	234	1·4
March ...	63	1,826	60	315	331	1·4
April ...	58	3,670	60	348	336	2·8
May ...	45	2,439	45	307	307	2·0
June ...	55	3,671	55	93	93	9·9
Total ...	296	12,905	285	1,266	1,301	2·5

N.B. * p'/p = Column (3) ÷ 4 × Column (6). $\lambda = 4$.

Assuming that larval mortality rate is directly proportional to the withdrawal rate, the above analysis indicates that the larval mortality on *Salvinia* is twice that

* The value of λ for the individual months, on the basis of the number of egg masses seen during each observation, was not calculated because the overall value for the entire observation period, together with the value obtained in the laboratory, was deemed sufficiently accurate for the general conclusions drawn. Further, as proved in Appendix I, the larger the number of observations the more precise the value of λ ($\varepsilon \to 0$, for large values of n).

on *Pistia* when the two plants grow separately. But when they grow together, the chances of larval mortality apparently are fairly equal. This observation, however, involves a fallacy because of the fact that in the mixed ponds the larvae, which have hatched out of the eggs laid on one of the two plants, can always stray on to the other and thus vitiate the actual withdrawal ratio.

TABLE XI.

Larval withdrawal ratio in Pistia *and* Salvinia *ponds (Group B).*

Month.	Number of observations.	Number of larvae on *Pistia.*	Number of larvae on *Salvinia.*	*p'/p.
1	2	3	4	5
April ...	60	621	303	1·0
May ...	45	1,567	779	1·0
June ...	55	1,328	532	1·25
Total ..	160	3,516	1,614	1·1

N.B. * p'/p = Column (3) ÷ 2 × Column (6). $\lambda = 2$.

The degree of hospitability of a host plant to *Mansonioides* must ultimately be measured by the output of adult mosquitoes from it. In the present studies, a limited attempt to guage this output in respect of the three groups of experimental ponds was made by using a floating mosquito trap, constructed for the purpose. Whereas the experimental groups of ponds A and B yielded emergent adults almost every day, Group C yielded none at all for 8 consecutive days, even though breeding was going on in the ponds of this Group as evidenced by the finding of eggs and larvae. This apparent paradox points to the presence of some inhibitory factor which prevented the successful emergence and escape of the imagoes. This is presumably to be found in the impervious matty growth of *Salvinia*, to which reference has already been made. In comparatively small collections of water, such as ponds, wherein the surface area is limited the horizontal expansion of the matty growth is contained, but since the vegetative activity of the plant continues unabated the mat tends to get thick, so thick sometimes that water birds could be seen to walk over it. This kind of dense growth would impede the emergence and escape of the imagoes. Probably with large expanses of water, like lakes and large tanks, this may not happen.

The results of field reconnaissance of infested areas have indicated that *Salvinia* by itself seldom promotes breeding of *M. annulifera*. Breeding was found mainly in situations where *Salvinia* was associated with *Pistia* and retting cocoanut husks.

CONCLUSION

The results of this study indicate that *Salvinia auriculata* is not a favourable host plant for *M. annulifera*, the main vector of *Brugia malayi*, in Kerala. The

fewer egg masses laid on *Salvinia*, the higher larval mortality and the diminished output of adults due to impervious growth of the fern in ponds, indicate that it is an unfavourable host plant for *M. annulifera*. The recent massive invasion of considerable areas of the coastal tract of Kerala by this weed is not likely to worsen the vector position. On the other hand, the indications are that as *Pistia stratiotes* has given place to *Salvinia auriculata* in many places, the incidence of *M. annulifera* in such areas has dropped.

SUMMARY

1. Using certain standardized methods of field observations, supplemented by laboratory experiments, the hospitability of *Salvinia auriculata* (a recently introduced pestiferous weed in Kerala) to *Mansonioides annulifera* was studied in comparison with that of *Pistia stratiotes*, its normal host plant.

2. It was found in the laboratory as well as field experiments that *M. annulifera* would oviposit on *Salvinia* even if *Pistia* be presented alongside. But the preference was always for *Pistia*. The preference ratio was 4 : 1 when they grew together.

3. In the laboratory, when *Pistia* and *Salvinia* were offered together for larval attachment, nearly 70 per cent of the larvae were found attached to *Pistia*.

4. The choice between *Pistia* and *Salvinia* by the gravid female of *M. annulifera* for oviposition, and by the larvae of the mosquito for attachment, is apparently a tentative process of trial and error. The adult mosquito and the larva do not seem to be capable of on-the-spot recognition of the more favourable host plant.

5. A statistical analysis of the data obtained from the field experiments has indicated a higher (about twice) larval mortality in pure *Salvinia* ponds as compared to pure *Pistia* ponds.

6. A limited series of observations on the output of adult *M. annulifera* from the experimental breeding places, by using a floating cage, showed that a very dense growth of *Salvinia* constitutes a physical barrier to the emergence and free escape of the adults. None of the pure *Salvinia* ponds yielded any adults for 8 consecutive days of collection whereas the ponds with *Pistia* and *Salvinia* growing together as well as those with pure *Pistia*, showed an average output of 2·5 and 4·2 adults per day per trap respectively.

7. A general reconnaissance of some *Salvinia*-infested areas showed breeding of *Mansonioides* only in a few ponds where this weed was associated with considerable growth of *Pistia* and retting cocoanut husks.

8. It is concluded that *Salvinia auriculata* is not a favourable host plant for *M. annulifera*.

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APPENDIX

BY

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Let x_i denote the number of fresh larvae added to the *Pistia* ponds, during the ith observation. Let p denote the average withdrawal rate of larvae (which includes both mortality and conversion to the pupa stage). The number of larvae during the different observations can be expressed, as in the following table, where $q = 1 - p$ which can be assumed to be small.

Serial number of observation	*Number of larvae*
1	x_1
2	$x_1q + x_2$
3	$x_1 q^2 + x_2q + x_3$

The total number of larvae in n observations

$$= x_1 + (x_1q + x_2) + (x_1q^2 + x_2q + x_3) + \ldots + (x_1q^{n-1} + \ldots + x_{n-1}\, q + x_n)$$

$$= x_1 (1 + q + \ldots + q^{n-1}) + x_2 (1 + q + \ldots + q^{n-2}) + \ldots\ldots\ldots + x_n$$

$$= \frac{1}{1-q} \{x_1 (1 - q^n) + x_2 (1 - q^{n-1}) + \ldots\ldots + x_n (1 - q)\}$$

$$= \frac{1}{1-q} \{(x_1 + x_2 + \ldots x_n) - (x_1q^n + x_2q^{n-1} + \ldots + x_nq)\}$$

$$= \frac{(x_1 + x_2 + \ldots + x_n)}{1-q} \left\{ 1 - \frac{(x_1q^n + x_2q^{n-1} + \ldots + x_nq)}{x_1 + x_2 + \ldots\ldots + x_n} \right\}$$

$$= \frac{\Sigma x_1}{1-q} (1 - \varepsilon) \text{ where } \varepsilon \to 0 \text{ for sufficiently large value of } n.$$

$$= \frac{\Sigma x_1}{1-q} \text{ for large } n = N, \text{ say.}$$

Similarly, total number of larvae in *Salvinia* ponds

$$= \frac{\Sigma x'_1}{1-q'} \text{ for large } n$$

$$= N', \text{ say}$$

where x'_1 is the number of fresh larvae found in *Salvinia* ponds and p' is the average withdrawal rate of larvae and $q' = 1 - p'$.

$$\frac{N}{N'} = \frac{1-q'}{1-q} \quad \frac{\Sigma x}{\Sigma x'_1} = \frac{p'}{p} \quad \frac{\Sigma x_1}{\Sigma x'_1}$$

But $\frac{\Sigma x_1}{\Sigma x'_1}$ is equal to the ratio of egg clusters or the oviposition preference ratio of the mosquito, assuming that the number of larvae is directly proportional to the number of egg clusters.*

$\therefore \frac{N}{N'} = \frac{p'}{p} \lambda$ where λ is the oviposition preference ratio of the mosquito.

* There was no reason to suppose that there could be any considerable mortality among eggs whether they were laid on *Pistia* or *Salvinia*. In any case the factors, that might cause mortality, could not have been different among the experimental ponds because the ecological factors (barring the vegetation present in them) were quite similar in all. It would, therefore, be safe to expect that the larval population in a pond will be directly related to the number of egg masses, once the ecological equilibrium conducive to active breeding has been established, and oviposition and hatching out of larvae have become a matter of regular day-to-day occurrence. (See also 'Discussion', page 324)

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STUDY ON THE USE OF CHYMOTRYPSIN IN CHRONIC FILARIAL LYMPHOEDEMA

BY

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AND

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[December 22, 1963.]

MANY drugs have been used from time to time in the treatment of chronic lymphoedema, but so far no single drug has been completely successful. To mention a few, diethylcarbamazine, sulphonamides, antibiotics, auto-vaccines, florocid (sodium fluoride), filocid (extract of vitex peduncolaries), thiazide diuretics, T.A.B. vaccine, steroid hormones, etc., have been tried by various authors, with only partial or no success (Jordan, 1960 ; Subramanian, 1953, 1961 ; Subramanian and Ranganathan, 1960 ; Subramanian and Sadasivam, 1959).

In fact, surgery offers the only certain way of reducing the swelling and in an established case, the operation is often formidable. Even after surgery, the cosmetic results are not fully satisfactory.

Filarial oedema is a major problem in Kerala, all along the coastal belt. Trivandrum is endemic for bancroftian filariasis and the incidence of elephantoid lesions of the limbs and scrotum is high. The study presented in this paper was undertaken with a view to determine whether proteolytic enzymes had any significant part to play in the treatment of filarial lymphoedema.

Chymotrypsin is the major component of the commercial preparation obtained from beef pancreas, processed by the Kuntz and Northop method. It has esterase and amidase activity, with an optimum pH range of 7 to 9. Chymotrypsin acts specifically on the internal peptide linkages, containing an aromatic amino acid nucleus. It acts upon the substrate from the carboxyl side of the protein bond (Movain, 1960).

Chymotrypsin has got anti-inflammatory activity. In the guinea-pig this effect is at least twice as great as that of Trypsin and is greater than the anti-inflammatory effects of cortisone acetate and salicylates (Davis *et al.*, 1959). It is also fibrinolytic (Stormorlein, 1956). Proteolytic enzymes help to reverse inflammation, resorb oedema and liquefy or localise suppurative exudate, by this biochemical

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action (Meulelm, 1938). Chymotrypsin has been used successfully in asthma and bronchitis, athletic injuries, plastic surgery, thrombophlebitis, and traumatic conditions. Its use is being extended to other conditions as well.

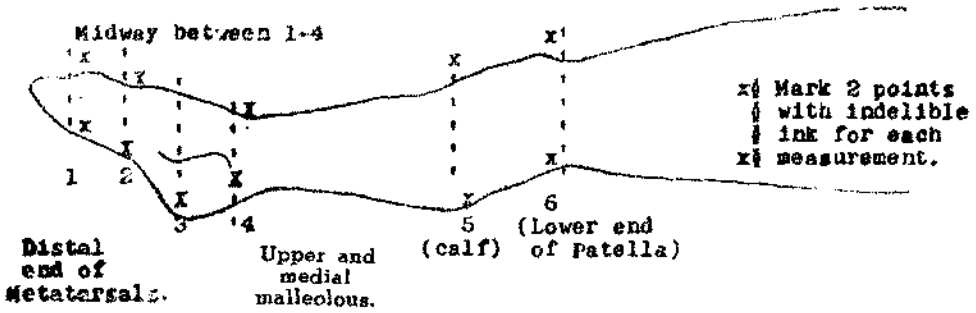
W.E. Adams reports favourably on the use of Chymotrypsin in filarial oedema. He got good to fair response in sixty-three out of seventy-eight patients employed in a controlled trial. Subjective improvement, like reduction of joint stiffness and swelling and feeling of well-being, was also noticed.

MATERIALS AND METHODS

Patients who had established oedema for one year or more were included in this study. They were examined clinically and adenitis, filarial fever, hydrocoele and elphantiasis of scrotum or other parts were taken as additional evidence of filariasis. Blood was repeatedly examined for microfilaria. Total and differential leucocyte counts and liver function tests were done before commencement of the trial and at its completion to find out whether there was any variation. Cases were selected from the patients attending the Medical Out-patients Department of the Medical College Hospital, Trivandrum. Three cases were positive for microfilaria in night-blood. Others were negative. Cases were taken for the trial after a course of standard anti-filarial treatment. They were given ten daily injections of Procaine penicillin and Diethyl-carbamazine 4 mg. per kg. body-weight per day for 21 days. This was supplemented with elastic crepe bandage to the affected limb and elevation in bed. Injections of Chymotrypsin were started ten days after this course of treatment. Seven cases were followed-up for periods of over three months. Three patients stopped visiting the hospital during the trial and so only the former have been included in the final analysis.

PLAN OF STUDY

The cases were observed for three days with daily measurements of the affected limb at various levels as indicated in the following diagram.



The same observer took the measurements on all days under identical conditions. Six sites were measured in each limb and the circumference at each point

TABLE I.

Measurement of lymphoedema of lower limbs.

Case number.	Age, in years.	Sex.	Duration of oedema, in years.	Microfilaria.	Measurement in cm.						Measurement on the 30th day of starting trial.						Measurement on the 90th day of starting trial.					
					1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
I	11	M	3	+	20·6	22·0	29·2	21·5	23·4	25·2	19·9	22·0	30·1	22	25·0	25·7	19·8	21·2	29·8	23·1	25·7	26·4
II	21	F	1	+	20·4	21·1	28·9	21·6	30·5	34·9	19·4	21·1	27·4	20·1	30·9	35·1	19·4	20·7	27·3	20·3	30·8	35·1
III	38	F	Left leg 2	—	19·0	20·0	27·2	20·6	33·5	36·8	19·2	20·4	27·2	21·2	33·8	37·9	19·0	20·1	27·1	20·4	33·5	37·8
			Right leg 2	—	22·0	23·0	32·2	26·7	37·1	38·0	20·6	22·4	30·2	25·6	36·8	38·9	20·3	21·2	30·0	25·5	36·7	38·4
IV	37	M	1	+	22·5	24·6	33·0	23·4	36·0	38·9	22·4	24·2	32·8	22·9	36·4	38·0	22·7	24·0	32·7	23·8	36·0	38·0
V	62	F	3	—	21·0	22·5	30·2	22·4	25·3	27·7	21·1	22·4	30·1	23·8	26·4	28·8	21·2	21·6	30·1	23·2	25·8	27·9
VI	33	F	6	—	19·5	22·7	30·2	24·6	28·9	29·5	19·4	21·4	29·4	23·9	28·4	29·5	19·6	20·7	28·7	22·7	28·7	30·1
VII	48	M	13	—	30·1	33·0	39·2	31·4	44·0	37·7	29·7	33·4	41·1	33·3	46·2	40·3	29·6	33·3	41·0	33·3	46·2	40·0

M = male ; F = female.

was noted. From the fourth day onwards, Chymotrypsin was given in a dose of 2 ml. (10,000 units) as deep IM injection daily for six days. Thereafter 2 ml. were given by the same route every alternate day for one month (15 injections). The measurements were taken daily and tabulated. No other form of therapy was given simultaneously. Table I shows the data of the measurements.

After the completion of this course of injections, the patients were observed at weekly intervals for periods exceeding three months and only the cases who persisted throughout this period have been included in the final analysis.

STATISTICAL REPORT OF THE TRIAL OF TRYPSIN IN FILARIAL EDEMA.

The observations for 7 patients have been analysed. Since one of the patients had edema on both the legs, he has been considered as two patients. Hence in toto there are 8 patients for whom the observation on six sites are available for a period of six months. Since the measurements did not show any material change after the first month, the analysis has been made for six periods spaced at an interval of six days. The "Analysis of Variance" technique has been used to separate the different variance components.

TABLE II.

Aneva.

Source of variation.	D.F.	S. S.	M. S.	Ratio.
Patient ...	7	4,453·82	636·26	...
Site ...	5	6,967·91	1,381·58	...
Period ...	5	2·05	0·41	...
Site × Patient ...	35	1,434·42	40·98	...
Site × Period ...	25	17·44	0·69	6·3
Patient × Period ...	35	44·42	1·30	11·8
Site × Patient × Period (Error) ...	175	19·00	0·11	...
Total ...	287	12,879·06	...	...

There is obviously tremendous variation among the different patients and among the different sites. We are not interested in the magnitude of these variations. The site-period interaction and period-patient interaction are highly significant ($P < .001$). This signifies that the different sites react differently during different periods. Similarly different patients react differently during different periods. Hence there is no consistency of response of the different sites and patients. The mean square for the period is less than either of the site-period and patient-period interaction. Hence it appears that there is no significant effect of the period over the magnitude of the edema. Since the effect of the period is the same as the influence of the drug, we conclude that the drug does not produce any significant reduction in the edema.

It was decided to see whether the drug produced any linear trend in the size of the edema. The regression analysis is shown in Table III.

TABLE III.

Regression analysis.

Source of variation.	D.F.	S.S.	M.S.	Ratio.
Period ...	(5)	(2·05)	(0·41)	
Linear regression ...	1	0·32	0·32	..
Deviation ...	4	1·73	0·43	..
Site × Period ...	(25)	(17·44)	(0·69)	..
Site × Period Linear	5	3·96	0·79	7·2
Deviation ...	20	13·48	0·67	..
Error ...	175	19·00	0·11	..

The interaction of site with the linear component of period is highly significant. This means that the linear component varies from site to site. The mean square for the linear component of period is less than the site-period (linear) interaction. This denotes the absence of any significant linear trend in the size of the edema.

DISCUSSION

The clinical effects of Chymotrypsin in helping to resorb filarial oedema have been studied in seven patients as a pilot study. The prior administration of a course of standard antifilarial treatment, before starting Chymotrypsin, enabled us to judge whether the latter had any advantage over conventional therapy. All cases tolerated the injections well. Case VII developed urticaria after the sixth injection. Skin testing with Chymotrypsin solution and the diluent separately showed that the diluent was at fault.

All cases observed loosening of the oedema and slight reduction in the size of the swelling by about the third week of starting treatment. Soon, this effect waned and the limbs regained their pre-treatment dimensions. There was no evidence of toxicity in any patient.

SUMMARY

Pilot-study of the effect of systematic administration of Chymotrypsin in filarial lymphoedema failed to show any consistent benefit.

There is high variability in the size of the edema among the different patients and at different sites.

The effect of the drug in reducing the edema is not statistically significant.

The different sites show differential response with the drug. There is no uniform linear trend in the differential response of the site.

There is no statistically significant trend in the variation of the size of the edema with the administration of the drug. Also there is no significant curvature of response to the drug.

ACKNOWLEDGEMENTS

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REVERSION OF DIELDRIN-RESISTANCE IN THE FIELD POPULATION OF *A. CULICIFACIES* IN MAHARASHTRA STATE (ERSTWHILE BOMBAY STATE), INDIA.

BY

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[December 22, 1963.]

PATEL *et al.* (1958) first detected appearance of fairly high resistance (about 60 fold) to dieldrin in *Anopheles culicifacies* in villages of Murbad Taluka, Thana District, Bombay State (now Maharashtra State) in the month of October, 1958, after three rounds of dieldrin spraying in that area. This resistance was accompanied with recrudescence of malaria, affecting about 60 per cent population of about 209 villages in 500 sq. miles (86,000 population), as reported by Patel *et al.* (1961). Dieldrin spraying was consequently withdrawn from the area and instead spraying with DDT was resorted to. A closely situated group of villages, *viz.* Potgaon, Nandni, Mohop, Asola and Wagholi, from about mid of the affected area mentioned above, were kept under continuous observations for periodical measurement of dieldrin-resistance after the withdrawal of dieldrin pressure. After the withdrawal of dieldrin pressure from the area, a partial reversion of resistance to dieldrin in *A. culicifacies* was noticed in a course of about a year and was reported by Rao *et al.* (1960). Dieldrin pressure was again applied in these observation villages by spraying these with two rounds of dieldrin at 25 mg. per sq. foot (0·25 gm. per sq. metre) once in August, 1959, and second time in December, 1959. As a result of these sprayings, the resistance of *A. culicifacies* to dieldrin was found again to shoot up to about 360-fold in March, 1960, and 760-fold in May 1960. Again partial reversion occurred in about a year's time when dieldrin pressure was withdrawn. A lapse of further two years, without dieldrin pressure, effected a complete reversal of dieldrin-resistance irrespective of the fact that DDT spraying was being continued in the area. Hence a complete reversal of dieldrin-resistance in the course of about three years time has been observed after withdrawal of dieldrin pressure in the field population of *A. culicifacies* and the results of these findings are reported in this paper. The susceptibility tests with dieldrin in these studies were carried out using the World Health Organization technique and Kit and with the impregnated papers supplied by them.

RESULTS

The results of periodical susceptibility tests, carried out with dieldrin against fully engorged wild-caught females of *A. culicifacies* of Potgaon village (one of the

TABLE

Dosage-mortality data of wild-caught females of A. culicifacies *with dieldrin,*

Exposure period—One hour

Serial number.	Period of test.	DIELDRIN DOSAGES TRIED AND THE ADJUSTED KILL PERCENTAGE :													
		Control		0·05		0·1		0·2		0·4		0·8		1·6	
		D/T	Per cent kill.	D/T	Per cent kill.	D/T	Per cent kill.	D/T	Per cent kill.	D/T	Per cent kill.	D/T	Per cent kill.	D/T	Per cent kill.
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	Oct. 12-15, 1958	1/88	1·4	2/65	3·5	8/66	12·6	18/82	22·3	25/82	30·9	27/103	26·4	37/94	39·7
2	Dec. 23-24, 1959	0/45	0·0	..	..	28/40	70·0	15/22	68·2	31/40	78·0	33/40	82·5	38/40	95·0
3	Mar. 8-13, 1960	0/49	0·0	..	..	..	..	..	..	2/16	12·5	1/18	5·6	8/41	19·5
4	May 2-6, 1960	0/105	0·0	..	..	..	..	5/120	4·2	12/120	10·0	13/108	12·3	15/100	15·0
5	Jun. 20-24, 1960	0/100	0·0	..	..	..	..	3/90	3·3	8/100	8·0	14/100	14·0	27/100	27·0
6	Jun. 28 to Jul. 2, 1960	0/97	0·0	..	..	..	..	9/102	8·8	12/102	11·7	17/104	16·3	46/145	31·7
7	Mar. 28-31, 1961	1/36	2·8	1/10	7·4	7/22	29·7	24/30	79·4	18/21	85·1	..	..	..	..
8	Jul. 20-22, 1961	1/22	4·5	..	..	11/23	45·3	7/10	68·6	24/24	100·0	..	..	..	..
9	Dec. 12-17, 1961	0/32	0·0	13/35	37·1	17/35	48·6	31/35	88·6	36/36	100·0	..	..	45/45	100·0
10	Apr. 10-14, 1963	2/50	4·0	17/35	46·5	32/40	79·1	60/60	100·0	..	..	..	..	..	..
11	Jun. 14-17, 1963	3/60	5·0	32/80	36·8	66/80	81·6	78/80	97·3	80/80	100·0	60/60	100·0	..	..

D = Number of mosquitoes found dead. T = Number of mosquitoes treated.

I.

of Potgaon Village of Murbad Taluka, Thana District, Maharashtra State.

Mortality observations taken 24 hours after exposure.

Log (dosage X 100) probit kill regression equation.	X^2 (degree of freedom).	Hetero-geneity.	LC_{50} Dield-rin per cent.	LC_{90} Dieldrin per cent.	Remarks.
17	18	19	20	21	22
Y=0·74x+3·1143	$X^2(4)=8·071$	N.H.	3·5	190·1	Resistance to Dieldrin found after 3 rounds of Dieldrin spraying.
Y=0·74364x+4·6610	$X^2(3)=2·1233$	N.H.	0·03	1·5	Partial reversion after 14 months. Even 1·6 per cent has not given 100 per cent kill.
Y=0·70626x+2·3937	$X^2(1)=1·0024$	N.H.	18·7	586·1	High resistance after re-spraying with Dieldrin twice, in Aug. 1959, and Jan. 1960.
Y=0·70686x+3·4061	$X^2(2)=5·7314$	N.H.	38·4	2477·0	High degree of resistance continues.
Y=1·4019x+1·2964	$X^2(2)=0·224$	N.H.	4·4	36·14	Reversion, perceptible 5 months after last round of spraying in January, 1960.
Y=0·9466x+2·3479	$X^2(2)=2·2491$	N.H.	6·3	142·6	
Y=2·3003x+3·2825	$X^2(3)=3·789$	N.H.	0·14	0·47	Fast reversion in the further 8 months, although DDT spraying substituted.
Y=2·9591x+1·8034	$X^2(1)=0·4191$	N.H.	0·11	0·304	Slow further reversion continues.
Y=2·8165x+2·5020	$X^2(3)=4·1247$	N.H.	0·08	0·22	Slow further reversion continues.
Y=3·7439x+2·2095	$X^2(1)=1·816$	N.H.	0·055	0·1222	Almost complete reversion of Dieldrin resistance to original level noticed after 3 years of withdrawal of Dieldrin spraying.
Y=4·0222x+1·8438	$X^2(3)=2·8214$	N.H.	0·069	0·1107	

N.H. = Not heterogeneous.

villages of a group of five, kept under observation for the purpose of measurement of susceptibility of *A. culicifacies* to dieldrin) of Murbad Taluka, District Thana, are shown in Table I, along with probit-analysis and the LC_{50} and LC_{90} values and the regression equation and Chi^2 values. As the Thana District (including Potgaon village) has been continuously under DDT spray from 1948 to 1956 inclusive, and then under dieldrin spraying during 1957 (2 rounds) and June, 1958 (3rd round), the normal susceptibility level of *A. culicifacies* of this area, prior to treatment, could not be determined. Hence the susceptibility levels of a normal strain of *A. culicifacies* from unsprayed areas of Maharashtra State (from 2 places) have been shown in Table II (A) and (B) for the purpose of comparison. The dosage mortality lines, obtained by actually connecting the observation points, are depicted in Chart I. This is done in order to present the normal and actual course of each line for clarity purpose. However, the log dosage probit kill regression graphs are also presented separately in Chart 2. Also LC_{50} and LC_{90} values, obtained at various intervals, are presented in Chart 3.

The Dieldrin-resistance in *A. culicifacies* of Thana District was first detected in October, 1958, by Patel *et al.* (1958 *loc. cit.*). Data, pertaining to the month of October 1958, reproduced from these authors' work, are shown in Table I. They had found LC_{50} value of 3·1 per cent by extra-polation, by the Litchfield and Wilcoxon method. For sake of uniformity, we have worked out the LC_{50} value of all data by probit-analysis (Finney, 1952), and the LC_{50} value of 3·5335 per cent by this method is nearly the same as that obtained by the Litchfield and Wilcoxon method. The LC_{50} value of a normal strain of *A. culicifacies* from unsprayed areas was found to be 0·019093 per cent in Chorbavli and 0·0505 per cent in Kada *vide* Table II (A) and (B). To err on the safer side we may, therefore, take 0·05 per cent Dieldrin as the LC_{50} value of a normal strain of *A. culicifacies* for comparison purpose. Hence a resistance level of a magnitude of 70-fold was first detected by Patel *et al.* (1958 *loc. cit.*) as per our calculations in October, 1958, in *A. culicifacies* of Potgaon village of Thana District. This resistance appeared in the strain after only three rounds of Dieldrin spraying, two rounds of which were applied in 1957 and the third round in June, 1958, at the rate of 25 mg. per sq. foot (0·25 gm. per sq. meter). Dieldrin pressure was then withdrawn. Potgaon village was first kept unsprayed after data for October, 1958, was obtained till June, 1959, but fearing the risk of fresh transmission starting again in the village in absence of any insecticide, it was decided to spray DDT at the rate of 1·00 gm. per sq. meter in the transmission season lasting from June to end of October. Hence a round of DDT spray was applied in the month of June, 1959. But in spite of this a partial reversion in Dieldrin-resistance was noticed by Rao *et al.* (1960 *loc. cit.*) in a course of about a year or so. The December 1959 data is from these latter authors. Although the LC_{90} value obtained in October 1959 fell down to that of a normal susceptible strain in about a year's time after withdrawal of Dieldrin pressure, the reversion was not complete as there was still about 25 to 30 per cent resistant population in *A. culicifacies* of the Potgaon area and 100 per cent kill could not be obtained even with

TABLE II.

Dosage-mortality data of wild-caught females of A. culicifacies *from unsprayed areas of Maharashtra State.*

Exposure period—One hour. *Mortality observations taken 24 hours after exposure.*

(*A*)

Serial number.	Village.	Period of test.	Dieldrin dosages tried and adjusted kill percentage: Control. D/T.	Control. Per cent kill.	0·0125 D/T.	0·0125 Per cent kill.	0·025 D/T.	0·025 Per cent kill.	0·05 D/T.	0·05 Per cent kill.	0·1 D/T.	0·1 Per cent kill.	0·2 D/T.	0·2 Per cent kill.	Log (dosage × 100) probit kill regression equation.	χ^2 (degree of freedom).	Heterogenity.	LC_{50} Dieldrin concentration, per cent.	LC_{90} Dieldrin concentration, per cent.	Remarks.
1	Chorbavali (Nagpur)	Aug. 5–6, 1957	0/35	0·0	18/39	46	16/34	47	22/29	76	34/34	100	18/18	100	$Y=2·0243X+4·4314$	$\chi^2(3)=3·809$	N.H.	0·019093	0·082	Unsprayed area

(*B*)

Serial number.	Village.	Period of test.	Dieldrin dosages tried and adjusted kill percentage: Control. D/T	Control. Per cent kill.	0·0078 D/T.	0·0078 Per cent kill.	0·0156 D/T.	0·0156 Per cent kill.	0·0312 D/T.	0·0312 Per cent kill.	0·0623 D/T.	0·0623 Per cent kill.	0·125 D/T.	0·125 Per cent kill.	0·25 D/T.	0·25 Per cent kill.	0·5 D/T.	0·5 Per cent kill.	Log (dosage × 100) probit kill regression equation.	χ^2 (degree of freedom).	Heterogenity.	LC_{50} Dieldrin concentration, per cent.	LC_{90} Dieldrin concentration, per cent.	Remarks.
2	Kada (Bhir)	July 23–26, 1957	0/35	0·0	1/36	3	3/33	9	10/48	21	21/36	58	18/19	94	24/24	100·0	21/21	100·0	$Y=2·943X+0·98696$	$\chi^2(5)=4·183$	N.H.	0·05	0·14	Unsprayed area

D = Number of mosquitoes found dead. T = Number of mosquitoes treated. N.H. = Not heterogeneous.

CHART 1.

Dosage-mortality lines for A. culicifacies, with Dieldrin.

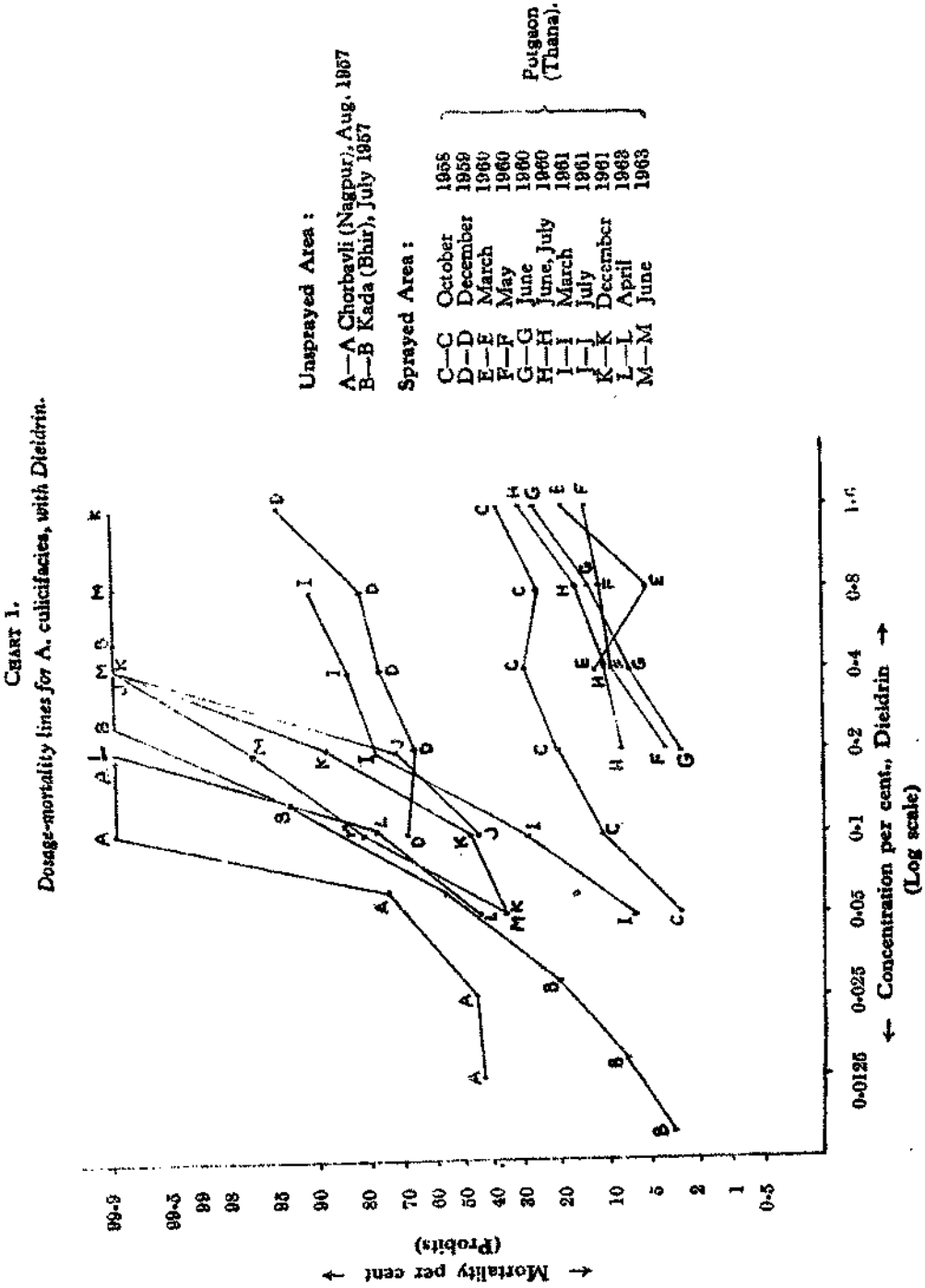


CHART 2.

Log dosage-probit mortality regression lines for A. culicifacies, with Dieldrin.

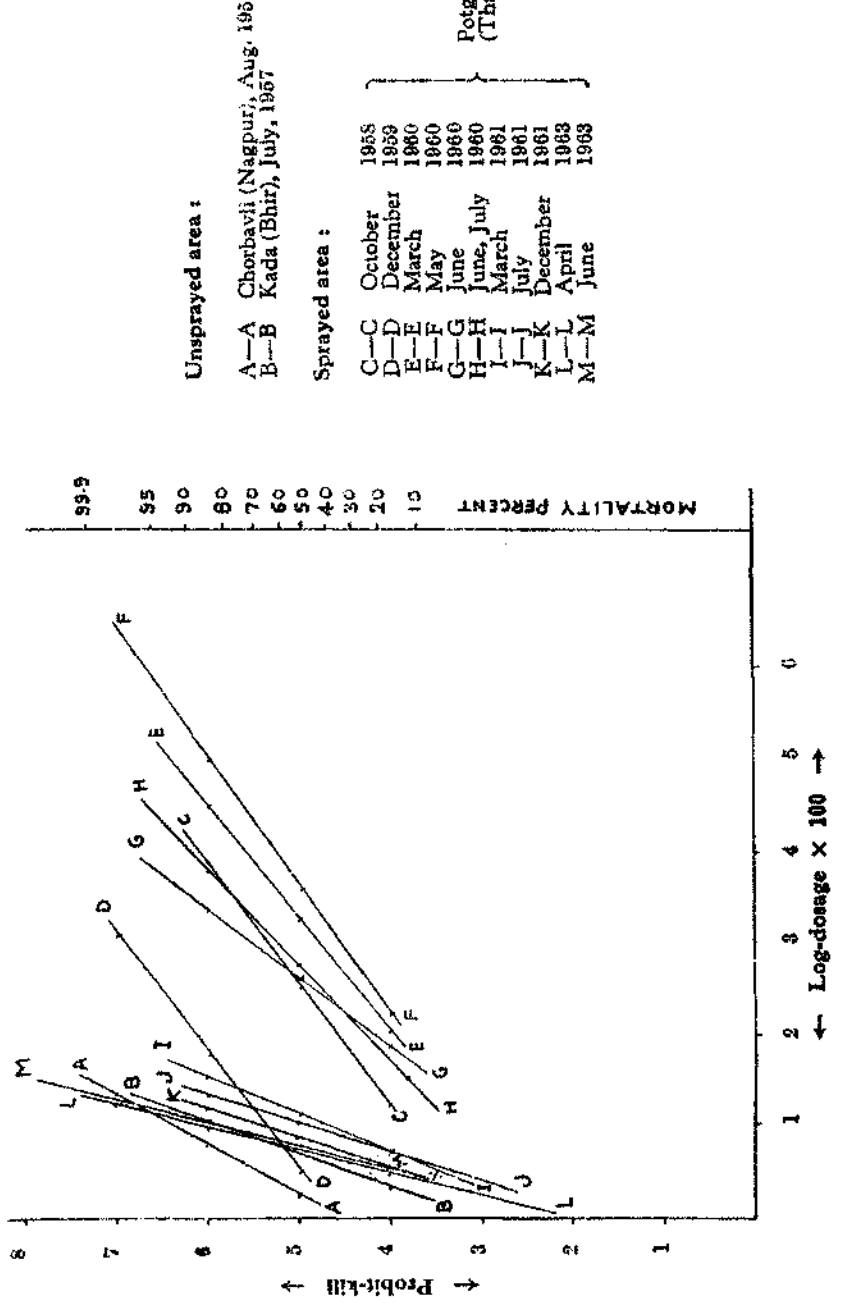
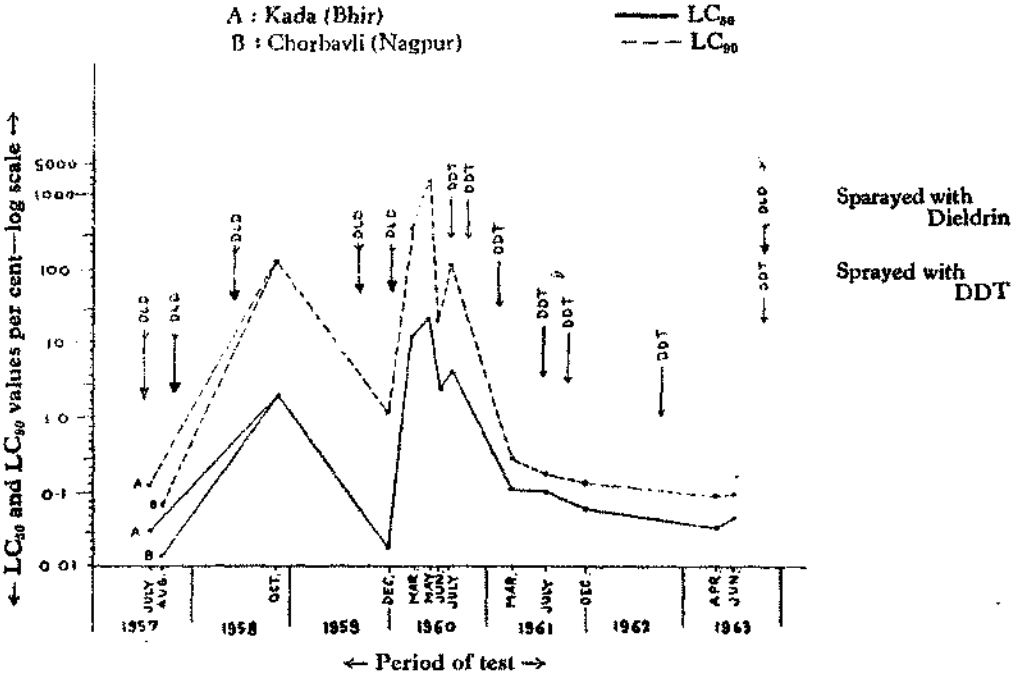


CHART 3.

LC_{50} and LC_{90} values of Dieldrin for A. culicifacies, *from Dieldrin sprayed area, Potgaon of Thana District. figures for the year 1957 are from unsprayed areas.*



1·6 per cent Dieldrin. As the reversion process was going on, it was decided to re-apply the Dieldrin pressure in the group of five villages mentioned above, including Potgaon, to see whether resistance to Dieldrin shoots up again or not. Therefore, one round of Dieldrin spraying was again applied in August 1959 at the rate of 0·25 gm. per sq. metre. This much pressure did not change the course of reversion as the data obtained in October, 1959 and December 1959 by Rao *et al.* (1960 *loc. cit.*) did not show any increase in resistance in these four months following the re-application of Dieldrin pressure. Then second round of Dieldrin was applied at the rate mentioned above in Potgaon and other four villages during January, 1960. This resulted in shooting up of the Dieldrin-resistance in *A. culicifacies* of the area to a very high degree. From Table I, LC_{50} value of *A. culicifacies* in March 1960 (about 2 months after the second round of Dieldrin spray) was calculated arithmetically, using the maximum likelihood method, and was found to be 18·759 per cent, suggesting an increase in resistance level of 360-fold the normal strain and it became 760-fold in the month of May, 1960. As no more Dieldrin-pressure was applied in the area under observation, the reversion process again started appearing and by the end of July 1960, LC_{50} dropped down to 6·3343 per cent (the June 1960 figure of LC_{50} is lower than that of July 1960). But still, these LC_{50} values were as high as about 80 to 120 times the LC_{50} value of the normal susceptible strain. The

reversion process further continued in spite of DDT spraying in the area and in March, 1961, *i.e.*, after about a year, LC_{50} value of 0·13705 per cent was recorded in the same area. Although LC_{50} values had completely reversed, LC_{90} value did not reach the level of normal strain. The rate of reversion slowed down further and it took almost a period of another two years to complete the process of reversion and attain the same susceptibility level as that of the normal susceptible strain. This is shown by the tests performed in July 1961, December 1961 and April 1963. The results of complete reversion in April 1963 were further verified and confirmed in June 1963. Thus it would be seen that a complete reversion of Dieldrin-resistance after the re-application of Dieldrin-pressure in January 1960 had taken three years to reverse to normal susceptibility. It may be again added here that this complete reversion in Dieldrin-resistance has occurred after withdrawal of Dieldrin-pressure in spite of the fact that DDT pressure was continued in this area up to the end of 1963. The LC_{50} and LC_{90} values plotted against the different periods of test also show clearly the process of reversion and the course it had taken. It would be seen that it took about 3 years to achieve complete reversion of Dieldrin-resistance in *A. culicifacies*, although a good deal of reversion did occur in about a year's time. It may be mentioned here that Rao and Bhatia (1957) had recorded MLC of DDT for *culicifacies* of Village Revti, which is about 5 miles from Potgaon, as 0·571 per cent in July 1956 which rose slightly to 0·84 per cent in October 1958 (Patel *et al.*, 1958). Patel *et al.* (1961) further recorded 95 per cent mortality of *culicifacies* of Potgaon Village with 2 per cent DDT in May 1959. *A. culicifacies* of the area continued to be susceptible up to March 1961 when 2 per cent DDT gave 79·1 per cent mortality. However, *culicifacies* of the area moved towards resistance to DDT in April 1963 when 2 per cent DDT gave only 28·5 per cent mortality and 4 per cent DDT gave 42·5 per cent mortality (*Unpublished data*).

DISCUSSIONS

Whereas Davidson (1963) has found the character of Dieldrin-resistance in *A. quadrimaculatus* to be of a semi-dominant nature like that in *A. gambiae*, etc., Rozeboom *et al.* (1961) have found the Dieldrin-resistance character in *A. albimanus* as dominant one. It is very difficult to colonize *A. culicifacies* in the laboratory. All the attempts of the previous workers have so far failed to colonize *A. culicifacies* in the laboratory. Hence the study of genetic constitution of Dieldrin-resistant strain of *A. culicifacies* seems to be extremely difficult, if not impossible. There is, therefore, no course left but to assume that Dieldrin-resistance in *A. culicifacies*, like that of anopheline mosquitoes so far studied, could probably be due to a dominant or semi-dominant gene. If this assumption is taken as correct, it is intriguing to find that the Dieldrin-resistance in *A. culicifacies* in the field population gets reversed when Dieldrin pressure is withdrawn, in spite of the fact that the character of Dieldrin-resistance is likely to be due to dominant or semi-dominant gene. The Hardy-Weinberg law (Sinnott, Dunn and Dobzhansky, 1958) of genetics of population would also suggest no reversion in Dieldrin-resistance of the field

population of *A. culicifacies* once the population has achieved genetic-equilibrium, provided random matings are occurring in the population and there is absence of the factors changing gene frequency, irrespective of the fact whether Dieldrin-resistance character is of dominant nature or a recessive one. Hence it is probable that the genetic equilibrium is not achieved in the field population of the resistant strain of *A. culicifacies* at the time of withdrawing of Dieldrin pressure or, probably the Dieldrin-susceptible population seems to be more adaptable to the environments than the Dieldrin-resistant population so that in course of time, after the withdrawal of Dieldrin pressure, the Dieldrin-susceptible population replaces completely the Dieldrin-resistant population, thus resulting in complete reversion of the Dieldrin-resistant strain of *A. culicifacies* to the original normal Dieldrin-susceptible strain. The other factors which would probably cause this reversion would be : (*i*) Mutation, (*ii*) Transgression of Dieldrin-susceptible population from adjoining areas, and (iii) Genetic drift. While the first two of these factors seem to be most unlikely in our case, the third factor could possibly play a fair part in bringing about this reversion. Whatever may be the reasons of reversion of Dieldrin-resistance in the field population of *A. culicifacies*, the occurrence of this phenomenon in the field is of some practical importance in the large-scale field application of the insecticides. Whether this observation, recorded by us, is of fundamental nature occurring in all places or is only applicable to this species in this particular area and under these particular circumstances, cannot be said. But such a thing has happened in some part of the world and is, therefore, of considerable importance.

The data presented has shown clearly that in spite of continuous DDT spraying after withdrawing Dieldrin pressure, the Dieldrin-resistance kept on reversing. This, therefore, suggests that the character of Dieldrin-resistance is quite independent of the character of DDT-resistance. Davidson (1963) also has found similar independent nature of DDT and Dieldrin-resistance in *A. quadrimaculatus* in the laboratory.

We have very limited experience on this phenomenon of reversion of resistance in case of DDT with the same species. We are not able to come to any definite conclusion with regards to reversion of DDT-resistance in *A. culicifacies*. However, we give the results of limited susceptibility tests carried out on DDT-resistant strain of *A. culicifacies* after the DDT pressure was withdrawn (Table III).

It would be observed from Table III that immediately after withdrawal of DDT-pressure and replacing it by BHC pressure a fair amount of reversion was noticed and *A. culicifacies* resistance to DDT came down to that of intermediate status. But there it has maintained itself for a further period of about two years and seems to be well stabilised at that level. There is no indication of its going down to normal susceptibility level so far. Similarly when DDT-pressure was withdrawn and no other insecticide was further used, it was observed that

TABLE III.

Results of susceptibility tests carried out on DDT-resistant strain of A. culicifacies.

Taluka.	Mortality rate : 2·0 per cent DDT D/T	2·0 per cent DDT Per cent kill.	4·0 per cent DDT D/T	4·0 per cent DDT Per cent kill.	Control. D/T	Control. Per cent kill.	Date of test.
Before DDT pressure was withdrawn in 1961 in Dhulia District.							
Dhulia	12/118	10·2	31/111	27·9	0/60	0·0	Dec. 6 and 7, 1960.
Shahada	15/60	25·0	32/60	53·3	0/20	0·0	Dec. 8, 1960.
Sakri	16/104	10·5*	43/118	30·6*	3/58	5·4	Dec. 5, 6 and 15, 1960.
Nandurbar	18/60	30·0	21/60	35·0	0/30	0·0	Dec. 8 and 9, 1960.
Akranimahal	15/134	11·2	28/126	22·2	0/60	0·0	Dec. 10, 11 and 16, 1960.
Akranimahal	..	..	11/65	16·9	1/22	4·5	Feb. 18, 1961.
Akranimahal	..	..	13/62	20·9	0/10	0·0	Feb. 18, 1961.
After DDT pressure was withdrawn in Dhulia District.							
Dhulia	25/39	64·0	21/24	87·5	0/10	0·0	Nov. 25, 1961.
Dhulia	21/60	35·0	43/60	71·7	0/35	0·0	Dec. 28, 29 and 30, 1962.
Dhulia	8/20	40·0	..	..	0/10	0·0	Feb. 20, 1963.
Shahada	32/51	62·7	22/30	73·3	0/15	0·0	Nov. 18, 1961.
Shahada	6/45	13·3	18/25	72·0	1/20	5·0	Feb. 26, 1963.
Sakri	33/40	82·5	24/25	96·0	0/10	0·0	Nov. 25, 1961.
Sakri	30/40	75·0	44/46	95·0	0/10	0·0	Nov. 26, 1961.
Sakri	8/11	72·7	..	..	0/10	0·0	Mar. 7, 1962.
Sakri	14/25	56·0	13/20	65·0	1/20	5·0	Feb. 22, 1963.
Nandurbar	51/60	85·0	..	..	0/15	0·0	Sep. 11, 1961.
Nandurbar	36/42	85·7	28/28	100·0	0/20	0·0	Mar. 6, 1962.
Nandurbar	34/40	85·0	20/20	100·0	0/10	0·0	Mar. 7, 1962.
Nandurbar	21/37	56·7	30/35	85·6	0/42	0·0	Oct. 24 and 25, 1962.
Nandurbar	26/60	43·3	48/60	80·0	0/40	0·0	Jan. 1 and 2, 1963.
Nandurbar	13/25	52·0	..	..	0/20	0·0	Feb. 22, 1963.
Akranimahal	30/44	68·1	20/25	80·0	0/20	0·0	Nov. 22 and 23, 1961.
Akranimahal	5/11	45·4	..	..	0/10	0·0	Mar. 4, 1962.
Akranimahal	7/13	53·8	..	..	0/10	0·0	Feb. 24, 1963.
Akranimahal	8/15	53·3	..	..	0/19	0·0	Feb. 25, 1963.

D/T = Number dead/Number treated.

* = Figures adjusted by applying Abbot's formula.

Note : BHC has been applied since 1961 and continued till the date of presenting this paper in December 1963.

resistance fell down to a very small extent only in course of a year as can be seen from the data of Buldhana District, reproduced in Table IV.

It would be seen from Table IV that the amount of reversion of DDT-resistance in *A. culicifacies* is very low, and the rate of reversion is extremely slow when DDT pressure is withdrawn, without substituting other insecticide in its place. It is doubtful whether at all it will reach the original susceptibility level of a normal susceptible strain in *A. culicifacies*.

DDT-resistance in *A. sundaicus* and other mosquitoes has been found to be of recessive nature. Assuming the similar nature of DDT-resistance in *A. culicifacies*, it is baffling to note that it is not completely reversing after withdrawal of

TABLE IV.

DDT susceptibility data in respect of Buldhana District, after various periods of the withdrawal of DDT pressure in October 1962 and without substituting any other insecticide.

Taluka / Village	Mortality rate : 2·0 per cent DDT		4·0 per cent DDT		Control		Date of test.
	D/T	Per cent kill.	D/T	Per cent kill.	D/T	Per cent kill.	
1	2	3	4	5	6	7	8
Mehekar / Mehekar	0/50	0·0	3/45	6·0	0/25	0·0	Jan. 23, 1963.
Mehekar / Mehekar	3/40	7·5	13/60	21·6	0/20	0·0	Oct. 10, 1963.
Chikhli / Dhad	1/30	3·3	0/30	0·0	1/20	5·2	Nov. 27 and 28, 1961.
Chikhli / Dhad	0/40	0·0	1/40	2·5	0/28	0·0	Jan. 21 and 22, 1963.
Chikhli / Dhad	..	..	6/50	12·0	0/20	0·0	Oct. 9, 1963.
Malkapur / Rajura	2/80	2·5	6/75	8·0	0/55	0·0	Nov. 28 to 30, 1961.
Malkapur / Rajura	1/60	1·6	2/60	3·3	0/40	0·0	Jan. 19 and 20, 1963.
Malkapur / Rajura	6/40	15·0	11/40	27·5	0/20	0·0	Oct. 8, 1963.

$\frac{D}{T} = \frac{\text{Number dead.}}{\text{Number treated.}}$

DDT pressure quickly to its original level of susceptibility. If probably it has achieved the degree of genetic equilibrium we may expect very slow rate of reversion or no reversion at all. But usually the field application of any insecticide, especially the residual intradomiciliary spraying unaccompanied by anti-larval insecticidal application, will always leave considerable room for dilution in the development of resistant population. Thus it is expected that the genetic equilibrium would probably be never achieved in the field populations of anopheline mosquitoes developing resistance to insecticides when intradomiciliary spraying is adopted. Thus there would always be a reversion of insecticidal resistance developed in anopheline mosquitoes in course of time after the withdrawal of the insecticidal pressure in such areas.

SUMMARY AND CONCLUSIONS

There was a considerable degree of reversion in the Dieldrin-resistance of field population of *A. culicifacies* after about one year of its manifestation in village

Potgaon, Thana District (Maharashtra State) after the Dieldrin pressure was withdrawn. It required a period of about three years to completely reverse the Dieldrin-resistance in the field population of the above mentioned species after the withdrawal of Dieldrin pressure. Such a reversion occurred in spite of the fact that DDT pressure was substituted in place of Dieldrin-pressure. This suggested the independent nature of Dieldrin-resistance to that of DDT-resistance. Also the appearance, disappearance and reappearance of Dieldrin-resistance in *A. culicifacies*, by applying Dieldrin pressure and withdrawing it and reapplying it in the field, was demonstrated.

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A NOTE ON THE NEED FOR MODIFICATION OF SPRAY SCHEDULE FOR MALARIA ERADICATION IN THE NORTHERN DISTRICTS OF MYSORE STATE ON THE BASIS OF SURVEILLANCE DATA

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SPRAY schedule for malaria eradication to be effective must be so drawn up as to intercept the transmission completely. The transmission season is generally determined by considering the following factors :—

1. Rainfall.
2. Density of vector mosquitoes.
3. Fever morbidity statistics in the dispensaries.
4. Infant parasite rate.
5. Gland/gut positivity in the vector mosquitoes.

In the attack phase units where malaria case finding mechanism is in progress, we also get another sensitive data showing the number of positive cases in each month. Epidemiological investigation of these positive cases reveals the precise dates of onset of the primary attack from which exact dates of transmission can be worked out easily. This has an additional advantage of supplying the information on low grade transmission whereas the infant parasite rate and positivity in the vector mosquitoes provide information usually when the transmission is more intense.

DDT spraying operation under the National Malaria Eradication Programme was commenced from 1958 in the endemic units and from 1959 in the hypoendemic units of Mysore State. The spray schedule adopted was on the data available at that time as per the information given in the *Manual of Malaria Eradication Operation—India (1960)*. Generally speaking, the response to this schedule was excellent in the southern half of the State but some transmission was persisting in the

northern districts of Mysore State even after 4 to 5 years of spray. Details based on the information given in the above mentioned *Manual* (1960) regarding the vector species, period of transmission, known at that time and the spray schedule adopted in these areas, are summarised in Table I.

TABLE I.

Summary of the then available information regarding the period of transmission and strategic spray calendar suggested in the Manual of Malaria Control Operation-India 1960. Each round covering a period of 2½ months ; dosage 100 mg. per sq. foot.

Unit	Vectors	Period of transmission	Spraying schedule
Bellary	*A. culicifacies* *A. fluviatilis*	July to November for areas coming under seasonal irrigation ; all through the year in perennial irrigation areas ; in other areas from July to October.	One round of spraying for the entire unit from second half of May, and second round in selected areas from October.
Belgaum	*A. culicifacies* *A. fluviatilis* *A. stephensi*	July to November and from July to March in areas near storage reservoirs.	First round for entire district from middle of June. Second round in selected areas from September.
Bijapur (Endemic and Hypoendemic)	*A. culicifacies* *A. fluviatilis* *A. stephensi*	July to November	First round for entire district from middle of June. Second round in selected areas from September.
Munirabad	*A. culicifacies* *A. fluviatilis*	July to November, and in the perennial irrigation areas all through the year.	First round for entire district from middle of June. Second round in selected areas from September.
Gulberga (Endemic and Hypoendemic)	*A. culicifacies* *A. fluviatilis*	July to November, and in certain hilly areas the season extends up to March.	First round for entire district from middle of June. Second round in selected areas from September.

A perusal of Table I would indicate that while in some areas two rounds of spray operation were undertaken, in others only one round was applied. This was on the assumption that the latter were hypoendemic areas with brief transmission season and that a single round would suffice.

The situation was reviewed in 1962 due to persistence of transmission, and the data collected through surveillance from 1960-1962 were analysed. From this analysis, monthwise distribution of positive cases and the peaks of malaria incidence were available. Careful study of these data showed that the period of transmission was not exactly the same as contained in Table I.

Summary of the data regarding the peaks of malaria incidence, probable period of transmission, amended spray schedule and the number of rounds recommended on the basis of these surveillance data, are mentioned in Table II.

TABLE II.

Actual peaks of malaria positive cases, probable period of transmission based on surveillance, and the amended spray schedule in the northern districts of Mysore State.

Units	Actual peaks of malaria parasite positive cases	Probable period of transmission.	AMENDED SPRAYING SCHEDULE : DATE OF COMMENCEMENT*.	
			First round. (2½ months each) From	Second round. From
Bidar (Gulberga) (Hypoendemic)	First one in July; second one in November.	May to September-October	1st March	15th July
Gulberga	First one in July ; second one in October-November.	April to September-October	1st March	15th July
Bijapur (Endemic)	One peak in March	May to September-October	1st March	15th July
Bijapur (Hypoendemic)	One peak in June ; another peak in November.	May to September-October	1st March	15th July
Belgaum	One peak in June ; another peak in October.	May to September-October	1st March	15th July
Manirabad	One peak in June ; another peak in November.	May to September-October	1st March	15th July
Bellary	One peak in June ; another in November	May to September-October	1st March	15th July

* Due to certain administrative difficulties, the first round was actually commenced from 1st April but completed by the middle of May.

It could be seen from Table II that two rounds of spray were advocated this time for all the areas in addition to the other changes suggested. This was necessary to cut the low transmission in the hypoendemic areas which was spread over 5 to 6 months. This modified programme was adopted during 1963 and the results were gratifying and practically the whole area has entered consolidation from 1964.

Ray (1963) scrutinised the spray schedule in the operationally difficult unit areas of different parts of India and suggested early commencement and shortening of the period of spray, mostly from the point of inaccessibility and difficulty of communication during monsoon. The main strategy was to avoid spray operation during heavy rains and yet the timing should be such as to retain the biological efficiency. However, he also suggested about early commencement of spray even in some plain areas. Thus it would appear that such changes are necessary both from the technical and operational point of view.

It is suggested that the transmission season and the spraying schedule be closely studied on the basis of surveillance data and epidemiological investigations in all the areas of persistent transmission and necessary changes be made without further delay to intercept the transmission completely.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. A.P. Ray, Director, National Malaria Eradication Programme, for his kind guidance and advice and to Dr. K.S. Nanjundiah, Deputy Director of Public Health, National Malaria Eradication Programme, Mysore, for many discussions and excellent co-operation extended by him.

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OBSERVATIONS ON URBAN FILARIASIS IN SITAPUR TOWN (UTTAR PRADESH)

BY

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[December 26, 1963].

INTRODUCTION

WHILE carrying out filaria surveys in the central zone of Uttar Pradesh, attempts were made to study the filariasis problem in urban and rural populations separately. Singh *et al.* (1963) have reported about the results of surveys in the rural areas of Sitapur District, while results of similar investigations in the urban area of Sitapur Town are presented here.

Sitapur Town is about fifty miles west of Lucknow and is situated on both banks of Sarayan River (Map 1). The town derives its name from Sita, the wife of Lord Rama, the holy God of Hindus, who is believed to have stayed there during her pilgrimage. This is almost a centrally located place in the district and lies on the upland plain. The town is badly effected by the devastating floods in wet years, otherwise the climate is generally healthy. The maximum temperature touches about 110°F during summer and reaches as low as 45°F during the winter months. The rainy season extends from the middle of June to end of September. The average rainfall for the district is about 45 inches. The relative humidity on an average is about 75 per cent.

PEOPLE AND THEIR OCCUPATION

The total population of the town is about 55,056 (1961 census) which is composed of people of various castes and sects engaged in business and government services. The town happens to be an important grain market with good facilities of transport, as it is connected both by rail and road with Bareilly and Lucknow. The town suffers from the handicap of having inadequate water supply and poor drainage system. A large number of dirty water collections are found along the streets and the road sides. The conditions prevailing, particularly in the old town, are very unhygienic.

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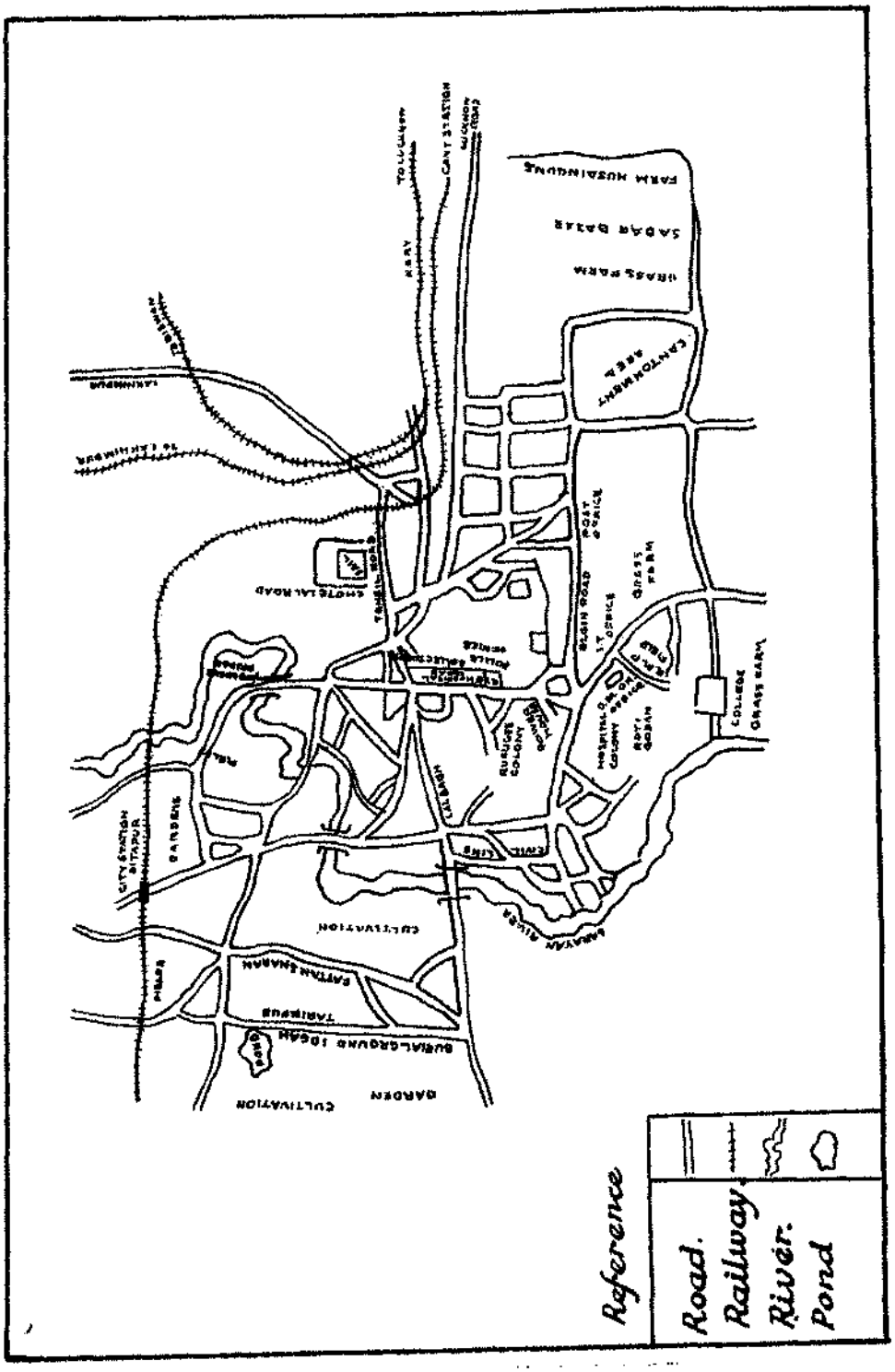

MAP OF SITAPUR TOWN.

MATERIAL AND METHODS

Attempts were made to inform people about the survey programme by distributing pamphlets and also by personal contacts by Insect Collectors during day-time while making mosquito collections. During the night between 8 p.m. to 12 p.m., thick blood smears (20 c.mm.) were collected from finger-tips of each individual examined. Cases showing disease manifestations were also examined at the same time. The dried blood films were stained the next day with J.S.B. Stain I (Jaswant Singh and Bhatacharji, 1944) and examined for microfilariae. Dissections of both *Culicine* and *Anopheles* mosquitoes were made for filarial infection. Collections of mosquito larvae were also made in order to study the types of breeding places.

RESULTS

During the present survey 4,216 persons of various age-groups and of both sexes were examined from the different mohallas of the town. The infection, disease and endemicity rates, average infestation per 20 c.mm. of blood and disease manifestations along with entomological observations, were recorded. The survey work was carried out during the year 1961-62.

Infection Rate.—The blood films obtained from 4,216 persons of all age-groups were examined and the results of examination, age-group wise, are shown in Table I.

TABLE I.

Infection rate and average infestation according to age.

Age-groups (Years).	Number of persons examined.	Number found positive for microfilaria.	Infection rate, per cent.	Average infestation per 20 c.mm. of blood.
0—1 ...	45	*Nil*	*Nil*	*Nil*
2—5 ...	202	1	0·49	1·00
6—10 ...	434	8	1·84	4·62
11—20 ...	1,076	57	5·29	5·21
21—30 ...	1,329	70	5·26	5·71
31—40 ...	637	46	6·83	5·15
41—50 ...	288	20	6·94	6·07
Above 50 ...	169	13	7·69	5·23
Total ...	4,216	215	5·09	5·46

Table I reveals that out of 4,216 persons examined, 215 showed presence of microfilariae in blood. The infection rate for all age-groups was 5·09 per cent, being highest in the age-groups of above fifty years (7·69 per cent). The average infestation per 20 c.mm. of blood was 5·46. The maximum number of microfilariae counted in a positive slide was 25. The lowest age at which the infection was detected was in a male child of four years. The type of infection detected was *W. bancrofti*.

DISEASE MANIFESTATIONS

Out of 4,216 persons examined, 116 were found having various disease processes of which hydrocoele and other genital affections were the most common, followed by elephantiasis of the limbs and chyluria. The disease rate works out to 2·75 per cent as shown in Table II. At the youngest age of four years, a female child was found having elephantiasis of the left lower limb.

TABLE II.

Disease manifestations according to various age-groups.

Age-groups (Years).	Number of persons examined.	Number of persons showing disease.	Disease rate (per cent).
0— 1	45	*Nil*	0·00
2— 5	202	2	0·99
6—10	434	*Nil*	0·00
11—20	1,076	22	2·04
21—30	1,329	41	3·08
31—40	637	31	4·59
41—50	288	12	4·16
Above 50	169	8	4·73
Total ...	4,216	116	2·75

The youngest age at which disease was noted was 4 years. The highest disease rate was noted in persons of 50 years and above.

The incidence of various disease manifestations observed in different age-groups and sexes is tabulated in Table III which shows that the hydrocoele and

TABLE III.

Disease manifestations by age-groups and sex.

Age-groups (years).	Disease manifestations: Hydrocoele and other genital lesions.		Right lower limb.		Left lower limb.		Right upper limb.		Left upper limb.		Chyluria.		Total.
	Male.	Female.	Male.	Female.	Male.	Female.	Male.	Female.	Male.	Female.	Male.	Female.	
0— 1	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
2— 5	1	..	..	..	1	..	..	..	..	..	..	..	2
6—10	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*
11—20	20	..	..	..	..	..	..	..	..	..	2	..	22
21—30	33	..	2	3	4	1	..	..	4	..	..	..	47
31—40	21	..	..	3	..	2	1	..	1	..	3	..	31
41—50	9	..	1	..	..	..	2	1	..	..	..	..	13
Above 50	7	..	..	..	..	..	..	..	1	..	..	..	8
Total ...	91	..	3	6	5	3	3	1	6	..	5	..	123*

* Different disease manifestations in the same individual have been counted separately, *i.e.* 116 persons have shown 123 disease manifestations.

other genital lesions were commonest, followed by affections of the limbs and chyluria. The apparent absence of genital affections in females is due to difficulty in making enquiries and examination of females.

ENDEMICITY RATE

On examining 4,216 blood films, collected from the town, 215 persons were found to have microfilaria in the blood while 116 persons exhibited disease manifestations of which 4 cases, all Hindus, had both disease as well as infection in their blood. It has been further noticed that a boy of 23 years was of the youngest age possessing the both. The incidence of simultaneous presence of the parasites and the disease manifestations in this town is highest in the age-group of 21 to 30 years. The average endemicity rate of the town is about 7·75 per cent. Details are presented in Table IV.

TABLE IV.

Endemicity rate according to different age-groups.

Age-groups.	Infection rate, per cent.	Disease rate, per cent.	Endemicity rate, per cent.
0—1 ...	0·00	0·00	0·00
2—5 ...	0·49	0·99	1·48
6—10 ...	1·84	0·00	1·84
11—20 ...	5·29	2·04	7·34
21—30 ...	5·26	3·08	8·12
31—40 ...	6·83	4·59	11·29
41—50 ...	6·94	4·16	11·11
Above 50 ...	7·69	4·73	12·42
Total ...	5·09	2·75	7·75

Further analysis of data of the infection, disease and endemicity rates amongst males and females, Hindus and Muslims, is shown in Tables V and VI.

It is further noted that the infection and endemicity rates are higher amongst males than the females. The infection rate being low in females by 47·7 per cent., as compared to males, is significant.

TABLE V.

Injection, disease, mixed and endemicity rates according to sex.

Sex.	Number of persons examined.	INFECTION RATE :		DISEASE RATE :		MIXED RATE :		Endemicity rate.
		Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	
Male	3,526	195	5·53	104	2·94	4	0·11	8·36
Female	690	20	2·89	12	1·73	..	0·0	4·63
Total	4,216	215	5·09	116	2·75	4	0·09	7·75

TABLE VI.

Infection, disease, mixed and endemicity rates according to religions.

Religion.	Numbers.	INFECTION RATE :		DISEASE RATE :		MIXED RATE :		Endemicity rate.
		Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	
Hindus	3,603	172	4·77	98	2·71	4	0·11	7·38
Muslims	613	43	7·01	18	2·93	*Nil*	0·00	9·95
Total ...	4,216	215	5·09	116	2·75	4	0·09	7·75

The above mentioned observations reveal that the infection rate, for the town as a whole, is higher amongst Muslims than Hindus.

ENTOMOLOGICAL FINDINGS

Mosquito collections, both adults and larvae, were made from various parts of the town and a total of 3,051 mosquitoes were obtained. Out of these, 1,889 were *Culex fatigans* while the rest were of different species of *Anopheles*. Dissections were made of the females of both the genera but out of 513 *C. fatigans* only 20 were found positive for infection. The infectivity rate was 0·97 per cent and infection rate 3·87 per cent. None of the *Anopheles* were found positive for infection. The results of dissections are shown in Table VII.

TABLE VII.

Number of mosquitoes caught, identified, dissected and found positive, and the stage of development.

Types of mosquitoes.	Number captured.	Number dissected.	Number found positive for infective larvae.	STAGES OF DEVELOPMENT :			
				I	II	III	IV
C. fatigans	1,889	513	20	*Nil*	7	11	5
Anopheles	1,162	401	*Nil*	*Nil*	*Nil*	*Nil*	*Nil*

Culex fatigans has been incriminated as the vector of the *W. bancrofti* infection in the town. Because of the inadequate water supply and poor drainage, numerous breeding places have been observed along the roads and near the houses. High larvae and adult density has been observed during the survey periods.

RECOMMENDATIONS

1. Anti-larval measures should be carried out in the town continuously throughout the whole year for the control of Bancroftian filariasis in the town.
2. Adequate under-ground drainage facilities should be provided.

SUMMARY

Filaria survey of Sitapur Town (Uttar Pradesh) was conducted during 1961-62 and 4,216 persons were examined for the presence of infection and disease.

The survey results reveal that Sitapur Town is moderately endemic for *W. bancrofti* infection. The infection rate worked out to 5·09 per cent. The average infestation per 20 c.mm. of blood was 5·46 and the highest number of microfilariae in a blood film was 25. The disease rate worked out to 2·75 per cent and the endemicity rate 7·75 per cent.

The main disease manifestations were of hydrocoele coupled with genital affections, followed by the elephantiasis of the limbs and chyluria.

Culex fatigans has been incriminated as the vector.

As worked out by Singh (1960) in the epidemiological surveys carried out in the State during the last ten years, the endemicity of *W. bancrofti* infection, which was believed to be originally restricted to eastern districts of the State, has now been found to be more widely distributed in the districts of central Uttar Pradesh also.

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Indian Journal of Malariology, **17**, 4, December 1963.

MORPHOLOGY OF THE ERYTHROCYTIC STAGE OF *PLASMODIUM INUI* HALBERSTADTER AND PROWAZEK, 1907, IN THE TOQUE MONKEY, *MACACA SINICA* FROM CEYLON.

BY

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[December 30, 1963.]

Four toque monkeys, *Macaca sinica*, were obtained from the Zoological Gardens, Colombo, for experimental work. These animals had been kept as pets before being sent to the zoo, so that it was not possible to trace the locality from which they had been caught. During the examination of blood films in February 1962, *Plasmodia* of *inui* type were seen in the blood of 3 of them. (Dissanaike, 1963, however was the first to report the presence of *Plasmodia* from monkeys, *Macaca sinica* and *Presbytis entellus thersites*, from Ceylon). Parasites were scanty in all the animals but all forms were present in the peripheral blood.

In this paper we have given a description of the erythrocytic form of the parasites from one of the monkeys (M3) in stained preparations.

METHODS

At first, the routine method of staining blood films for malaria for 10 minutes with Leishman's stain, using distilled water buffered to pH 7·4, was employed. These films showed a quartan type of parasite in cells which were free of stippling. Blackish brown granules of pigment, together with a more diffuse yellow pigment, were very obvious in parasites stained for this length of time, but stippling was not observed. Staining with Leishman's stain was therefore tried for a longer period of 15-20 minutes to determine whether stippling was present. This method revealed stippling of parasitised cells, but made the presence of pigment less obvious. Staining with Giemsa, however, showed that both stippling and pigment were well demonstrated.

For studying the morphology a minimum number of 50 cells of each stage was examined and the following description is based on these studies.

MORPHOLOGY

The smallest rings are 1·6 μ in diameter and have a fine hairlike ring of cytoplasm surrounding a small vacuole. The larger rings are up to 4·6 μ in diameter. The nuclear chromatin, in the majority, appeared as a rounded mass which projected beyond the ring ; in others it consisted of a large oval mass and in a few it formed part of the circumference of the ring (Plate I, Figs. 5-7). Approximately

20 per cent of rings had double chromatin dots, which were present at varying distances side by side or on opposite sides of the ring. Occasionally forms with projections from the cytoplasmic ring were seen, namely the stellate forms of Sinton (1934), *see* Plate I, Fig. 14. Multiple rings (two) were seen in approximately 6 per cent of cells. Sixty per cent of cells did not show stippling at this stage. Stippling was usually associated with large ring forms or with multiple rings, (Plate I, Figs. 4, 13 and 15); it had the appearance of clefts in some (Plate I, Figs. 7 and 12) and a few showed a fine stippling which was more profuse (Plate I, Fig. 6).

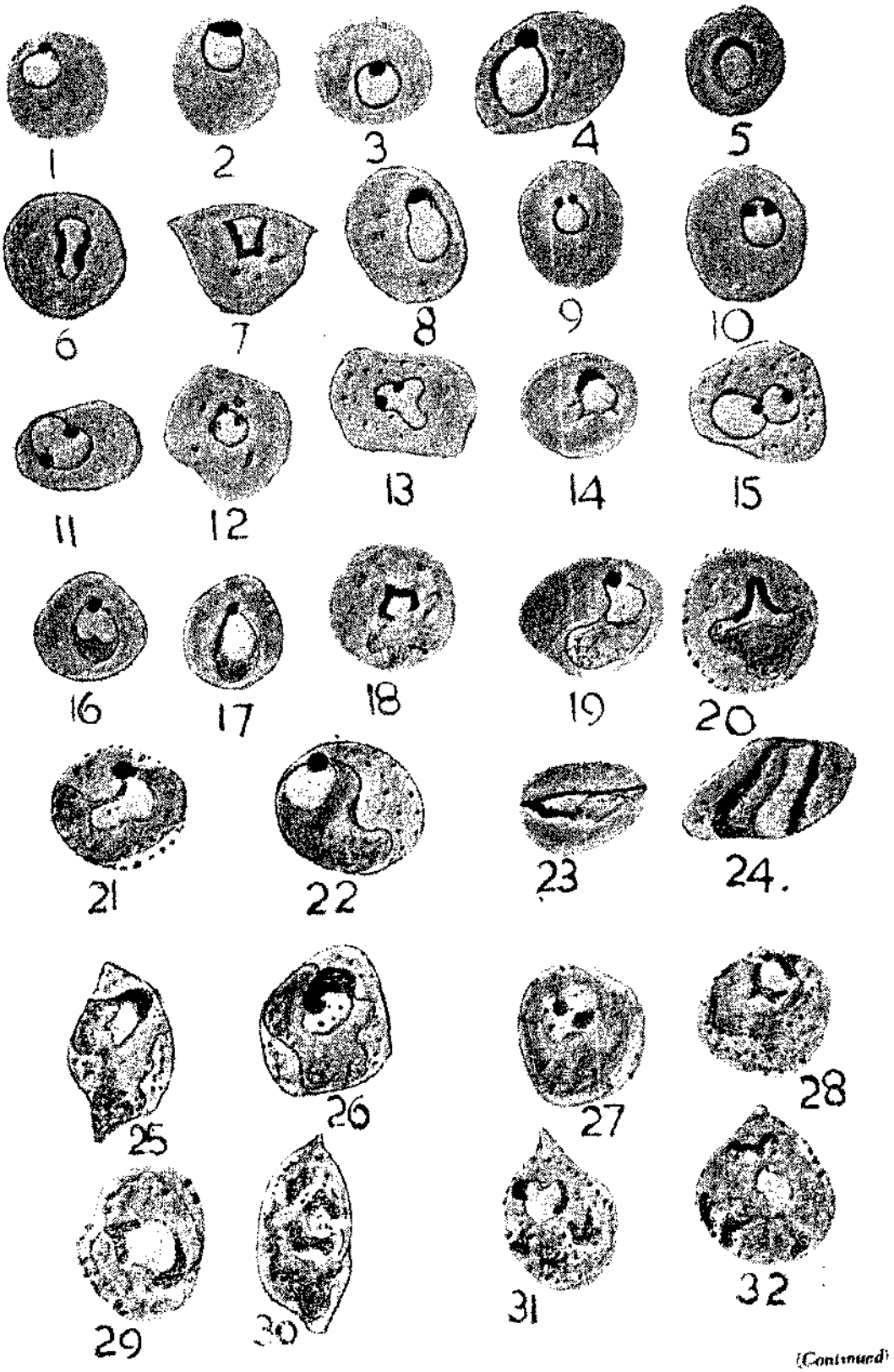
In the amoeboid stage the nuclear chromatin is increased gradually. The cytoplasm increases more rapidly and noticeably on the side opposite the nucleus. A common form seen at this stage consists of an arc of cytoplasm which narrows at the two ends to meet the nuclear chromatin. The middle of the parasite is occupied by a large vacuole—the nuclear vacuole. A pseudo vacuole in the cytoplasm was not seen by us probably due to light staining of the cytoplasm.

The wide blunt projections of cytoplasm described by Sinton (1934) were also seen in some parasites (Plate I, Fig. 18 and Plate II, Fig. 10). Fine granules of dark-brown to black pigment are visible at this stage and are typically at the periphery of the cytoplasmic ring. Prolonged staining with Leishman's stain, which was employed to bring out stippling, resulted in the pigment being less visible. Stippling was present in 94 per cent of cells examined at this stage, and consisted of large well circumscribed dots which, however, did not obscure the parasite. At this stage enlargement of parasitised cells was observed though not marked. This was because non-parasitised cells showed anisocytosis (Plate III) so that comparison with them did not help to detect enlargement of parasitised cells.

It is difficult to detect the beginning of schizogony owing to the presence of multiple broods of parasites. Cells with parasites, showing early division of chromatin, showed some degree of enlargement. The early projection of nuclear chromatin into the nuclear vacuole, as described by Sinton (1934), was not observed, but pairing of merozoites was seen in some in the early stages of division. Mature schizonts generally consisted of 16 merozoites and the parasite completely occupied the enlarged cell (Plate I, Fig. 36 and Plate II, Fig. 17). Some mature schizonts had only 8 merozoites and these did not occupy the entire cell; maturity was confirmed by the presence of rupturing cells (Plate I, Fig. 37 and Plate II, Fig. 18). Diffuse yellow pigment was seen usually in the centre of the parasites. Blackish-brown granules of pigment were also present which were larger than those in the amoeboid forms. Stippling of the cell was well-marked and large purplish red staining granules were present generally on the surface of the cell and thus, in some parasites, demarcating the peripheral cell wall. Typical daisy head arrangement of merozoites was not observed.

L. G. Jayewardene.

PLATE I.



(Continued)

PLATE 1 (*Concluded*).

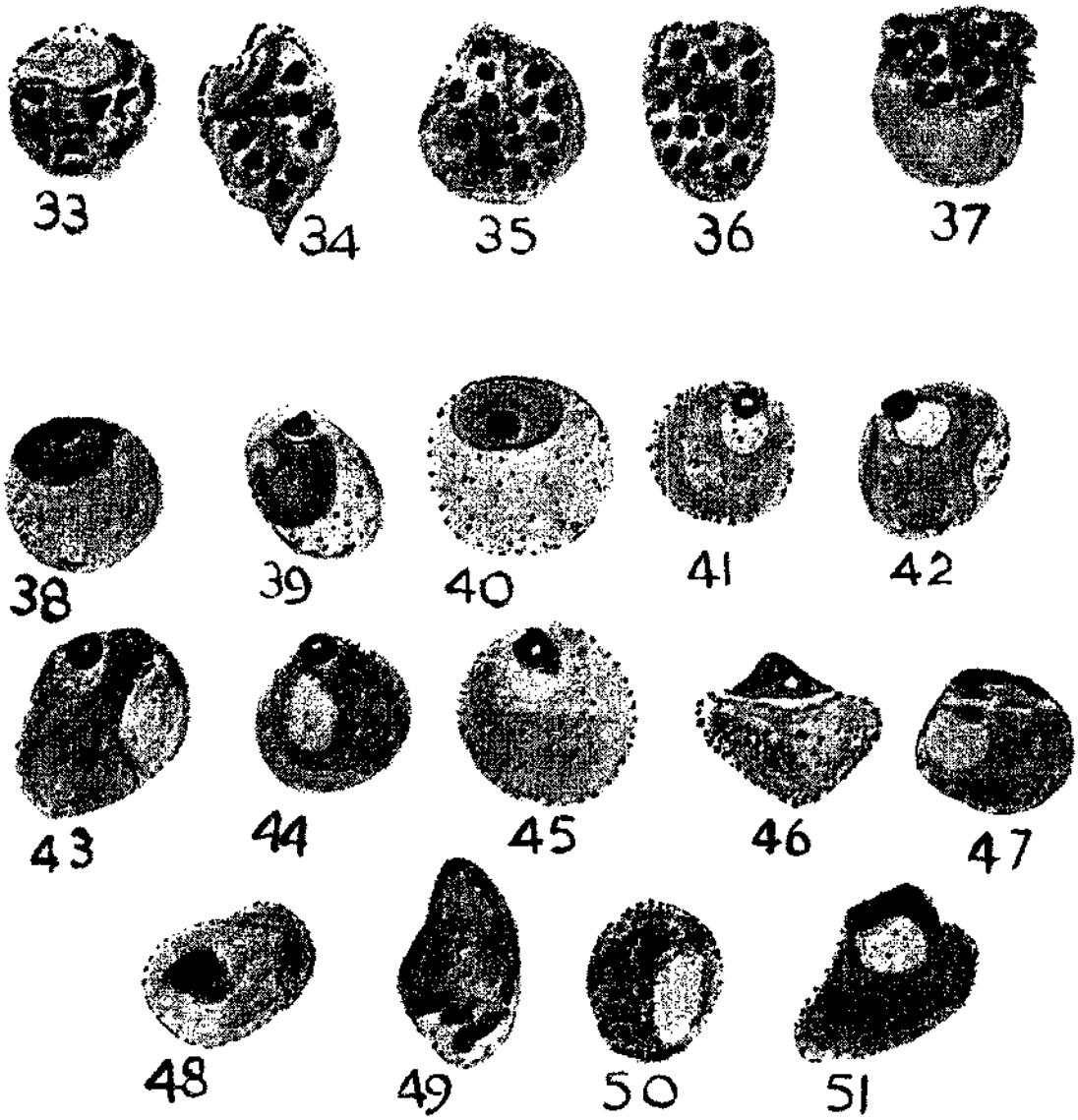


FIG. 1—15. Show variations in the morphology of the ring forms.
FIG. 1. Shows a typical ring.
Fig. 14. A 'stellar' form.
FIG. 16—17. Show increase of cytoplasm opposite the nucleus.
FIG. 18. Parasite with blunt wide pseudopodia.
FIG. 19—22. Growing forms with peripheral distribution of pigment in parasites.
FIG. 23—24. Small and large 'band' forms.
FIG. 25—26. Larger growing forms with increased nuclear chromatin.
FIG. 27—29. Early schizonts.
FIG. 30—35. Developing schizonts.
FIG. 36. Mature schizont with 16 merozoites.
FIG. 37. Rupturing schizont with 8 merozoites.
FIG. 38—40. Young gametocytes.
FIG. 41—45. Macrogametocytes.
FIG. 46—50. Microgametocytes.
FIG. 51. Macrogametocyte with arc shaped chromatin.

PLATE II.

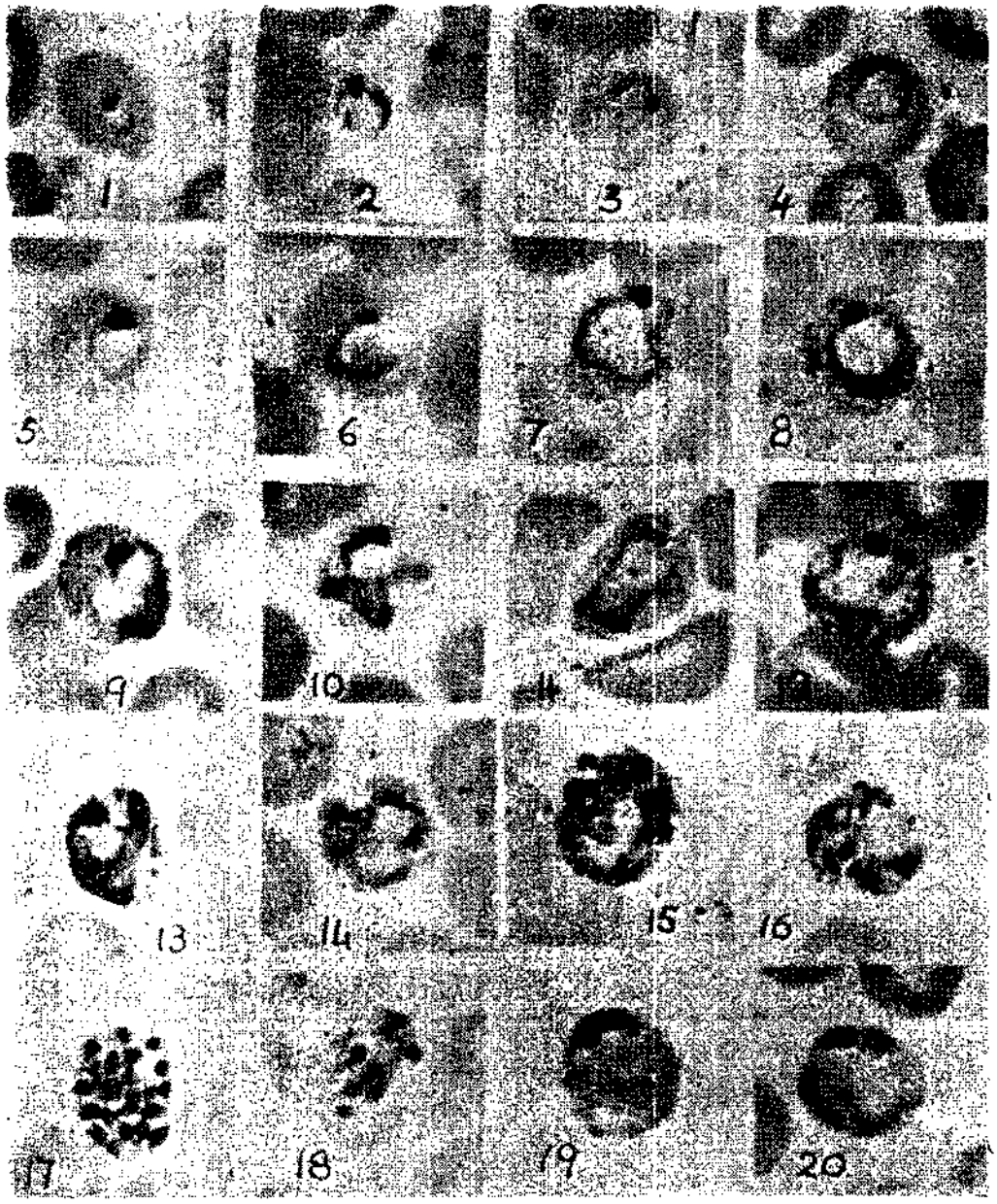
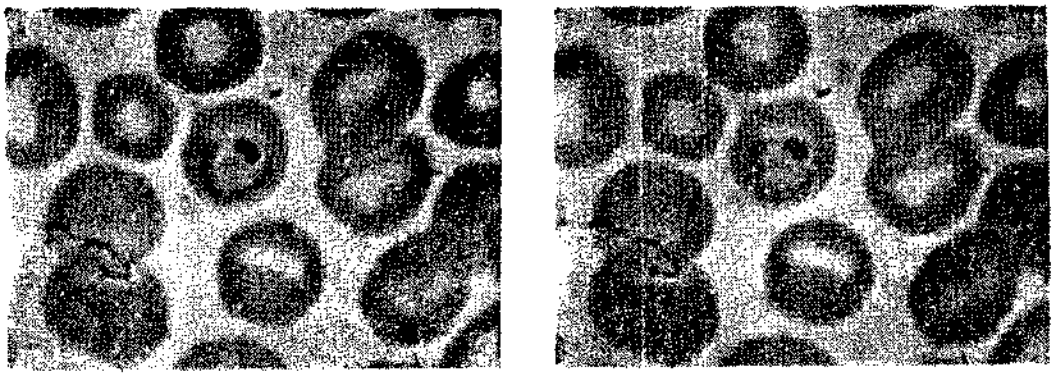


FIG. 1. Early ring.
FIG. 2. Larger ring with accessory chromatin.
FIG. 3— 4. Rings with double chromatin dots.
FIG. 5. Ring with large oval mass of nuclear chromatin, and stippling of red cell.
FIG. 6— 7. Large rings showing increase of cytoplasm.
FIG. 8— 12. Amoeboid forms.
FIG. 8. A typical crescentic form.
FIG. 10. Parasite with blunt wide pseudopodia.
FIG. 13—16. Early and developing schizonts.
FIG. 17. Mature schizonts with 16 merozoites.
FIG. 18. Mature schizont with 8 merozoites.
FIG. 19. Microgametocyte.
FIG. 20. Macrogametocyte.

PLATE III.



Blood film showing anisocytosis of red cells.

Female gametocytes were seen more often than male. They had a compact dense staining chromatin which was eccentrically placed and generally spherical in shape (Plate I, Figs. 41-45), sometimes the chromatin was arc-like (Plate I, Fig. 51). Often a small vacuole was seen close to the nucleus. The cytoplasm was not of an intense blue. Diffuse yellow and dark-brown pigment granules were well seen in mature gametocytes. Heavy stippling of the cell was also seen at maturity. Male gametocytes had a diffuse staining chromatin which was more often situated at the periphery (Plate I, Figs. 46, 47, 49 and 50) and less often in the centre of the parasite (Plate I, Fig. 48). Pigment and stippling was similar to that seen in the macrogametocytes. Developing gametocytes were recognised by the presence of compact dense staining cytoplasm and by the absence of the large vacuole so conspicuous in trophozoites (Plate I, Figs. 38-40). The average diameter of 18 cells containing mature macrogametocytes was 7·2 μ and that of 12 microgametocytes was 7·1 μ. Young forms were seen more often in microcytes.

DISCUSSION

We have not attempted to differentiate our parasite into a sub-species on the morphology of the stained erythrocytic forms. Eyles (1963) states that species differentiation was not possible by examination of blood films even by experienced observers. Moreover films, for comparison of the already described strains, were not available to us. Eyles (1963) reports that young forms of both the Sinton and South Indian strains were present in small cells (7·5 μ), older parasites were present in larger cells (7·8 μ). Enlargement of parasitised cells was also observed by us, the difference being that our young forms were present in microcytes averaging 6·5 μ in diameter and older parasites in larger cells of 7·3 μ in diameter. It may be possible to explain this difference in cell size by the marked anisocytosis of the cells present in our animal. This anisocytosis may have been due, in turn, to the chronicity of infection in the animal. Sinton's strain and the South Indian strain were

purified by serial passage so that infection was relatively new in the experimental animal for cell changes to be obvious. Our animals have had a low grade infection with multiple broods of parasites over several years, which we have been observing. Sinton and Mulligan (1933) have stated that "the diameter of red cells in apparently healthy specimens of *S. rhesus*, as determined in fresh and stained preparations, varied from 6·75 to 7·75 μ.....It is not, however, uncommon to find a considerable degree of anisocytosis in such animals". Our findings are similar. As Eyles (1963) emphasised, Sinton (1934) had observed enlargement of parasitised cells by comparing cells with young and old parasites and not by comparing parasitised with non-parasitised cells. Sinton (1934) states that young forms were common in microcytes and that this had also been observed by Leger and Bouilliez (1913). He states that enlargement of the cell was present at the time stippling appeared, which was in the amoeboid stage. Stippling was seen earlier with our parasites but enlargement of the cell was not obvious until development was more advanced. Sinton (1934) observed that "in old chronic infections where the parasites are scanty and anisocytosis is present, the change is much less strikingly shown". This is what was present in our animal.

Quartan periodicity of this parasite was established by counting the different stages present in serial blood films taken at 2 hourly intervals.

The infection did not appear to disturb the animal much though occasionally a febrile reaction with accompanying listlessness was observed.

The white cell counts of these animals have been observed over several years and have ranged from 11,000 to 21,000 per c.mm. The monocytes have been within normal limits, being from 1 to 2 per cent.

We think that this parasite is likely to be the South Indian strain of *Plasmodium inui*, namely *P. inui* subspecies *shortti*, owing to the relatively large discrete masses of stippling seen in cells with mature parasites. This should be confirmed but we are unable to do so at present.

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THE DISCIPLINE AND DYNAMICS OF ACTIVE CASE DETECTION PROCEDURE UNDER SURVEILLANCE OPERATION IN A MALARIA ERADICATION PROGRAMME

BY

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THE GENERAL BACKGROUND

SURVEILLANCE operation, which is generally introduced during the third year of the attack phase of a Malaria Eradication Campaign, embraces a number of activities like detection of malaria in the community by screening of all fever cases, institution of radical treatment of such patients with a view to sterilising the infection, investigation of the source of infection and undertaking such remedial measures as are necessary with the utmost speed and efficiency. In view of the very nature of activities like detection and mopping up of residual cases, surveillance operation helps in the acceleration of the pace programme. Besides, it provides an excellent tool in the measurement of malaria in a continuous way and, therefore, at the same time it can furnish valuable information in determining the end point of transmission and the time when the programme should be switched over from one phase to another. Ideally this operation could perhaps be initiated from the commencement of the attack phase to ensure early completion of the task. But under hyper-meso-endemic conditions this is hardly feasible as the entire machinery is likely to be disrupted on account of the enormous number of positive cases required to be dealt with at the initial stage of a campaign. Nevertheless, for known hypoendemic areas these activities could be ushered in along with the spray campaign from the beginning of the attack phase to hasten the process of malaria eradication.

As expressed earlier the activities envisaged under the surveillance operation are manifold and each aspect is interdependent on the other. Mere collection of blood smears by screening of fever cases would be meaningless unless there are efficient laboratory services for their rapid examination. Again epidemiological studies are of real value only when investigations are initiated promptly as and when a case is detected and remedial measures required to be taken must be prompt and of appropriate nature. But these are dependent on the accuracy and efficiency of epidemiological services. Furthermore, much depends on the thoroughness of screening of fever cases and the rapidity with which positive cases are detected. It would, therefore, be evident that the entire operation is not only complex in nature but needs a very dynamic approach.

THE BASIC PRINCIPLES

The basic philosophy behind the case detection procedure under an eradication programme would appear to be simple, though cannot be considered as original. In the past the experts on tropical diseases had persistently advocated examination of blood smears for malaria parasites in respect of all fever cases and that unless successive smears on three consecutive days showed negative results, all fever cases in the tropics were to be suspected as due to malaria in the first instance. That had been the teachings in this country as well as in many others. More or less this very principle is applied in an eradication programme though in an extended scale and in a comprehensive manner. If systematic screening is undertaken in respect of all fever cases in a community it should be possible to detect in course of a few years every malaria carrier. In the absence of fresh cases, as should be expected after application of residual insecticides like DDT over a period of 3 to 4 years, such mopping up operation under surveillance operation would finally result in the uprooting of the disease.

THE MECHANISM INVOLVED

Essentially a case detection mechanism aims at the collection of blood smears from every fever case and examination of the blood smears as amplified earlier. Normally such collection should not pose any problem through static agencies like hospitals, dispensaries, maternity and child health centres, rural health centres etc. This procedure, often called passive surveillance, has been given considerable importance in many countries where the medical and health facilities are extensive and adequate. While the same may be true under urban conditions in under-developed or developing countries, the facilities in rural areas are often few and far between. This means that fever cases have to cover long distances before they can find the necessary aid. In practice, however, only a few such patients could be expected to undertake such a journey for treatment of uncomplicated fever, particularly at the time of a bout of fever. Where communication and commutation facilities are meagre such instances are rare. As a rule they are either represented by friends or relatives at the dispensaries, particularly when the fever is of long duration. More often, however, they consult the local "medicine man" or quacks. Initially many take recourse to self medication. Observations made by a number of workers in India and Ceylon on the sphere of service and influence of the rural medical and health agencies, would indicate that nearly 90 per cent of the fever cases reporting to these agencies reside within a radius of $1\frac{1}{2}$ to 2 miles, beyond which the percentage of cases rapidly dwindles down to zero between 4 to 5 miles (Rao, 1961; Rao *et al.*, 1963; Mehta, 1963; Fredricksen, 1963; Kesavalu, 1963). Therefore, in a country like India there are vast tracts with millions of people who are left to their own resources for treatment of ailments like fever, as the agencies are located far apart from one another. Obviously a case detection procedure, based on the static agencies alone, could at best be localised and be of patchy nature, covering only a fraction of the entire population.

It may be stressed here that according to the definition, malaria eradication is said to have been accomplished when there is cessation of transmission for at least three years, of which at least two years must be in consolidation phase. This also emphasises the need for determining the end point of transmission, the most crucial stage of the operation. But it is not possible to do so unless the search has been careful, systematic and continuous, covering the entire community. It is for this reason that even a zero infant parasite index ceases to be of much significance as such a malariometric survey includes only a very small section of the community. As to the others, like child spleen and parasite indices, they are not sensitive enough to determine the transmission status in an area. Undoubtedly, therefore, there is the need for a machinery to cover every part of the country with a view to ensuring total coverage of the population in the search for malaria cases through a system of screening every fever patient.

This is provided by active case detection procedure through a system of domiciliary service at periodic intervals. Ideally speaking such domiciliary visits should be daily. But for reasons of economy and other factors this is not feasible. Therefore, a well balanced surveillance operation makes provision not only for case detection in active but also in passive forms. The latter is a continuous process and caters for those personnel who are likely to get fever in between the visits of the house visitors, even though the sphere of influence of the static agencies is of limited nature. Assistance through voluntary agencies like the medical practitioners, school teachers, etc., is also helpful in support of the procedures indicated above. But for reasons explained above, the active case detection mechanism is by far the most important of all for countries where the sphere of service of the static agencies does not extend over the entire community.

For regular and systematic domiciliary services it is essential to conduct a thorough geographical reconnaisance which should include enumeration of the people, recording and numbering of every single house of each rural and urban area. Particular stress is to be laid to include all hamlets in respect of the rural parts and the slum areas and peripheral belt of the urban areas. Data should also be collected on the method of approach to each section, the distance involved, communication and commutation facilities in dry season as well as during the rains, areas subject to periodic floods and alternate approach to such areas and similar other relevant information. These are of basic importance to determine the workload based on the number of houses and population as well as for preparation of time schedule for the house visitors and inspectors.

As to the duties of these personnel, the task is normally well defined. Each house is required to be visited at periodic interval as per determined time tables. During such visits enquiries are to be made whether any member of the house is suffering from fever or had history of recent fever in between the visits. If the answer is in the affirmative blood smears are to be taken of such patients and these are to be despatched to the laboratory assigned for microscopic examination.

Epidemiological investigations are required in respect of all positive cases which are also to be treated radically without the least possible delay. In every organisation it is essential to provide supervisory personnel at different echelons to ensure smooth and efficient running of the manifold activities. Considerable attention should also be focussed to the efficiency of laboratory services.

THE INDIAN PROGRAMME — ORGANISATIONAL PATTERN

The organisational pattern under the Indian Programme provides for a house visitor for every 10,000 people or approximately 2,000 houses, to be visited twice a month in a 12 day cycle. However, for operationally difficult terrain the work load is lessened and the reduction is proportional to the problems involved. Under extreme conditions the load is reduced to one tenth of the normal. The ambit of operation for each house visitor (surveillance worker) is called a section. Four such sections constitute a sector under supervision of a surveillance inspector. Normally there are 6 to 7 such sectors in a sub-unit under a Senior Malaria Inspector (assisted by a Junior Inspector). Four such sub-units constitute a unit (under a Unit Officer assisted by another, usually a non-medical officer), having its ambit of operation over a million population. Thus in a unit there are two Officers, 4 Senior Malaria Inspectors, 4 Junior Inspectors, 25 Surveillance Inspectors and 100 Surveillance Workers per million population. Units, which have a population slightly more than a million, have been provided with additional staff proportional to the extent of population. Each domiciliary worker has a fixed day-to-day time schedule and normally he is expected to visit about 150 to 160 houses a day. Besides the usual enquiries about fever cases or those giving history of fever, a few other questions are to be asked if the answers to the two primary questions are in the negative. In such cases enquiries should be made whether there had been no illness *at all* in the family during the period of 2 weeks. A fourth question is also asked about the presence of any other person in the house, perhaps a guest or a relative. However, the most important of all is the first and the second questions, that is, if any one is suffering from fever and whether there is any history of fever. It may also be added that the collection of smears is often reduced to half or even less if the second question is omitted. Such omission constitutes a great risk of missing the positive ones.

THE PROCEDURE

Prima facie it would seem to be an extremely difficult task for a surveillance worker to visit 150 to 160 houses a day, make the necessary enquiries in every house, take blood smears of all the fever cases encountered and maintain the records as prescribed. It is also true that collection of a blood smear is a break or handicap in the movement of the worker and, therefore, an abnormally high fever rate in an area may completely upset the time schedule. But normally this is within the physical limits in plains area where the population density is high. After the initial rounds the inmates of the houses (usually the housewife) know exactly the

purpose of the visit of the worker and the questions he is likely to ask. After several rounds of visits of the surveillance workers in an area it is a common experience to hear 'no' 'no' from the housewife even before the questions have actually been asked. Thus when the public relations between the surveillance staff and the beneficiaries to the programme are well established the workload assigned to each worker approaches the physical limits, particularly when the houses are not far apart. It may also be mentioned here that on an average the ambit of operation of a house visitor extends over about 15 villages. In a 12 day cycle he is, therefore, required to visit mostly one village a day and only on a few occasions more than one.

A Surveillance Inspector is required to visit every village once a month and under special conditions once in 6 weeks. During such visits he is to ensure the regularity of the visits of the House Visitors as per time schedule, that blood smears are actually collected from fever cases, the questionnaires are correct and so on. For this, he is to make concurrent and consecutive supervision and establish contacts with each of his four House Visitors once in five days and normally he is expected to visit every village once a month. But since he is required to cross-check a ten per cent of sample, that is, one in ten houses, the task assigned is within his physical limits. The Inspector is also primarily responsible for administration of primaquin for five days for radical treatment and compilation of data at peripheral level.

Prompt despatch of blood smears is also part of the responsibility of the Surveillance Worker. Normal procedure followed under the Indian Programme is to despatch the blood smears by post twice a week. This is also supplemented by despatch through other agencies like the passenger buses, messengers, etc. The Surveillance Inspector is required to check this aspect as well.

THE DISCIPLINE

At this stage it may be worthwhile to stress on the importance of maintaining strict time schedules for each individual House Visitor or Surveillance Inspector. Experience would show that for technical and administrative considerations, active case detection through domiciliary visits can be established within the shortest possible time when geographical reconnaissance has been thorough and the time-table worked for the surveillance staff is followed strictly. Since under the Indian Programme this has been found to be the essence for the stability of the machinery some further elaboration on this issue may be profitable for those programmes which are contemplating institution of such a procedure. During preparation of the time schedule attention should be focussed in full measure to the workload, physical capacity, distance involved, communication facilities or difficulties during the different seasons of the year in respect of each day of work and of each worker. If necessary, such time-table may need certain adjustments in the initial stages. But once finalised in consultation with the staff it must be strictly followed. Normally a fifteen minute margin is permissible in case there has been a delay in

the visit of such staff to a particular area. But for obvious reasons the staff must not be permitted to leave his work site before the actual scheduled time even if they might have finished their assignments earlier.

Thus it would be evident that domiciliary visits have to be brought to a discipline not only to facilitate regular and periodic visits according to routine laid down but also from the point of view of the beneficiaries of the programme who often expect and not infrequently await the arrival of the House Visitor. From detailed observations and also from interrogations the author firmly believes that when domiciliary visits are irregular and eratic or there is too frequent turn over of staff the people would soon lose interest in the whole procedure and would be reluctant to extend the necessary cooperation. But when such a service is well stabilised through a well-planned time schedule, many begin to depend on it and their cooperation is not wanting. In rural India, where in many parts medical and health facilities are few and far between, regular and periodic visits of the surveillance staff are welcome and important events in the placid life of the community. It is also well known that after several months the local House Visitor and the Inspector are considered as members of the community who are then prepared to depend on them. This also answers some of the questions which are often asked about the adequacy of cooperation from the public in a programme which involves such frequent visits for the same purpose, month after month and year in and year out.

THE METHOD OF CROSS-CHECKING AND SUPERVISION

In a complicated operation of this nature, particularly when an army of surveillance staff is employed (about 50,000 under the National Malaria Eradication Programme), it should be obvious that there must be an effective method and a suitable machinery for supervision. There are various indirect methods for assessing the activities of the staff, the most important of all is the rate of collection per house visitor per section per month, besides the total monthly collection in each sector, sub-unit and unit. For the fever rate in the community in the different parts of the country, as has been observed through surveillance operation for several years, it is now well established that the minimum collection should be 50 blood smears per month per surveillance worker with a workload of 10,000 population (60 fever cases per 1,000 per year). A rate below 50 indicates that the machinery needs some toning up. Immediate investigation is necessary when the range is between 30 to 40. Experience would also show that in such cases the questionnaire, particularly regarding the history of fever, is defective. This is usually attributed to lack of proper supervision. A rate lower than 30 smears per month, with a two weekly frequency, indicates that the visits are "irregularly irregular" (Ray, 1961) and large sections in the periphery are rarely visited. Such had been the observations in most of the units during the initial phase of the operation and also in a few areas in later stages where the discipline had broken down for one reason or other.

There are also a number of other indirect methods to evaluate the progress of active case detection. But in the final analysis direct cross-checking, through the entire supervisory echelons in the field, would appear to be inescapable not only as a method for actual verification but also to understand the inherent defects which may develop from time to time. Direct checking is carried out on sample basis through a cross-section of the community living in different parts of the urban or rural area, with particular emphasis laid on the verification of domiciliary visits in the periphery and slum areas of the towns or cities and in the hamlets of rural parts. In this endeavour attention is focussed to areas which are not approachable by motor transport or even on bicycle, but only on foot. Towards this end considerable guidance has been given earlier (Ray, 1961 *loc. cit.*).

It may also be of interest to note that enquiries from the men or children about the visits of the surveillance staff do not usually furnish accurate information regarding domiciliary visits. As men are often away from home and most school-going children are in schools they may corroborate the visit of the staff to the village or the ward of the town or city but as a rule they are uncertain about the frequency or periodicity of domiciliary visits. In such circumstances one is likely to hear very different versions of the case, one contradicting the other. On account of this, and in view of the volumes of experience gained, great stress has been laid under the Indian Programme to approach the housewife who usually furnishes the most accurate picture including the frequency of visits, if any visit has been missed by the staff, the types of questions asked, whether blood smears have been actually taken and if antimalarials either for presumptive or for radical treatment have been administered or left with the housewife. It is on this account that the author had indicated earlier that "while the history of spray operation is written on the ceilings of houses, the story of surveillance is in the lips of women."

For the above procedure, it is always necessary to institute a system of concurrent and consecutive supervision in which the latter must play a dominant role. Details of the procedure have been amply elaborated in *"Technical Directives and Administrative Guidance"* (Ray, 1963).

FREQUENCY OF DOMICILIARY SERVICES

While some attempt has been made to elaborate the dynamics and discipline of active case detection procedure, the picture will remain incomplete without a reference to the frequency of its domiciliary services. Although the need and the principles of such activities are well-recognised there would appear to be a great degree of divergence on the execution. In some programmes the active case detection machinery is operative right through the year even though the frequencies may vary, while in others the procedure is followed only during certain parts of the year, usually during the transmission season. The variations in the frequency of visits would seem to range from 5 to 10 days in respect of some country to as long as 3 months as followed by some programmes. However, in many countries the

domiciliary visits are at intervals of 2 to 8 weeks, the most common being 2 to 4 weekly visits (Pampana, 1963).

Although discussions on the relative merits of the pattern followed in different countries are not within the purview of the present paper, it may be worthwhile to analyse the rationale of the frequency to be adopted under active case detection procedure. It has been explained earlier that, besides evaluation of the effectiveness or otherwise of the insecticide, protective coverage and the prevailing epidemiological conditions in the area of operation, the case detection procedure in the attack phase lends support to the spray campaign in reducing the parasitic load in the community and thus hastening the process of interruption of malaria transmission. Therefore, at this stage it may simply be stressed that the sooner a case is detected and adequately dealt with the better it is, particularly in areas where transmission is prolonged or perennial. But in the consolidation phase the frequency of domiciliary visits, and the rapidity with which a case should be detected, have considerable significance. In the absence of insecticide protective coverage it is likely that the vector density will increase gradually or rapidly, depending on the vector involved. In respect of a vector like *A. culicifacies* the build-up is rapid and in the presence of a few positive cases the critical density is often high enough for resumption of transmission. In areas previously subjected to periodic epidemics, even a few undetected positive cases may give rise to major focal outbreaks. Such episodes have actually been observed in a few instances under the Indian Programme.

Thus in such circumstances it is not merely enough to indicate that case detection should be rapid but the time element should be clearly stipulated and that a case should be detected within the limit and necessary remedial measures taken forthwith. According to some authors, like Pampana (1962), the ideal frequency should be a daily domiciliary visit whereas in the other extreme there are programmes which follow a 8 or even 12 weekly frequency pattern. It is possible the financial implications have, to some extent, been responsible for the low frequency pattern adopted. It is obvious that the more frequent are the visits the more costly would be the programme. In view of these, the inevitable questions which may arise are whether, between the two extreme limits, there is any room for compromise and what should be the criteria to determine the optimum frequency ?

According to most authorities (Macdonald, 1957 ; Pampana, 1963, and others) the incubation intervals in respect of *P. vivax* and *P. falciparum* are 20 and 35 days respectively beyond which period, other conditions permitting, one should expect secondary cases. In other words an undetected or untreated *P. vivax* case would constitute a risk to the community beyond a period of 20 days. Such a risk in respect of *P. falciparum* would be after about 35 days. Therefore, purely on technical grounds, it should be necessary for a programme to set up such a machinery so as to enable it not only to detect a positive case within a period of

3 weeks but also to ensure thorough epidemiological investigation and the necessary remedial measures within that period. A frequency of 3 to 4 weekly intervals, as is adopted in some countries, may be considered strictly outside the permissible limit of 20 days, the incubation interval of *P. vivax*. Further it may be borne in mind that if one visit is missed for one reason or other the next would be after about 8 weeks. During transmission season, this will definitely constitute a risk to the community. However, it is usually argued that, under certain epidemiological conditions, in a four weekly visit the risk involved is not quite considerable and moreover it is more economical than a two-weekly frequency. The relative merits of a three and a four weekly visit are about the same though the latter should be more economical. As to the one and two weekly patterns, both are within the safety limits and, therefore, the latter should be preferred on the grounds of economy.

The question of frequency has been the subject of some degree of controversy for a long time and in order to find an answer, the World Health Organization had set up special teams, two in India and one in Ceylon. The frequency studies were related to two and four weekly patterns in comparatively small communities. However, there was no attempt to compare the financial aspects as the workload of the House Visitors remained the same in both groups. The data did not present any significant dfference between the two frequency groups. But since these studies were undertaken under special conditions in smaller communities with extraordinary facilities for supervision, it was considered necessary that the National Malaria Eradication Programme organization should institute large-scale investigations under the existing normal working conditions. Accordingly in 1962 a network of such studies was developed under which in every group of ten units, with 40 sub-units, one sub-unit followed a four-weekly pattern while the rest continued the standard two-weekly frequency. In order to understand the economical implications the workload was doubled in the 4-weekly areas. While the details are being published elsewhere, the observations may be summed up as follows : In the 2-weekly frequency areas, the rate of collection was 1·35 to 1·46 times higher as compared to the 4-weekly areas. What is perhaps more significant was the higher parasite rate in the two-weekly areas which varied from 4 to 5 times. The factors responsible for the wide difference are attributable to "memory lapses" in respect of the past fever on account of the long interval between the two visits under the 4-weekly pattern and also to some extent to the failure to contact cases. If one visit was missed in a four-weekly visit the next contact could be made only after 2 months. The chances of "memory lapses" would be much more under such a situation.

Since the main principle behind the case detection procedure is to collect blood smears from every fever case and as all such cases are to be presumed to be suffering from malaria as explained earlier, it should be obvious that there must exist a direct correlation between fever and positive cases. This would imply that the more the fever cases are detected and blood smears examined the more would

be the chance of finding positive cases unless the stage has reached when malaria has been eradicated from an area. Therefore, as a corollary it should be enunciated that unless such a stage is reached, the more fever cases are missed the more would be the chances of missing positive cases. Since in a two-weekly frequency the collection of fever cases is significantly higher than that in the four-weekly group, the merit of a two-weekly frequency is unquestionable and, moreover, it is also within the safe limits of incubation intervals. At this stage it may be stressed that the incubation interval factor has considerable significance under Indian conditions because, in areas where malaria is unstable, *A. culicifacies* is generally the vector and its build-up is extremely rapid, particularly after interruption of insecticide spray operation or, where conditions permit, fairly high density of vector during major part of the year. In areas where malaria is stable and endemic or hyperendemic conditions prevail, the transmission is usually prolonged, and the vector may be *A. minimus* or *A. fluviatilis* having a much lower critical density. Further in such areas often *A. culicifacies* may also play some role along with *A. fluviatilis*. In view of these factors, a frequency of 2-weekly visit under the Indian Programme would appear to be inescapable.

LABORATORY SERVICES

Since collection of blood smears from fever cases is as important as their microscopic examination and diagnosis of the species of plasmodium in positive cases, laboratory services constitute an integral element of the case detection procedure. While detailed discussion on the subject is outside the scope of the present communication, it is worthwhile to lay emphasis on certain aspects. The need of adequate strength of microscopists (depending on the workload) and their training status should be appreciated from the beginning so that smears could be examined as fast as they are collected and received in the laboratories. A back-log of unexamined blood smears will not only delay the institution of radical treatment of positive cases, but also the epidemiological services on which depend the various remedial measures necessary. Further, the laboratory should have enough potentialities to deal with sudden additional load of blood smears collected during mass blood survey or on account of abnormal increase in the incidence of fever in a particular community. Delay in such cases will render the entire surveillance procedure infructuous.

It may also be stressed that, however, efficient a laboratory might be there should be adequate and outside cross-checking agencies. The need for such counter-checking increases appreciably as the programme advances. At this stage the chances of inadvertant missing of a few odd positive cases are far too many since day after day the technicians have to examine more and more negative smears. Introduction of positive blood smears in the field collection, without the knowledge of the technicians, is adopted by many workers as a supplementary method to keep the technicians on their toes.

The Indian experience would also show that even the best laboratory needs constant supervision in all its activities, including the dehaemoglobinisation, proper

flushing, staining, etc. It has been found too often that the better the laboratory services and facilities for cross-checking the less are the chances of missing positive cases.

EPIDEMIOLOGICAL SERVICES

It is customary in all programmes to initiate epidemiological studies from the beginning, commencing from the preparatory phase. Although different countries adopt various procedures, most pin their faith on the classical malariometric survey during the initial stages including the early attack phase. This is usually supported by morbidity data from hospitals, dispensaries, etc. Besides, certain basic entomological investigations are also undertaken. Such studies are primarily to determine the efficacy of insecticide application.

However, much more accurate information is available after initiation of the surveillance operation through which it is possible to determine the actual number of positive cases from each locality, sector or section within a short time. Detection of confirmed cases also facilitates rapid epidemiological investigation to determine the source of infection, and the various remedial measures necessary in respect of each case. Special investigations like mass blood survey, routine, stratified or biased, constitute an essential feature of surveillance operation, so also certain entomological studies. Biased mass blood surveys have been recently introduced under the Indian Programme with the deliberate object of trying to detect positive cases in certain vulnerable or doubtful areas. In the absence of an intricate and close network of medical and health agencies, active case detection provides the maximum opportunity not only for early detection of positive cases, but also for undertaking specific epidemiological investigations and prompt remedial measures.

In the absence of spray protective coverage as in the consolidation phase the performance has to depend mainly on the case detection organisation and it is obvious that concentrated attention should be focussed on surveillance operation and a high standard of epidemiological services. Careful epidemiological investigation and classification of every confirmed case at this stage must be ensured to facilitate taking up prompt remedial measures so necessary for the success of this campaign.

SUMMING UP

From the foregoing it would be amply evident that the activities under active case detection procedure are manifold, complex and need a dynamic approach. The necessity of routine periodic visits to the same houses in the same locality, month after month and for a number of years, calls for a high degree of discipline. As the task becomes often monotonous and as the staff may become gradually complascent when positive cases are few and far between, supervision of the entire operation needs to be tightened up more and more, so also the cross-checking of blood smears. Further the lacunae and the pitfalls in this operation are so many

that only a continuous and intensive vigilence can ensure an effective service. It may also be stressed that the financial implications are quite considerable, particularly when the frequency of visits is short.

However, it should be borne in mind that it is only the active procedure which can ensure total coverage in respect of every section and sector. This is not feasible in passive surveillance for obvious reasons. Moreover, the patients who are very ill or those having slight temperature are not likely to attend the hospitals and dispensaries even though they may be adequate in number. It may also be stressed that the task of convincing each and every medical practitioner about the need for taking blood smears from all cases with fever or giving history of fever, is not easy. It is often necessary for the malaria eradication staff to approach these agencies frequently and also to assist them in microscopic examination of smears. In some instances the staff are required to assist the overburdened clinicians to take blood smears, a procedure often termed "activated passive surveillance". In view of these problems it would not be wise to depend wholly on passive case detection even if a country may have developed a net-work of such agencies. In any case since these agencies are not employed under the malaria eradication organisation, strict supervision of the activities is needed (Pampana, 1963 *loc. cit.*).

In view of these considerations and on the basis of the experience gained during the past few years, most workers in the field have come to the conclusion that in developing and under-developed countries active case detection mechanism must play a dominant role and it should be supported by passive surveillance. Even if there is a net-work of such passive case detection agencies, active case detection would be essential to ensure total coverage in space. As regards the frequency of domiciliary services, technical considerations like incubation interval, epidemiological features, the speed of the laboratory and epidemiological services and similar other factors should have over-riding priority over all other issues in order to ensure rapid eradication of the disease.

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